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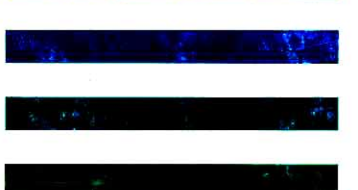

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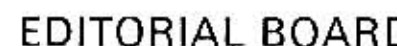

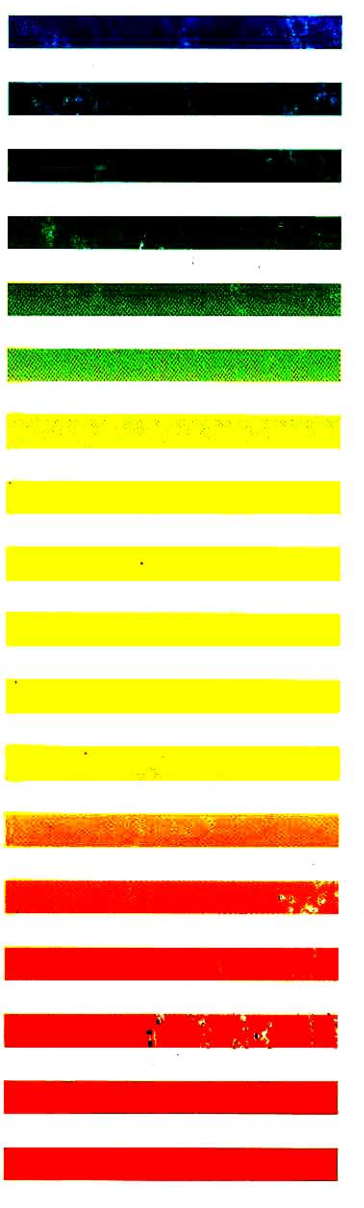

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For Contents, see p. VII

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CHROMATOGRAPHY IN ENVIRONMENTAL ANALYSIS

PART II

The papers submitted for the Special Volume on *Chromatography in Environmental Analysis* are published in two consequtive volumes of the *Journal of Chromatography*: Vols. 642 and 643 (1993). The Preface only appears in Vol. 642. A combined Author Index to both Vol. 642 and 643 only appears in Vol. 643.

VOL. 643 NOS. 1+2 JOURNAL OF CHROMATOGRAPHY JULY 23, 1993

CONTENTS

CHROMATOGRAPHY IN ENVIRONMENTAL ANALYSIS, PART II

APPLICATIONS

Chromatographic techniques used to determine benz[*c*]acridines in environmental samples (Review)
by N. Motohashi and K. Kamata (Tokyo, Japan) and R. Meyer (Irvine, CA, USA) 1

Air pollution

Sampling and analysis of volatile organic compounds in estuarine air by gas chromatography and mass spectrometry
by A. P. Bianchi (Milton Keynes, UK) and M. S. Varney (Southampton, UK) 11

Practical method for monitoring polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in the atmosphere
by K. Kuwata, Y. Yamashita, S. Nakashima, Y. Nakato, T. Kohno, S. Tanaka, T. Okumura and Y. Yamaguchi (Osaka, Japan) 25

Determination of volatile amines in air by diffusive sampling, thiourea formation and high-performance liquid chromatography
by R. Lindahl, J.-O. Levin and K. Andersson (Umeå, Sweden) 35

Selective clean-up for polynuclear aromatic compounds in airborne particles and soil
by T. Spitzer (Helmstedt, Germany) 43

Evaluation of a modified Marcali technique with high-performance liquid chromatography–ultraviolet detection for the determination of 2,4-toluene diisocyanate in air
by M. Colli and L. Zabarini (Monza, Italy) and G. V. Melzi d'Eril and R. Marchetti (Pavia, Italy) 51

Identification and determination of biogenic and anthropogenic volatile organic compounds in forest areas of Northern and Southern Europe and a remote site of the Himalaya region by high-resolution gas chromatography–mass spectrometry
by P. Ciccioli, E. Brancaleoni, A. Cecinato, R. Sparapani and M. Frattoni (Monterotondo Scalo, Italy) 55

Assessment of ambient volatile hydrocarbons from tobacco smoke and from vehicle emissions
by G. Barrefors and G. Petersson (Gothenburg, Sweden) 71

Evaluation of capillary gas chromatography for the measurement of C_2–C_{10} hydrocarbons in urban air samples for air pollution research
by J. Y. K. Lai, E. Matisová, D. He, E. Singer and H. Niki (North Ontario, Canada) 77

Study of polychlorinated dibenzodioxins and furans from municipal waste incinerator emissions in the Netherlands: analytical methods and levels in the environment and human food chain (Review)
by A. P. J. M. de Jong, A. K. D. Liem and R. Hoogerbrugge (Bilthoven, Netherlands) 91

Detection of airborne cocaine and heroin by high-throughput liquid-absorption preconcentration and liquid chromatography–electrochemical detection
by S. Zaromb, J. Alcaraz and D. Lawson (Argonne, IL, USA) and C. S. Woo (Cedar Falls, IA, USA) 107

Water pollution

Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water (Review)
by D. Barceló (Barcelona, Spain) 117

Pollutants in drinking water and waste water
by H. Fr. Schröder (Aachen, Germany) 145

Determination of ethylenethiourea in water samples by gas chromatography with alkali flame ionization detection and mass spectrometric confirmation
by J. M. van der Poll, G. G. Versluis-de Haan and O. de Wilde (Zeist, Netherlands) 163

Determination of alkylbenzenesulphonates in environmental water by anion-exchange chromatography
by Y. Yokoyama, M. Kondo and H. Sato (Yokohama, Japan) 169

Screening methods for asulam, oxine-copper and thiram in water by high-performance liquid chromatography after enrichment with a minicolumn
by T. Suzuki, K. Yaguchi and I. Kano (Tokyo, Japan) 173

ห้องสมุดกรมวิทยาศาสตร์บริการ

1 7 ส.ค. 2536

Chromatographic methods for the analysis of size-classified and individual raindrops
by K. Bächmann, I. Haag, T. Prokop, A. Röder and P. Wagner (Darmstadt, Germany) 181

Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia
by P. D. Nichols, R. Leeming, M. S. Rayner and V. Latham (Hobart, Australia) and N. J. Ashbolt and C. Turner (West Ryde, Australia) 189

Determination of pesticides in river water by gas chromatography–mass spectrometry–selected-ion monitoring
by H. Kobayashi, K. Ohyama, N. Tomiyama, Y. Jimbo, O. Matano and S. Goto (Ibaraki, Japan) 197

Solid-phase extraction followed by high-performance liquid chromatographic analysis for monitoring herbicides in drinking water
by A. Balinova (Kostinbrod, Bulgaria) 203

Determination of resin acids by gas chromatography and high-performance liquid chromatography in paper mill effluent, river waters and sediments from the upper Derwent Estuary, Tasmania
by J. K. Volkman and D. G. Holdsworth (Hobart, Australia) and D. E. Richardson (Boyer, Australia) 209

Determination of the potent mutagen 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX) in water by gas chromatography with electron-capture detection
by S. Ogawa, H. Kita, Y. Hanasaki and S. Fukui (Kyoto, Japan) and H. Kami (Shiga, Japan) 221

Determination of chlorophenoxy and other acidic herbicide residues in ground water by capillary gas chromatography of their alkyl esters formed by rapid derivatization using various chloroformates
by S. Butz and H.-J. Stan (Berlin, Germany) 227

Gas chromatographic determination of halogenated organic compounds in water and sediment in the Skagerrak
by K. Abrahamsson and A. Ekdahl (Gothenburg, Sweden) 239

Residue analysis

Determination of benomyl and its degradation products by chromatographic methods in water, wettable powder formulations, and crops (Review)
by R. P. Singh (St. Catharines, Canada) and M. Chiba (Vineland Station, Canada) 249

Use of insoluble polyvinylpyrrolidone and isoelectric focusing in the study of humic substances in soils and organic wastes (Review)
by C. Ciavatta and M. Govi (Bologna, Italy) 261

Thin-layer chromatographic methods for use in pesticide residue analysis (Review)
by H. S. Rathore and T. Begum (Aligarh, India) 271

Chromatographic methods in the determination of herbicide residues in crops, food and environmental samples (Review)
by J. Tekel' and J. Kovačičová (Bratislava, Slovak Republic) 291

Residue levels of polynuclear aromatic compounds in urban surface soil from Japan
by T. Spitzer (Helmstedt, Germany) and S. Kuwatsuka (Nagoya, Japan) 305

High-performance liquid chromatographic method for the determination of oxolinic acid residues in crops
by N. Shiga and O. Matano (Tokyo, Japan) 311

Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high-performance liquid chromatography with the use of fluorescence detection and a single *o*-phthalaldehyde post-column reaction
by V. A. Simon, K. S. Pearson and A. Taylor (Jacksonville, FL, USA) 317

Thin-layer chromatographic behaviour of carbamate pesticides and related compounds
by H. S. Rathore and T. Begum (Aligarh, India) 321

Separation of some chlorophenoxyacetic acid congeners on a porous graphitized carbon column
by T. Cserháti and E. Forgács (Budapest, Hungary) 331

Determination of diflubenzuron residues in water by solid-phase extraction and quantitative high-performance thin-layer chromatography
by J. Sherma and C. Rolfe (Easton, PA, USA) 337

Efficient screening method for determining base/neutral and acidic semi-volatile organic priority pollutants in sediments
by W. M. Davis, J. A. Coates, K. L. Garcia, L. L. Signorella and J. J. Delfino (Gainesville, FL, USA) 341

High-performance liquid chromatographic analysis of carbofuran residues in tomatoes grown in hydroponics
by C. F. Ling, G. P. Melian and F. Jiminez-Conde (Las Palmas de Gran Canaria, Spain) and E. Revilla (Madrid, Spain) 351

Mobility of cadmium as influenced by soil properties, studied by soil thin-layer chromatography
by M. Sánchez-Camazano and M. J. Sánchez-Martín (Salamanca, Spain) 357

Application of solid-phase partition cartridges in the determination of fungicide residues in vegetable samples
by A. Di Muccio, R. Dommarco, D. A. Barbini, A. Santilio, S. Girolimetti, A. Ausili, M. Ventriglia, T. Generali and L. Vergori (Rome, Italy) 363

Liquid chromatographic analysis of antibacterial drug residues in food products of animal origin (Review)
by B. Shaikh and W. A. Moats (Beltsville, MD, USA) 369

Organic micropollutants in Swiss sewage sludge
by P. Frost (Zurich, Switzerland), R. Camenzind and A. Mägert (Belp, Switzerland), R. Bonjour (Liebefeld-Berne, Switzerland) and G. Karlaganis (Berne, Switzerland) 379

Biochemical analysis

Chromatographic determination of volatile solvents and their metabolites in urine for monitoring occupational exposure (Review)
by A. Astier (Créteil, France) 389

Environmental analysis of polychlorinated terphenyls: distribution in shellfish from the Ebro Delta (Mediterranean)
by M. T. Galceran, F. J. Santos, J. Caixach, F. Ventura and J. Rivera (Barcelona, Spain) 399

Rapid isolation of polychlorinated biphenyls from milk by a combination of supercritical-fluid extraction and supercritical-fluid chromatography
by A. G. Mills and T. M. Jefferies (Bath, UK) 409

Determination of paraquat in rat brain by high-performance liquid chromatography
by M. T. Corasaniti and G. Nisticò (Rome, Italy) 419

Ion chromatographic investigation of brown algae (*Fucus vesiculosus*) of the German Environmental Specimen Bank
by V. D. Nguyen and M. Rossbach (Jülich, Germany) 427

Plane tree bark as a passive sampler of polycylic aromatic hydrocarbons in an urban environment
by A. Sturaro, G. Parvoli and L. Doretti (Padova, Italy) 435

Identification and quantification of 18-nor- and 19-norditerpenes and their chlorinated analogues in samples of sediment and fish
by P.-Å. Hynning, M. Remberger and A. H. Neilson (Stockholm, Sweden) and P. Stanley (Jealott's Hill, UK) 439

AUTHOR INDEX VOLS. 642 AND 643 453

Journal of Chromatography, 643 (1993) 1–10
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Review

Chromatographic techniques used to determine benz[*c*]acridines in environmental samples

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ABSTRACT

Benz[*c*]acridine and many of its related compounds have been shown to exhibit carcinogenic activity. Unfortunately, these compounds are continually being found in many natural and environmental samples in widely divergent geographical locations. A review of chromatographic methods for mainly benz[*c*]acridine and its analogues is presented.

1. Introduction 2
2. Urban air 3
 2.1. Urban air particulates 3
 2.2. Additional air pollution source effluents 4
 2.3. Automobile exhaust 4
3. Petroleum distillates 5
 3.1. Coal-tar pitch 5
 3.2. Creosote 6
 3.3. High-boiling petroleum distillates 6
 3.4. Coal liquefaction products 6
4. Tobacco (smoke) 7
5. Sediments 7
 5.1. Lake sediment 7
 5.2. River sediment 7
 5.3. Marine sediment 7
 5.4. Groundwater 8
6. Food (ham) 9
References 9

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1. INTRODUCTION

Investigations into the composition of urban air are a recent phenomenon. It was not until the early 1950s that the 3,4-benzopyrene content of the urban atmosphere was reported [1,2]. Later, benzacridines of azaarenes were found in airborne particulates (Fig.1). Because of a lack of standard benzacridines with various attached alkyl groups, research in the biochemical and pollution fields lagged behind. This is important because the presence of a methyl group in a benzacridine molecule can cause a drastic change in its carcinogenic activity. For example, benz[*c*]acridine (**1**) and benz[*a*]-acridine (**2**) are inactive as carcinogens on mouse skin, whereas their 7-methyl derivatives demonstrate very strong activity. Many of the other methyl derivatives also show carcinogenic activity [3,4]. In addition to skin adsorption, inhalation and ingestion may serve as routes of entry of these compounds. Consequently, the metabolism of these benz[*c*]acridines may lead to active carcinogens. Mutagenicity studies on the metabolites of these compounds, in bacteria and mammalian cells, indicate that the position of the nitrogen heteroatom can markedly affect the mutagenic activity [5]. Among dibenzacridines, dibenz[*a,j*]acridine (**4**) and dibenz[*a,h*]acridine (**5**) have been shown to be weak carcinogens [6,7], and dibenz[*c,h*]acridine (**6**) has also been shown to be carcinogenic [6].

Many methods for the separation of benz[*c*]acridines have been investigated. For example, thin-layer chromatography (TLC) on silica gel 60 GF_{254} with benzene was used for the separation of a methyl-substituted benz[*c*]acridine mixture [8]. Reversed-phase high-performance TLC (RP-HPTLC) was also used for the same purpose [9]. Both gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) were applied for the separation of methyl-substituted benz[*c*]acridines [10]. Benz[*c*]acridines were separated by cation-exchange HPLC (CE-HPLC) with an ion-exchange column (Partisil 10 SCX) [11].

This paper reviews chromatographic methods for mainly benz[*c*]acridine (**1**) and analogues of these potentially carcinogenic and mutagenic benzacridines and dibenzacridines. Methods for the syntheses as standard benzacridines are not discussed here [12].

1. Benzacridines

benz[*c*]acridine (**1**)
(1,2-benzacridine)

benz[*a*]acridine (**2**)
(3,4-benzacridine)

2. Dibenzacridines

dibenz[*a,c*]acridine (**3**)
(1,2,3,4-dibenzacridine;
phenophenanthracridine)

dibenz[*a,j*]acridine (**4**)
(1,2,7,8-dibenzacridine;
3,4,6,7-dinaphthacridine;
β-naphthacridine)

dibenz[*a,h*]acridine (**5**)
(1,2,5,6-dibenzacridine;
α-N-β-dinaphth-
acridine)

dibenz[*c,h*]acridine (**6**)
(3,4,5,6-dibenzacridine;
α-naphthacridine;
α-N-a-dinaphthacridine)

Fig. 1. Structures of benzacridines and dibenzacridines.

2. URBAN AIR

2.1. Urban air particulates

Over the years, many researchers have presented methods for and results of air pollution studies. For example, benzacridines in air particulates from the effluents of air pollution sources were separated by column chromatography (CC) and TLC. By the use of the appropriate bands in their spectra, the benzacridines were determined by a baseline technique in combination with spectrofluorimetry of TLC fractions. Air pollution effluents of coal used for room heating showed that benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) were measured at concentrations of 15 and 18, 26 and 7.70, 17 and <0.12, and 2 and <0.015 mg per 1000 m^3 of gas, respectively [13]. Another study of an air pollution source of industrial effluents showed also benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**). These compounds were determined at concentrations of 60, 18, 0.7 and 1.8 mg per 1000 m^3 of gas, respectively [13].

Another study, using a Technicon time-flow fraction collector, demonstrated the CC separation of organic airborne particulate fractions. For detection of benzacridines in a 1-year composite airborne particulate sample from downtown Nashville, TN, USA, TLC was used with dimethylformamide (DMF)–water (35:65) on cellulose after alumina CC fractions of the basic fraction were collected. The fluorescent spot of benzacridines was treated with trifluoroacetic acid fumes. By this method, benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) were identified. Additionally, some of the alkyl derivatives of benz[*c*]acridine (**1**) and dibenz[*a,j*]acridine (**4**) could be carcinogenic (Table 1) [14].

Azaarenes, including benzacridines, were determined by gas chromatography (GC) with a glass capillary column coated with SE-52. The instrument was equipped with dual-channel flame ionization and electron-capture detectors, with a dual electrometer connected to the dual channels. Samples of *ca.* 0.3–0.5 g of dust collected from 1000–2000 m^3 of air were extracted in a Soxhlet extractor for 12 h with 100 ml of cyclohexane. Benz[*c*]acridine (**1**), 8,10-dimethylbenz[*c*]acridine (**7**), 10-methylbenz[*c*]acridine (**8**), 10-methylbenz[*a*]acridine (**9**) and 1,10-dimethylbenz[*a*]acridine (**10**) in the extracts were detected by GC on SE-52 [15].

Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**), dibenz[*a,j*]acridine (**4**) and their alkylated counterparts were found as potentially carcinogenic benzacridines in six downtown urban atmospheres in the USA [16].

A simple TLC procedure was used to determine benz[*c*]acridine (**1**) in pollutants associated with airborne particulates. Benz[*c*]acridine (**1**) was separated from crude benzene-soluble compounds using pentane–diethyl ether (19:1) on TLC plates coated with equal volumes of aluminium oxide G and silica gel G. Benz[*c*]acridine (**1**) was measured spectrofluorimetrically ($\lambda_{em.}$ 290 nm, $\lambda_{em.}$ 470 nm) in trifluoroacetic acid. Crude benzene-soluble compounds of particulates collected from 51 American cities in January–June, 1966, were analysed and concentration

TABLE 1

BENZACRIDINES IN AN AVERAGE AMERICAN URBAN ATMOSPHERE

Compound	Benzene-soluble fraction (μg/g)	Airborne particulates (μg/g)	In 1000 m^3 of air (μg)
Benzacridines			
Benz[*c*]acridine (**1**)	50	4	0.6
Benz[*a*]acridine (**2**)	20	2	0.2
Dibenzacridines			
Dibenz[*a,h*]acridine (**5**)	7	0.6	0.08
Dibenz[*a,j*]acridine (**4**)	4	0.3	0.04

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ranges of 0–1.5 μg per 1000 m^3 of air for benz[*c*]-acridine (**1**) were determined [17].

Airborne particulate samples from a residential town were collected on Whatman GFA glass-fibre filters and extracted in a Soxhlet extractor with benzene. The benzene solution was separated into a basic fraction. For separation of the residue, GC was performed on a 5-m packed column containing 4% Dexsil 300 on Gas Chrom Q (100–120 mesh) support. From the relative retention times on the column and gas chromatography–mass spectrometry (GC–MS), azabenz[*a*]anthracenes such as benz[*c*]-acridine (**1**) and benz[*a*]acridine (**2**), methylbenz-acridines and dibenz[*a,h*]acridine (**5**) were detected down to a concentration of 4 ppm [18].

Airborne particulates in Taiyuan area (province) of China were analysed for organic pollutants by flexible quartz capillary column GC. One component was found at a concentration of 1.5–20 μg/m^3. This compound, with a molecular mass of 229 and one nitrogen atom, showed carcinogenicity and was tentatively identified as benz[*c*]acridine (**1**) [19].

Azaarenes were extracted from airborne particulate samples by toluene with sonication. The basic azaarenes in the toluene phase were then extracted with 8.25 *M* phosphoric acid. After this step, the toluene phase still contained various arene molecules. The phosphoric acid phases were combined and adjusted to pH 14 with 11 *M* potassium hydroxide. The azaarenes were extracted from this alkaline phase with dichloromethane. By capillary column GC (utilizing an Ultra-1 fused-silica column with a nitrogen-sensitive detector), benz[*a*]-acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]-acridine (**4**) were detected in the air of a suburban residential area and in a busy street in Copenhagen, Denmark, in the Februarys of 1976–82 at concentrations of 0.09 and 0.17, 0.2 and 0.08 and , 0.2 and 0.07 ng/m^3, respectively. Remarkably, the concentrations of dibenz[*a,j*]acridine (**4**) and dibenz[*a,h*]-acridine (**5**) were 2.7 times higher in the residential area than in the busy street. This suggests that perhaps the heating of homes is a major source of dibenz[*a,j*]acridine (**4**) and dibenz[*a,h*]acridine (**5**), and that the emission rate of these compounds is much higher in combustion gases from home furnaces and stoves than that in car exhausts [20].

The azaarene fraction of an urban atmospheric particulate matter extract collected in Tokyo has been measured by HPLC on Zorbax ODS using acetonitrile–water (7:3). Benz[*a*]acridine (**2**), dibenz-[*a,c*]acridine (**3**), dibenz[*a,j*]acridine (**4**), and dibenz-[*a,h*]acridine (**5**) were detected at average concentrations of 3.3, 0.43, 0.29, and 0.36 mg/g, respectively [21].

GC–MS was used for the detection of azaarenes in the air of Calcutta, India. Benz[*a*]acridine (**2**) (1.06–4.76 ng/m^3), dibenz[*a,j*]acridine (**4**) (7.27–13.16 ng/m^3) and dibenz[*a,h*]acridine (**5**) (7.27–13.16 ng/m^3) were detected at higher levels than those found in European or American cities [22].

2.2. *Additional air pollution source effluents*

Samples of urban airborne particulates or air pollution source effluents were extracted with benzene–diethylamine (4:1) in a Soxhlet extractor. The residue, dissolved in dichloromethane, was placed on an alumina TLC plate and developed with pentane–diethyl ether (19:1). Benz[*c*]acridine (**1**) was determined spectrofluorimetrically ($\lambda_{ex.}$ 288 nm, $\lambda_{em.}$ 472 nm). The detection limit of benz[*c*]acridine (**1**) was 40 ng/ml [23].

2.3. *Automobile exhaust*

A basic fraction was chromatographed using pentane containing increasing 8% multiples of diethyl ether, up to 64%, on an alumina TLC column. Carcinogenic benzacridines, such as dibenz-[*a,h*]acridine (**5**), dibenz[*a,j*]acridine (**4**) and the alkyl benz[*c*]acridines were detected in automobile exhaust at concentrations lower than 0.3 μg per gram of the benzene-soluble fraction, indicating that the carcinogenic effect of automobile exhaust in terms of these compounds is negligible compared with some other sources of pollution [24].

In Japan, benzacridines were detected in both diesel and gasoline engine vehicle exhausts in air samples taken in a road tunnel. The base extracted tars were collected and fractionated with a Soxhlet extractor. The solution was chromatographed on a two-dimensional TLC plate coated with alumina G + Kieselghur G [2:1% (w/w) and 26% acetylated cellulose]. 7,9-Dimethylbenz[*c*]acridine (**11**), 7,10-dimethylbenz[*c*]acridine (**12**), and dibenz[*a,h*]-acridine (**5**) were identified. The average emission rates (μg/h per vehicle) of the three benzacridines

TABLE 2

AVERAGE EMISSION RATES OF BENZACRIDINES FROM DIESEL AND GASOLINE ENGINE VEHICLES

Compound	Average emission rate (μg/h)	
	Diesel	Gasoline
Benzacridines		
7,9-Dimethylbenz[*c*]acridine (**11**)	2.1	0.18
7,10-Dimethylbenz[*c*]acridine (**12**)	6.0	1.2
Dibenzacridine		
Dibenz[*a,h*]acridine (**5**)	54	6.5

from heavy-duty diesel engine vehicles were detected in larger amounts than those from light-duty gasoline engine cars, especially for 7,9-dimethylbenz[*c*]acridine (**11**). (Table 2) [25].

HPLC methods were developed for detecting benzacridines in atmospheric aerosols and particulates emitted by diesel and gasoline engines with a view to speed and high sensitivity (pg) by using fluorimetric detection ($\lambda_{ex.}$ 313 nm, $\lambda_{em.}$ 375 nm and $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 425 nm) (Table 3) [26].

By GC with a 25-m cross-linked SE-54 fused-silica capillary column, benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) in diesel fuel were detected at concentrations of < 1 ppm [27].

TABLE 3

CONCENTRATIONS OF BENZACRIDINES AND DIBENZACRIDINES IN ATMOSPHERIC AEROSOLS FROM DIESEL AND GASOLINE ENGINES

Compound	Concentration (ng/m^3)	
	Diesel	Gasoline
Benzacridines		
Benz[*c*]acridine (**1**)	5.80	0.07
Benz[*a*]acridine (**2**	6.10	0.19
2-Methylbenz[*a*]acridine (**13**)	1.12	0.01
7-Methylbenz[*a*]acridine (**14**)	0.49	0.01
Dibenzacridines		
Dibenz[*a,h*]acridine (**5**)	0.82	
Dibenz[*a,j*]acridine (**4**)	0.84	
Dibenz[*c,h*]acridine (**6**)	0.72	

3. PETROLEUM DISTILLATES

3.1. Coal-tar pitch

Alumina CC was used for the separation of airborne particulate samples polluted by coal-tar pitch. The collected coal-tar polluted atmosphere was Soxhlet extracted with benzene. The basic fraction was separated and then chromatographed on an alumina column with pentane–diethyl ether, pentane–acetone, diethyl ether and then methanol. The fractions were separated further using DMF–water (35:65) on TLC plates coated with MN-300 G cellulose powder. Benz[*c*]acridine (**1**), alkylated benz[*c*]acridines, dibenz[*a,h*]acridine (**5**), benz[*a*]acridine (**2**), alkylated benz[*a*]acridines and dibenz[*a,j*]acridine (**4**) were all detected in coal-tar pitch in air pollution source effluents [28].

Dibenz[*a,h*]acridine (**5**) was detected spectrofluorimetrically ($\lambda_{ex.}$ 308 nm, $\lambda_{em.}$ 450 nm) from a CC fraction obtained from air polluted with coal-tar pitch. Prior to the scan, the sample was separated by cellulose TLC with DMF–water (35:65). The spots were treated with trifluoroacetic acid fumes before scanning [29].

A basic fraction of coal-tar pitch was analysed by two-dimensional TLC [solvent 1 = cyclohexane–ethyl acetate (19:1); solvent 2 = DMF–water (35:65)] on alumina–cellulose (2:1). Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**) and dibenz[*a,h*]acridine (**5**) were detected in the coal-tar pitch [30].

A method for the rapid determination of benz[*c*]acridine (**1**) in urban airborne particulates and air pollution source effluents involves preliminary separation by one-dimensional TLC using pentane–diethyl ether (19:1) on alumina or two-dimensional TLC using pentane–diethyl ether (19:1) followed by DMF–water (35:65) on alumina–cellulose (2:1). Either preliminary separation step was followed by direct spectrofluorimetric detection, elution and spectrofluorimetric detection, or elution and filter fluorimetric detection [31].

After pretreatment by alkali extraction, cation-exchange chromatography on Amberlyst 15, LC on a polar bonded-phase silica OPN–Poral C and co-oxidation chromatography on $FeCl_3$–Chromosorb W for each azaarene, benz[*c*]acridine (**1**) was determined by GC on a 40-m glass support-coated open-tubular (SCOT) capillary column coated with

SP-2250 (50% methyl-, 50% phenylsilicone) stationary phase and GC–MS [32–34].

Exhaustive Soxhlet extractions of pitch performed near the boiling points of the solvents *n*-hexane and subsequently benzene yielded the following extracts: *n*-hexane solubles (HS), benzene solubles (BS) and *n*-hexane insolubles (HI). The concentrates were separated by gel permeation chromatography on a glass column filled with Sephadex LH-20 with tetrahydrofuran as eluent. The eluates were analysed using a UV detector at 254 nm. The basic concentrates were characterized by mass spectrometry using direct introduction of a sample into the ion source. From analysis of the HS-extract, benzacridine of average molecular mass 238 was detected at a concentration of 8.5% (v/v), and from analysis of BS and HI extracts, benzacridine of average molecular mass 239 was detected at a concentration of 7.1% (v/v) [35].

3.2. Creosote

Creosote oil was chromatographed using methanol–dichloromethane (1:4) on a silica gel–alumina column to obtain fractions enriched in benzacridines. The benzacridines were characterized by GC with a thermionic (nitrogen–phosphorus-specific) detector and a GC–MS system. Two benzacridines (isomers with a molecular mass of 229 but with different retention indices of 392.0 and 397.9) were detected at concentrations of 4900 and 600 μg/g, respectively. Three methylbenzacridines (isomers with a molecular mass of 243 but with different retention indices of 408.0, 409.9 and 416.6) were detected at concentrations of 71, 110 and 98 μg/g, respectively. The reference compound, dibenz[*a,j*]acridine (**4**) (molecular mass 279; calculated retention index 489.6), showed a concentration of 0.62 μg/g with a measured retention index of 488.9 [36].

After selective enrichment including liquid–liquid acid (base) partioning, CC on Sephadex LH 20 and ion-exchange chromatography, basic fractions of a creosote oil were analysed by HPLC with fluorescence detection and subsequent capillary column GC with a thermionic detector. Additionally, a basic fraction was separated by HPLC and RP-HPTLC. The subfractions were followed by GC–MS and spectrofluorimetry. The concentration of benz[*c*]acridine (**1**), 9-methylbenz[*c*]acridine (**15**) and 10-methylbenz[*c*]acridine (**8**) found in creosote oil were 7.7, 18.4 and 192.7 μg/g, respectively [37].

3.3. High-boiling petroleum distillates

A base fraction was separated through a column containing Cellex-P cation-exchange cellulose. The non-reactive bases of the eluate from the Cellex-P column were passed through an acidic alumina column. The weakly held bases of each fraction were then placed on a basic alumina column. Subfractions showed partial fluorescence excitation and fluorescence emission spectra of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) together with the corresponding spectra of a typical Wilmington petroleum sample. Comparison of the petroleum sample spectra with the spectra of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) suggested that the petroleum sample contains a mixture of the two benzacridines [38].

3.4. Coal liquefaction products

Basic fractions in coal liquefaction products were analysed by GC with flame ionization detection (FID). The GC conditions consisted of a 40-m SGE glass SCOT capillary column coated with SP-2250 (50% methyl-, 50% phenylsilicone) stationary phase using hydrogen as the carrier gas for FID. Benz[*c*]acridine (**1**) was subsequently identified by GC–MS [39].

The azaarene fraction from a neutral alumina adsorption step was separated on a silicic acid adsorbent using benzene and diethyl ether eluents to provide an enriched azaarene fraction. A Hewlett-Packard (HP) Model 5880 gas chromatograph with a DB-5 fused-silica capillary column was used, with FID and ^{63}Ni electron-capture detection (ECD). Qualitative analysis of each fraction was also performed on an HP 5982A capillary column GC–MS system operated in the electron impact mode. Benz[*c*]acridine (**1**) was measured at 578, 1237 and 2357 ppm on 371–399°C, 399–427°C and 427–454°C fraction cuts from a solvent refined coal liquefaction process, respectively (SRC II) [40].

The basic fraction from asphaltene of a flash pyrolysis tar of Millmerran coal in Australia was fractionated by adsorption chromatography on silica gel. The column was eluted successively with light

petroleum (b.p. 40-60°C), light petroleum (b.p. 40-60°C)–toluene, toluene, chloroform and methanol. Benz[*c*]acridine (**1**) and dibenz[*a,h*]acridine (**5**) were identified in the basic fraction from the asphaltene on the basis of the similarity of their fluorescence emission and excitation spectra with those of known compounds. In contrast, the basic fraction from New Waterfield hydrogenation oil in South Africa was separated using Amberlyst 15 cation-exchange resin and fractionated by chromatography on acidic and then basic alumina. Benz[*a*]-acridine (**2**) was identified by comparison of the fluorescence excitation spectra in both neutral and acidic media with the reported UV spectra [41].

4. TOBACCO (SMOKE)

Hueper *et al.* [42] presented evidence that cigarette smoking is only one of many factors (including air pollution) that play a significant role in causing lung cancer. Wynder and Hoffman [43] cited evidence that cigarette smoke plays the major role in the etiology of lung cancer.

For analysis of tobacco pyrolysed at 850°C for azaarenes of the atmosphere, a scheme was designed to fractionate the tobacco smoke condensate. First, for bases, GC was applied using a stainless-steel column containing 15% Carbowax 20M on 60–80-mesh Chromosorb W. Second, TLC of azaarenes was carried out on glass plates coated with silica gel G using ethyl acetate–methanol–formic acid (80:10:10) and benzene–methanol (95:5) as eluents. Benzacridines were detected in tobacco and nicotine pyrolysates and tobacco smoke condensates [44].

Snook *et al.* [45] investigated benzacridine concentration in the basic fraction of cigarette smoke condensate. After gel chromatography on Bio-Beads S-X12 of cigarette smoke condensate in benzene, each individual gel fraction was submitted to GC on a column packed with 6% OV-17 on 100–120-mesh Chromosorb G/HP. The separated components of GC cuts were determined by GC–MS. No benzacridines were detected under these chromatographic conditions.

For extraction, basic compounds were collected by S-Sepharose ion-exchange chromatography, Sephadex LH-20 and again with S-Sepharose. GC with a fused-silica column of SE-54 with N-FID was used for the detection of particulate-bound azaarene extracts from mainstream smoke and sidestream smoke. Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**) and 7-methylbenz[*c*]acridine (**16**) were detected in mainstream and sidestream smoke when compared with standard benz[*c*]acridines [46].

HPLC with a reversed-phase ODS column was used for azaarene extract separation. The best isocratic elution was with acetonitrile–water (75:25). Benz[*c*]acridine (**1**) and 9-methylbenz[*c*]acridine (**15**) were detected in cigarette smoke condensates [47].

5. SEDIMENTS

5.1. Lake sediment

Major peaks with estimated concentrations of identified benzacridines in two surface sediments and street dust were compared using GC and GC–MS. It was found that the levels of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) were almost the same in Lake Zurich samples, whereas benz[*a*]acridine (**2**) was much more abundant than benz[*c*]acridine (**1**) in Lake Lucerne and street dust samples. A similar but more dramatic variation was observed for dibenz[*a,c*]acridine (**3**; 1,2,3,4-dibenzacridine) and dibenz[*a,j*]acridine (**4**, 1,2,7,8-dibenzacridine) (Table 4) [48].

5.2. River sediment

Samples were subjected to Soxhlet extraction with dichloromethane. The extract was preseparated on a Sephadex LH-20 column eluted with 2-propanol. The fraction containing benzacridines was analysed by fused-silica capillary GC and GC–MS. Benz[*c*]acridine (**1**; M_r 229), benz[*a*]acridine (**2**; M_r 229), dibenz[*a,c*]acridine (**3**; M_r 279) and dibenz-[*a,h*]acridine (**5**; M_r 279) were all identified from GC retention times and by molecular mass methods by comparison with standard samples [49].

5.3. Marine sediment

Organic extracts of marine sediments from Eagle Harbor, Puget Sound, WA, USA, were eluted using methanol–dichloromethane (1:4) on a silica gel–alumina column to obtain fractions enriched in benz-

TABLE 4

BENZACRIDINES AND DIBENZACRIDINES IDENTIFIED AND DETERMINED IN TWO SURFACE SEDIMENTS AND STREET DUST

Compound	Estimated concentration (ng/g)		
	Lake surface sediment		Street dust
	Zurich	Lucerne	
Benzacridines			
Benz[*c*]acridine (**1**)	45	3.9	525
Benz[*a*]acridine (**2**)	50	0.8	220
Dibenzacridines			
Dibenz[*a,c*]acridine (**3**) or dibenz[*a,h*]acridine (**5**)	35	5.0	260
Dibenz[*a,j*]acridine (**4**)	37	0.7	56

acridines. The benzacridines were characterized by GC with a thermionic (nitrogen–phosphorus-specific) detector and GC–MS. Two benzacridines (isomers of molecular mass 229 with retention indices of 392.0 and 398.3) were detected at concentrations of 7.7 and 8.5 μg/g, respectively. Three methylbenzacridines (isomers of molecular mass 243 with retention indices of 408.0, 410.0 and 416.6, respectively) were detected at concentrations of 1.0, 1.2 and 1.4 μg/g, respectively. The reference compound, dibenz[*a,j*]acridine (**4**; M_r 279; calculated retention index 489.6), showed a concentration of 0.62 μg/g with a measured retention index of 488.9 [36].

5.4. Groundwater

Groundwater samples contaminated by coal-tar wastes was analysed for azaarenes. GC with a wall-coated open-tubular, fused-silica capillary column coated with SE-54 and MS were utilized for the separation and identification of the basic extract after isolation by partitioning. Each isomer of benzacridines and dibenzacridines was identified in the oily-tar phase of groundwater [50].

Benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) in groundwater from Beijing, China, were separated by TLC with a mobile phase of pentane–diethyl ether (19:1) on silica gel G and identified by TLC

TABLE 5

BENZ[*c*]ACRIDINES AND BENZ[*a*]ACRIDINES IDENTIFIED IN GROUNDWATER IN BEIJING

Sampling point	Sampling point depth (m)	Benz[*c*]acridine (**1**, μg/l)[a]			Benz[*a*]acridine (**2**, μg/l)[b]		
		1	2	3	1	2	3
S1	46 (control)	0.059	0.023	0.083	D[d]	D	D
S2	70	0.360	0.049	0.090	D	D	D
S3	60–70	0.130	0.045	N.D.[c]	D	D	D
S4	12	0.039	0.010	N.D.	D	D	D
S5	60–70	Trace	0.015	0.102	Trace	D	D
S6	River water	0.069	0.056	0.193	D	D	D

[a] Recovery 65 ± 3%.
[b] Qualitative determination.
[c] N.D. = not detected.
[d] D = detected.

and MS. Concentrations of 0.01–0.40 μg/l of benz[*c*]acridine (**1**) were determined by spectrofluorimetry at 384 nm with recoveries of 65 ± 3% (Table 5) [51].

6. FOOD (HAM)

A method for the determination of benzacridines in meat was investigated by liquid–liquid partitioning (dimethylformamide–water–cyclohexane). Extraction of benzacridines was accomplished with sulphuric acid, re-extraction after neutralization by cyclohexane or, alternatively, by non-adsorbing ion-exchange chromatography. Further purification was performed by CC on Sephadex LH-20. Benzacridines were separated by capillary column GC and measured by comparing the corresponding peak areas with those of an internal standard such as 10-azabenzo[*a*]pyrene. The detection limit of this method ranges from 0.1 to 0.4 ng for benzacridines. The relative standard deviations (R.S.D.s) for reference benz[*c*]acridine (**1**), 8,10-dimethylbenz[*c*]acridine (**7**), dibenz[*c,h*]acridine (**6**), dibenz[*a,h*]acridine (**5**), and dibenz[*a,j*]acridine (**4**) in comparison with 10-azabenzo[*a*]pyrene as internal standard were 13.6, 4.0, 5.5, 5.2 and 5.8% for the analysis, respectively. The R.S.D.s for benz[*c*]acridine (**1**), dibenz[*c,h*]acridine (**6**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) in spiked meat samples were 10.4, 22.3, 10.7 and 25.4%, respectively [52,53].

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Sampling and analysis of volatile organic compounds in estuarine air by gas chromatography and mass spectrometry

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ABSTRACT

The spatial and temporal distribution of volatile organic compounds (VOCs) in air samples taken beside a semi-industrialised estuary was intensively studied over a two-year period. Samples were collected by trapping VOCs onto multi-bed traps packed with graphitised carbon black sorbents capable of selectively trapping a wide range of volatile substances. Samples were then analysed using an automated thermal desorption technique followed by capillary column gas chromatography with simultaneous detection of volatile eluates using flame ionisation and ion trap detectors. The lower limit of detection was circa 0.5 ng m^{-3} for most compounds. The major groups of organic compounds collected were C_1–C_4 alkylbenzenes, alkanes and alkenes, and chlorinated solvents. Terpenes were also found in high concentrations in forested areas which fringe the estuary. Significant seasonal differences in the distribution and concentration of VOCs occur between summer and winter. A case study from the VOCs research in and around the Southampton Water estuary on the central southern coastline of England is presented as an example of the method application.

INTRODUCTION

Airborne volatile organic compounds (VOCs) have been the subject of interest for some years, particularly those associated with emissions from gasoline and diesel powered engines. Although vehicle exhausts have long been recognised as a major source of pollutant VOCs in urban air [1,2] along with petroleum and solvent-based industries [3], forests and woodlands were also recognised as a significant source of biogenic VOCs, particularly those emited directly from trees and vegetation [4,5]. Of more immediate concern is the harmful effects that toxic airborne VOCs impose on human health, particularly the known and suspected carcinogenic properties of VOCs (*e.g.* alkylbenzenes). Volatile compounds are highly mobile and are capable of being inhaled by people living and/or working in proximity to vehicle emissions and specific industrial activities [6–8]. As yet, environmental health professionals have insufficient data on the behaviour and long-term concentration trends of airborne VOCs in order to accurately assess their chronic effects on health and air quality. It is therefore not surprising that increasing emphasis should be placed on long-term sampling studies of airborne VOC concentrations.

In this study, another important reason for long-term study of VOCs was to integrate data on airborne concentrations with those previously measured in estuarine and coastal waters, principally in order to examine source and sink relationships be-

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tween air and sea. Methods for the analysis of VOCs in estuarine water and sediments were reported in previous papers [9,10], along with typical concentration data for key volatile compounds in the aforementioned sample matrices.

Within the last few years, technical approaches towards the sampling and analysis of low-level airborne VOCs have taken on new dimensions. Popular alternatives include trapping volatile compounds in glass vessels, followed by cryogenic trapping and thermal desorption [11]. Other methods rely on cryotrapping VOCs directly from air, followed by injection onto the chromatographic column via multi-port gas valves [12]. Increasingly, commercial manufacturers are developing remote sampling and analytical GC systems for semi-continuous analysis of urban air [13].

Historically, conventional methods use low-flow sampling pump to trap airborne VOCs onto adsorbent beds consisting of activated charcoals [14], porous polymers *e.g.* Tenax-TA [15], or combinations of sorbents *e.g.* charcoal and polyurethane foam [16]. However, recent developments in the production of thermally modified carbon blacks and carbon molecular sieve adsorbents for quantitatively trapping VOCs has been reflected in their superior performance over "traditional" sorbents such as activated charcoal and porous polymers which tend to lack uniform adsorbent characteristics necessary for adsorbing and retaining the different physicochemical properties of VOCs encountered within airborne environments. Deleterious effects include poor retentivity for certain functional groups, susceptibility to water vapour, or poor desorption characteristics for strongly retained compounds [17]. Instead, the use of non-specific, Type I sorbents such as the graphitised carbon blacks and carbon molecular sieves have overcome concerns about which functional group(s) an adsorbate possesses. The application of such adsorbents has minimised problems with contamination and artefact formation [18,19], characteristics which render them attractive alternatives for sampling VOCs in quite different environments, and which were extensively used in this study.

A newer alternative for sampling VOCs is the use of Summa passivated canisters [20]. Although canisters represent an excellent option, until quite recently, they have not been readily available outside of the USA. In a recent paper on Summa canisters the authors quoted a lower limit of detection of 1–2 ppb (10^{-9} v/v) per component (or lower in full-scan MS mode) and possible problems with excess moisture [20]. Despite the advantages Summa canisters offer over adsorbent-type sampling, current questions over their use for prolonged sampling periods, the inability of the method to attain the desired limit of detection (*i.e.* 5 ng m^{-3} minimum), and concerns over moisture-initiated contamination with salt-laden sea air only add further potential complexities.

In this study we report the use of triple-packed adsorbent tubes using a combination of carbon black and molecular sieve sorbents, followed by thermal desorption of the volatile eluates from the adsorbent tubes using a Perkin-Elmer automated thermal desorber (ATD-50) (Perkin-Elmer, Beaconsfield, UK). The method was successfully used to analyse over 300 samples collected from ground-level sites around the estuary over a 2-year period.

EXPERIMENTAL

Reagents and materials

Standards were prepared using both analytical and high-purity spectrophotometric-grade materials (Aldrich, Wimborne, UK). Stock standard (liquid) mixtures were prepared gravimetrically in all-glass flasks according to both EPA [21] and CONCAWE [22] methods and spiked onto standard sorbent tubes. Certified calibration gas standards (*i.e.* for substances which are gaseous at room temperature) were purchased externally on a custom order specification (Air Products, Bracknell, UK; and Electrochem Specialty Gases, Stoke-on-Trent, UK) and quantitatively spiked onto adsorbent tubes via all-glass cylinders fitted with motorised hypodermic syringes.

Adsorbent tubes (stainless-steel, 90 mm × 5 mm I.D.) obtained from Supelco (Supelco, Bellefonte, PA, USA) were packed with Carbotrap 300 specification sorbents [*i.e.* Carbotrap C (250 mg) 20/40 mesh; Carbotrap B (175 mg) 20/40 mesh; Carbosieve S-III (105 mg) 60/80 mesh]. Carbotrap B and C are graphitised carbon black sorbents and Carbosieve S-III is a carbon molecular sieve. The principle behind the packing (and sampling) regime is that the most volatile components (*i.e.* C_2–C_5) will slow-

ly migrate through the carbon black sorbents and are eventually trapped on the carbon molecular sieve. Compounds of progressively higher molecular weight are successively trapped on the carbotrap adsorbents, an arrangement which theoretically serves to quantitatively adsorb a range of VOC from the highest to the lowest volatility across the entire length of the sampling tube. The theoretical (and experimental) details which underpin this multi-sorbent trapping arrangement have been presented in detail by Betz and Gisch [17]. A second sorbent tube was attached *via* 1/4-in. (0.64 cm) Swagelock unions "in-series" to each sampling tube to check that breakthrough had not occurred during sampling. The use of a back-up tube also offers the analyst the opportunity to carry out a semi-quantitative analysis should breakthrough of organic compounds occur due to breakdown of the first sorbent bed, or due to massive overload in the event of an extreme pollution event. Small silanised and head-treated glass-wool plugs were then inserted into the front end of each sampling tube as a pre-filter for coarse airborne particles (*i.e.* dusts, grits, pollen). The adsorbent tubes were prepared freshly by being conditioned overnight (*i.e.* by passing a stream of ultra-pure helium at 100 ml min^{-1} through each tube for 8 h at 280°C). The tubes were then analysed in the normal way to guarantee the absence of artefacts or contamination. The tubes were then capped with Swagelok end-caps and stored in sealed glass jars immediately prior to use, normally within 12 h of preconditioning.

Sampling

Sample pumps [low-flow (Accuhaler 808 Model): MDA, Lincolnshire, IL, USA] and low/medium-flow [(Flo-Lite): MSA, Pittsburg, PA, USA] pumps were attached to individual sorbent tubes using 30 cm lengths of clear, inert polythene tubing suitable for low-level organics sampling (BDH-Merck, Eastleigh, UK). Each pump/tube assembly was then calibrated using a bubble flow meter (SKC UK, Blandford Forum, UK) in order to attain precise flow-rates. The pumps were adjusted to sample at 50 ml min^{-1} (*i.e.* MDA pumps) and 500 ml min^{-1} (*i.e.* MSA pumps), respectively. Pairs of pump/tube assemblies were then mounted onto heavy duty tripods (12 off, 1.5 m height) using locating brackets. Each tripod was capped with a curved aluminium sheet "umbrella" (40 cm radius × 2 mm thick) for weather protection. The tripods were then placed in fixed sampling locations for 1.0 (MDA) and 4.0 (MSA) h time periods, respectively, and successively replaced by fresh pump/tube assemblies on an ongoing basis (*i.e.* 4, 8, 12, 16 and 24 h) depending on the sampling schedule. Individual flow-rates were rechecked at the end of the sampling period in order to obtain assurance about the integrity of the pump flow-rate and the total sampling volume. Tubes were then sealed with Swagelok end-caps, sealed in all-glass jars with ground-glass stoppers, cooled to approximately −10°C and analysed within 24 h of sampling. Measurements of air temperature (dry bulb), barometric pressure, relative humidity (%) and wind direction were made at regular intervals (*i.e.* hourly and four-hourly). In some cases, a platform-mounted thermohygrograph (Casella, London, UK) was used to continuously monitor and record temperature and relative humidity in the sampling zone.

Instrumentation and capillary column

The Perkin-Elmer automated thermal desorber (ATD-50) was connected to a Perkin-Elmer 8700 gas chromatograph via a 1 m length of deactivated fused-silica transfer line, 0.22 mm I.D., held at 150°C. The ATD-50 is a multi-functional instrument designed for the analysis of organic vapours at low concentrations (sub-ppm). An integral two-stage desorption facility is available whereby organic compounds desorbed from adsorption tubes, within an oven held at 150°C, are then re-trapped inside an electronically cooled cold-trap packed with a secondary adsorbent bed, at a temperature down to −30°C. The cold-trap is then electronically heated at a rate exceeding 1000°C min^{-1} to an upper limit of 300°C, sending a discrete band of concentrated sample through the fused-silica transfer line to the gas chromatographic (GC) capillary column where the transferred components are chromatographed. The eluted components were then passed through an effluent splitter for simultaneous flame ionisation and ion trap detection.

The gas chromatograph was fitted with a cradle-mounted, 50 m × 0.22 mm I.D. aluminium-clad BP-1 wall coated open-tubular capillary fused-silica capillary column, 0.25 μm film thickness (SGE, Milton Keynes, UK). The exit point of the column was

connected to a twin-hole split ferrule permitting 50% of the column eluent to be routed to a flame ionisation detector. The remaining 50% is swept via a second 1 m length of transfer line at 250°C into an ion trap detector (Finnigan MAT). The repeatability, reproducibility and linearity of response of this system was checked *via* intercalibration excercises and the use of multi-range standards.

Carbon dioxide gas for cooling the chromatograph oven below ambient temperature was piped into the rear of the oven via a 4 m length of 3.0 mm O.D. copper tubing. The feed rate for carbon dioxide gas is gauged by a microprocessor-controlled valve in the gas-chromatograph.

Analytical operating conditions

The analytical operating conditions were similar to those already described by Bianchi and Cook [23].

Carrier gas: ultrapure helium 5.5 grade (Air Products, Basingstoke, UK); carbon dioxide (Air Products, Hythe Depot, Southampton, UK). ATD-50: cold-trap packing, 20 mg Chromosorb-106; cold-trap low temperature, −30°C; cold-trap high temperature, 250°C; split ratio (combined), 50:1; desorption oven temperature, 250°C; adsorbent tube desorption time, 10 min; desorption gas-flow through tube, 10 ml min^{-1}, carrier gas pressure, 170 kPa.

Gas chromatograph. Detector temperature, 300°C; carrier gas flow-rate, 1 ml min^{-1} (20 cm s^{-1} at 10°C). Temperature conditions: oven temperature, −35°C; isothermal time 1, 7.0 min; ramp rate 1, 20°C min^{-1}; oven temperature 2, 60°C, isothermal time, 0.1 min; ramp rate 2, 5°C in^{-1}, oven temperature 2, 300°C; final hold time 2, 1.0 min.

Ion trap detector. Ionisation voltage, 70 eV; s/scan, 1.0; mass range, 45–300 mass units; transfer temperature, 250°C; ion source temperature, 250°C; multiplier delay, 120 s; mass defect, 100 molecular mass units (m.m.u.)/100 u; acquire time, 60 min.

Compound quantitation and method performance

The qualitative and quantitative identification of compounds was carried out by direct comparison of the specific GC retention data (*i.e.* from the flame ionisation detector) in tandem with the confirmatory mass spectral data. Identifications were also carried out by co-injection of authentic standards and subsequent comparison with an "in-house" retention index library containing over 1000 separate compounds (*i.e.* for non-polar, chemically bonded methylsilicone capillary columns). Absolute concentrations of individual compounds were calculated in terms of the mass (μg) per unit volume (1.0 m^3) per component. Whereas ratio expressions (*i.e.* ppb) are idenpendent of pressure and temperature, weight/volume (w/v) expressions vary according to the gas laws (*i.e.* according to temperature and pressure). Using the calibration and standard spiking protocols previously discussed, individual response factors for each component were determined at the ng l^{-1} level (*i.e.* external standard method). These values, expressed in ng, were then divided by the total pumped air volume through the adsorbent tube, and corrected for ambient temperature (K) and pressure (mmHg) to yield the corrected μg m^{-3} concentration per component. Further details of the procedure and calculations are provided in the CONCAWE guidance document 8/86 [22].

Specific data on the sorbent characteristics and the field performance of the graphitised carbon blacks and the carbon molecular sieves has been previously reported [17–19]. Our results were generally consistent with those reported by Betz and co-workers [18,19]. The short term precision (*i.e.* repeatability) of our air sampling method is expressed as the relative standard deviation (R.S.D.), and is presented in Table I. The data show that R.S.D. is generally in the order of ± 10% except for the most highly volatile compounds (*e.g.* ethene, 14%) and the least volatile compounds (*e.g.* *n*-heptadecane, 15%). In contrast, the R.S.D. for aromatics (*i.e.* among the most toxic of the airborne VOCs, and those with a high potential for producing ground-level ozone) is excellent *e.g.* benzene, 3.3%; 1,2,3-trimethylbenzene, 3.9%). The overall accuracy of the method (*i.e.* recovery + precision) was within ±25% for all components. The absolute detection limit was between 0.1 and 0.4 ng m^{-3} for the volatile aromatic and organohalogen compounds, and between 0.3 and 1.0 ng m^{-3} for the remaining compounds listed in Table I, comparable with the absolute detection limits reported by Rosell *et al.* [16] in their analysis of Barcelona air. In our study, these detection limits were found to be at least a factor of 1000 times more sensitive than typical minimum VOC concentrations observed in real samples, ren-

TABLE I

THE REPEATABILITY OF MODEL VOLATILE ORGANIC COMPOUNDS SAMPLED FROM AMBIENT AIR IN THE SOUTHAMPTON WATER ESTUARY

Short term precision is expressed as % relative standard deviation (R.S.D.) (n = 10). (Note that the compound list is presented according to their general elution order from the capillary column.)

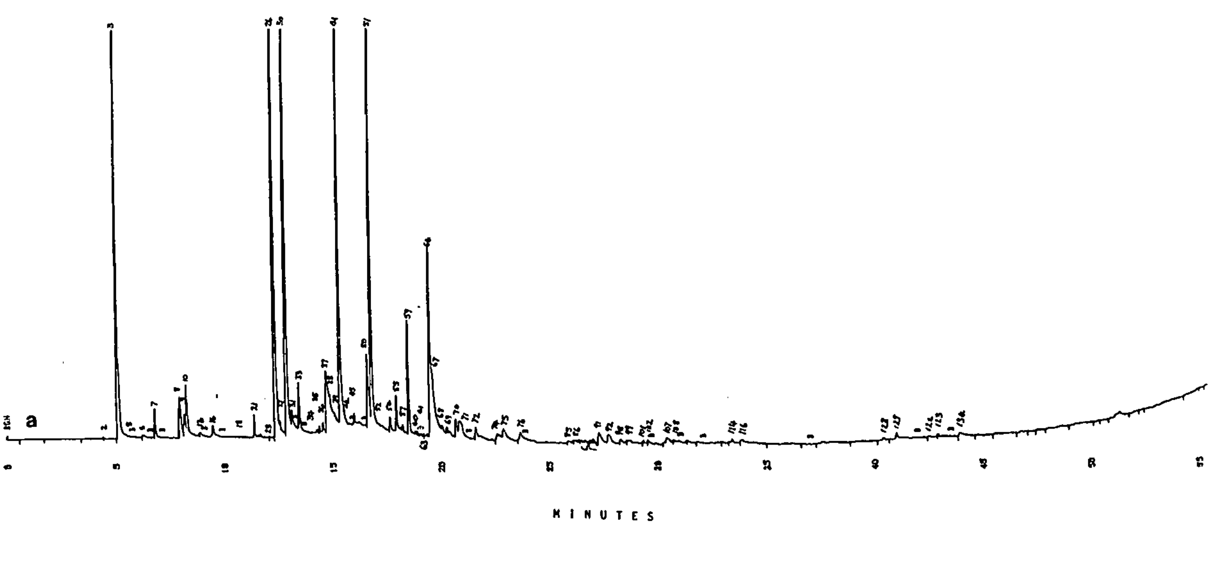
Compound Name	% R.S.D.	Compound Name	% R.S.D.
Ethene	14.1	*n*-Hexane	5.4
Ethane	10.3	Trichchloromethane	4.7
Propene	10.5	1,2-Dichloroethane	4.7
Propane	9.6	1,1,1-Trichloroethane	4.6
Chloromethane	10.1	Benzene	3.3
Cyclopropane	9.2	Carbon tetrachloride	4.3
iso-Butane	9.3	*n*-Butanol	9.7
n- and iso-Butene	8.2	Cyclohexane	5.4
1,3-Butadiene	9.3	Trichloroethylene	4.9
Methanethiol	7.6	2-Methylhexane	7.7
n-Butane	8.9	Pentanal	8.7
trans-Butene-2	7.2	Cyclohexene	7.9
2,2-Dimethylpropane (neopentane)	7.6	Trichloroethylene	5.0
Chloroethane	8.3	Heptene-1	6.8
cis-Butene-2	7.7	2,2,4-Trimethylpentane (iso-octane)	5.3
Cyclobutene	8.3	3-Pentanone (diethyl ketone)	9.0
3-Methylbutene-1	8.4	*n*-Heptane	6.4
Cyclobutane	9.8	Dimethyldisulphide	6.6
iso-Pentane	6.2	Methylcyclohexene	6.7
Pentene-1	6.3	Toluene	3.6
2-Methylbutene-1	8.5	*n*-Octane	5.5
2-Methyl-1,3-Butadiene (isoprene)	6.7	Chlorobenzene	4.3
n-Pentane	5.7	Ethylbenzene	3.4
Fluorotrichloromethane	6.7	1,1,3-Trimethylcyclohexane	5.6
trans-1,2-Dichloroethylene	6.8	1,3-Dimethylbenzene (*p*-xylene)	4.0
2-Methylbutene-2	7.3	1,4-Dimethylbenzene (*m*-xylene)	4.2
Dichloromethane	4.6	Styrene	5.0
Dimethylsulphide	4.9	1,2-Dimethylbenzene (*o*-xylene)	4.3
tert.-Butanol (2-methylpropan-2-ol)	6.5	*n*-Nonane	6.7
Carbon disulphide	6.2	Isopropylbenzene (cumene)	3.9
Freon-113 (1,1,2-trichloro-1,2,2-trifluoroethane)	4.3	α-Pinene/dipentene terpinene	7.8
Cyclopentadiene	5.6	β-pinene	7.8
2,2-Dimethylbutane	7.3	*n*-Propylbenzene	3.9
Cyclopentene	8.3	Benzaldehyde	8.5
4-Methylpentene-1	7.9	1,3,5-Trimethylbenzene (mesitylene)	3.9
3-Methylpentene-1	7.6	*n*-Decane	7.9
2,3-Dimethylbutene-1	8.3	*n*-Undecane	9.2
Cyclopentane	8.9	1,2,3,5-Tetramethylbenzene	4.4
2,3-Dimethylbutane	9.0	Naphthalene	6.9
Methyl-*tert*.-butyl ether [m-TBE]	7.8	*n*-Tridecane	9.3
2-Methylpentane	5.8	*n*-Tetradecane	9.4
3-Methylpentane	5.8	*n*-Pentadecane	9.9
Methyl ethyl ketone (2-butanone)	9.3	Tridecanal	8.9
n-Butanal	9.0	*n*-Hexadecane	10.0
2-Butanol	7.9	Pristane	9.4

dering the pursuance of greater analytical sensitivity and uneconomic and unnecessary exercise.

RESULTS AND DISCUSSION

The Southampton Water case study

Southampton Water is a semi-industrialised estuary on the coastline of sourthern central England. It accomodates a wide range of industries and activities including a large petrochemical complex and an electric power generating plant on its southwestern shore, plus three large marinas on its western, northern and eastern shores, respectively. An assemblage of boat-building industries are dotted along its coastline. In summer, it plays host to intense watersports which are often in conflict with free access of merchant ships en-route to this busy port. Both city and housing development have mushroomed in size over the last 20 years, with doubling of traffic density and sewage flows to the estuary. Taken together, such development has placed the estuary under intense environmental pressure. Across the United Kingdom, deteriorating air quality is a formidable manifestation of simi-



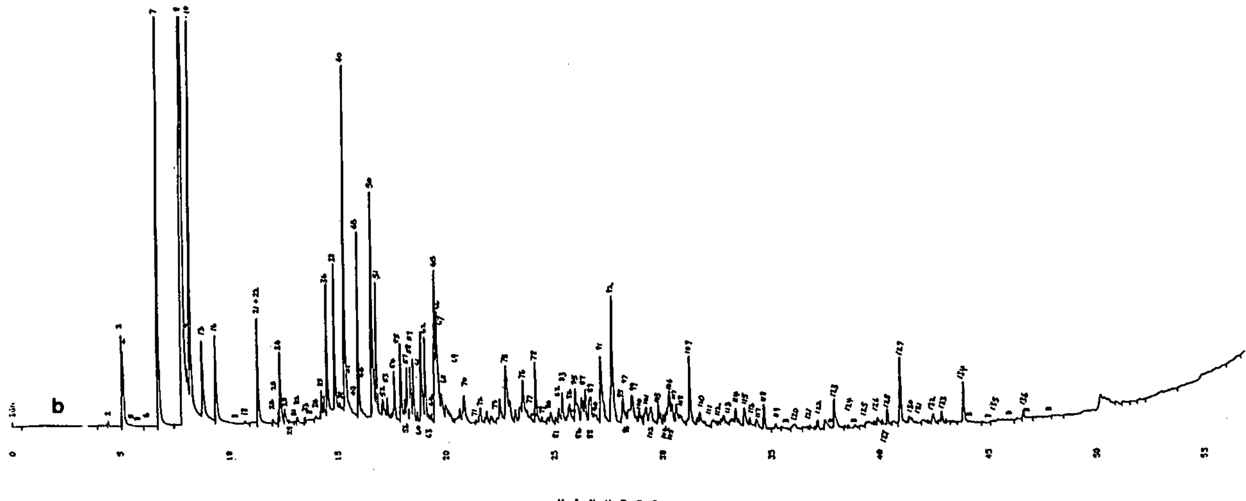


Fig. 1.

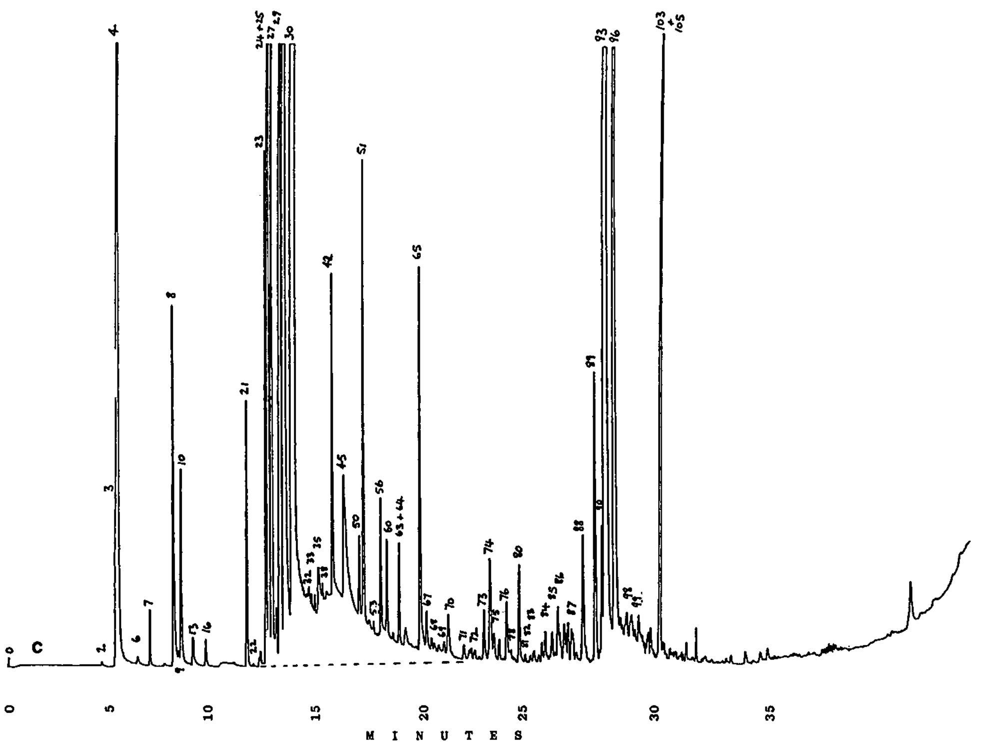


Fig. 1. (a) A representative gas chromatogram obtained from analysis of ambient air taken from a sampling station 2 km downwind from the Southampton Water petrochemical complex (July 1991). Range: × 1; attn.: × 32. (b) A representative gas chromatogram obtained from analysis of ambient air taken near the rural village of Beaulieu in the New Forest, Hampshire, UK (August 1991). See component key for compound assignations. Range: × 1, attn.: × 8. (c) A gas chromatogram obtained from analysis of ambient air taken 0.5 km downwind of a ship-repairers yard. The chromatogram reveals a large number of solvent hydrocarbons associated with glass-fibre moulding and construction activity at the yards. Range: × 1; attn.: × 64. See Table II for compound assignations.

lar pressures, and levels of ozone, nitrogen oxides and sulphur dioxide have reached all-time maxima within the past two years, and are attributed as major causative factors in the steady increase of respiratory disorders such as asthma within the local populace [24–26].

Total VOC concentrations in Southampton Water airborne environments are quite high, ranging from 100 μg m^{-3} to 5000 μg m^{-3} in over 250 air samples taken in and around the Southampton Water estuary over a two year period. In a 2-km zone downwind of the petrochemical complex itself, C_1–C_4 alkylbenzenes, *n*-alkanes and *n*-alkenes were the major constituents of the VOCs. Conversely, in forested locations which lie about 18 km west of the estuary (*e.g.* Beaulieu village), less than 10% of the typical urban levels of VOCs were found. Although some VOCs attributable to air pollution were found within forest air, many more compounds were of biogenic origin (*e.g.* volatile terpenes and aldehydes such as isoprene, limonene and *n*-pentanal). In locations such as the Southampton City Centre and its industrial fringes (*i.e.* which lie about 20 km north west of the petrochemical complex), high levels of airborne contamination were found. Typical (summertime) chromatograms from the aforementioned locations are shown as Fig. 1a, b and c, respectively. Fig. 1c reveals particularly high concentrations of solvent hydrocarbons in ambient air sampled about 0.5 km downwind of ship-repairers yards specialising in fibre-glass work.

Concentrations of volatile organohalogens, particularly common chlorinated solvents (*i.e.* 1,1,1,-trichlorethane, tetrachloroethylene and carbon tetrachloride) were consistently high near shipbuilding and repairing factories regardless of season *i.e.* up

TABLE II

COMPONENT KEY FOR FIGS. 1 AND 2

Peak no.	Compound	Peak no.	Compound
1	Ethene	54	3-Methylhexane
2	Ethane	55	Pentanal
3	Propene	56	Trichoroethylene
4	Propane	57	3-Ethylpentane
5	Chloromethane	58	2,2,4-Trimethylpentane
6	Cyclopropane	59	*trans*-Heptene-2
7	iso-Butane	60	*n*-Heptane
8	*n*-Butene	61	Methylcyclohexane
9	iso-Butene	62	Ethylcyclopentane
10	1,3-Butadiene	63	2,5- + 2,4-Dimethylhexane
11	Methanethiol	64	2,3,4-Trimethylpentane
12	*n*-Butane	65	Methylbenzene (toluene)
13	*trans*-Butene-2	66	4-Methylcyclohexanone
14	2,2-Dimethylpropane (neopentane)	67	4-Methylheptane
15	Chloroethane	68	Hexanal
16	*cis*-Butene-2	69	Tetrachloroethylene
17	2-Methylbutane	70	*n*-Octane
18	Cyclobutene	71	3-Methyl-2-heptene
19	Pentene-1	72	Ethylcyclohexane
20	2-Methylbutene-1	73	Ethylbenzene
21	*n*-Pentane	74	1,2- + 1,4-Dimethylbenzene
22	2-Methyl-1,3-butadiene (isoprene)	75	Heptanal
23	Fluorotrichloromethane	76	1,3-Dimethylbenzene
24	*trans*-Pentene-2	77	3,3,4-Trimethylhexane
25	*cis*-Pentene-2	78	Styrene
26	2-Methylbutene-2	79	*n*-Nonane
27	Dichloromethane	80	Isopropylbenzene
28	Dimethylsulphide	81	Benzaldehyde
29	*tert.*-Butanol (2-Methylpropan-2-ol)	82	α-Pinene
30	Freon-113 (1,1,2-Trichloro-1,2,2-trifluoroethane)	83	a dipentene (terpene?)
31	2,2-Dimethylbutane	84	Cyclooctene
32	Cyclopentene	85	*n*-Propylbenzene
33	Ethylcyclopropane + cyclopentane	86	Camphene
34	2,3-Dimethylbutane	87	Carene
35	Methyl-*tert.*-butyl ether [mTBE]	88	1-Methyl-3- + 1-methyl-4-ethylbenzene
36	2-Methylpentane	89	1,3,5-Trimethylbenzene
37	2-Butanone	90	Methyl-2-ethylbenzene
38	3-Methylpentane	91	β-Pinene
39	*n*-Butanal + 1-Hexene	92	Octanal
40	*sec*-Butanol	93	1,2,4-Trimethylbenzene
41	*n*-Hexane	94	*sec*-Butylbenzene
42	Trichloromethane	95	*n*-Decane
43	*trans*-2-Hexene	96	1,2,3-Trimethylbenzene
44	*cis*-2-Hexene	97	*p*-Cymene
45	2- + 3-Methylpentene-2	98	Indane
46	Methylcyclopentane	99	Linonene
47	2,4-Dimethylpentane	100	1H-Indene
48	1,1,1-Trichloroethane	101	β-Phellandrene
49	3-Methyl butanal	102	*n*-Butylbenzene
50	Benzene	103	1,4-Dimethyl-2-ethylbenzene
51	Cyclohexane	104	2-Nonenol-1
52	2-Methylhexane	105	1-Methyl-2-propylbenzene
53	2,3-Dimethylpentane	106	γ-Terpinene

TABLE II (*continued*)

Peak no.	Compound	Peak no.	Compound
107	1,3-Dimethyl-2- + 1,2-Dimethyl-4-ethylbenzene	123	*n*-Tridecane
108	Dimethylethylbenzene	124	a biphenyl ether
109	Nonanal	125	Dodecanal
110	*n*-Undecane	126	n-Tetradecane
111	1,2,4,5-Tetramethylbenzene	127	2-Ethylnaphthalene
112	1,2,3,5-Tetramethylbenzene	128	Acenaphthalene
113	2,3-Dihydro-5-methyl-1H-indene	129	C_{14} alkene (?)
114	4-Terpineol	130	1,3- + 1,4-Dimethylnaphthalene
115	Naphthalene	131	*n*-Pentadecane
116	α-Terpineol	132	Tridecanal
117	Decanal	133	Tetradecanal (?) (a C_{14} aldehyde)
118	*n*-Dodecane	134	*n*-Hexadecane
119	2-Methylnaphthalene	134	*n*-Pentadecane
120	a C_{11} aldehyde	135	Pristane
121	3-Methylnaphthalene	136	a C_{15} alkene (?)
122	Geraniol		

to a maximum of 50.0 $\mu g\ m^{-3}$ per component. Volatile freons such as 1,1,2-trichloro-1,2,2-trifluoroethane (*i.e.* Freon-113) were also commonly found in air samples. Rigorous preparation and checking of blank sample tubes (*i.e.* including those taken to and from sampling sites) confirmed that the freons were neither contaminants nor artifacts but regular constituents of the airborne environment.

Volatile aromatics are also ubiquitous in ambient air, particularly those sampled in and around Southampton City where traffic volume is highest. On both sides of the estuary aromatics such as benzene, methylbenzene, di- and tri-methylbenzenes prevail. Benzene concentrations vary from about 1–50 $\mu g\ m^{-3}$ in summer to about 5–500 $\mu g\ m^{-3}$ in winter. Methylbenzene and dimethylbenzene concentrations tended to be between 1.3 and 2.0 times higher than benzene concentrations. These levels may be considered to be of prime environmental interest, particularly the higher wintertime concentrations. For example, as a result of the perception of a potential health risk to the public, ambient air quality standards for benzene have recently been proposed in Germany and in the Netherlands of 2.5 $\mu g\ m^{-3}$ and 10 $\mu g\ m^{-3}$, respectively. Our data suggest that ambient air from the South Hampshire area would not fall well below these proposed limits, particularly during the winter.

Interestingly, no major differences in VOC concentrations were found when comparing air quality near the petrochemical complex with air from the city centre and some of its suburbs, and total VOCs in both locations appeared to co-vary with season rather than any other clearly identifiabe factor. This is highlighted in Table III which lists typical summer and winter concentration ranges for a spectrum of VOCs regularly found in estuarine air samples. On an annual basis, 75–80% of the prevailing winds are from the south-west (*i.e.* which tracks directly from the petrochemical complex towards the eastern suburbs of the city some 20 km away). However, even when the prevailing wind direction is from the opposite direction, the ambient air VOC concentrations in the city do not differ widely, and wintertime concentrations are frequently higher than levels downwind of the complex. The dominance of season over concentration is illustrated more clearly in Fig. 2a and b, which shows the total VOC concentrations at (a) a fixed sampling point adjacent to a yacht marina, (about 10 km south-west of Southampton City), and (b) a sampling point beside a busy (4-lane) road-bridge, 4 km west of Southampton City. Fig. 2 reveals the co-variance of total volatile aromatics (*i.e.* shown as the crossed points) with total VOCs (*i.e.* shown as the filled circles) from October 1990 through October 1991. In both diagrams, February was the worst month (typical air temperature, 2°C) whereas lowest VOC con-

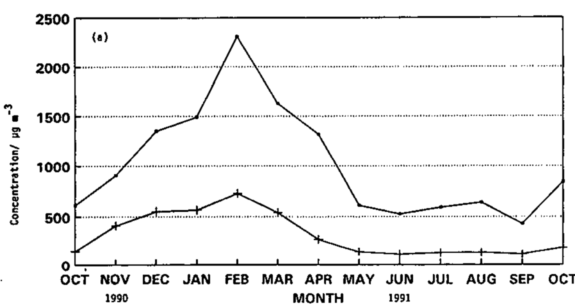
TABLE III

TYPICAL AIRBORNE CONCENTRATION RANGES OF SELECTED VOCs IN AMBIENT AIR FROM THE SOUTHAMPTON WATER ESTUARY DURING SUMMER AND WINTER

All results are expressed as $\mu g\ m^{-3}$

Atmospheric conditions	Summer	Winter
Temperature	19–32°C	−12–10°C
Relative humidity	38–98%	55–100%
Barometric pressure	749–790 mmHg	721–782 mmHg
VOCs	**Min–Max**	**Min–Max**
Ethane	1.6–18.4	2.9–155.6
Ethene	2.0–20.5	2.6–169.6
Propane	0.9–23.2	3.2–289.4
Propene	3.2–27.3	4.7–398.2
iso-Butane + *n*-Butane	3.7–34.5	5.3–605.3
n + iso-Butene	5.8–44.3	5.3–497.3
trans- + *cis*-Butene-2	2.2–24.3	4.3–338.5
n-Pentane	0.4–50.3	3.3–570.1
n-Hexane	0.3–44.5	3.0–449.3
n-Heptane	0.3–39.6	2.7–250.7
n-Nonane	0.6–34.4	2.3–147.2
n-Decane	0:3–37.7	4.3–153.5
n-Undecane	0.3–24.3	0.9–138.6
n-Dodecane	0.6–24.5	0.5–132.1
2,2-Dimethylbutane	3.4–22.4	4.5–198.9
2,3-Dimethylbutane	3.1–26.4	4.0–230.3
2-Methylpentane	4.6–58.4	8.5–296.9
3-Methylpentane	6.4–67.4	5.8–355.7
2,4,4-Trimethylpentane	2.3–55.3	4.3–389.4
Benzene	1.1–53.2	5.3–498.4
Methylbenzene (toluene)	4.6–76.6	10.3–944.3
1,3-Dimethylbenzene	4.5–84 4	7.8–895.7
1,2-Dimethylbenzene	2.3–77.8	5.6–800.4
Ethylbenzene	1.2–67.9	5.3–797.5
1,3,5-Trimethylbenzene	1.4–55.4	4.5–532.8
1,2,3-Trimethylbenzene	1.2–53.8	3.4–433.6
1,2,4-Trimethylbenzene	0.9–47.9	4.0–450.2
Isopropylbenzene	0.6–40.3	3.4–410.1
n-Propylbenzene	0.4–38.3	3.2–390.3
1,2,3,5-Tetramethylbenzene	0.4–23.2	2.2–260.2
Naphthalene	0.2–19.2	4.6–155.3
Dichloromethane	0.1–76.6	1.2–96.5
1,1,1,-Trichloroethane	0.2–85.5	1.0–90.4
Tetrachloroethene	0.1–24.5	0.4–33.6
Freon-113	3.2–66.8	5.5–438.7
2-Butanone	0.5–57.7	0.9–110.2
2-Pentanone	0.4–45.4	0.4–54.7
4-Methylcyclohexanone	0.5–66.8	0.8–87.8
Methylisobutylketone	0.3–37.7	0.6–68.6
n-Butanol	<0.1–6.4	0.4–10.2
2-Butanol	<0.1–5.4	0.5–9.6
Hexanal	<0.1–9.7	0.7–43.4
Octanol		

centrations were typically found from June to September (*i.e.* in two successive years, 1990–91) when typical air temperatures are much higher (*i.e.* 19°C–32°C) Despite higher volumes of traffic which occur during the peak summer tourist season, faster volatilisation rates combined with rapid photodegradation of volatile products partly explains why VOC levels are lower in summer. During wintertime, cooler air temperatures and slower photodegradation rates (*i.e.* high cloud cover, fog etc.) combined with greater inputs of combusted fossil-fuel products (*i.e.* aromatics) are major contributory factors to high VOC concentrations in winter months. Similar observations were made by Broddin *et al.* [27] in urban environments. Airborne hydrocarbons in aerosol fractions have also been identified further afield in North Sea atmospheres. Preston and Merrett [28] have shown that saturated hydrocarbons and PAH reach their highest levels in air which has been transported from terrestrial sources, particularly during winter.



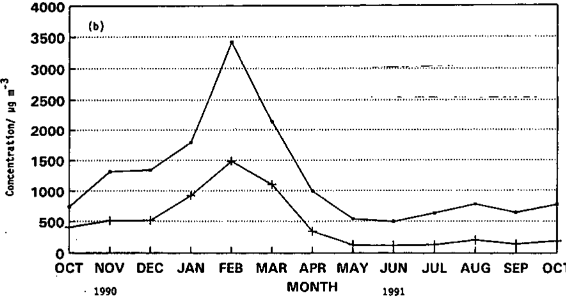


Fig. 2. (a) The monthly variation of total VOCs (●) and total aromatics (+) in ambient air at Hythe Marina in Southampton Water. This sampling station accommodates approximately 100 yachts and motor cruisers and is located approximately 10 km south-west of Southampton City. (b) The monthly variation of total VOCs (●) and total aromatics (+) in ambient air at the Redbridge 4-lane roadbridge 4 km west of Southampton City. This roadbridge typically accommodates over 50 000 vehicle movements in a single 24-h period.

Another important medium where the significant seasonal differences manifest themselves is in the estuarine waters of Southampton Water. As Table IV shows, higher levels of VOCs occur in the sea water during winter than in summer, and the estuary itself has a complex source-sink relationship with the atmosphere. Sauer [29] determined that reservoir and material fluxes of VOCs to and from sea water could be appropriately estimated using a stagnant film model developed by Broecker and Peng [30]. The model predicts that the flux, F, of gas (VOCs) from sea water to the atmosphere is dependent on the molecular diffusivity of the gas and the thickness of the stagnant diffusion-controlled boundary layer, z:

$$F = D_i\,(\mathrm{d}c/\mathrm{d}z)\ (i.e.\ F = K_i\,\Delta C_i)$$

where $K_i = D_i/z$, D_i = coefficient of molecular diffusion ($cm^2 s^{-1}$), z = film thickness (cm), and ΔC_i = concentration difference across the film layer, $C_{il} - C_{ig}$ ($mol\,l^{-1}$). C_{ig} is the gas concentration at equilibrium with the overlying air, $C_{ig} = \alpha p$, where α is the solubility of gas and p is the partial pressure of gas in the atmosphere (*i.e.* in these flux estimates, C_{ig} is assumed to be negligible). C_{il} is the concentration of gas in the aqueous mixed layer. Fluxes are determined on the assumption that there is no con-

TABLE IV

TYPICAL CONCENTRATION RANGES OF SELECTED VOCs IN THE WATER COLUMN OF SOUTHAMPTON WATER DURING SUMMER AND WINTER

Note that these concentrations refer to estuarine water from the head of the estuary. All results are expressed as $\mu g\,l^{-1}$.

VOCs	Summer	Winter
n-Hexane	0.08–0.91	0.55–1.03
n-Decane	0.04–0.20	0.19–1.10
Methylcyclohexane	0.01–0.29	0.36–0.92
Benzene	0.05–0.51	0.44–1.57
Methylbenzene (Toluene)	0.43–1.33	1.24–4.23
1,2-Dimethylbenzene	0.07–1.01	1.23–2.92
Ethylbenzene	0.09–0.92	0.81–1.77
1,2,4-Trimethylbenzene	0.02–0.18	0.14–0.83
Naphthalene	0.05–0.44	0.17–4.33

TABLE V

EQUILIBRIUM CONCENTRATIONS (ng l^{-1}) IN SEA WATER EQUILIBRATED WITH AIRBORNE CONCENTRATIONS OF 1 AND 50 ppb (v/v)

Numbers in parentheses beside each component represent the 1 and 50 ppb (v/v) concentrations expressed respectively in $\mu g\ m^{-3}$ units. *e.g.* 1 ppb (v/v) of benzene = 3.2 $\mu g\ m^{-3}$ in air. See text for details.

VOCs	M.W.	Hi_R [a]	C_i (ng l^{-1}) [b]	
			1 ppb (v/v)	50 ppb (v/v)
n-Hexane	86	47	0.063 (3.5)	3.8 (176)
n-Decane	142	252	0.024 (5.4)	1.2 (270)
Methylcyclohexane	98	11.4	0.36 (4.03)	18.0 (201.5)
Benzene	78	0.12	27.0 (3.2)	1370.0 (160)
Methylbenzene	92	0.18	21.0 (3.8)	1070.0 (189)
1,2-Dimethylbenzene	106	0.24	19.0 (4.3)	930.0 (217)
Ethylbenzene	106	0.25	18.0 (4.3)	890.0 (217)
1,2,4-Trimethylbenzene	130	0.20	27.0 (4.9)	1370.0 (246)
Naphthalene	128	$8.4 \cdot 10^{-3}$	640.0 (5.2)	3200.0 (260)

[a] Effective Henry's Law constant derived from vapour pressure and solubility data [35].
[b] $(P_i/Hi_R RT) \cdot (M.W._i) \cdot 10^9$; C_i = concentration in sea water (ng l^{-1}), P_i = concentration in the atmosphere (atm), $R = 82.05 \cdot 10^{-3}$ (atm l g mol^{-1} K^{-1}), and T = 290K.

tribution from the atmosphere. However, if VOCs are appreciable in the atmosphere, $C_{ig} \neq 0$ and the flux from marine waters will be reduced due to the decrease in the concentration difference, $C_{il} - C_{ig}$. Near urban areas atmospheric concentrations are appreciable enough to retard the flux from the water column and, if high enough, will contribute VOCs to the water column. Using Sauer's model, equilibrium concentrations of key VOCs in Southampton air and in its estuary were calculated and the results are presented in Table V for atmospheric concentrations varying from 1 to 50 ppb (v/v). The equivalent mass per unit volume concentration for each component is presented in parentheses for direct comparison with its v/v concentration. By applying Southampton sea water concentration data published previously by Bianchi and co-workers [9, 10,31–34] to the equilibrium model developed by Sauer [35], and calculating the equilibrium concentrations (presented in Table V), it is possible to see that atmospheric concentrations of VOCs in Southampton Water will contribute VOCs to surface sea water, especially during colder winter months. For example, a 50 ppb (v/v) (160 $\mu g\ m^{-3}$) concentration of benzene in estuarine air could conceivably yield a single component equilibrium concentration of 1.37 $\mu g\ l^{-1}$ in surface water.

CONCLUSION

The sampling and analytical method has proved to be very reliable in routine use. Despite new developments in sampling methodology, low-flow sampling pumps used in tadem with the Carbotrap and Carbosieve adsorbents have proved to be a sustainable and cost-effective option for taking over 300 individual samples. The use of thermal desorption ensures that all volatile compounds are quantifiable, minimising the disadvantages incurred with solvent-based desorption *i.e.* elution of components under the solvent peak, contamination from impurities in the solvent. Unlike a similar study of volatile hydrocarbons in ambient air within the city of Rome by Ciccioli *et al.* [36], no problems with water build-up on the Carbosieve S-III were found in this study, even when relative humidities were high (*i.e.* > 90%), permitting efficient sampling, thermal desorption and GC–MS analysis of every sample taken over the 2-year period.

This brief discussion about airborne VOCs in the ambient air of the Southampton Water region reveals a complex picture. Furthermore, the long-range aspect of this study has shown a clear and repeatable seasonal variability in the total VOC concentration profile. VOC concentrations are gen-

erally high, and when compared to similar studies carried out in cities such as Barcelona [16] and Rome [36], the concentrations of toxic compounds such as benzene appear to be slightly higher. However, since no seasonally varying data from the aforementioned surveys has been published, it is impossible to compare these studies directly. At minimum we believe that Southampton City would find it difficult to comply with the airborne standards for benzene concentrations proposed by the German and Dutch governments. We suspect that this conclusion would also apply to many other major British cities. The seasonal variation in VOC concentrations is also important because equilibrium models predict that many VOCs will partition into and out of surface sea water (depending on the concentration gradient across the air–sea interface for example). Previous long-term studies carried out on the Southampton Water estuary by the author(s) have already revealed marked increases in marine volatile aromatic concentrations during winter months which parallel increases in local ambient air concentrations, an observation which can be substantiated by Sauer's predictive model. This sampling and analytical exercise also reinforces the concept of a dynamic balance in VOC concentrations taking place at the air–sea interface and suggests that marine VOCs are unlikely to be in equilibrium with VOCs in the local atmosphere on a year-round basis. New air–sea exchange models are continuously being developed, particularly those based on dual tracer techniques [37], which will indoubtedly enhance our understanding of these complex processes now and in the future.

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Practical method for monitoring polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in the atmosphere

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ABSTRACT

A practical method is described for monitoring tetra- to octachlorodibenzo-*p*-dioxins (T_4–O_8CDDs) and tetra- to octachlorodibenzofurans (T_4–O_8CDFs) in atmospheric samples at ground level. The substances in air were sampled on quartz fibre and polyurethane foam plugs by using a high-volume air sampler. The sample congeners were extracted with acetone, washed with sulphuric acid after transfer into a hexane layer, fractionated by silica gel and alumina column chromatography and subsequently analysed by gas chromatography–mass spectrometry. Thirty three peaks found for 47 congeners out of 49 T_4–O_8CDDs and 58 peaks found for 82 congeners out of 89 T_4–O_8CDFs could be monitored.

INTRODUCTION

Many reports have been published on sources, toxicity, mechanisms of formation and analytical methods for polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). As PCDDs and PCDFs are amongst the most hazardous chemicals found in the environment [1–6], monitoring has recently been conducted even for airborne PCDDs and PCDFs in order to investigate their toxcity to human health and carcinogenic risks [7–14]. However, various congeners present in ambient air are at ultra-trace levels corresponding to 1/100–1/1000th of the concentrations in emission gas from an incinerator, and ambient air could include a great number of organics that interfere in the analysis. More recently, since the toxicity of the individual congeners has been evaluated by using the toxicity equivalence quantity (TEQ) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-T_4CDD), all the congeners of tetra- to octachlorodibenzo-*p*-dioxins (T_4–O_8CDDs) and dibenzofurans (T_4–O_8CDFs) could be simultaneously determined in ambient air. In field investigations, a number of samples should be sampled at a time at various sites and accurately analysed in a limited period. In addition, a practical method must be consistent with low analysis costs, a simple procedure and easy maintenance of analytical instruments. Conventional techniques for analyses for PCDDs and PCDFs are time-consuming, laborious and not completely satisfactory.

In this paper, a convenient method is proposed for monitoring trace levels of T_4–O_8CDDs and T_4–O_8CDFs in the atmospheric environment at ground level. Air is sampled by using a high-volume air sampler on a quartz fibre filter (QFF) and polyurethane foam plugs (PUFPs). The trapped sample is extracted, washed with sulphuric acid, purified by silica gel and alumina column chromatography and then analysed by gas chromatography–mass spectrometry (GC–MS). The method has been successfully used for monitoring PCDD and PCDF con-

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geners in the atmospheric environment at ground levels for several years.

EXPERIMENTAL

Standard reagents and materials

^{13}C-labelled and unlabelled PCDDs and PCDFs as 10 μg/ml solutions (see Table I) were purchased from Cambridge Isotope Laboratories (Cambridge, MA, USA). Another standard mixture was prepared in toluene by extracting fly ash from a incinerator [15] for adjusting and/or confirming the analytical conditions and identifying PCDD and PCDF congeners. *n*-Hexane, acetone and dichloromethane were of pesticide residue analysis grade from Wako (Osaka, Japan) and other solvents or reagents were of either chromatographic or special grade from Wako.

Silica gel for silica gel column chromatography (Si-CC) was Wakogel (Wako) activated by heating at 130°C for 4 h. A 3-g amount of the silica gel was slurry packed into a 30-cm × 10-mm I.D. glass column. The top of the gel was covered with a 10-mm layer of anhydrous sodium sulphate. Alumina for alumina column chromatography (A1-CC) was of basic type with activity 1 from Merck (Darmstadt, Germany), activated by heating at 130°C for 4 h. A 5-g amount of the alumina was slurry packed into a 30-cm × 10-mm I.D. glass column. The top of the column was covered with a 10-mm layer of anhydrous sodium sulphate. The PUFP was cut as a 50-mm × 90-mm diameter piece from a polyurethane foam sheet (0.020 g/cm^3 density and 50 mm thick; Ether-type) available from Bridge Stone (Tokyo, Japan) and washed with acetone for 24 h. The QFF was QR-100 from Advantic Toyo (Tokyo, Japan).

TABLE I

UNLABELLED AND ^{13}C-LABELLED PCDD AND PCDF CONGENERS USED AS STANDARDS

The analytical responses to the native congeners in the SIM mode were corrected by using relative response of the listed ^{12}C-labelled congeners to the equivalent ^{13}C isotopes.

[^{12}C]- and [^{13}C]PCDDs	[^{12}C]- and [^{13}C]PCDFs
2,3,7,8-T_4CDD	2,3,7,8-T_4CDF
1,2,3,7,8-P_5CDD	1,2,3,7,8-P_5CDF
1,2,3,6,7,8-H_6CDD	1,2,3,4,7,8-H_5CDF
1,2,3,4,6,7,8-H_7CDD	1,2,3,4,6,7,8-H_7CDF
1,2,3,4,6,7,8,9-O_8CDD	1,2,3,4,6,7,8,9-O_8CDD

Apparatus

The apparatus for sampling PCDDs and PCDFs is shown in Fig. 1 [16]. The sampling apparatus was attached to a Kimoto Electric (Osaka, Japan) HV-120 high-volume air sampler equipped with a Kansai Gas Meter (Osaka, Japan) N2-K838 integrating gas flow meter. An Ogasawara (Tokyo, Japan) A-1250 automatic recording thermometer with a platinum thermo-sensor was set next to the sampling apparatus.

The specification and operating conditions of the gas chromatograph–mass spectrometer used are given in Table II. For GC–MS analysis the selected ion monitoring (SIM) mode was used. The resolution of the mass spectra was 3000–5000. The number of mass ion monitoring channels was four for both PCDD and PCDF congeners in one analytical run.

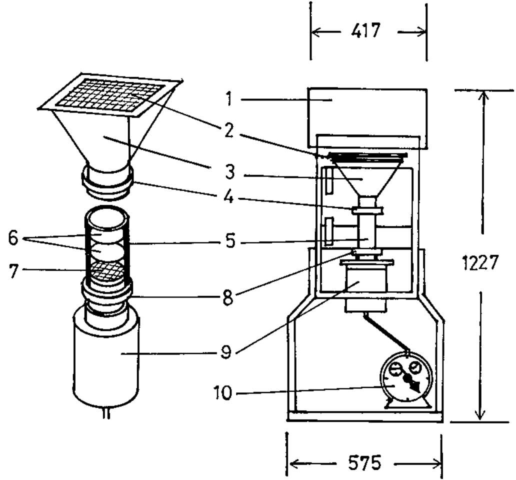

Fig. 1 Sampling apparatus for sampling PCDD and PCDF congeners in air. 1 = Shelter; 2 = filter and stainless-steel wire net (5 mm mesh); 3 = filter holder; 4 = screw clasp with PTFE packing; 5 = PUFP holder (200 mm × 84 mm I.D. aluminium tube); 6 = two PUFPs (50 mm × 90 mm diameter in series); 7 = stainless-steel wire mesh (50 mm mesh); 8 = screw clasp with PTFE packing; 9 = high-volume air-suction pump (Kimoto Electronic HV-120); 10 = integrating gas flow meter (Kansai Gas Meter N2-K838). The dimensions of the apparatus are indicated in millimeters.

TABLE II

ANALYTICAL CONDITIONS FOR GC–MS

Apparatus: Hewlett-Packard (Avondale, PA, USA) model 5790A gas chromatograph and Japan Electronic (Tokyo, Japan) DX303/DA5000 mass spectrometer.

Parameter	T_4-, P_5- and H_6C congeners			H_7- and O_8C congeners		
Column	Supelco (Bellefonte, PA, USA), 30 m × 0.25 mm I.D., O.20 μm film thickness, SP-2331 chemically bonded fused-silica capillary column (63% cyanopropyl polysiloxane)			Hewlett-Packard, 25 m × 0.31 mm I.D., 0.52 μm film thickness, Ultra-1 chemically bonded fused-silica capillary column (non-polar, cross-linked methylsilicone)		
Injection	Splitless (90 s)			Splitless (90 s)		
Injection temperature	260°C			260°C		
Column head pressure	1.0 kg/cm^2			1.0 kg/cm^2		
Column conditions	160°C for 2 min, programmed at 8°C/min to 200°C and 3°C/min to 265°C and held at 265°C for 30 min			160°C for 2 min, programmed at 20°C/min to 200°C and 5°C/min to 310°C and held at 310°C for 30 min		
		^{12}C	^{13}C		^{12}C	^{13}C
Mass number of selected ion monitor (SIM)	T_4CDDs	320, 322	332, 334	H_7CDDs	424, 426	436, 438
	T_4CDFs	304, 306	316, 318	H_7CDFs	408, 410	420, 422
	P_5CDDs	356, 358	368, 370	O_8CDDs	460, 462	470, 472
	P_5CDFs	340, 342	350, 352	O_8CDFs	442, 444	452, 454
	H_6CDDs	390, 392	402, 404			
	H_6CDFs	374, 376	386, 388			
Voltage of ion multiplier		2.0 kV			2.0 kV	
Electron ionization voltage		70 eV			70 eV	

Sampling

Ambient air was sampled at 0.6–0.7 m^3/min for 24 h from 10 a.m. to 10 a.m. the next day. The average temperature was calculated from the temperature recorded continuously throughout the day.

Clean-up of sample

The QFF and the PUFPs were each extracted with 500 ml of acetone in a Soxhlet extractor. The extracts were combined, concentrated to 5 ml in a Kuderna–Danish (KD) concentrator and evaporated to 1 ml by slowly flushing nitrogen over the sample from a needle.

The concentrated extract was mixed in a separating funnel with 150 ml of *n*-hexane and then with the internal standard solutions individually containing 7.5 ng of 10 ^{13}C-labelled PCDDs and PCDFs (see Table I). The mixed sample was repeatedly washed with 5 ml of sulphuric acid until the sample became colourless (normally three or four washings were required). The hexane layer was then washed three times with 50 ml of distilled water, dehydrated with 2 g of anhydrous sodium sulphate, evaporated to 5 ml in a KD concentrator and reduced to 3 ml by flushing nitrogen over it.

The sample was poured onto the silica gel columns and eluted with 250 ml of *n*-hexane. The eluate was evaporated to 5 ml and then reduced to 3 ml by slowly passing nitrogen over the sample.

The sample was poured onto the alumina column, washed with 20 ml of *n*-hexane and eluted with 50 ml of *n*-hexane–dichloromethane (1:1). The eluate was reduced to 3 ml in a similar way as above. The alumina column chromatographic purification was conducted twice for samples from heavily air-polluted sites. The sample was concentrated to 100 μl by flushing nitrogen over it, mixed with 200 μl of toluene and then concentrated to 50 μl by flushing nitrogen over it. The sample was then ready for analysis by GC–MS.

GC–MS analysis

A 3-μl volume of the sample was introduced into the GC–MS instrument with a microsyringe. In the GC–MS analysis (see Table II for the analytical

conditions), two mass ion channels were selected for an isomer gorup. The GC–MS analyses were performed five times for each sample. The first run was for T_4C and H_6C congeners (after T_4C congeners had been eluted, the channels were switched to those in the analysis of H_6C congeners), the second for P_5C congeners, the third for the fly-ash extract for identification of the T_4–H_6C congeners, the fourth for H_7C and O_8C congeners and the last for the fly-ash extract for identification of the H_7C and O_8C congeners. PCDD or PCDF congeners were identified by matching the retention times of the congeners with those of the corresponding congeners in the fly-ash extract, and positively quantified by peak areas in the cases that (1) the ratio of the relative peak areas of the two major characteristic ions monitored for a particular congener corresponded within $\pm 30\%$ to that resulting for the corresponding standard and (2) the signal-to-noise ratio was greater than 3 [12]. The analytical responses to the native congeners in the SIM mode were corrected by using the relative responses of the ^{12}C congeners to the equivalent ^{13}C isotopes listed in Table I.

Calculation of concentration of PCDDs and PCDFs

PCDDs and PCDFs were individually monitored as concentrations in pg/m^3 at 20°C under 1 atm for the atmospheric samples. The concentration of PCDD and PCDF congeners are presented as total concentrations of the individual PCDD and PCDF congeners, respectively. The TEQ calculation was made by the method of the North Atlantic Treaty Organization [12].

RESULTS AND DISCUSSION

Sampling

Since the PCDDs and the PCDFs are present at ultra-trace levels in the atmosphere, as much air sample as possible should be sampled to ensure detection of the congeners. The sampling apparatus, capable of sampling a large volume of air (*ca.* 1000 m^3/day), was similar to that for sampling polyclic aromatic hydrocarbons or PCDDs and PCDFs in ambient air [7,9,16]. The sampling rate (0.6–0.7 m^3/min) and the total sample volume were strictly checked and confirmed by an integrating gas flow meter calibrated before the sampling. The T_4–O_8C congeners were usually trapped at above 99% with the QFF and the first PUFP attached to the high-volumne air sampler [7,9]. In this instance two PUFPs were used, considering the high sampling temperature and the heavy air pollution near a busy road. However, very small amounts of T_4–O_8C congeners, leaving the QFF during the sampling process, were detected on the second PUFP.

Selection of internal standards

The use of as many ^{13}C-labelled standards as possible is desirable for minimizing analytical errors, but none of the congeners are commercially available, In addition, commercially available ^{13}C-labelled standards are much too expensive for routine work. Under the assumption that PCDD and PCDF isomers behaved in the clean-up procedure in the same way as the corresponding ^{13}C-labelled internal standards [12,17–20], five ^{13}C-labelled PCDDs and five ^{13}C-labelled PCDFs (see Table I) were used as internal standards.

Clean-up of air sample

The extracts from the QFF and the PUFPs were heavily contaminated with organic and inorganic substance and showed high viscosity. The viscous matter seemed to come mainly from the PUFPs but were effectivley reduced by the washing with sulphuric acid to remove fatty, basic and other organics. The extracts from ordinary samples should be washed at least three times until the the extracts become colourless. For samples from the heavily air-polluted areas, the extracted samples often included cotton-like suspended matter that must be removed by filtration after addition of the internal standards. Up to six washings with sulphuric acid were necessary until the extracts became colourless.

In conventional analysis [9–11,13–15,18,20,21], Al-CC and reversed-phase liquid chromatography (RPLC) using a silica-ODS column have been used for the purification of dioxin samples. However, the PCDD and PCDF congeners in air samples could not be determined, except for the O_8C congeners, because of interfering organics if all the congeners were collected in one fraction by RPLC. In addition, the RPLC procedure is laborious and time consuming and the samples often acquire contaminants from the injection port. It was found that Si-CC [19,21–24], which efficiently removes polar and

TABLE III

ELUTION ORDER OF PCDD CONGENERS ON THE SP-2331 AND THE ULTRA-1 COLUMNS

No.[a]	T_4CDDs[b]	No.[a]	P_5CDDs[b]	No.[a]	H_6CDDs[b]
1	1,3,6,8	1	1,2,4,6,8	1	1,2,3.4,6,8
2	1,3,7,9		1,2,4,7,9		1,2,4,6,7,9
3	1,3,7,8	2	1,2,3,6,8		1,2,4,6,8,9
4	1,2,4,7/1,2,4,8	3	1,2,4,7,8	2	1,2,3,6,7,9
	1,3,6,9	4	1,2,3,7,9		1,2,3,6,8,9
5	1,2,6,8	5	1,2,3,4,7	3[d]	1,2,3,4,7,8
6	1,4,7,8		1,2,4,6,9	4[d]	1,2,3,6,7,8
7[d]	2,3,7,8	6[d]	1,2,3,7,8	5	1,2,3,4,6,9
8	1,2,3,4/1,2,3,7	7	1,2,3,6,9	6[d]	1,2,3,7,8,9
	1,2,3,8/1,2,4,6	8	1,2,4,6,7	7	1,2,3,4,6,7
	1,2,4,7		1,2,4,8,9		
9	1,2,3,6/1,2,7,9	9	1,2,3,4,6	No.[a]	H_7CDDs[c]
10	1,2,7,8/1,4,6,9	10	1,2,3,6,7		
11	1,2,3,9	11	1,2,3,8,9	1	1,2,3,4,6,7,9
12	1,2,6,9			2[d]	1,2,3,4,6,7,8
13	1,2,6,7				
14	1,2,8,9			No.[a]	O_8CDDs[c]
				1[d]	1,2,3,4,6,7,8,9

[a] Elution order (or peak order).
[b] Separation on the SP-2331 capillary column.
[c] Separation on the Ultra-1 capillary column.
[d] 2,3,7,8-Substituted congeners used for calculating the toxicity equivalence quantity (TEQ) of 2,3,7,8-T_4CDD by the method of the North Atlantic Treaty Orgnization [12].

coloured substances, was effective in cleaning up the air samples. Hence the combined Si-CC and Al-CC system was useful for cleaning up the samples without multiple fractionation for the T_4–O_8CDD and T_4–O_8CDF congeners.

The recoveries of the standard PCDD and PCDF congeners using the clean-up procedure were ususally 70–90% though they differed depending on the level of contamination of the samples, the number of washing times with sulphuric acid and the number of Al-CC steps applied. Increased washings with sulphuric acid and Al-CC steps may have adverse effects on the recovery of the compounds. Heavily polluted samples required six washings and two Al-CC steps for clean-up and, as a result, the recoveries often decreased to *ca.* 50%. A larger Al-CC column might be used if elution data are confirmed for the PCDD and PCDF congeners.

GC–MS analysis

Recently, several analytical columns, such as DB-Dioxin, Quadrex DXN and Quadrex 23, have become commercially available with excellent resolution for PCDD and PCDF congeners, but it was difficult to use them owing to insufficient retention data for all of the congeners. The SP-2331 column was most useful for the analysis of the T_4–H_6C congeners as the resolution was excellent and the retention data had been clarified for these congeners [8,12,17,20,25–27]. However, the SP-2332 column showed heavy bleeding and low analytical accuracy at the elution temperature of the O_8C congeners. A non-polar Ultra-1 column (cross-linked methylsilicone) was therefore used to determine the H_7C and O_8C congeners.

The analytical mass numbers for SIM, listed in Table II, were selected so as to minimize the effects of interfering substances in the air samples especially in the analysis for P_5CDF, H_6CDF, O_8CDD and O_8CDF congeners.

Identification and determination of the PCDD and PCDF congeners were basically effected using EPA Method 8290 [12]. A standard sample for identification was prepared by extracting a fly-ash

sample from a city incinerator [12,15]. Tables III and IV and Fig. 2 show retention data for the PCDDs and the PCDFs from the fly-ash sample used for identification of the PCDD and PCDF congeners in air samples. The fly-ash sample contained much higher concentration levels of all the congeners than found in air, but with similar chromatographic patterns. The fly-ash sample was also useful for the adjustment and confirmation of the column and gas chromatographic conditions.

The detection limit for each PCDD and PCDF congener was 2 pg in GC–MS analysis and the minimum detectable concentration of each congener was 0.5 pg/m^3 for a 1000-m^3 air sample.

Analysis of atmospheric samples

The method was applied to monitoring of the PCDD and PCDF congeners in the atmospheric environment at industrial, commercial, residential and background sites in the Osaka prefecture in August and December 1988–92. The total number of samples analysed was 108. The PCDDs and the PCDFs were detected in all samples, even those from the countryside. However, 2,3,7,8-T_4CDD and 2,3,7,8-T_4CDF were difficult to determine even in heavily contaminated samples, although they were detected as trace peaks. The total concentrations of the PCDD and PCDF congeners were 2–100 and 2–200 pg/m^3, respectively. The TEQs were

TABLE IV

ELUTION ORDER OF PCDF CONGENERS ON THE SP-2331 AND THE ULTRA-1 CAPILLARY COLUMNS

No.[a]	T_4CDFs[b]	No.[a]	P_5CDFs[b]	No.[a]	H_6CDFs[b]
1	1,3,6,8	1	1,3,4,6,8	1	1,2,3,4,6,8
2	1,3,7,8/1,3,7,9	2	1,2,4,6,8	2	1,3,4,6,7,8
3	1,3,4,7	3	1,3,6,7,8		1,3,4,6,7,9
4	1,4,6,8	4	1,3,4,7,8	3	1,2,4,6,7,8
5	1,2,4,7/1,3,6,7	5	1,3,4,7,9/1,2,3,6,8	4	1,2,4,6,7,9
6	1,3,4,8	6	1,2,4,7,8	5[d]	1,2,3,4,7,8
7	1,2,4,8/1,3,4,6	7	1,2,4,7,9/1,3,4,6,7		1,2,3,4,7,9
8	1,2,4,6/1,2,6,8	8	1,2,4,6,7	6[d]	1,2,3,6,7,8
	1,2,3,7/1,4,7,8	9	1,2,3,4,7/1,4,6,7,8	7	1,2,4,6,8,9
	1,3,6,9	10	1,3,4,6,9	8	1,2,3,4,6,7
9	1,2,3,4/2,3,4,9	11[d]	1,2,3,4,8/1,2,3,7,8	9	1,2,3,6,7,9
10	1,2,3,6/1,2,3,8	12	1,2,3,4,6	10	1,2,3,4,6,9
	1,4,6,7/2,4,6,8	13	1,2,3,7,9		1,2,3,6,8,9
11	1,3,4,9	14	1,2,3,6,7	11[d]	1,2,3,7,8,9
12	1,2,7,8	15	1,2,4,6,9/2,3,4,8,9	12	1,2,3,4,8,9
13	1,2,6,7/1,2,7,9	16	1,3,4,8,9	13[d]	2,3,4,6,7,8
14	2,3,6,8/1,4,6,9	17	1,2,4,8,9		
	1,2,4,9	18	1,2,3,6,9	No.[a]	H_7CDFs[c]
15	2,4,6,7	19	2,3,4,6,8		
16	1,2,3,9/2,3,4,7	20	1,2,3,4,9	1[d]	1,2,3,4,6,7,8
17	1,2,6,9	21[d]	2,3,4,7,8	2	1,2,3,4,6,7,9
18[d]	2,3,7,8/2,3,4,8	22	1,2,3,8,9	3	1,2,3,4,6,8,9
19	2,3,4,6	23	2,3,4,6,7	4[d]	1,2,3,4,7,8,9
20	2,3,6,7/3,4,6,7				
21	1,2,8,9			No.[a]	O_8CDFs[c]
				1[d]	1,2,3,4,6,7,8,9

[a] Elution order (or peak order).
[b] Separation on the SP-2331 capillary column.
[c] Separation on the Ultra-1 capillary column.
[d] 2,3,7,8-Substituted congeners used for calculating the TEQ.

0–0.6 pg/m^3 for the PCDD congeners and 0–1.2 pg/m^3 for the PCDF congeners, although a few unresolved congeners overlapped with 2,3,7,8-T_4CDF and 1,2,3,7,8-P_5CDF (see Table IV).

Table V presents typical results of monitoring PCDD and PCDF congeners in the atmosphere in Osaka prefecture and Fig. 3 shows chromatograms for a sample from a central urban site close to busy roads. A number of peaks, appearing close to the isomers of T_4CDDs and T_4CDFs, might adversely affect the measurment of the T_4C congeners at ultra-trace levels as seen in Fig. 3. Thirty three peaks for 47 congeners out of 49 PCDDs and 58 peaks for 82 congeners out of 87 PCDFs, most of which were close to the detection limit (0.5 pg/m^3), could be quantitatively monitored in the atmospheric samples and the concentrations of PCDDs and PCDFs were represented as total concentrations of the individual PCDD and PCDF congeners, respectively. Thus, the analytical results could become significantly different unless the analytical conditions are precisely confirmed around the detection limits. More effective clean-up procedures and/or higher MS resolution may be necessary to determine the T_4-O_8CDD and T_4-O_8CDF congeners at levels lower than 0.5 pg/m^3 without effects of interfering substances.

TABLE V

TYPICAL MONITORING DATA OF PCDD AND PCDF CONGENERS IN THE ATMOPSHERIC ENVIRONMENT IN OSAKA

Sample	Congeners	Concentration of PCDDs and PCDFs (number of peaks, number of isomers)[a]					Total (TEQ)
		T_4C-	P_5C-	H_6C-	H_7C-	O_8C-	
Sample 1[b,g]	DDs	2.5(3, 4)	3.7(3, 5)	5.4(3, 6)	8.3(2, 2)	6.2(1, 1)	26.1(–)
	DFs	14.4(12, 22)	14.1(15, 20)	13.3(8, 11)	8.4(4, 4)	3.1(1, 1)	53.3(–)
Sample 2[b,h]	DDs	9.4(7, 8)	15.9(9, 12)	13.9(5, 8)	23.1(2, 2)	20.5(1, 1)	82.8(0.5)
	DFs	28.8(17, 31)	45.0(19, 24)	35.2(10, 13)	31.9(4, 4)	15.0(1, 1)	155.9(1.2)
Sample 3[c,g]	DDs	1.9(2, 2)	5.4(6, 9)	2.2(2, 5)	5.0(2, 2)	3.9(1, 1)	18.4(–)
	DFs	11.6(11, 25)	8.7(12, 17)	6.5(7, 10)	3.8(2, 2)	2.1(1, 1)	32.7(–)
Sample 4[c,h]	DDs	4.7(2, 2)	5.5(4, 5)	5.8(3, 6)	16.3(2, 2)	8.8(1, 1)	41.1(–)
	DFs	24.6(15, 30)	27.0(16, 21)	22.2(8, 11)	26.6(4, 4)	21.3(1; 1)	121.7(0.4)
Sample 5[d,g]	DDs	15.7(4, 10)	42.8(9, 12)	5.8(3, 6)	6.0(2, 2)	3.3(1, 1)	73.6(–)
	DFs	23.4(14, 29)	23.7(16, 21)	12.4(8, 11)	11.2(3, 3)	4.9(1, 1)	75.6(0.3)
Sample 6[d,h]	DDs	9.6(8, 12)	8.3(5, 8)	9.2(4, 7)	18.1(2, 2)	15.4(1, 1)	60.6(–)
	DFs	20.9(15, 32)	29.0(17, 22)	27.6(10, 13)	40.3(4, 4)	21.6(1, 1)	139.4(0.5)
Sample 7[e,g]	DDs	2.3(2, 2)	9.1(5, 7)	4.2(3, 6)	11.4(2, 2)	9.6(1, 1)	36.6(–)
	DFs	8.0(8, 20)	18.8(14, 19)	11.4(8, 11)	4.8(3, 3)	5.5(1, 1)	48.5(0.1)
Sample 8[e,h]	DDs	4.2(2; 2)	10.6(5, 7)	7.9(6, 9)	11.5(2, 2)	18.4(1, 1)	52.6(–)
	DFs	22.1(17, 32)	42.0(18, 23)	19.4(10, 13)	17.2(4, 4)	8.5(1, 1)	109.2(0.4)
Sample 9[f,g]	DDs	0.9(1, 1)	1.3(2, 3)	ND[i](–, –)	1.8(1, 1)	1.8(1, 1)	5.8(–)
	DFs	ND(–, –)	0.5(1, 1)	4.2(3, 4)	8.7(3, 3)	1.0(1, 1)	14.4(–)
Sample 10[f,h]	DDs	0.9(1, 1)	1.2(2, 3)	ND(–, –)	0.6(1, 1)	0.9(1, 1)	3.6(–)
	DFs	2.5(3, 5)	ND(–, –)	3.5(2, 3)	ND(–, –)	ND(–, –)	6.0(–)

[a] Daily average concentration in pg/m^3 at 20°C and 1 atm; the number of peaks and the number of isomers quantified in the SIM analysis are given in parentheses.
[b] Sampling site: adjacent to traffic roads in the urban central area.
[c] Sampling site: near to a traffic road in the urban residential area not far from the coast.
[d] Sampling site: north-inland residential area.
[e] Sampling site: coastal industrial area.
[f] Sampling site: south mountainous area.
[g] Sampling time: August 1990.
[h] Sampling time: December 1990.
[i] ND = not detected (the concentration of each congener less than 0.5 pg/m^3).

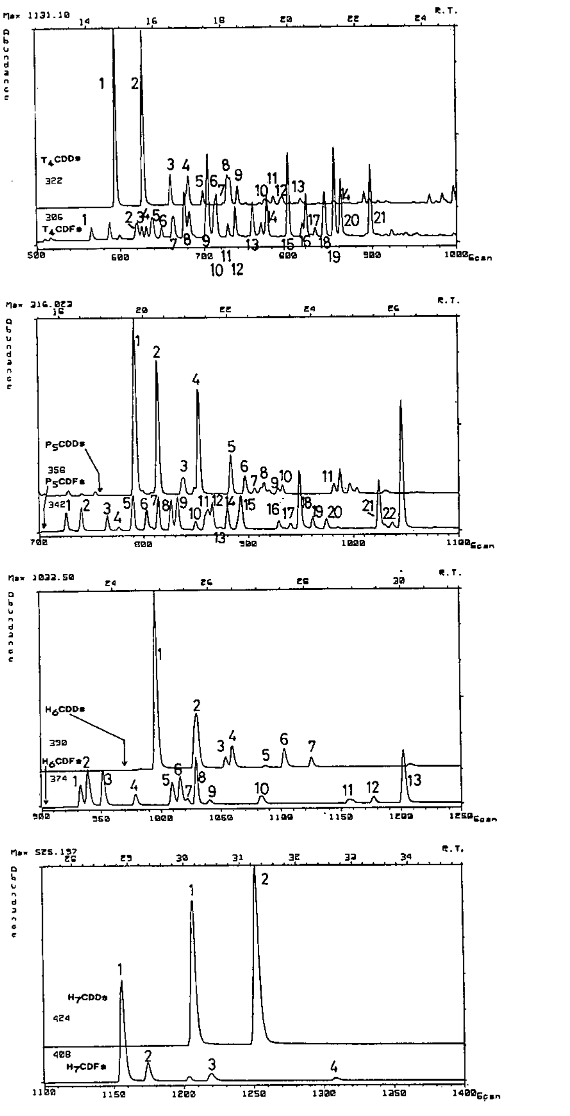

Fig. 2. SIM chromatograms showing elution orders of the PCDD and PCDF congeners in the fly ash extract. See Tables III and IV for peak numbers. R.T. = Retention time in min.

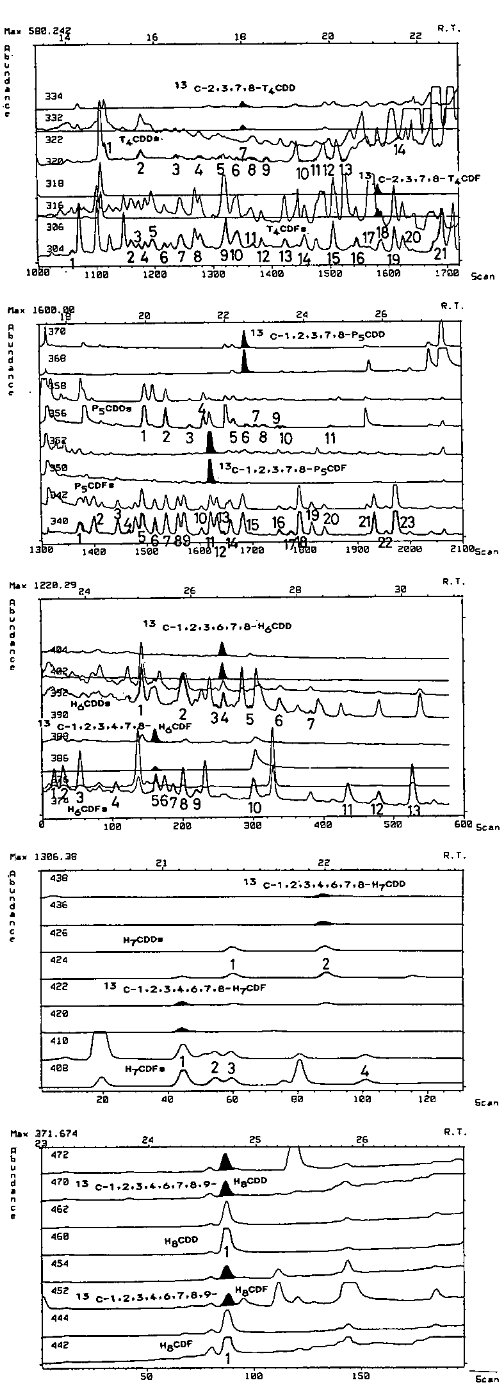

Fig. 3. Typical SIM chromatograms for the PCDD and PCDF congeners in urban air. See Tables III and IV for peak numbers. R.T. = Retention time in min.

CONCLUSIONS

The proposed method may be convenient for sampling high-volume air samples and determining the T_4–O_8CDD and T_4–O_8CDF congeners. The combined Si-CC and Al-CC system, after washing the samples with sulphuric acid, was effective in cleaning up the samples without multiple fraction-

ations for all the congeners. A fly-ash sample was useful for identification of the congeners and for adjustment of the column and GC–MS conditions. GC–MS analysis may be reasonable with a 3000–5000 resolution by selecting suitable internal standards and analytical mass numbers for SIM that are less affected by interfering substances. The method may be useful for analysing large numbers of airborne sample within a reasonable time.

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CHROMSYMP. 2722

Determination of volatile amines in air by diffusive sampling, thiourea formation and high-performance liquid chromatography

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ABSTRACT

A diffusive sampling method for the determination of primary and secondary amines in air was evaluated. The sampler consists of a filter, impregnated with naphthyl isothiocyanate, in a polypropylene housing. A substituted thiourea is formed *in situ* during sampling, which is desorbed and determined by high-performance liquid chromatography. The sampler was validated for the sampling of methylamine, allylamine, isopropylamine, *n*-butylamine and dimethylamine using standard amine atmospheres, and the uptake rates for the five amines were determined. The effect on uptake rate of amine concentration, sampling time and relative humidity was investigated and found to be small. The detection limits for the amines studied are below 50 $\mu g/m^3$ for an 8-h sample.

INTRODUCTION

For the monitoring of low-molecular-mass aliphatic amines in air, several methods are available involving impingers or gas dispersion bottles, acidic solvents and gas chromatographic (GC) determination. These "wet" sampling methods have disadvantages, especially with personal sampling, and dry methods are preferred. Polar solid sorbents, such as silica gel, can be used for the sampling of amines, followed by chromatographic determination of free amines in solution, but these methods are usually not very sensitive [1]. For the sampling of more reactive primary and secondary amines, sorbents are usually not suitable unless coated with a reagent. This derivatization may enhance the sensitivity for the GC or high-performance liquid chromatographic (HPLC) analysis [2,3]. We have previously reported the use of a 1-naphthyl isothiocyanate-coated XAD-2 sorbent for the determination of ethylenediamine and gaseous polyamines and of diethylamine in air [4–6]. Primary and secondary amines react rapidly and quantitatively and form stable thiourea derivatives. This method has high sensitivity when reversed-phase HPLC and UV detection are used.

Diffusive sampling has become an important method as an efficient alternative to pumped sampling in occupational hygiene [7]. We have previously reported the development of a diffusive sampler, designed to contain a reagent-coated filter for the sampling of reactive compounds. The sampler has been validated for formaldehyde, with a 2,4-dinitrophenylhydrazine-coated filter [8], and for diethylamine, with a 1-naphthyl isothio-cyanate-impregnated filter [6]. We have now validated the sampler for determination of methylamine, isopropylamine, *n*-butylamine, allylamine and dimethylamine in air.

EXPERIMENTAL

Diffusive sampler

The diffusive sampler is shown in Fig. 1. The housing, measuring 60 × 30 × 5 mm, is made of

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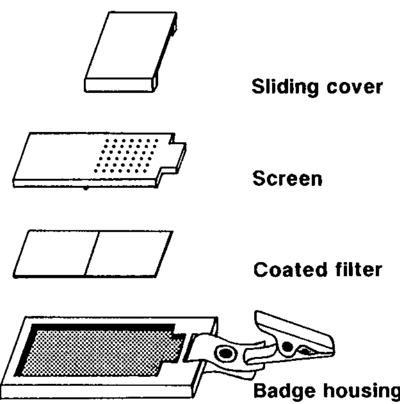

Fig. 1. Diffusive sampler for primary and secondary amines.

polypropylene. The impregnated filter, 20 × 45 mm, is placed beneath a 2.9-mm thick screen of the same size. Within an area 20 × 20 mm, the screen has 112 holes with a diameter of 1.0 mm. The filter part beneath the holes is used for sampling (sampling filter) and the other half is used to determine the filter blank (control filter). The tape is divided into the two sections by a small ridge on the back of the screen plate. A sliding cover is used to seal the holes when the sampler is not in use. The sampler is available from GMD Systems, (Hendersonville, PA, USA).

Chemicals

Solvents used for the HPLC analysis were acetonitrile (HPLC grade; Rathburn, Walkerburn, UK) and water (purified with Milli-RQ system; Millipore, MA, USA). Certified amine gas was used for the dynamic generation (methylamine 795 ± 40 ppm, isopropylamine 503 ± 25 ppm, allylamine 161 ± 5 ppm, *n*-butylamine 300 ± 15 ppm and dimethylamine 609 ± 12 ppm in nitrogen; Aga Specialgas, Lidingö, Sweden).

1-Naphthyl isothiocyanate (NIT) analytical-reagent grade; (Sigma, St Louis, MO, USA) was purified by recrystallization from absolute ethanol. The reference thiourea derivatives were synthesized from the different amines and NIT. To a solution of 500 mg of NIT a small excess of amine in 2 ml of ethanol was added dropwise at room temperature. The solution was heated for 10 min and then allowed to cool, then 7 ml of water was added, dropwise, until crystallization started. After filtration, the crystals were recrystallized from hot 50% ethanol. These thiourea derivatives were used for preparing analytical standards in acetonitrile. The solutions are stable for several months if stored in a refrigerator.

Sample analysis

The filters from the diffusive samplers were cut into two pieces for separate analysis of the sampling and control filter parts. These parts were transferred into 4-ml glass vials and shaken for 30 min with 3.0 ml of acetonitrile. The analyses were performed with a Millipore Waters (Milford, MA, USA) M-710 B automatic injector, two Millipore Waters M-6000 pumps, an M-440 absorbance detector and a computer with a Millipore Waters Maxima control and evaluation program. Volumes of 10 μl were injected and the column was a Millipore Waters Nova-Pak C_{18} (100 × 5 mm I.D., 4-μm particles). The flow-rate was 0.8 ml/min and the mobile phase was water–acetonitrile (1:1). The thiourea derivatives were detected at 254 nm with detection limits of about 0.5 ng (signal-to-noise ratio = 4:1). Chromatograms with the different amine derivatives and a blank filter are shown in Fig. 2 . The amine content of the control filter was always subtracted from that of the sampling filter when calculating air concentrations.

Laboratory validation

Standard atmospheres of amines were generated in a dynamic system by dilution of certified amine gas. The generation system and the exposure chamber were essentially the same as those used for the generation of formaldehyde [8]. The samplers were exposed six at a time, to amine levels from 0.5 to 25 mg/m^3, with sampling periods between 15 min and 8 h. The relative humidity was varied between 10% and 80%. Samplers were oriented parallel to the air stream. The air velocity was about 0.25 m/s. For confirmation of amine levels in the exposure chamber, pumped sampling with gas dispersion bottles (1 l/min) containing 15 ml of 0.02 *M* HCl was used. The solution from the gas dispersion bottles were analysed by isotachophoresis [9] or HPLC [10].

RESULTS AND DISCUSSION

To be able to meet functional criteria, a sampling

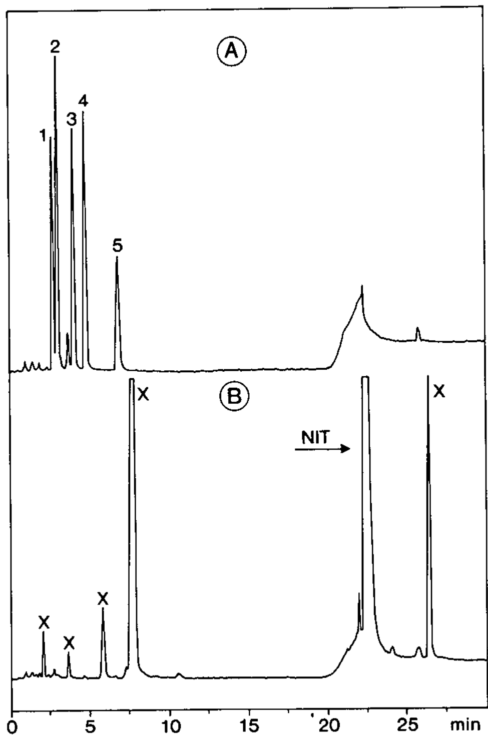

Fig. 2. HPLC of (A) amine standard solution and (B) filter blank. The urea derivatives are from (1) methylamine, (2) dimethylamine, (3) allylamine, (4) isopropylamine and (5) *n*-butylamine. The amounts of the urea derivatives are from 21 to 30 ng. X = impurities. The mobile phase was water–acetonitrile (1:1) for 15 min, then changed to acetonitrile in 0.5 min, to reduce the retention time of the unreacted naphthyl isothiocyanate (NIT), which under these conditions elutes in 23 min.

method must be thoroughly validated. Several procedures for the evaluation of diffusive samplers have been proposed. The latest and most important is the procedure described by the Commitè Europeén de Normalisation (CEN) [11]. The diffusive sampler was validated according to the protocol published by CEN. The effects of concentration, sampling time, relative humidity (RH), zero exposure and storage were investigated. The effects of wind velocity and sampler orientation were studied in connection with validation of the sampler for formaldehyde [8]. In that study the wind velocity at the sampler face was varied between 0.05 and 1.0 m/s. The uptake rate was constant within the wind velocity range studied. Most personal sampling conditions give wind velocities of about 0.1 m/s [7]. Perpendicular orientation of the sampler resulted in a slight increase in the uptake rate at high wind velocity. At wind velocities below 0.02 m/s the sampler performed well, allowing static area sampling of indoor air [8]. As wind velocity and sampler orientation effects are parameters associated with the sampler and not the analyte, these effects were not studied further.

The amine concentration in the exposure chamber was calculated from the dilution of the certified amine gas. Good agreement (within 10%) was generally obtained between the concentrations given by the reference method and the calculated concentrations.

In the validation experiments with dimethylamine, the concentrations were varied between 1.87 and 18.7 mg/m^3 (1 and 10 ppm), the sampling time between 30 and 480 min and the relative humidity between 10% and 80%. Table I gives the uptake rate of dimethylamine calculated from the different experiments.

The statistical analysis was performed with the use of multiple regression [12]. The parameters concentration, sampling time and relative humidity were scaled in the interval -1 to $+1$. The intercept of the regression curve gives the mean uptake rate and the parameter estimates give an indication of the influence on the uptake rate by the different variables. For significance the estimate has to be greater than the standard error. The results of the regression analysis of the data for diethylamine are given in Table II. As can be seen, no influence of concentration and relative humidity can be detected. The effect of time is statistically significant but small and will not significantly influence the overall uncertainty of the method. The mean uptake rate was 15.8 ml/min (R.S.D. = 6%, n = 54).

To evaluate the stability of the thiourea derivative during sampling, a zero exposure test was performed. Amine exposure for 30 min was followed by a zero concentration (clean air) exposure for 7.5 h . As shown in Table I, a small but significant decrease in uptake rate can be noted. Compared with the mean uptake rate, the decrease was only 4%. As can be seen from Table I, the derivative was stable during storage for 14 days at 18°C.

The validation experiments with *n*-butylamine were performed with various concentrations between 1.52 and 24.2 mg/m^3 (0.5 and 8 ppm), sampling times between 15 and 480 min and relative

TABLE I

UPTAKE RATE OF DIFFUSIVE SAMPLER AT VARIOUS DIMETHYLAMINE CONCENTRATIONS, RELATIVE HUMIDITIES AND SAMPLING TIMES

Face velocity = 0.3 m/s. RH = relative humidity; R.S.D. = relative standard deviation; N = number of determinations.

Concentration (mg m^{-3})	Sampling time (min)	RH (%)	Uptake rate (ml min^{-1})	R.S.D. (%)	n
18.7	30	10	15.6	3	6
18.7	30	80	17.8	2	6
1.87	480	10	15.4	6	6
1.87	480	80	14.8	2	6
18.7	480	10	15.6	1	6
18.7	480	80	15.5	5	6
18.7	240	45	15.6	3	6
1.87	240	45	16.4	3	6
9.4	120	45	15.6	2	6
18.7	30	80	15.1[a]	3	3
9.4	120	45	15.7[b]	3	6

[a] Exposure for 30 min followed by exposure to zero concentration for 7.5 h.
[b] Stored for 14 days at 18°C after exposure.

humidity between 10% and 80%. Table III shows the uptake rate of the diffusive sampler with these parameter variations. The mean uptake rate was 11.2 ml/min with an R.S.D. of 11% (n = 51). The statistical evaluation showed a small but significant influence of concentration and time. The zero exposure and the storage tests showed no instability of the n-butylamine derivative (Table III).

Diffusive sampling of isopropylamine was validated with various concentrations between 1.23 and 24.5 mg/m^3 (0.5 and 10 ppm), sampling times between 30 and 480 min and relative humidities between 10% and 80%. In Table IV the uptake rate of isopropylamine in the different experiments is shown. The mean uptake rate was determined to be 10.2 ml/min with an R.S.D. of 9% (n = 60). The statistical analysis showed a small but significant influence on uptake rate from concentration and relative humidity. The zero exposure test shows a small decrease in uptake rate (8%) compared with the mean uptake rate (Table IV). The isopropylamine derivative was stable according to the storage test which is shown in Table IV.

TABLE II

MULTIPLE REGRESSION ANALYSIS ON THE INFLUENCE OF CONCENTRATION, SAMPLING TIME AND RELATIVE HUMIDITY ON UPTAKE RATE OF DIMETHYLAMINE

Variable	Parameter estimate (ml/min)	Standard error
Uptake rate (intercept)	15.8	0.11
Concentration	0.12	0.12
Time	−0.51	0.14
Relative humidity	0.23	0.20

The diffusive sampling of methylamine was validated with various concentrations from 1.29 to 25.8 mg/m^3 (1 to 20 ppm), sampling times from 30 to 480 min and relative humidity from 10% to 80%. The uptake rate of methylamine in the different measurements is shown in Table V. The mean uptake rate was determined to 17.4 ml/min and the R.S.D. was 14% (n = 54). The statistical analysis showed a small negative influence from the concentration and a larger negative influence from time. This is probably due to a reversible reaction from the thiourea derivative to methylamine and 1-naphthyl isothiocyanate. This theory is supported by the result from the zero exposure test which shows a 36% decrease in uptake rate compared with the mean uptake rate when exposed to zero concentration for 7.5 h (Table V). The diffusive sampler can still be used for exposure measurements of methyla-

TABLE III

UPTAKE RATE OF DIFFUSIVE SAMPLER AT VARIOUS n-BUTYLAMINE CONCENTRATIONS, RELATIVE HUMIDITIES AND SAMPLING TIMES

Face velocity = 0.3 m/s.

Concentration (mg m^{-3})	Sampling time (min)	RH (%)	Uptake rate (ml min^{-1})	R.S.D. (%)	*n*
24.2	15	10	9.7	7	5
24.2	15	80	9.4	2	5
1.52	480	10	10.5	3	6
1.52	480	80	10.6	9	6
24.2	480	10	12.7	1	5
24.2	480	80	11.5	2	6
24.2	240	45	11.4	3	6
1.52	120	45	12.9	2	6
12.9	120	45	12.1	2	6
24.2	30	80	11.4[a]	5	5
12.9	120	45	12.3[b]	2	6

[a] Exposure for 30 min followed by exposure to zero concentration for 7.5 h.
[b] Stored for 14 days at 18°C after exposure.

mine but with a higher standard deviation. The storage test shows no instability or reversible reaction, as can be seen from Table V.

In the validation experiment with allylamine, concentrations were varied between 0.47 and 9.5 mg/m^3 (0.2 and 4 ppm), sampling time between 30 and 480 min and relative humidity between 10% and 80%. Table VI gives the uptake rate of allylamine in the different measurements. The mean uptake rate was determined to 14.9 ml/min with an R.S.D. of 8% (n = 53). The statistical analysis gave a significant influence on uptake rate from concentration and relative humidity. This influence is small and can be disregarded. The zero exposure test

TABLE IV

UPTAKE RATE OF DIFFUSIVE SAMPLER AT VARIOUS ISOPROPYLAMINE CONCENTRATIONS, RELATIVE HUMIDITIES AND SAMPLING TIMES

Face velocity = 0.3 m/s.

Concentration (mg m^{-3})	Sampling time (min)	RH (%)	Uptake rate (ml min^{-1})	R.S.D. (%)	*n*
24.5	30	10	11.8	4	6
24.5	30	80	10.0	3	6
1.23	480	10	11.2	2	6
1.23	480	80	10.1	1	6
24.5	480	10	10.3	5	6
24.5	480	80	10.0	3	6
24.5	240	45	10.0	3	6
1.23	240	45	9.6	11	6
12.3	120	45	9.6	9	12
24.5	30	80	9.4[a]	4	3
12.3	120	45	10.3[b]	5	6

[a] Exposure for 30 min followed by exposure to zero concentration for 7.5 h.
[b] Stored for 14 days at 18°C after exposure.

TABLE V

UPTAKE RATE OF DIFFUSIVE SAMPLER AT VARIOUS METHYLAMINE CONCENTRATIONS, RELATIVE HUMIDITIES AND SAMPLING TIMES

Face velocity = 0.3 m/s.

Concentration ($mg\ m^{-3}$)	Sampling time (min)	RH (%)	Uptake rate ($ml\ min^{-1}$)	R.S.D.	n
25.8	30	10	20.3	5	6
25.8	30	80	20.4	2	6
1.29	480	10	18.7	3	6
1.29	480	80	16.5	7	6
25.8	480	10	14.3	3	6
25.8	480	80	14.1	1	6
25.8	240	45	16.0	2	6
1.29	240	45	17.5	9	6
6.25	120	45	19.0	4	6
25.8	30	80	15.7[a]	1	3
12.3	120	45	20.6[b]	2	5

[a] Exposure for 30 min followed by exposure to zero concentration for 7.5 h.
[b] Stored for 14 days at 18°C after exposure.

gives a decrease in uptake rate of 25% compared with the mean uptake rate (Table VI). This is probably the same effect as can be seen with methylamine, *i.e.*, a reversible reaction of the thiourea derivative. However, this effect for allylamine is smaller and cannot be seen from the other experiments. No instability of the derivative was evidenced by the storage experiments, as can be seen in Table VI.

TABLE VI

UPTAKE RATE OF DIFFUSIVE SAMPLER AT VARIOUS ALLYLAMINE CONCENTRATIONS, RELATIVE HUMIDITIES AND SAMPLING TIMES

Face velocity = 0.3 m/s.

Concentration ($mg\ m^{-3}$)	Sampling time (min)	RH (%)	Uptake rate ($ml\ min^{-1}$)	R.S.D. (%)	n
9.5	30	10	14.0	2	6
9.5	30	80	15.5	9	5
0.47	480	10	15.7	4	6
0.47	480	80	15.2	7	6
9.5	480	10	14.6	2	6
9.5	480	80	14.0	2	6
9.5	240	45	14.1	2	6
0.47	240	45	14.5	3	6
4.7	120	45	16.7	7	6
9.5	30	80	11.1[a]	14	3
4.7	240	45	13.4[b]	3	3

[a] Exposure for 30 min followed by exposure to zero concentration for 7.5 h.
[b] Stored for 14 days at 18°C after exposure.

CONCLUSIONS

The present diffusive sampler is meant to be used for sampling of reactive compounds. It was specially designed for use with reagent-coated filter tape. With the use of 1-naphthyl isothiocyanate as reagent the sampler can be used for sampling of primary and secondary amines in air. The uptake rates

TABLE VII

UPTAKE RATES AND DETECTION LIMITS FOR THE DIFFERENT AMINES

Amine	Uptake rate (ml/min)	R.S.D. (%)	*n*	Detection limit (mg/m^3)[a]
Methylamine	17.4	14	54	0.4
Isopropylamine	10.2	9	60	0.7
n-Butylamine	11.2	11	51	0.3
Allylamine	14.9	8	53	0.2
Dimethylamine	15.8	6	54	0.4
Diethylamine[b]	12.0	6	35	0.5

[a] Corresponding to 30-min diffusive sampling.
[b] From Ref. 6.

for the different amines studied are summarized in Table VII. The influence on uptake rates of concentration, sampling time and relative humidity was generally not detectable or small. The only exception was methylamine, where a probable reverse reaction from the thiourea occurred, which resulted in a larger negative influence of the sampling time. However, the effect was small enough not to reduce significantly the utility of the method.

The chromatographic determination of the thiourea derivatives is highly sensitive and specific. Short-time sampling is possible with detection limits below 1 mg/m^3, as can be seen from Table VII. After proper validation, the sampler may also be used for other primary and secondary amines. Previously, the sampler has been shown to perform well at very low wind velocities. Overall, the sampler is suitable for both static and personal monitoring of low levels of primary and secondary amines in air.

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CHROMSYMP. 2688

Selective clean-up for polynuclear aromatic compounds in airborne particles and soil

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ABSTRACT

Urban air particulate matter, internal combustion engine particulate emissions, gas turbine particulate emissions and soil were analysed for polynuclear aromatic compounds. Polynuclear aromatic compounds were isolated by adsorption chromatography on XAD-2. Polynuclear aromatic hydrocarbons (PAHs), polynuclear aromatic ketones and nitro-PAHs were obtained together in the aromatic fraction, while interfering compounds were absent. Glass capillary gas chromatography with the stationary phases OV-1, SE-54, Dexsil 300 and OV-25 was performed, and benzo[*a*]pyrene, 7-*H*-benz[*de*]anthracene-7-one and related compounds were identified. Differences between PAH profiles are discussed. It is concluded that cyclopenta[*cd*]pyrene and benzo[*ghi*]fluoranthene are rapidly decomposed in the atmosphere. 7-*H*-Benz[*de*]anthracene-7-one and 6-*H*-benzo[*cd*]pyrene-6-one are degraded during atmospheric exposure at a similar rate to benzo[*a*]pyrene, and nitro-derivatives are not formed in substantial amounts.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are lipophilic substances, and carcinogenic properties are attributed to many compounds of this class [1]. Determination of these compounds involves solvent partition, liquid chromatography or HPLC, and a large number of such methods have been reported and reviewed [2].

While PAHs are non-polar, derivatives of PAHs containing oxygen, sulphur or nitrogen atoms will have a higher polarity. PAHs and their derivatives or structurally related compounds thus form the larger group of polynuclear aromatic compounds (PACs). A method of determination should therefore deal with PACs as a group of compounds having one property in common: the polynuclear aromatic structure. Thus, an analytical method based on this common property was developed. It takes advantage of selective adsorption chromatography of PACs on XAD-2 followed directly by capillary GC or further fractionation by liquid chromatography [3,4].

7-*H*-Benz[*de*]anthracene-7-one (benzanthrone) and other polynuclear aromatic ketones (PAKs) are present in particulate emissions of combustion sources at concentrations similar to PAHs. Furthermore, polar derivatives are found during exposure of particulate-adsorbed PAHs to an environment of air and light. All such compounds are not detected if standard isolation procedures of PAHs by clean-up on silica gel are employed [5–8].

Multistep procedures of isolation are required if PAHs are determined along with their carbonyl and nitro derivatives [9]. Research described in the present study ameliorates this situation. It is demonstrated that PAHs can be conveniently characterized along with PAKs in a one-step procedure that involves one uniform fraction of column eluate from the isolation procedure.

EXPERIMENTAL

Reagents and standards

Solvents were glass-distilled through a 40-cm-long column. XAD-2 of particle size 150–200 μm was supplied by Serva (Heidelberg, Germany). Stationary GC phases were purchased from Phase Separa-

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tions (Queensferry, UK). Chemicals and standards were obtained from Merck (Darmstadt, Germany), Wako (Tokyo, Japan) or Tokyo Kasei (Tokyo, Japan).

Particulate matter and soil

Air particulate matter was obtained by high-volume sampling at a sampling rate of 50–100 m^3/h. Membrane filters (Type ST 69, 1.2 μm pore size, Schleicher & Schüll, Germany) were used. Total sampling time was 24 h for air particulate matter. Gas turbine particulates and internal combustion engine particulates were obtained by high-volume sampling for 60 min at a rate of 50–100 m^3/h using glass fibre filters (Type 6, Schleicher & Schüll). The sampling apparatus was placed behind the turbine or exhaust ducts. Soil (Anjo soil, Japan, mineral soil, 1.8% carbon) was spiked with nitro derivatives of PAHs and subjected to analysis.

Extraction of PACs

Extraction of particulate matter was carried out in a Soxhlet apparatus. Pure toluene was used as a solvent, and total extraction time was 4 h, which corresponded to sixteen Soxhlet cycles. Soil samples were dried over silica gel for 48 h, and also extracted in a Soxhlet apparatus with 5% ethanol in toluene.

Isolation of PACs

XAD-2 of 150–200 μm particle size was slurry-packed into a glass column of 9 cm × 1.4 cm I.D. The column was washed with 20 ml of toluene, followed by 50 ml of ethanol. The concentrated (0.5 ml or less) crude sample solution was transferred to the top of the column. Elution was carried out with 25 ml of ethanol (fraction A), 10 ml of *n*-pentane and 10 ml of ethanol (fraction B), and 12 ml of toluene and 10 ml of ethanol (fraction C). The column was then eluted with an additional 20 ml of toluene, followed by 20 ml of ethanol, and could then be used for the next analysis. Fraction C was concentrated to 0.5 ml using a rotary evaporator, and aliquots of the concentrated solution were injected into the gas chromatograph.

Gas chromatography

GC was carried out with Hitachi or Carlo Erba instruments. They were modified for capillary column use and equipped with split–splitless injectors.

TABLE I

RECOVERIES OF SOME COMPOUNDS IN THE TOLUENE ELUATE FROM XAD-2

Substance	Recovery (%)
Benzo[*e*]pyrene	95
Benzanthrone	98
1-Nitropyrene	98
1,2,7,8-Dibenzacridine	70
6-Nitroquinoline	0
Diethylphthalate	0
n-Eicosane	0

The temperature programmes were 110–260°C, and the heating rate was 4°C/min. Injector temperature was 280°C, and detector temperature was 300°C Capillary columns were home-made Pyrex columns coated with SE-54, OV-25 and Dexsil 300, and one fused-silica column coated with OV-1.

RESULTS AND DISCUSSION

Table I lists recoveries of some components representing different classes of PACs and other substances. These data demonstrate that the suitability of clean-up on XAD-2 is not limited to parent PAHs. Oxygenated PAHs have been found together with PAHs in urban air particulate matter [10,11] and in particulate emissions of combustion sources [12,13]. Nitro derivatives of PAHs have also been found in particulate matter [14]. Nitro-PAHs can be determined conveniently with negative-ion chemical ionization MS coupled with GC, while PAHs can be present in a 10–100 fold excess in the sample [15,16]. Thus, capillary GC coupled with different detectors successfully provides qualitative and quantitative information on PAHs and their derivatives without prior separation.

Urban air particulate matter

Fig. 1 displays two chromatograms of PACs isolated from urban air particulate matter by adsorption chromatography on XAD-2. All major peaks are due to PAHs except two substances. These are 7-*H*-benz[*de*]anthracene-7-one (benzanthrone, peak A) and 6-*H*-benzo[*cd*]pyrene-6-one (peak B, co-eluted with perylene). 6-*H*-Benzo[*cd*]pyrene-6-one

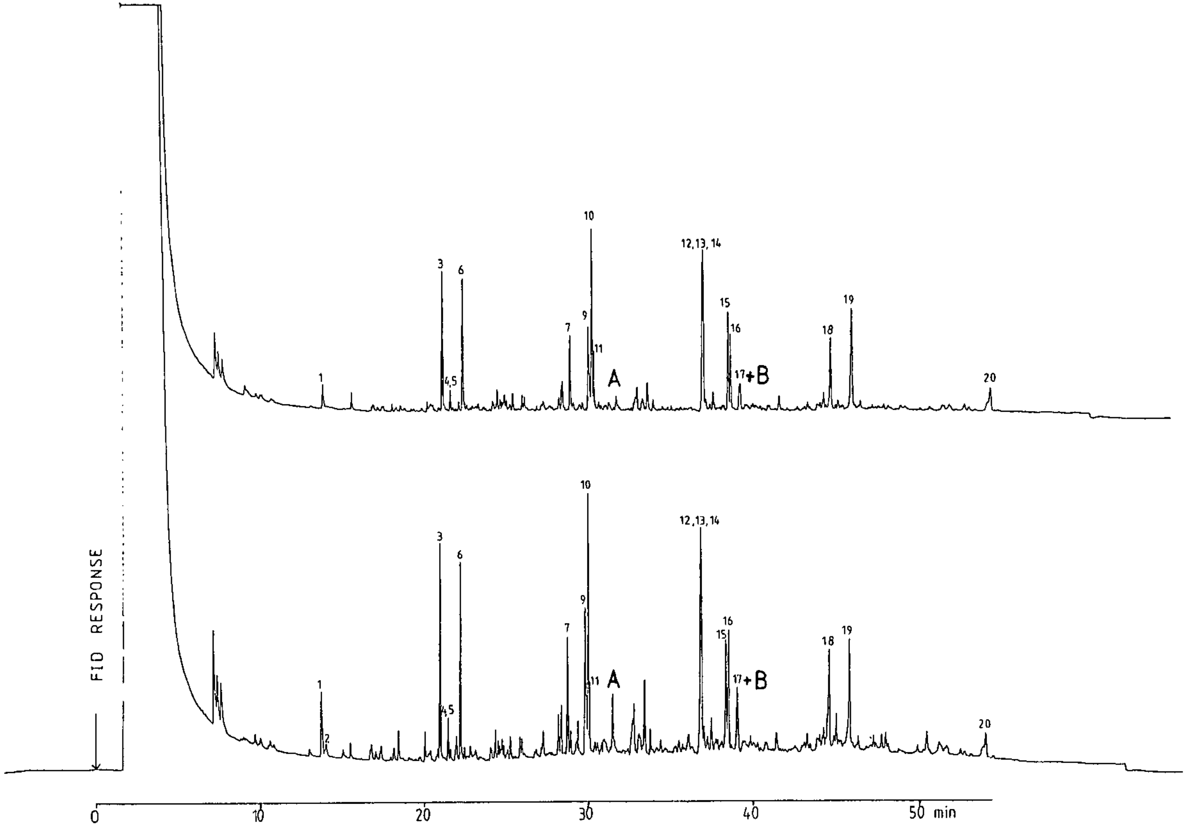

Fig. 1. Gas chromatograms of PACs from two samples of urban air particulate matter. Column: 40 m × 0.3 mm I.D., Dexsil 300. Peaks: 1 = phenanthrene; 2 = anthracene; 3 = fluoranthene; 4 = acephenanthrylene; 5 = aceanthrylene; 6 = pyrene; 7 = benzo[*ghi*]fluoranthene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = triphenylene; 12 = benzo[*b*]fluoranthene; 13 = benzo[*j*]fluoranthene; 14 = benzo[*k*]-fluoranthene; 15 = benzo[*e*]pyrene; 16 = benzo[*a*]pyrene; 17 = perylene; 18 = indeno[1,2,3*cd*]pyrene; 19 = benzo[*ghi*]perylene; 20 = coronene; A = benzanthrone; B = 6-*H*-benzo[*cd*]pyrene-6-one.

can be identified by additional GC analysis on SE-54 (co-elution with benzo[*a*]pyrene) or GC–MS (MW = 254).

The upper chromatogram in Fig. 1 reveals an excess of benzo[*e*]pyrene (peak 15) over benzo[*a*]-pyrene (peak 16). Concentrations of benzo[*ghi*]-fluoranthene and benz[*a*]anthracene are close to the level of triphenylene (peak 11). Benzanthrone (peak A) and 6-*H*-benzo[*cd*]pyrene-6-one (peak B) are minor components.

A slight excess of benzo[*a*]pyrene over benzo[*e*]-pyrene is found in the lower chromatogram. The relative amounts of benzanthrone (peak B) and 6-*H*-benzo[*cd*]pyrene-6-one are higher, and benzo[*ghi*]-fluoranthene (peak 7) and benz[*a*]anthracene (peak 9) are in a substantial excess over triphenylene.

PACs in the lower chromatogram have undergone less atmospheric change than PACs in the upper chromatogram. This can be deduced from the fact that the amounts of benzo[*ghi*]fluoranthene, benz[*a*]anthracene or benzo[*a*]pyrene relative to benzo[*e*]pyrene are lower than the amounts found in major sources [17] of PAC emission (*e.g.*, combustion engines, gas turbines, residential heating). Other important indicators of atmospheric degradation are benzanthrone and 6-*H*-benzo[*cd*]pyrene-6-one. Both components disappear at a slightly higher rate than benzo[*a*]pyrene during exposure to light and

atmosphere. Thus, determination of these two compounds is important in providing information on the chemical fate PAHs have undergone in the atmosphere. Since atmospheric conditions in the summertime are more PAH-degrading than atmospheric conditions in the wintertime, a comparison of PAH profiles obtained in summer and winter can give some information on the stability of PAHs in the atmosphere [18].

Finally, both chromatograms in Fig. 1 prove that nitro derivatives of PAHs are not present as major components.

Internal combustion engine particulate emissions

Fig. 2 displays a profile of PACs isolated from internal combustion engine exhaust by clean-up on XAD-2.

OV-25 was chosen as a stationary phase in order to separate isomeric benzofluoranthenes (peaks 12–14). On SE-54, benzofluoranthenes are also separated, but resolution on OV-25 is better [19,20]. Cyclopenta[*cd*]pyrene is revealed as a major compound among PAHs emitted. However, this compound rapidly decays during exposure to the atmosphere. Benzanthrone (peak A) and 6-*H*-benzo[*cd*]-pyrene-6-one (peak B) are also released. Nitroderivatives of PAHs would also appear in this chromatogram if they are present. These compounds appear at much lower concentrations than PAHs [9] and are not visible as major peaks in this chromatogram. Benzo[*ghi*]perylene (peak 19) has a substantial excess over indeno[1,2,3*cd*]pyrene (peak 18). These data are in accordance with results published elsewhere [21], but additional information on PAKs is provided here.

Aircraft turbine particulate emissions

Fig. 3 displays a profile of PACs obtained from aircraft turbine particulate emissions after clean-up on XAD-2.

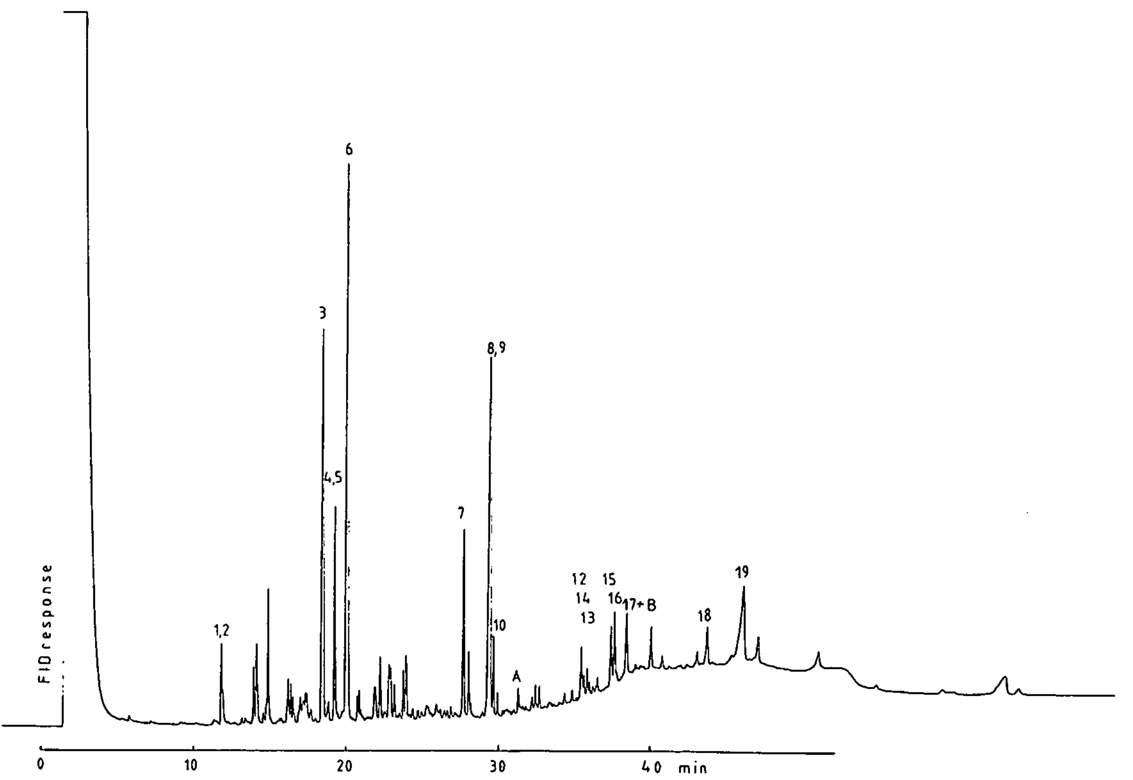

Fig. 2. Gas chromatogram of PACs from internal combustion engine particulate emissions. Engine: 1200 cm^3 air cooled (VW motor works, Wolfsburg, Germany). Column: 25 m × 0.3 mm I.D., OV-25. Peak: 8 = cyclopenta[*cd*]pyrene; other peaks as in Fig. 1.

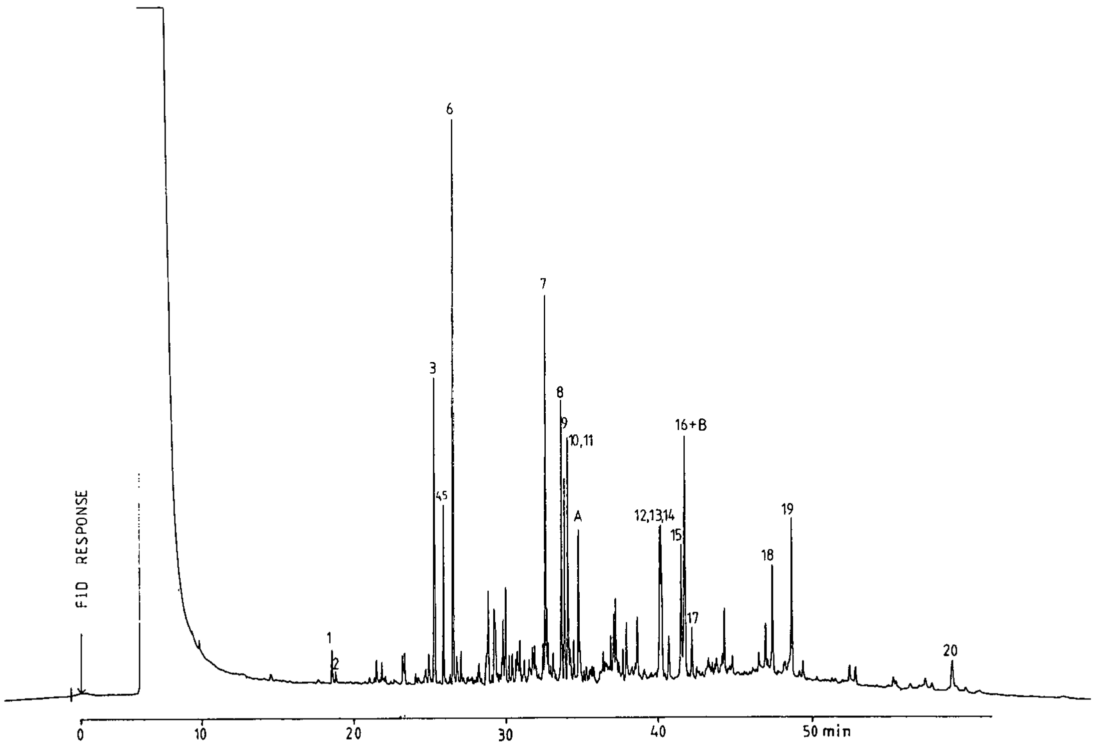

Fig. 3. Gas chromatogram of PACs from aircraft turbine particulate emissions. Engine: Pratt & Whitney JT3D3 gas turbine. Column: 70 m × 0.3 mm I.D., SE-54. Peaks as in Figs. 1 and 2.

SE-54 was chosen as a stationary phase for GC separation of PAHs. There is a partial separation of benzofluoranthenes (peaks 12–14) because of the high efficiency of the capillary column. 6-*H*-Benzo-[*cd*]pyrene-6-one is co-eluted with benzo[*a*]pyrene (peak 16 + B). Identification of this compound can be achieved by additional chromatography on OV-1 (co-elution with benzo[*e*]pyrene occurs) or by GC–MS. Benzanthrone is also present as a major component. The relative amount of cyclopenta[*cd*]pyrene is lower than encountered in internal combustion engine particulate emissions. Indeno[1,2,3*cd*]pyrene and benzo[*ghi*]perylene are present in similar amounts.

Soil

1-Nitronaphthalene, 2-nitronaphthalene, 2-nitrofluorene, 3-nitrofluoranthene, 1-nitropyrene and 6-nitrochrysene were added to soil, and the soil was then analysed by Soxhlet extraction with toluene and isolation of PACs by clean-up on XAD-2. The column eluate containing the aromatic fraction was further analysed by capillary GC with electron-capture detection.

The resulting gas chromatogram is shown in Fig. 4. This time a fused-silica capillary column coated with OV-1 was used in analysis. There are almost no interfering compounds giving rise to signals in the electron-capture detector. The concentration of 6-nitrochrysene added to the soil was 110 μg/kg.

CONCLUSION

Isolation of PACs by adsorption chromatography on XAD-2 is a simple chromatographic separation based on selectivity and not high efficiency of the separation column. Isolation of PACs is achieved

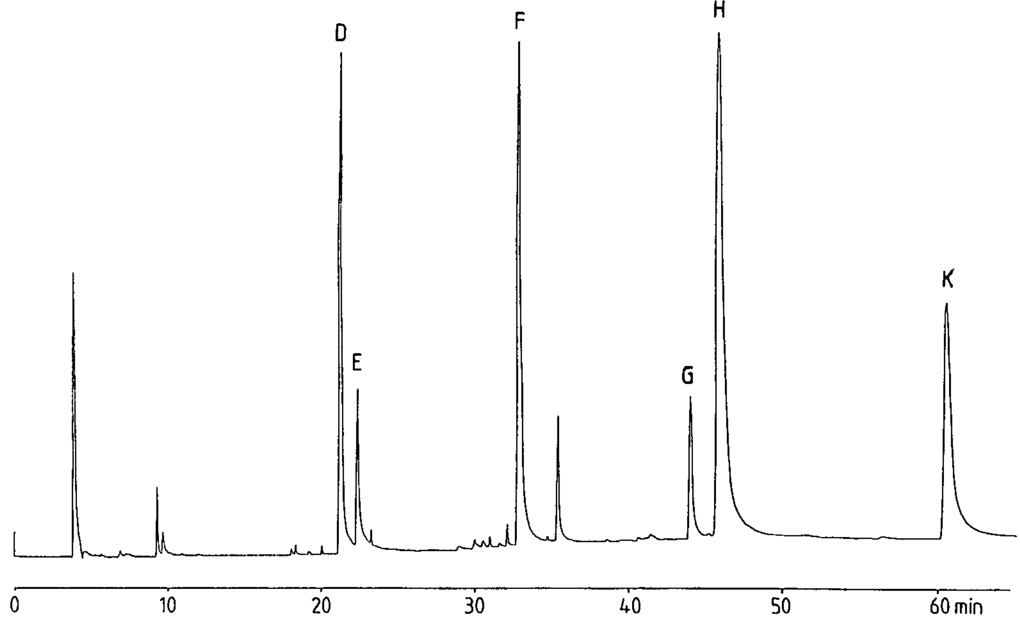

Fig. 4. Gas chromatogram of nitroarenes recovered from soil. Column: 25 m × 0.3 mm I.D., OV-1. Peaks: D = 1-nitronaphthalene; E = 2-nitronaphthalene; F = 2-nitrofluorene; G = 3-nitrofluoranthene; H = 1-nitropyrene; K = 6-nitrochrysene.

within 30 min, and solvent consumption is 70 ml for one sample. A simple glass column is used instead of an HPLC apparatus. Direct capillary column GC with the aromatic fraction reveals the qualitative nature of PAHs, nitro derivatives, PAKs and other PAH derivatives, and their quantitative ratios. The presence of PACs other than PAHs in the aromatic eluate from the XAD-2 column provides an additional information about decomposition reactions that might have occurred during exposure of PACs to the environment.

Comparing the profiles of PACs in the emission samples (Figs. 2 and 3) to the profiles of PACs in urban air particulate matter (Fig. 1), cyclopenta[*cd*]-pyrene can be seen to have disappeared completely. Benzo[*ghi*]fluoranthene also decreased in concentration. Important indicators of degradation are 7-*H*-benz[*de*]anthracene-7-one and 6-*H*-benzo[*cd*]-pyrene-6-one, which disappear at similar rates to benzo[*a*]pyrene during atmospheric exposure.

The choice of stationary phases for analysis of PAHs was Dexsil 300, OV-25, SE-54 and OV-1 in this work. Different stationary phases including liquid crystal phases can also be applied successfully [22,23]. However, because of the large number of components present among PACs, a stationary phase able to separate all compounds is unlikely to exist. Instead of only one purpose-designed phase, at least two or three stationary phases of different polarity should be applied in order to recognize random overlap of components [24].

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CHROMSYMP. 2694

Evaluation of a modified Marcali technique with high-performance liquid chromatography–ultraviolet detection for the determination of 2,4-toluene diisocyanate in air

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ABSTRACT

This work describes an method for the determination of 2,4-toluene diisocyanate concentration in air. Traps containing 20–40 mesh silica gel coated with phosphoric acid are used. After the aspiration of air, sodium hydroxide is added to the silica gel, which is subsequently eluted with methanol. The amine formed is then separated on a C_{18} column using a mobile phase of phosphate buffer–methanol (60:40, v/v). This can be performed in less than 4 min. The effluent is monitored with a UV detector at 235 nm. The detection limit based on a 20-l air sample is 0.2 $\mu g/m^3$. Complete analysis requires about 30 min.

INTRODUCTION

Polyurethanes, produced from toluene diisocyanate monomers, are used extensively in the coating and plastic industry. In fact, sprayed-in-place polyurethane foam has become a popular form of thermal insulation in the construction industry. These foams are used in the building of roofs, storage tanks, barns, walk-in coolers and new homes. Consequently, a large number of different types of workers are potentially at risk of occupational isocyanate exposure. They include polyurethane foam producers, textile processors, foam converters, wire-enamelling workers, paint sprayers, diisocyanate resin production workers, organic chemical synthesizers, and workers in the rubber, varnish and adhesive industries [1]. As a result of their widespread use, respiratory problems and allergic reactions have been reported by workers exposed to isocyanates. The symptoms resulting from the inhalation of vapour, aerosol or fine particles of isocyanate include eye and mucous membrane irritation, coughing fits and dyspnoea [2]. Chronic exposure may lead to allergies such as asthma. Sensitization reactions do not occur in all individuals, but they can be observed with exposure to very low concentrations.

The treshold limit value (TLV) of the American Conference of Governmental Industrial Hygienists [3] for 2,4-toluene diisocyanate (TDI) as time-weighted average (TLV-TWA) is 36 $\mu g/m^3$ with a

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140 μg/m^3 short-term exposure limit (STEL). The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) is 40 μg/m^3, similar to the above limit, with a 150 μg/m^3 action level [4].

In addition to spectrophotometric methods [5,6], used also in band-type monitors for continuous monitoring [7,8] a number of other procedures based on chromatographic techniques (HPLC, GC, TLC) have been developed to determine isocyanate content in air [9–13]. Most of these procedures, however, are both long and tedious, which severely limits their use in routine monitoring of industrial environments and controlled atmospheres where a rapid response is necessary to ensure air quality.

This paper describes the preliminary results of a promising method, which is simple and quick, for the analysis of TDI in air. The isocyanate was collected by reacting in an acid medium, like that of the traditional Marcali procedure [5], and the corresponding amine, after separation by reversed-phase liquid chromatography, was measured with a UV detector. The goal for the detection limit for a 20-l air sample was two orders of magnitude below the isocyanate TLV-TWA. This corresponded to 0.36 μg/m^3.

EXPERIMENTAL

Reagents and standards

TDI, 98% pure, was purchased from Kodak (Rochester, NY, USA), whereas 2,4-toluene diamine (TDA), 99% pure, a derivative of the hydrolysis of TDI, was purchased from Merck (Darmstadt, Germany). Methanol and water were of HPLC grade (Merck), while all other chemical were of analytical-reagent grade.

The mobile phase was phosphate buffer–methanol (60:40, v/v). The buffer was composed of 10 mmol/l dipotassium hydrogenphosphate adjusted to a pH of 7.0 with 1 mol/l phosphoric acid. Stock solutions of TDI were prepared at 1 mmol/l by diluting a known amount of the isocyanate with the proper amount of acetone, while stock solutions of TDA were prepared at the same concentrations weighing a known amount of amine with the proper amount of acetone. Working standard solutions of 0.3 mmol/l TDI and TDA were prepared by serial dilutions with acetone. TDI solution is not stable and must be prepared daily; TDA solution is stable for 10 days when stored at 4°C.

Equipment

The liquid chromatograph consisted of a Perkin-Elmer series 400 pump, a Perkin-Elmer Model ISS 100 autosampler, a Merck 25 cm × 4.6 mm I.D. RP-18 column with 5-μm particles and a Perkin-Elmer Model LC 95 (3-μl cell) detector that monitored at 235 nm. Chromatograms were recorded and the peaks integrated on a Shimadzu Model CR 6A (attenuation 3, speed 5 mm/min) integrator.

Procedure

The absorber medium was prepared by mixing 0.6 ml of 10% H_3PO_4 (v/v) orthophosphoric acid with 200 mg of silica gel. After water evaporation at 75°C in a rotary evaporator (30 min), the silica gel was dried overnight in an oven at 80°C. Glass tubes, 85 mm × 5 mm I.D., were filled with 200 mg of the acidified absorber medium and closed at each end with glass wool plugs. Prepared traps will remain unaltered for at least 6 months if kept at room temperature. To prepare the calibration curve and to study the collection efficiency, known concentrations of TDI were added to the trap using a microsyringe. This was done in accordance with the NIOSH evaluation of sampling parameters. Air samples were sucked trough the trap using an MWG (Neuberger, Freiburg, Germany) membrane pump. The sampling rate was of the order of 0.5 l/min.

The contents of the trap were transferred to a glass test tube, while 0.5 ml of methanol and 100 μl of 10 mol/l sodium hydroxide were added to the silica gel. The mixture was first sonicated for 10 min and then centrifuged at 3300 *g* for 5 min. The supernatant was concentrated to 100 μl. A 20-μl volume was then injected into the HPLC system. The separation of the amine was carried out at a flow-rate of 1 ml/min.

RESULTS

The trapping efficiency of the orthophosphoric acid absorber medium for TDI was assessed by using two traps in series. To the first trap, 0.3 μmol of TDI were added with a microsyringe, while 20 l of air were sucked through both traps. In three differ-

ent experiments TDI was not detected in the second trap. This indicated that the trapping efficiency was essentially 100%, and that only one trap would be required for collection in the field.

Test performed with sampling tubes spiked with 3 μmol of TDI showed that virtually 100% of the TDA was recovered using 500 μl of methanol. To concentrate this volume to 100 μl, a vacuum centrifuge proved very useful. In comparison with a rotary evaporator, the vacuum centrifuge has two advantages: bumping is avoided and several samples can be handled at the same time.

Under the experimental conditions described above, good separation of TDA was achieved within 4 min. Typical chromatograms of a standard solution of TDI and of an air sample are shown in Fig. 1. Quantitation of TDA was performed using a calibration curve based on the measured absorbance at 235 nm.

In order to prepare the calibration curve, 1, 3, 5, 7 and 10 μl of working standard solution (0.3 mmol/l TDI) were introduced with a microsyringe in the traps. In this manner, standards of 2.6, 7.8, 13.0, 18.2, 26.0 μg/m^3 (for a 20-l air sample) were obtained. Analysis of the standard gave a calibration curve with $y = 4.5$ x, where y is the quantity of TDI and x is the peak area.

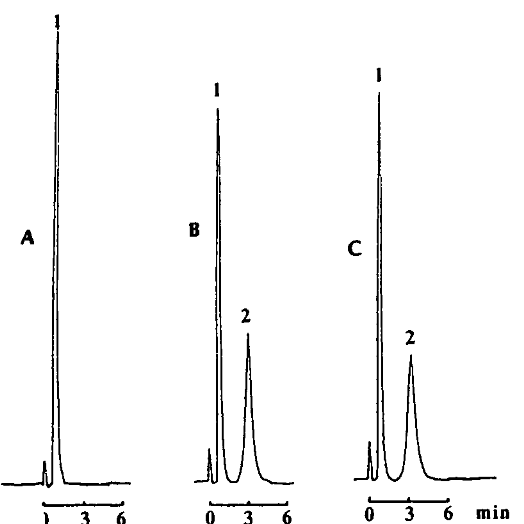

Fig. 1. HPLC profiles obtained by injecting methanolic eluate of the absorber: not treated, *i.e.* used as a blank (A), with a standard solution of 0.3 nmol of TDI (B), and after air sampling of a concentration of 2.5 μg/m^3 (C). Peaks: 1 = front; 2 = TDA.

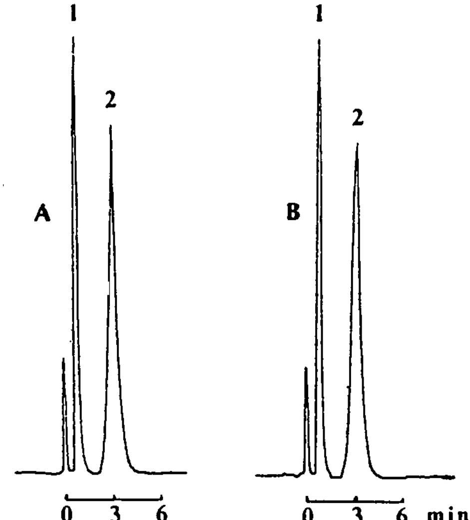

Fig. 2. HPLC profiles of 0.6 nmol of TDI standard solution added to the trap (A) and 0.6 nmol of TDA standard solution injected directly into the column (B). Peaks: 1 = front; 2 = TDI (A) or TDA (B).

Fig. 2 shows a chromatogram of 0.6 nmol of TDI added to a trap and that of the same amount of TDA added to another trap. The retention times and the areas of the peaks are identical, thus confirming the complete transformation of TDI into TDA.

The absolute retention time was reproducible within 4%. Reproducibility was also determined for the quantitative analyses of the calibration curve. Triplicate injections of standard sample at the four different concentrations gave an R.S.D. of less than 3%. The precision of the method was evaluated with both within-run assays (twelve equal samples of a standard solution added to the traps and analysed on the same day) and between-run assays (sample of equal concentration added to traps and analysed on ten consecutive days). These results are reported in Table I.

As is customary, the detection limit of the procedure was defined as the concentration derived from a signal three times the noise level. With an air sample of 20 l, the TDI derivative (TDA) showed a detection limit of 0.2 μg/m^3.

The linearity of the assay was verified by determining increasing amounts of TDI standards (up to 3 nmol). The response was linear over the range

TABLE I

PRECISION OF THE TDI ASSAY

n	x (nmol)	S.D.	R.S.D. (%)
Within-run precision			
12	0.3	0.013	4.2
12	1.5	0.046	2.9
Between-run precision			
12	3	0.023	7.0
12	15	0.080	4.9

investigated. Often it is not practical to analyse a sample for a number of days. In the present case, storage studies indicated that samples should be desorbed within 10 days for maximum recovery when they are stored at 4°C in the dark. Losses up to 5% can occur after 15 days. Recovery of TDA was unchanged up to 10 days for refrigerated desorbed solutions.

DISCUSSION

The principal advantage of this method is its considerably reduced sample handling prior to the chromatographic step. This can be done without significant sacrifice in sensitivity (30 pmol). The simplicity of the method is the result of the very rapid and complete one-step sample preparation as well as the fact that unreacted orthophosphoric acid does not interfere in the subsequent chromatographic step. Our method is linear up to at least 3 nmol, which is 100 times above the detection limit. Complete determination, including sample preparation and analysis, can be performed in less than 30 min.

The equipment required is relatively inexpensive and readily available. This enables on-site analysis to be carried out by most factories.

Since TDA is also present in the environment it can be trapped in the silica gel as well. As a result, its presence can simulate that of TDI. In order to determine the environmental level of TDI alone, we took advantage of the fact that TDI does not collect on silica gel without acid. This was done by preparing two type of traps: one containing acid, which collected both TDI and TDA, and another without acid, which collected only TDA.

Other amines (hexamethylendiamine; 4,4′-diaminodiphenylmethane) and 2,6-toluene diisocyanate do not interfere since they have different retention times. We feel that the present method could be extended to other isocyanates (4,4′-diphenylmethane diisocyanate, hexamethylene diisocyanate, isophorone diisocyanate) found in polluted air if the accuracy of the quantitative determination of the compounds can be confirmed.

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CHROMSYMP. 2696

Identification and determination of biogenic and anthropogenic volatile organic compounds in forest areas of Northern and Southern Europe and a remote site of the Himalaya region by high-resolution gas chromatography–mass spectrometry

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ABSTRACT

More than 100 volatile organic compounds of natural and man-made origin were identified and determined in air samples collected in forest and remote areas. The combined use of carbon adsorption traps and high-resolution gas chromatography–mass spectrometry made possible the selective determination of polar compounds such as organic acids, alcohols and carbonyl compounds by selected-ion detection. A comparison between the distribution and composition of volatile organic components recorded in Northern Europe, the Mediterranean basin and the Himalaya region seem to be indicative of the ubiquitous occurrence of some polar organic compounds of biogenic origin. The determinations carried out in the Italian Station built by the National Research Council (CNR) of Italy at the foot of Mount Everest show clearly that, under favourable conditions, substantial amounts of organic pollutants of man-made origin can be transported over unpolluted areas.

INTRODUCTION

Emission of large amounts of chemical compounds into the atmosphere as a result of anthropogenic and biogenic processes results in a complex array of chemical transformations ultimately leading to diverse effects on man and the environment. Among them, photochemical air pollution, acid deposition, changes in the stratospheric ozone layer and global weather modifications are the most important [1]. For a long time, volatile organic compounds (VOC) have been recognized to be important primary pollutants, acting as precursors of atmospheric pollution either in tropospheric and stratospheric layers [1]. Because of the great complexity of chemical reactions and emission, transport and deposition processes, computer models have been developed to predict the possible adverse effects associated with increased emission of VOC into the atmosphere [1–2].

In the last 15–20 years, validation of the chemical mechanisms by computer models has been regarded as one of the fundamental tasks to be pursued in order to make model predictions sufficiently accurate to be used for a targeted decrease in atmo-

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spheric pollution [2,3]. The comparison of observations with predictions implies, however, that both the composition and concentrations of VOC present in different air parcels are known in great detail and with high accuracy. Particularly important is the capability to detect biogenic components whose emission from vegetation and microbial processes is still largely unknown [4]. This is possible only if analytical techniques capable of detecting both precursors and products of atmospheric reactions at trace levels (pptv) are available.

Recently, the use of adsorption traps filled with graphitic carbons combined with high-resolution gas chromatography–mass spectrometry (HRGC–MS) has been proposed as a suitable method for investigating the composition of the organic fraction with carbon numbers ranging between 4 and 14 [5]. More than 140 compounds exhibiting different polarities (mainly alkanes, alkenes, arenes, alcohols, aldehydes and ketones) were identified in samples collected in urban and suburban areas of the Italian peninsula and a pine forest located in Central Italy [5,6]. To test further the accuracy of this method and extend its capability to the identification and determination of components emitted by natural sources or formed by their photochemical oxidation in air, additional experiments were carried out in relatively unpolluted areas of Northern Europe, the Mediterranean basin and in the Himalaya region close to the Mount Everest where a permanent station has recently been set up by the National Research Council of Italy (CNR).

The analysis of these samples confirmed that the method proposed is suitable for evaluating in a single run a large number different classes of organic components useful to assess the relative importance of biogenic *vs.* man-made emission, investigate atmospheric processes and detect the influence of transport in remote areas. In this paper the full methodology for determining the various classes of organic components that can be present in the atmosphere is described. The observations made are discussed on the light of the present knowledge on VOC emission and reactivity. Evidence for the importance of transport in determining the levels of organic pollutants in remote areas of the Himalaya region is presented.

EXPERIMENTAL

Adsorption Traps

VOC present in volumes of air varying from 1 to 6 l were collected on two-stage traps consisting of glass tubes (15 cm × 0.3 cm I.D.) filled with Carbotrap C (0.034 g) and Carbotrap (0.17 g) particles ranging between 20 and 40 mesh. Both adsorbents were supplied by Supelco (Bellefonte, PA, USA). Graphitic carbons were preferred to more adsorbing carbon materials (such as Carbosieve III) as their hydrophobic surface and low specific surface area prevented the adsorption of large amounts of water and carbon dioxide on the traps, thus allowing the mass spectrometric identification of volatile components with carbon numbers higher than 2 [5]. Before sample collection, the traps were cleaned by passage of a stream of pure helium at a flow-rate of 300 ml/min for 10 min at 285°C. After purging, the traps were sealed with metal connectors equipped with PTFE ferrules, wrapped in aluminium foil and stored in a tightly closed glass container (3 l) in the presence of open cartridges filled with a desiccant and active charcoal to prevent contamination of the trapping materials during transport. One sealed trap in each container was used as a blank to check whether accidental exposure of the container to contaminants would have caused passive collection of hydrocarbons in the sampled traps.

A high precision, battery-operated sampling device (Genesis Air Sampler; DuPont, Kenneth Square, PA, USA) was used for the parallel collection of the same volume of air in two different traps. After sampling, the traps were sealed and stored in the glass container for shipment to the laboratory. By adopting this procedure, VOC adsorbed on traps were found to be stable for more then 2 months.

Desorption unit, GC–MS apparatus and columns

Hydrocarbon components retained on the carbon traps were transferred to the GC unit by thermal desorption. A Chrompack (Middelburg, Netherlands) purge and trap injector was adapted for this purpose by eliminating the purging and drying units. The unit operates according to a two-step mechanism involving a cryofocusing process at the column inlet to prevent band broadening of the GC peaks in the capillary column. After the trap has

been back-flushed at a flow-rate of 10 ml/min for 1 min, the gas stream is diverted to the cryofocusing unit (−150°C) containing a fused silica liner. The flow-rate is then increased to 20 ml/min and maintained for 5 min through both the trap and liner while the temperature of the trap is ballistically increased to 250°C. The desorbed sample enriched on the cryofocusing unit is subsequently transferred to the GC column by increasing the temperature of the fused-silica liner from −150 to 230°C.

All separations were performed on a 60 m × 0.32 mm I.D. capillary column (J&W Rancho Cordoba, CA, USA) coated with a 0.25-μm film of DB-1. After transfer of the sample, the column oven was maintained at 5°C for 3 min, programmed to 50°C at 3°C/min and then to 220°C at 5°C/min. A Model 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) connected to a Hewlett-Packard Model 5970 B mass spectrometer (mass-selective detector, MSD) was employed for all GC–MS determinations. Positive identification of the various components was carried out by combining the information obtained through the analysis of mass spectra with those acquired through the determination of the elution sequence determined by measuring the retention indices of a large number of pure components. When standards were not available, retention indices reported in the literature were used for peak identification [7]. Selected-ion detection was preferred for identification and quantification purposes whenever eluted compounds were characterized by fragmentation patterns where specific ions could have been used for unambiguous determinations.

Sites investigated

Forest samples were collected in eastern Germany and Central Italy. Eastern Germany was selected because it is covered by large wooded areas highly representative of the vegetation present in the northern hemisphere at high latitudes (*i.e.*, Northern Europe, USA and Canada).

Sampling was carried out inside a large pine forest located at Storkow, 30 km south east of Berlin and not far from the Polish border. More than 30 samples were collected during daytime and at night during the second half of July.

The site located in Central Italy was representative of the "Mediterranean Macchia", a wooded area mainly characterized by deciduous trees mixed with short plants growing near the sea shore. The presence of pine trees (*Pinus pinea, Pinus aleppensis, Douglasia*) is also frequent. This type of forest, common in Southern Europe from Spain to Greece, gives rise to an intense smell associated with a strong emission of VOC caused by the presence of aromatic and scented herbs and the occurrence of the high temperatures typical of temperate regions. Sampling was performed in a large, protected area located 25 km west of Rome. Also in this case, a large number of samples were collected during the winter season when reduced impact from the urban area occurred.

The VOC distribution existing in remote areas was investigated by collecting air samples at the Italian Station installed by CNR in Nepal. Established since 1990, it is located at an altitude of 5050 m at the foot of Mount Everest (8848 m) in the middle of the National Park of Sagarmatha. It can be reached from the Lobuche camp in a 6-day trek and only during the favourable season (end of the monsoon season). The station consists of a glass pyramid built on a small hill next to a gorge between two large glaciers. As power generation is partly ensured by solar cells covering the glass windows and partly by a small hydroelectric generator, no direct emission of man-made VOC could have affected the determinations. Air samples were taken during the 1991 expedition that started at the beginning of September and ended in the middle of October. This activity was part of a larger programme (Ev-K2-CNR) involving research in geological, environmental and biological fields and studies on advanced technologies.

RESULTS AND DISCUSSION

Figs. 1–3 show the total ion current (TIC, m/z 34–200) profiles obtained by submitting to GC–MS analysis air samples collected at the three different sites. The peak numbers refer to the compounds listed in Table I, where the retention time, retention index and amount detected are also reported for each component identified in the various samples.

The VOC are grouped into classes in order to distinguish better compounds released by anthropogenic sources (alkanes, arenes and partly alkenes) from those suspected to be associated with biogenic

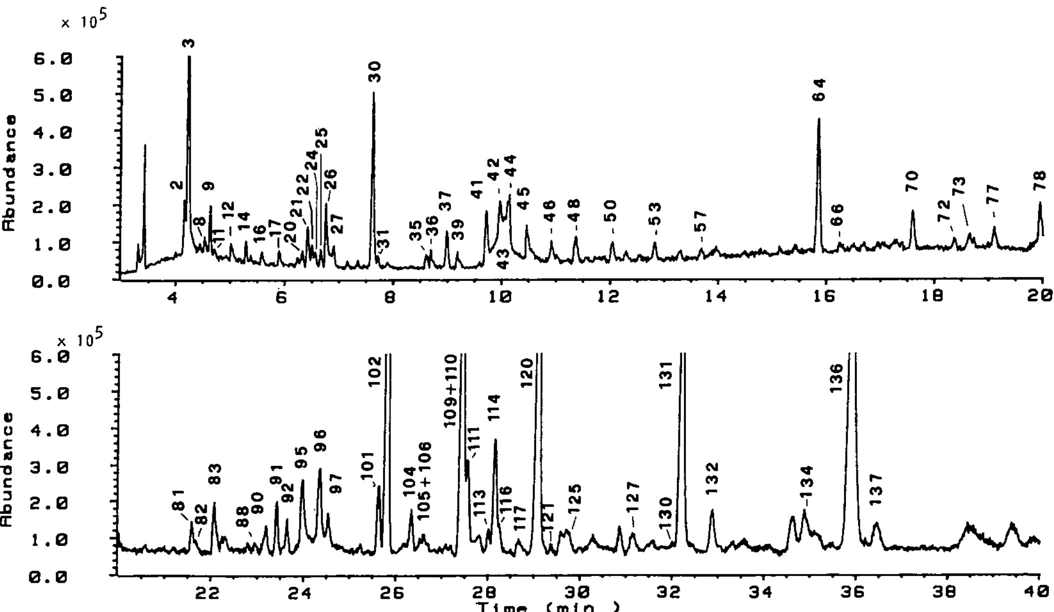

Fig. 1. GC–MS profile of a sample collected in a Northern European pine forest (Storkow, Germany). The trace is the reconstructed chromatogram obtained by using a mass window ranging from m/z 34 to 200. Peak numbers refer to the compounds listed in Table I.

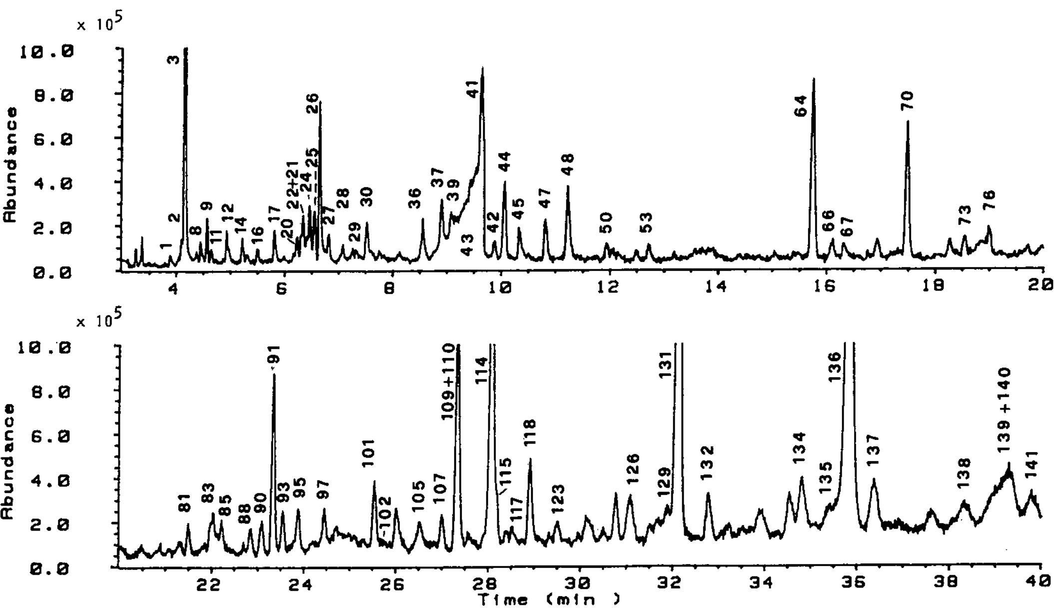

Fig. 2. GC–MS profile of a sample collected in a "Mediterranean Macchia" in Central Italy (Castel Porziano). The trace is the reconstructed chromatogram obtained by using a mass window ranging from m/z 34 to 200. Peak numbers refer the compounds listed in Table I.

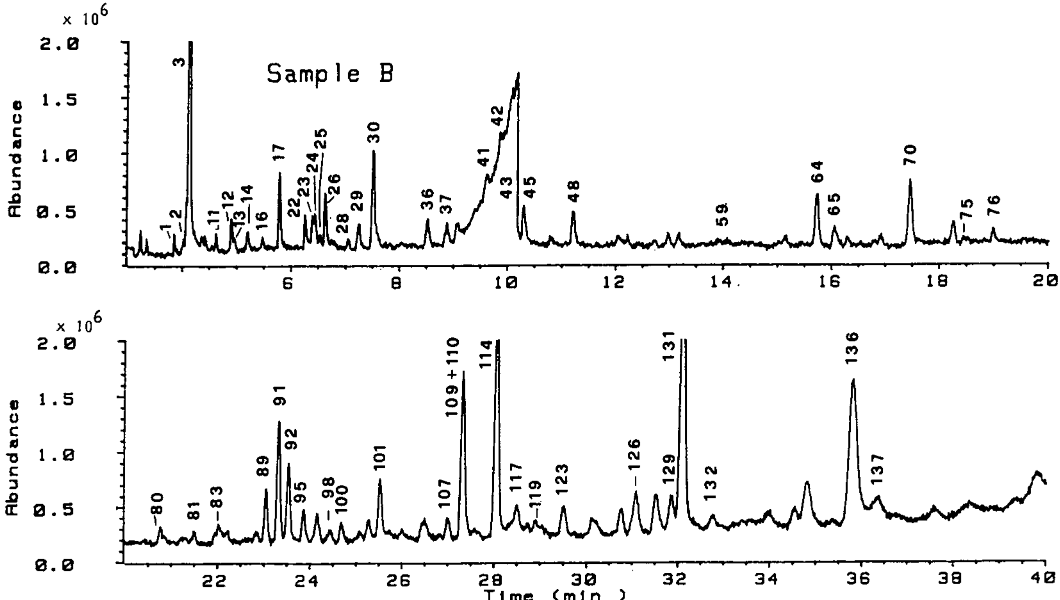

Fig. 3. GC–MS profile of a sample collected at the Italian Station in Nepal. The trace is the reconstructed chromatogram obtained by using a mass window ranging from m/z 34 to 200. Peak numbers refer to the compounds listed in Table I.

emission (some alcohols, aldehydes and ketones). Monoterpenes are listed separately as together with isoprene they are the most abundant organic components emitted by trees, being present in large amounts in the oil cells of leaves [8]. In Table I, the number of individual components identified in each class into which the VOC were grouped is reported and the net and relative contributions of the various classes to the whole organic fraction are also given.

The GC–MS profiles in Figs. 1–3 were accurately selected from more than 70 samples in order to highlight the complexity of the VOC distribution found in each of the areas investigated. As both natural and man-made emissions and photochemical reactions taking place in the atmosphere change rapidly from day- to night-time, and are strongly influenced by the meteorological conditions and seasonal variations, it is not surprising that the greatest complexity of the VOC distribution of the samples shown in Figs. 1–3 was realized at different times of the day. For the pine forest sample, the largest number of components was recorded ed at night when polar components remaining from the daytime hours were still present in the air together with monoterpene compounds not removed by reaction with OH radicals and ozone. In the "Mediterranean Macchia", where monoterpene emission was restricted to α-pinene released by some pine trees, the greatest complexity was rather realized in the middle of the day when high temperatures promoted substantial emissions of polar components from plants and/or photochemical reactions led to their formation. In this instance, low emission combined with fast removal by reaction with OH radicals can reasonably explain the low levels of α-pinene found in this sample. Although some day-to-day variations occurred in the VOC concentrations in forest samples collected in Northern and Southern Europe, they were mainly affecting the anthropogenic fraction and thus are attributed to the variable extent to which man-made emissions and their transport were influencing the sites. Daily profiles of natural components were, instead, fairly regular with a high abundance of monoterpene compounds at night and maximum levels of polar compounds around noon.

Within the above-mentioned limitations, the mass chromatograms shown in Figs. 1 and 2 can be

TABLE I

VOC IDENTIFIED AND DETERMINED BY GC–MS IN THE PINE FOREST AREA OF STORKOW (GERMANY) THE "MEDITERRANEAN MACCHIA" OF CASTEL PORZIANO (ITALY) AND THE ITALIAN STATION LOCATED AT THE FOOT OF EVEREST (K2,CNR-PYRAMID, 5050 m) IN NEPAL

Peak No	Compound	Retention time (min)	Retention index	VOC ($\mu g/m^3$)					
				Storkow (July 18, 1991, 3 a.m.)	Castel Porziano Feb. 27, 1992, noon)	K2-A (Sept. 28, 1991, 7 p.m.)	K2-B (Oct. 3, 1991, 7.30 a.m.)	K2-C1 (Oct. 4, 1991, 8 a.m.)	K2-C2 (Oct. 4, 1991, 6 p.m.)
	Alkanes								
9	n-C_5	4.555	500.00	0.38	0.53	3.74			
15	Butane, 2,2-dimethyl-	5.292	524.77			0.09			
19	Butane, 2,3-dimethyl-	6.147	553.48			1.54			
21	Pentane, 2-methyl-	6.338	559.88	0.41	0.71	10.32			0.31
27	Pentane, 3-methyl-	6.818	576.01	0.23	0.51	10.54			0.34
30	n-C_6	7.532	600.00	1.85	0.45	25.84	2.60	1.04	0.86
46	Hexane, 2-methyl-	10.778	662.46	0.31					
50	Pentane, 2,2,4-trimethyl-	11.942	684.86	0.29	0.25	1.38			
53	n-C_7	12.729	700.00	0.27	0.50	1.88			
76	n-C_8	18.998	800.00		0.49	2.36	0.35		
97	n-C_9	24.449	900.00	0.40	1.01	0.88	1.47		
119	n-C_{10}	28.908	1000.00		2.71	0.82	0.68		0.22
132	n-C_{11}	32.768	1100.00	1.24	1.74	0.78	1.53		
137	n-C_{12}	36.359	1200.00	0.89	2.78	0.79	3.22		
141	n-C_{13}	39.795	1300.00		1.53	0.61			
Total amount				6.27	13.21	61.57	9.85	1.04	1.73
% of total				6.9	7.7	23.0	5.7	0.8	7.7
No. of compounds				10	12	14	6	1	4
	Alkenes								
6	1-Pentene	4.351					0.92		
8	1-Butene, 2-methyl-	4.441		0.19	0.35				
11	Isoprene	4.631	502.55	0.12	0.23	0.12	0.33	0.34	0.16
28	1-Hexene	7.057	584.04		0.26	1.47	0.24	0.15	0.27
38	2,4-Hexadiene	9.050	629.21					0.36	0.10
51	1-Heptene	12.070	687.33			2.53			
55	1-Pentene, 2,2,4,-trimethyl-	13.203	707.57			1.86			
58	1,4-Heptadiene, 3-methyl-	13.691	715.35					0.49	
72	1-Octene	18.269	788.38	0.26		2.70			
Total amount				0.57	0.84	9.6	0.57	1.34	0.53
% of total				0.6	0.5	3.6	0.3	1.1	2.3
No. of compounds				3	3	6	2	4	3

	Arenes								
41	Benzene	9.615	640.08	0.54	3.08	1.99	0.42	0.27	0.50
64	Toluene	15.778	748.64	1.87	4.02	31.38	1.80	0.28	0.14
81	Ethylbenzene	21.488	845.68	0.47	0.66	2.09	0.51		
83	(*m* + *p*)-Xylene	22.009	855.24	0.77	1.54	3.48	1.99		
88	Vinylbenzene	22.851	870.69	0.16	0.79	1.44			
90	*o*-Xylene	23.095	875.16	0.41	0.95	0.93			
99	Isopropylbenzene	24.797	907.81			0.07			
100	Benzene, 1-methoxy-4-methyl-	25.077	914.09				0.42	1.32	
103	*n*-Propylbenzene	26.146	938.06			0.33			
105	Benzene, 1-methyl-3-ethyl-	26.531	946.41	0.11	1.14	0.38			
106	Benzene, 1-methyl-4-ethyl-	26.536	946.81	0.14					
108	Benzene, 1,3,5-trimethyl-	26.859	954.05			0.30			
112	Benzene, 1-methyl-2-ethyl-	27.268	963.22			0.09			
113	Benzene, 1,2,4-trimethyl-	27.925	977.96	0.39					
121	*p*-Cymene	29.355	1011.58	0.06					
122	Indane	29.410	1013.00	0.07					
Total amount				4.99	12.18	42.48	5.14	1.87	0.64
% of total				5.5	7.1	15.9	3.0	1.5	2.8
No. of compounds				11	7	11	5	3	2
	Monoterpenes								
102	α-Pinene	25.707	928.22	9.28	0.05	0.84			
104	Camphene	26.238	940.13	0.61		0.51			
111	β-Pinene	27.540	969.32	1.77					
116	Myrcene	28.240	985.02	0.33		0.09			
118	Terpene	28.583	992.71			0.63			
120	Δ^3-Carene	29.071	1004.22	9.08		0.20			
124	β-Phellandrene	29.591	1017.69	0.41					
125	Limonene	29.661	1019.51	0.34		1.16			
130	α-Terpinolene	32.004	1080.20	0.51					
133	Camphor	33.408	1117.81			2.66			
Total amount				22.33	0.05	6.09	0	0	0
% of total				24.6	0.0	2.3	0.0	0.0	0.0
No. of compounds				8	1	7	0	0	0
	Alkyl halides and CFCs								
4	CFC 11[b]	4.224				0.06			
14	CFC 113[b]	5.206	521.87	0.18	0.34	0.04	0.47	0.24	0.25
32	Chloroform[b]	7.577	600.86	0.12		1.40			0.22
37	1,1,1-Trichloroethane	8.890	626.13	0.49	0.83	1.48	0.52	0.37	0.40
42	Carbon tetrachloride	9.873	645.05	0.29	0.31	0.29	0.03	0.72	0.67
49	Trichloroethene	11.731	680.80			0.36			
73	Tetrachloethene	18.549	792.84	0.30	0.45	1.60			
Total amount				1.38	1.93	5.23	1.02	1.33	1.54
% of total				1.5	1.1	2.0	0.6	1.1	6.8
No. of compounds				5	4	7	3	3	4

(Continued on p. 62)

TABLE I *(continued)*

Peak No	Compound	Retention time (min)	Retention index	VOC ($\mu g/m^3$)					
				Storkow (July 18, 1991, 3 a.m.)	Castel Porziano Feb. 27, 1992, noon)	K2-A (Sept. 28, 1991, 7 p.m.)	K2-B (Oct. 3, 1991, 7.30 a.m.)	K2-C1 (Oct. 4, 1991, 8 a.m.)	K2-C2 (Oct. 4, 1991, 6 p.m.)
	Sulphur compounds								
13	Carbon disulphide[b]	4.957	513.50			1.03	0.28		
54	Methane isothiocyanate[b]	12.984	704.08			1.10			
Total amount				0	0	2.13	0.28	0	0
% of total				0.0	0.0	0.8	0.2	0.0	0.0
No. of compounds				0	0	2	1	0	0
	Alcohols								
1	Ethanol[b]	3.857			0.19	0.46	0.31	0.12	0.06
7	2-Propanol[b]	4.408				1.42		0.71	
12	2-Propanol, 2-methyl-	4.914	512.06	0.22	0.54	2.32	0.60	0.24	0.33
18	1-Propanol	5.913	545.62			0.86			
31	3-Buten-2-ol, 2-methyl-	7.533	600.00					0.84	
36	1-Propanol, 2-methyl-	8.537	619.33	0.23	0.77		0.79	0.25	
45	1-Butanol	10.439	655.95	0.52	0.67	21.13	0.90	0.11	0.38
63	1-Butanol, 3-methyl-	15.712	747.59			0.35			
67	1-Pentanol	16.355	757.85		0.44	0.92		0.36	
85	1-Hexanol	22.138	857.61		0.68	0.25		0.22	
87	2-Propanol, 1,3-dichloro-	22.418	862.75			0.45			
95	Ethanol, 2-butoxy-	23.800	888.09	1.25	0.40	0.10	1.35	1.59	
96	2,4-Pentanediol, 2-methyl-	24.344	898.08	1.34					
107	1-Heptanol	26.996	957.11		0.87		0.92	0.68	
123	1-Hexanol, 2-ethyl-	29.494	1015.18	0.44	0.90	0.28	1.76	1.06	
126	1-Octanol	31.079	1056.24		2.28		2.78	2.07	
134	1-Nonanol	34.802	1156.62	0.77	2.41		3.74	1.92	
138	1-Decanol	38.327	1257.28		2.12				
Total amount				4.77	12.27	28.54	13.15	10.17	0.77
% of total				5.3	7.1	10.7	7.6	8.3	3.4
No. of compounds				7	12	11	9	13	3
	Aldehydes								
2	2-Propenal[b]	4.044		0.49	0.27	0.08	0.25		0.15
16	2-Propanal, 2-methyl-	5.479	531.03	0.16	0.29	0.74	0.29	0.13	0.10
17	2-Butenal	5.791	541.52	0.19	0.49	3.32	1.41	2.19	0.24
24	Butanal	6.444	563.45	0.17	0.84	4.29	0.18	1.52	0.12
39	Butanal, 3-methyl-	9.073	629.65	0.24	0.13	2.16			
48	Pentanal	11.228	671.12	0.41	1.52	7.96		0.93	
61	Pentanal, 2-methyl-	14.299	725.05			0.19			

65	2-Pentenal	16.052	753.01				0.85	1.60	
70	Hexanal	17.511	776.28	0.65	2.71	17.71	2.15	2.22	0.08
74	2-Furancarboxyaldehyde	18.814	797.07			0.19			
80	2,4-Hexadienal	20.764	832.40				0.54	0.50	
84	1-Formylcyclopentene	22.131	857.48			0.82			
91	Heptanal	23.331	879.50	0.72	4.10	5.21	4.17	3.30	
101	Benzaldehyde	25.531	924.27	1.16	1.91	1.72	2.25	0.98	1.84
114	Octanal	28.054	980.85	2.04	9.91	1.81	9.41	4.17	
131	Nonanal	32.102	1082.74	7.41	21.53	1.77	14.59	3.58	
136	Decanal	35.809	1184.67	11.45	24.76	0.95	12.59	2.35	
140	Undecanal	39.290	1285.31		5.11				
Total amount				25.09	73.57	48.92	48.68	23.47	2.53
% of total				27.7	42.8	18.3	28.3	19.1	11.2
No. of compounds				12	13	15	12	12	6
	Ketones								
3	2-Propanone[b]	4.119		3.52	6.05	2.66	14.38	15.68	1.12
22	3-Buten-2-one	6.364	560.77	0.20	0.28		0.54	1.42	0.17
26	2-Butanone	6.627	569.61	0.77	2.22	4.28	0.93	1.00	0.12
40	2-Butanone, 3-methyl-	9.392	635.79			0.72			
47	2-Pentanone	10.823	663.32		0.81	1.10			
60	2-Pentanone, 4-methyl-	13.998	720.25			0.39			
62	3-Pentanone, 2-methyl-	14.740	732.09			1.10			
66	Cyclopentanone	16.110	753.94	0.02	0.49				
69	2-Hexanone	16.976	767.75					0.24	
78	2-Pentanone, 4-hydroxy-4-methyl-	19.941	817.31	0.68					
86	2-Heptanone	22.222	859.15					0.01	
89	5-Hexen-2-one, 5-methyl-3-ethylene-	23.043	874.21				1.91	3.60	
92	2-Heptanone, 3-methyl-	23.527	883.09	0.37			3.14	2.30	
98	2(5*H*)-Furanone, 5,5-dimethyl-	24.673	904.12				0.79	0.92	0.91
109	5-Hepten-2-one, 6-methyl-	27.323	964.46	7.86	6.27	1.03	6.69	3.31	0.21
117	2*H*-Pyran-2-one, tetrahydro-	28.504	990.94	0.20	0.77		1.32		
128	3,5-Heptadien-2-one, 6-methyl-	31.857	1076.39				2.01	5.06	
Total amount				13.62	16.89	11.28	31.71	33.54	2.53
% of total				15.0	9.8	4.2	18.4	27.4	11.2
No. of compounds				8	7	7	9	10	5
	Ethers and furans								
5	Furan	4.243							0.24
20	Propane, 2-methoxy-2-methyl-	6.224	556.06	0.18	0.40				
29	Furan, 2-methyl-	7.253	590.60		0.14	6.03	0.66	0.78	0.21
33	Furan, 3-methyl-	7.634	601.96			0.70			
52	Furan, 2,5-dimethyl-	12.173	689.31					0.32	
93	Furan, 2,3-dihydro-5-methyl-	23.535	883.24		1.10				
Total amount				0.18	1.64	6.73	0.66	1.1	0.45
% of total				0.2	1.0	2.5	0.4	0.9	2.0
No. of compounds				1	3	2	1	2	2

(Continued on p. 64)

TABLE I (*continued*)

Peak No.	Compound	Retention time (min)	Retention index	VOC ($\mu g/m^3$)					
				Storkow (July 18, 1991, 3 a.m.)	Castel Porziano Feb. 27, 1992, noon)	K2-A (Sept. 28, 1991, 7 p.m.)	K2-B (Oct. 3, 1991, 7.30 a.m.)	K2-C1 (Oct. 4, 1991 8 a.m.)	K2-C2 (Oct. 4, 1991, 6 p.m.)
	Acids								
43	Acetic acid	10.011	(648)[a]	2.90	12.12	8.55	25.46	20.24	0.40
59	Propanoic acid	13.731	(716)	0.12	0.59	0.70	0.41	0.26	
75	Butanoic acid	18.819	(797)	0.09	1.14	0.99	0.09	0.23	
82	Butanoic acid, 3-methyl-	21.766	(851)	0.20	0.85	0.14	0.01	0.20	
94	Pentanoic acid	23.792	(888)	0.01	1.09	0.24	0.27	0.22	
115	Hexanoic acid	28.076	(981)	0.17	2.11	0.31	0.76	0.73	
129	Heptanoic acid	31.903	(1078)	0.12	1.66	0.15	0.79	0.77	
135	Octanoic acid	35.475	(1175)	0.39	2.65	0.39	2.29	1.14	
139	Nonanoic acid	39.082	(1279)	0.66	2.20	0.48	3.72	0.86	
Total amount				4.66	24.41	11.95	33.8	24.65	0.4
% of total				5.1	14.2	4.5	19.6	20.1	1.8
No. of compounds				9	9	9	9	9	1
	Esters								
10	Formic acid, ethyl ester	4.619	502.17			0.30			
23	Acetic acid, ethenyl ester	6.400	561.97				0.16		
34	Acetic acid, ethyl ester	7.728	603.77			0.51			
56	Formic acid, butyl ester	13.389	710.54			0.67			
68	Acetic acid, 2-methylpropyl ester	16.584	761.50			0.82			
77	Acetic acid, butyl ester	19.101	801.90	0.40					
Total amount				0.4	0	2.3	0.16	0	0
% of total				0.4	0.0	0.9	0.1	0.0	0.0
No. of compounds				1	0	4	1	0	0
	Cycloalkanes								
35	Methylcyclopentane	8.533	619.26	0.19		7.11			0.49
44	Cyclohexane	10.061	648.66	0.36	1.44	7.99			0.10
57	Methylcyclohexane	13.591	713.76	0.18		0.79			
71	Butylcyclopropane	17.873	782.06						0.27
79	Ethylcyclohexane	20.451	826.66			0.46			
127	Butylcyclopentane	31.145	1057.95	0.74					
Total amount				1.47	1.44	16.35	0	0	0.86
% of total				1.6	0.8	6.1	0.0	0.0	3.8
No. of compounds				4	1	4	0	0	3

	Phenols								
110	Phenol		27.323	964.46	0.67	0.33	0.35		
Total amount				0.67	0.33	0.35	0	0	0
% of total				0.7	0.2	0.1	0.0	0.0	0.0
No. of compounds				1	1	1	0	0	0
	Alkylsilanols								
25	Trimethylsilanol	6.537	566.56	0.17	0.80	0.55	0.13	0.05	
Total amount				0.17	0.8	0.55	0.13	0.05	0
% of total				0.2	0.5	0.2	0.1	0.0	0.0
No. of compounds				1	1	1	1	1	0
Total VOC amount				90.72	171.87	267.29	172.10	122.56	22.57
Total VOC identified				86.57	159.51	254.07	145.15	98.56	11.98
Total VOC not identified				4.15	12.36	13.22	26.95	24.00	10.59
Total number of VOC identified				81	74	101	59	58	33

[a] Retention indices are reported in parentheses to indicate the uncertainty associated with peak overloading.

[b] Compounds characterized by a break-through volume lower than 1 l. Only qualitative determination was possible. Values in the table refer to the portion retained on the trap.

taken as reasonably representative of the areas investigated. In particular, the monoterpene and carbonyl fractions observed in the German forest seem to reflect well the composition that can be found in almost any pine forest, being similar to that measured in the wooden area of Monti Cimini Park (Central Italy), also characterized by a high density of pine trees [5].

It was difficult to define a "typical" situation for the samples collected in Nepal. At an altitude of 5050 m the vegetation is restricted to musks and lichens, the fauna is scarce and anthropogenic emissions are almost non-existent, hence the composition and levels of VOC are mainly determined by transport phenomena and are thus strongly influenced by the meteorological conditions occurring during sampling. In the 1991 expedition, some days were dominated by strong ascending currents carrying VOC emitted by natural and man-made sources located far down in the valley up to the station (southern winds), whereas other days were characterized by the descent of cold air masses moving from the mountains downward to the valley (northern winds). In the latter instance, a substantial removal of VOC away from the sampling site was taking place. Owing to the dramatic difference in the VOC composition and concentration associated with these two circulation patterns, we have reported in Fig. 3 the mass chromatogram of a sample collected when only light winds (<1–2 m/s) were blowing (sample B, 7.30 a.m.). Under these conditions, the concentration of VOC in air was mainly determined by local emissions and the organic species remaining from the previous days.

To give an idea of the levels associated with the different air mass circulation occurring at the Himalaya station, we have also reported in Table I the VOC concentrations measured when prevailing winds were blowing from the southern (sample K2-A, 7 p.m.) and northern sectors (sample K2-C2, 6 p.m.).

Although the number of individual components found in forest and remote sites was usually lower than that observed in urban areas [5,6], all samples were characterized by a higher complexity than that existing in heavily impacted airsheds because of the numerous classes of polar organic compounds present in the atmosphere. In addition to alkanes, alkenes, arenes, aldehydes, ketones, isoprene and some monoterpene components also detected at different levels in many urban and suburban samples, a complex array of free acids, esters, alcohols and furans was found. Although some of these polar components were sometimes found in urban and suburban airsheds [5,6], their abundance was not such that specific detection was required.

The huge amounts of acids present in the chromatograms in Figs. 2 and 3 giving rise to a large, overloaded peak in the first part of the chromatogram (peak No. 43), coupled with the constant and detectable presence of alcohols from ethanol to hexanol and numerous aliphatic esters in the samples collected in the "Mediterranean Macchia" and the Himalaya region, suggested that selected-ion detection was necessary to elucidate better the complex array of polar and non-polar components present in forest and remote areas. This approach was found to be particularly useful for investigating the presence of organic acids, aldehydes, alcohols and terpenes.

An example of the information provided by mass spectrometry with selected-ion detection is reported in Fig. 4, where the total ion current profile of a sample collected at the Himalaya station (K2-A, 7 p.m.) is displayed together with the mass chromatograms used for the identification of some specific classes of components listed in Table I. The ion at m/z 31, corresponding to the oxonium ion ($[CH_2OH]^+$) formed by the cleavage of the carbon–carbon bond next to the α-carbon atom, was specific for the identification of many primary and secondary alcohols whereas the ion at m/z 44 ($[CHCHOH]^{+\cdot}$), coming from the rearrangement of the γ-hydrogen available for transfer to the carbon oxygen followed by the cleavage of the carbon bond in the β-position (McLafferty rearrangement), allowed the selective determination of all aldehydes with carbon numbers >3. The ion at m/z 60 corresponding to the molecular ion of acetic acid and to a fragment coming from the McLafferty rearrangement ($[CH_2C(OH)_2]^{+\cdot}$) typical of aliphatic acids with carbon numbers >4 was used for the selective detection of many acidic components. Recording of the molecular ion (m/z 74 not displayed in Fig. 4) was, instead, necessary for the identification and evaluation of propionic acid. The HRGC–MS profile of the cyclic ion at m/z 93, derived from the sequential loss of aliphatic chains from the terpen-

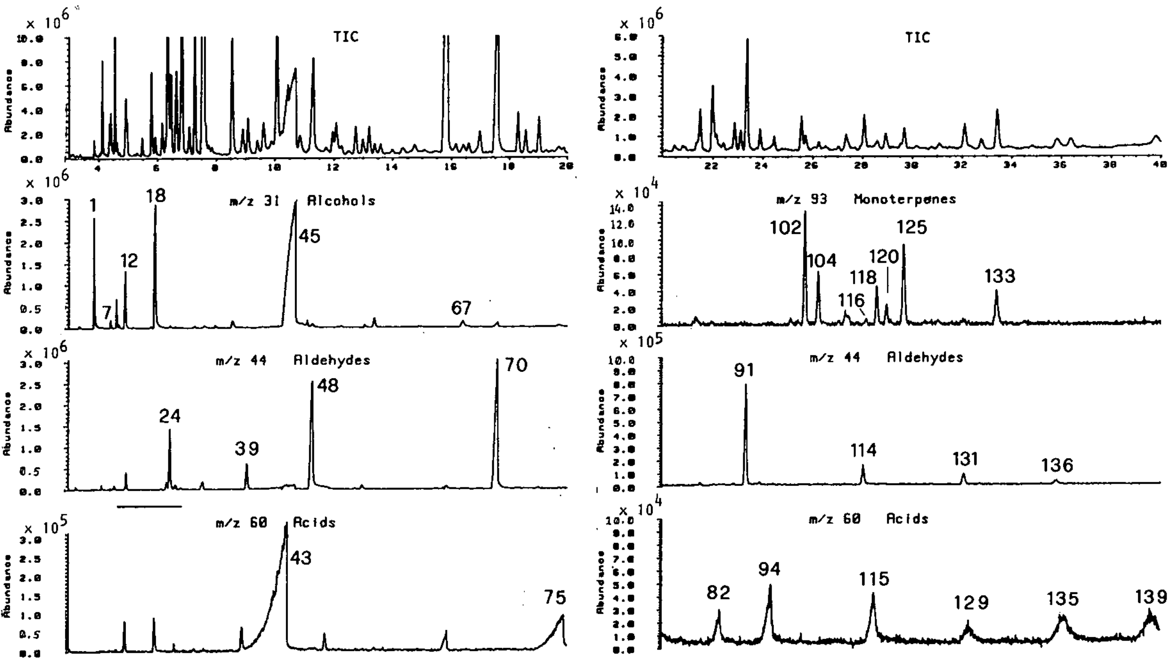

Fig. 4. Total ion current (m/z 34–200) and selected-ion detection profiles used for the identification of alcohols, aldehydes, acids and monoterpene compounds present in a sample collected at the Italian station in Nepal. The profile refers to sample K2-A in Table I. For peak identification, see Table I.

oid structure, allowed the specific detection of monoterpene compounds present at trace levels in the air samples.

Selected-ion detection was also extensively applied to the determination of other co-eluted components of natural and man-made origin (*i.e.*, alkylbenzene isomers or 6-methyl-5-hepten-2-one and phenol). Profiles relative to these organic species are not shown in Fig. 4 as the approach followed was the same as that used for investigating urban and suburban airsheds and examples of selected-ion mass chromatograms already published [5].

The analysis of samples characterized by substantial amounts of polar components was extremely useful from the methodological viewpoint as it showed the capability of carbon traps to retain and release in a quantitative way very polar organic compounds (particularly alcohols, aldehydes, ketones, esters and free acids) with a wider range of carbon numbers than reported previously (C_2–C_3, depending on the functional group). It also suggested that HRGC–MS determination of aliphatic acids might be competitive with methods based on denuder collection followed by liquid ion chromatography [9], as it makes it possible to detect all homologues members from acetic to nonanoic acid and to distinguish between isomeric components (see the peaks 82 and 94 in Fig. 4). Of course, a different column from that used for generating the mass chromatograms in Fig. 4 would be necessary for better identification and quantification purposes.

A detailed knowledge of the VOC distribution and composition present in forest and remote areas was also useful for assessing the possible origin of individual components. The high abundance of semi-volatile aldehydes, ketones (particularly 6-methyl-5-hepten- 2-one, known to be emitted by flowers and fruits), esters, alcohols and acids with respect to isoprene and monoterpene components of natural origin, alkanes, alkenes, arenes, chlorofluorocarbons and trimethylsilanol of anthropogenic origin and benzaldehyde of photochemical origin seem to be highly indicative that the largest proportion of polar components comes from natural processes. Whereas carbonyl compounds, esters

and alcohols are common constituents of the essential oils and fragrances extracted from plants and flowers [10] and some of them have been already detected in natural emission [4,11–13] and forest environments [5,14], organic acids might arise from either photochemical oxidation or microbial degradation of organic compounds [15].

The prevalence of aliphatic acids with an even number of carbon atoms found in many of the samples investigated and the low concentrations of ozone present in the European forest during the monitoring campaign seem to be highly suggestive of microbial origin. Also, the presence of carbon disulphide in one of the samples collected at the Himalaya site is not surprising as it is emitted together with carbonyl sulphide from different soils and plants [16]. It is more difficult to assign a definite source to 2-methylfuran, although the lack of this component in the urban and suburban airsheds [5,6] might be highly indicative of its biogenic origin.

Although the classes of polar VOC and levels measured in the wooden areas of Northern and Southern Europe were basically the same as those found at the Himalaya station, different distributions of the individual components present in each class were observed. This difference is particularly evident for aldehydes, as nonanal and decanal were the most abundant components recorded in the European forests whereas an almost Gaussian distribution centred on hexanal was found at the Himalaya station. This difference might be attributed either to the type of biogenic emission prevailing in the various sites (*i.e.* trees *vs.* short vegetation) or condensation processes that selectively removed high-boiling components from the air masses carrying VOC to the Himalaya station. The first hypothesis is supported by some laboratory experiments showing that aldehydes and ketones with carbon numbers ranging from 4 to 8 can be emitted by short vegetation growing under the canopy of Northern European forests [11] or plants growing in the California basin [4]. However, these results are somehow in contrast with the aerometric determinations carried out in forest areas [5,6,14] showing that, similarly to the results in Figs. 1 and 2, nonanal and decanal are the most abundant carbonyl components present in air and they account for a large proportion of the whole organic fraction. In our opinion, the predominance of hexanal at the samples collected in the Himalaya station can be better explained by the progressive depletion of high-boiling components taking place into the air masses during their travel towards the site. It is likely that the lowering of temperature associated with ascent of the air masses from the valley to the mountains, where daytime temperatures are close or below to 0°C, caused an efficient conversion of high boiling VOC into particles, thus promoting their removal from the airshed by wet and dry deposition processes. Evidence of transport of VOC into the station is provided by the data recorded when southern winds were blowing over the monitoring site. If we consider the concentrations measured on September 28th at the Himalaya station (sample K2-A in Table I, the chromatogram of which is reported in Fig. 4), we can see that levels of man-made emitted VOC (alkanes, alkenes, arenes, trimethylsilanol and some chlorinated hydrocarbons) much higher than those existing in the European forests were reached during the afternoon. This, combined with the occurrence of substantial amounts of monoterpene hydrocarbons known to be emitted by pine trees not growing at such high altitudes, can be taken as highly indicative of the fact that VOC present in the samples from the Himalaya station were actually emitted far away from the site where natural emission from forests was mixed with organic emissions released by anthropogenic processes (mainly combustion of biomass fuels for house heating and charbroiling or meat-cooking operations).

The dramatic difference with the VOC composition measured when Northern winds were removing VOC from the station is clear if we compare the results for the sample collected on September 29th with that taken on October 4th at 6 p.m. (sample K2-C2 in Table I). In the latter instance a total VOC content one order of magnitude lower than that measured when polluted airsheds reached the site was measured. No presence of monoterpene hydrocarbons was detected and negligible amounts of arenes (benzene and toluene) were present in the sample. The major components were oxygenated compounds of natural origin whereas much lower levels of halogenated hydrocarbons of man-made origin were detected. Trimethylsilanol was below the detection limit. A comparison with the levels

obtained on October 4th in the morning (sample K2-Cl in Table I) when low circulation occurred seems to suggest that the prevalence of northern winds in the afternoon hours caused a substantial removal of VOC from the sampling site by transporting them back to the valley. Although the observation that pollution levels recorded in the remote Himalaya station can be much higher than those measured in forest areas of heavy industrialized countries of Europe might appear surprising, it is perfectly in line with the observations of Davidson *et al.* [17], who found that the excessive per capita use of biomass fuels in Nepalese houses gives rises to quite high levels of pollution in the Himalaya region. Owing to the high indoor concentrations of organic compounds existing in Nepal, levels as high as 5 and 8 $\mu g/m^3$ were measured in outdoor samples collected outside one house in Sundarjial. The indications given by the sample collected on September 28th, 1991, seem to suggest, however, that anthropogenic emission might be even higher than that measured by Davidson *et al.*, giving rise to toluene concentrations as high as 30 $\mu g/m^3$. It is also possible that further injection of organic pollutants into polluted air masses coming from the Indian peninsula might be responsible for the levels reached at the Himalaya station when southern winds were dominating the air mass circulation. The observation that levels of benzene and toluene measured on the following days were close to those expected to be present in unpolluted atmospheres (*ca.* 0.5 and 0.6 $\mu g/m^3$, respectively) highlights the importance of transport phenomena in affecting the air quality of remote Himalaya regions. Whatever the cause (local emissions, long-range transport or a combination of both) leading to high levels of organic pollutants is, it must be regarded a serious source of concern for the preservation of the Everest environment owing to the possible formation of photochemical pollution and enhanced acid deposition arising from the exposure of such pollutants to the intense UV radiation existing at high altitudes.

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CHROMSYMP. 2715

Assessment of ambient volatile hydrocarbons from tobacco smoke and from vehicle emissions

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ABSTRACT

Quantitative proportions of C_2–C_8 alkenes, alkadienes, alkynes, alkanes and arenes were determined for indoor smoky air and for air inside a private car. Samples were taken on adsorbent cartridges and analysed by gas chromatography on an aluminium oxide column. The proportions of more than twenty reported alkenes, alkadienes and alkynes were demonstrated to be very similar in a smoky room and in sidestream cigarette smoke. Isoprene, ethene and propene are major components. Urban air polluted by petrol-fuelled vehicles differs mainly by having much lower proportions of isoprene and much higher proportions of petrol alkanes and alkylbenzenes. The total concentration of C_2–C_8 hydrocarbons was found to be similar in a smoky room and in a car in urban traffic.

INTRODUCTION

Increasing emphasis is being given to health hazards arising from the well-known volatile hydrocarbons ethene [1], 1,3-butadiene [2] and benzene [3]. Other volatile alkenes, alkadienes and arenes are likely to constitute significant but less well-known hazards. Non-smoking average people are exposed to these hydrocarbons mainly through environmental tobacco smoke and emissions from petrol-fuelled vehicles.

A few volatile hydrocarbons have been reported in a comprehensive experimental study [4] and in an overview [5] of environmental tobacco smoke. A recent study of in-vehicle air pollutants [6] was based on volatile petrol alkanes and arenes. The whole range of C_2–C_8 hydrocarbons from vehicles can be sampled on adsorbent cartridges and assessed by gas chromatography on a single column, as demonstrated recently for a road tunnel [7] and for urban air near to traffic [8]. This study uses a similar technique to compare volatile hydrocarbons in environmental tobacco smoke and vehicle-polluted urban air. A critical purpose of the study is to assess quantitative proportions for a wide range of the potentially hazardous C_2–C_8 hydrocarbons characterizing air polluted by the two different sources.

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EXPERIMENTAL

Several samples of environmental tobacco smoke were taken on different occasions in Junggrens Café, located along the Parade Avenue in Gothenburg, and well known for its delicious sandwiches. The volume of the room is *ca.* 150 m^3 and about ten smoking and ten non-smoking customers were present during sampling. The absence of prominent contributions from room-specific sources in the café was ascertained by comparisons with samples from other smoky indoor places and with background samples taken before the opening of the café.

Sidestream cigarette smoke was obtained by dropping a lit cigarette into a 100-ml glass vessel. Samples of gas were taken after 2 min. The volume of the glass vessel was not critical with respect to the resulting hydrocarbon composition. The commercial brands studied were Blend Ultima (Swedish) and Marlboro Light.

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In-vehicle samples were taken in a private car, a 1988 Nissan Micra, with threeway catalyst equipment. The exhaust CH_x level was well below 100 ppm, which is the upper limit permitted in the compulsory Swedish yearly car test. The fuel was unleaded RON 95 commercial petrol from the OK Petroleum chain. Samples were taken on two occasions during urban driving in Gothenburg from Chalmers University of Technology to the Central Railway Station and back again. The driving time of 20 min included 10–20 stops at traffic lights and intersections. The engine was warm from the start, the windows were closed, and the fan was set to intermediate speed. The ambient temperature was 10–20°C and the air was moderately turbulent. The absence of non-typical contributions to specific hydrocarbons was ascertained by comparisons with a variety of urban samples of traffic-polluted air.

Samples were taken on triple-layer cartridges (glass, 150 mm × 4 mm I.D.) with Tenax TA (0.6 ml, 60/80 mesh, Chrompack), Carbotrap (0.4 ml, 20/40 mesh, Chrompack) and Carbosieve S-III (0.4 ml, 60/80 mesh, Chrompack) as adsorbents. The sampling volumes were *ca.* 1 ml for sidestream smoke and ~500 ml for ambient air. The analytical separations were performed on a 50 m × 0.32 mm I.D. fused-silica PLOT column (Chrompack) with aluminium oxide–5% potassium chloride as the stationary phase. The system for sampling, thermal desorption and gas chromatography has been described previously [9]. The analytical results were checked with respect to the proportions of specific hydrocarbons by comparisons with duplicate samples. Ethene and the C_3 hydrocarbons were sampled without breakthrough losses [9]. Alkadienes and the alkenes with more than two alkyl groups adjacent to the double bond appeared to be sensitive to losses by chemical decomposition when using the triple-layer cartridges. Their quantitative proportions were checked by duplicate samples taken on Tenax or Tenax + Carbotrap cartridges. Response differences of the flame ionization detector were disregarded, although a correction for the somewhat higher response of benzene [7,8] and alkylbenzenes [7] was previously applied for urban air.

Mass spectra, total-ion chromatograms and single-ion chromatograms were obtained from a Varian Saturn II ion trap mass spectrometer, coupled to an aluminium oxide column in the GC unit. Gas samples of sidestream cigarette smoke were injected directly onto the GC–MS system without adsorbent sampling.

RESULTS AND DISCUSSION

Chromatographic separation

In Fig. 1, the separation achieved for C_2–C_8 hydrocarbons from a smoky café room is demon-

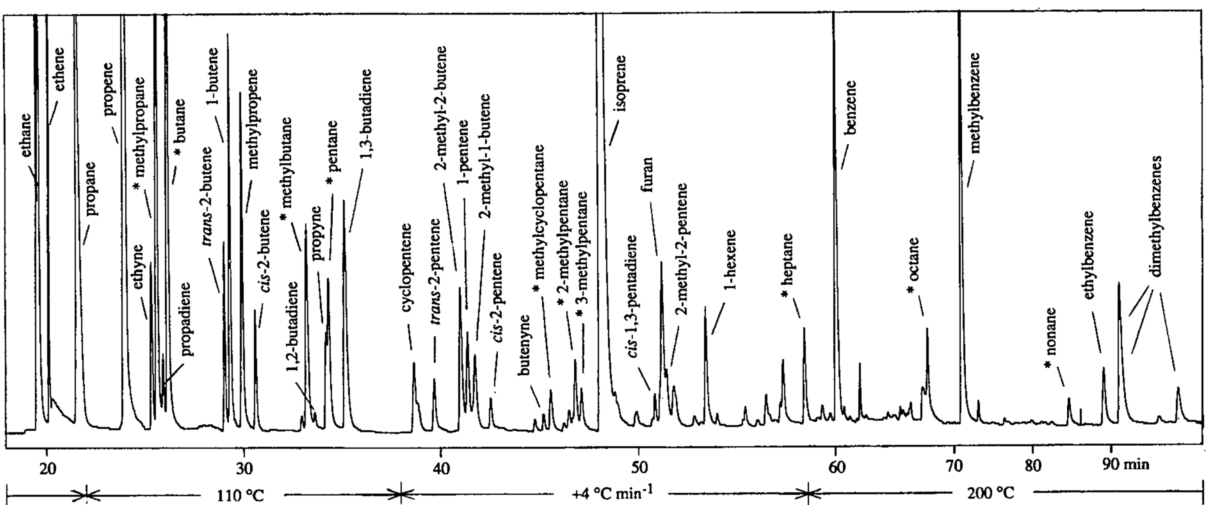

Fig. 1. Gas chromatographic separation of ambient volatile hydrocarbons from tobacco smoke (Junggrens Café, Gothenburg, April 15th, 1992; prominent components originating mainly from sources other than cigarettes are marked with an asterisk; the time scale includes the initial desorption period, and a programmed lowered chart speed in two steps after 60 min total time).

strated. The identities of the hydrocarbons marked in the chromatogram were determined from retention data and from mass spectra of the corresponding species appearing in the chromatogram of sidestream cigarette smoke. A major advantage of the aluminium oxide column is the clear-cut position of C_n alkenes between the C_n and C_{n+1} alkanes. The C_n alkadienes elute later than the C_n alkenes because of the larger induced polar interactions with the stationary phase. The non-linear temperature programme (Fig. 1) further improved the separation of the hydrocarbons.

In studies of hydrocarbons, the aluminium oxide column offers the advantage that polar compounds are not eluted from the column. The only oxygen-containing species observed from environmental tobacco smoke were furan and methylfurans. These are disregarded in the results given for hydrocarbons. Nitrogen-containing and other polar compounds from environmental tobacco smoke can be analysed on conventional columns after proper adsorbent sampling [10].

Hydrocarbon composition

The quantitative proportions of prominent C_2–C_8 hydrocarbons are given in Table I with emphasis on a wide coverage of unsaturated species. Smoky ambient air is compared with sidestream smoke from a cigarette, with air inside a car during urban driving, and with vehicle-polluted air in a road tunnel.

The percentage proportions of alkenes, alkadienes and alkynes are remarkably similar for the café and for the sidestream smoke. The proportion of the carcinogenic 1,3-butadiene is notably higher than in vehicle emissions. Isoprene (methyl-1,3-butadiene) is the major component, in sharp contrast to vehicle-polluted air. The extensive release of isoprene is likely to be linked to the high content of linear isoprene polymers in tobacco [11]. The higher proportion of isoprene in sidestream smoke than in the smoky room may be due partly to a lower than average oxygen supply during the experimental combustion. The alkenes from tobacco and from vehicle emissions are the same, but their quantitative proportions are higher in tobacco smoke. Cigarette smoke contains ethene and propene in similar high amounts, whereas vehicles emit considerably more ethene than propene as combustion products. Among the isomeric C_4–C_6 alkenes, the proportions of unbranched 1-alkenes and of 2-methyl-2-alkenes are relatively higher for cigarette smoke. The proportions of the 1,3-pentadienes and other incompletely resolved species were confirmed by mass spectrometric single-ion monitoring for sidestream smoke samples.

The proportions of alkylbenzenes and of C_4–C_8 alkanes are much higher in vehicle-polluted air than in cigarette smoke. These hydrocarbons are prominent components of petrol and are emitted mainly as unburnt exhaust hydrocarbons [8]. The volatile C_4–C_5 alkanes are also major components of petrol vapours. From the results given in Table I, it is concluded that C_4–C_6 alkanes originate mainly from traffic emissions even in smoky rooms. Although there is little traffic near to the café, a significant contribution from vehicles is also indicated for benzene and the C_7–C_8 alkylbenzenes. Background samples taken before the opening of the café confirmed the presence of C_4–C_6 alkanes and C_6–C_8 arenes. On the other hand, the results for sidestream cigarette smoke demonstrate that not only benzene but also significant amounts of methylbenzene (toluene) and C_8 alkylbenzenes are formed as combustion products from tobacco.

With regard to rooms polluted by cigarette smoking, it is concluded that alkadienes, alkenes and several other volatile hydrocarbons orginate predominantly from tobacco smoke. Mainstream smoke contributes, although the yields are higher for sidestream smoke [5]. A comparison between sidestream and mainstream smoke indicated a similar composition for the C_2–C_8 hydrocarbons. A wide range of volatile hydrocarbons in mainstream smoke is known from early studies [12]. The hydrocarbon proportions in sidestream smoke from a Swedish cigarette (Blend) were found to be similar to those given in Table I (Marlboro). Sidestream smoke from different cigarettes is known to differ less in composition than mainstream smoke [13]. The tabulated hydrocarbon proportions for sidestream smoke and smoky indoor air compare well with results previously reported for a few of the most prominent hydrocarbons [4]. Although the proportions of hydrocarbons were found to be similar in different smoky rooms, specific indoor sources and sinks may give partly deviating results.

With regard to in-vehicle volatile hydrocarbons, it

TABLE I

COMPOSITION (%) OF C_2–C_8 HYDROCARBONS IN AIR POLLUTED BY TOBACCO SMOKE AND TRAFFIC EMISSIONS

		Smoky café April 15th, 1992	Sidestream smoke Marlboro	Car September 23rd, 1992; 08.20–08.40 h	Road tunnel [7] February 19th, 1992; 08.10–08.40 h
Alkenes		*31*	*38*	*10*	*14*
C2	Ethene	8.9	11.1	4.7	7.8
C3	Propene	11.6	9.6	2.3	2.8
C4	*trans*-2-Butene	0.8	1.0	0.3	0.3
	1-Butene	2.1	2.8	0.4	0.6
	Methylpropene	1.9	2.7	0.9	1.1
	cis-2-Butene	0.6	0.7	0.2	0.2
C5	Cyclopentene	0.2	0.3	0.1	0.1
	3-Methyl-1-butene	0.5	0.7	0.1	0.1
	trans-2-Pentene	0.3	0.4	0.2	0.2
	2-Methyl-2-butene	1.2	2.7	0.3	0.3
	1-Pentene	0.8	1.2	0.2	0.2
	2-Methyl-1-butene	0.7	1.3	0.2	0.2
	cis-2-Pentene	0.2	0.3	0.1	0.1
C6	1-Hexene	0.8	1.3	0.1	0.1
	2-Methyl-2-pentene	0.3	0.4	0.1	0.1
Alkadienes		*20*	*34*	*2*	*1*
C3	Propadiene	0.2	0.2	0.1	0.2
C4	1,2-Butadiene	0.1	0.1	0.0	0.0
	1,3-Butadiene	1.9	3.2	0.4	0.7
C5	Isoprene	16.7	29.2	1.5	0.0
	cis-1,3-Pentadiene	0.2	0.2	0.0	0.0
	trans-1,3-Pentadiene	0.4	0.4	0.0	0.0
Alkynes		*2*	*2*	*2*	*5*
C2	Ethyne	0.9	0.8	2	5
C3	Propyne	0.4	0.4	–	–
C4	Butenyne	0.1	0.2	0.0	0.0
Alkanes		*28*	*12*	*46*	*35*
C2	Ethane	6	3	1.4	0.9
C3	Propane	6	4	0.8	0.5
C4	Methylpropane	4.0	0.5	5.1	2.8
	Butane	4.3	1.7	11.8	4.8
C5	Methylbutane	1.5	0.3	9.1	7.1
	Pentane	1.1	0.5	3.3	3.2
C6	Methylcyclopentane	0.3	0.0	2.0	1.8
	2-Methylpentane	0.5	0.0	2.7	2.8
	3-Methylpentane	0.3	0.0	2.0	2.4
	Hexane	–	0.0	2.2	2.0
Arenes		*19*	*14*	*40*	*45*
C6	Benzene	4.8	3.9	9.6	9.1
C7	Methylbenzene	8.4	6.4	16.0	17.5
C8	Ethylbenzene	1.0	0.7	3.1	3.7
	Dimethylbenzenes	4.3	2.8	11.6	14.5

is concluded that they originate almost exclusively from traffic emissions. The results from the road tunnel represent outside-vehicle concentrations at low ambient temperature. The higher proportions of butanes in the private car may be due to petrol vapours from other vehicles and from the sampling

TABLE II

CONCENTRATIONS ($\mu g/m^3$) OF HAZARDOUS HYDROCARBONS IN A SMOKY ROOM AS COMPARED WITH A PRIVATE CAR

The first two samples represent high indoor levels of cigarette smoke, and the following two samples moderate and fairly high levels of vehicle-emitted pollutants inside a car during urban driving. The last column gives rush hour levels from previous measurements in an urban road tunnel [7].

	Café April 15th, 1992; 13.25–13.55 h	Café May 12th, 1992; 10.30–11.00 h	Car September 23rd, 1992; 08.20–08.40 h	Car September 24th, 1992; 07.50–08.10 h	Tunnel February 19th, 1992; 08.10–08.40 h
CH_x (C_2–C_8)	*640*	*570*	*390*	*630*	*3600*
Ethene	56	42	18	30	280
Propene	73	37	9	15	100
Benzene	30	38	37	55	330
Methylbenzene	53	40	62	110	630

car itself. The observed proportions of benzene and alkylbenzenes are higher than reported for US vehicles [6], because of the higher proportion of arenes in urban air in Sweden [8], reflecting a high content in petrol. The elevated concentration of isoprene in the car is likely to be explained by isoprene in exhaled air from the two persons in the car. The isoprene content in exhaled air may approach 1000 $\mu g/m^3$ [14]. Differences in net human uptake between the hydrocarbons may also give rise to minor differences in concentrations. Thus benzene is known to be excreted through breath to a greater extent than alkylbenzenes [15].

Human exposure

In Table II, exposure levels for selected hazardous hydrocarbons are compared. The recorded concentrations are of the same order of magnitude in the smoky room as in the private car during urban driving. The alkene levels tend to be highest for cigarette smoke and the arene levels tend to be highest for traffic pollution. The café is regarded as being smokier than average cafés, but less smoky than many pubs. The lower in-vehicle level corresponds to moderate wind and traffic, whereas the upper level corresponds to weak wind and peak traffic. The upper in-vehicle concentrations were similar to those observed for volatile arenes in a recent study of hydrocarbons in commuter cars [16]. The road tunnel concentrations correspond to peak traffic and are 5–10 times higher than the in-vehicle levels. This is explained mainly by the limited dilution of the traffic emissions in the tunnel.

It has previously been demonstrated that the concentrations of traffic-emitted hydrocarbons are higher in private cars during urban driving than in the breathing zone of cyclists [17]. The concentrations in cars were also observed to be higher than on the pavement, and several times higher than in off-traffic places where official urban air pollution levels are often measured. Similar observations were made in a recent extensive study of in-vehicle air pollutants [6]. The obvious explanation is that cars run straight into air pollutants from the vehicles ahead, whereas the pollutants are rapidly diluted with increasing distance from the stream of vehicles.

It is evident that the concentrations of volatile hydrocarbons and other air pollutants are considerably higher in vehicles and in smoky rooms than in most other urban environments. The daily exposure time exceeds 1 h for many people. Children are often exposed in vehicles and in public and private smoky rooms. It is concluded that exposure in smoky rooms and in vehicles should be primary targets of efforts to decrease health hazards due to urban volatile hydrocarbons. In Sweden, prohibition of smoking in hospitals and various public indoor environments are examples of means of reducing exposure. Current extensive plans for new urban road tunnels represent an environmental change that may result in greatly increased exposure in vehicles [7].

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Evaluation of capillary gas chromatography for the measurement of C_2–C_{10} hydrocarbons in urban air samples for air pollution research

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ABSTRACT

A method for the determination of speciated non-methane hydrocarbons (C_2–C_{10} range) in ambient air samples was evaluated. The method involves a two-step cryogenic procedure: preconcentration (air sample volumes of 100–1000 cm^3) and prefocusing combined with capillary GC with flame ionization detection. GC columns were used under optimized temperature programming conditions (GC oven starting at above ambient temperature). Two systems were used to monitor hydrocarbon concentrations in urban air. The first system uses a megabore capillary column with a thick film of bonded non-polar silicone stationary phase (5 μm, SPB-1), which was found to be suitable for the chromatographic separation of hydrocarbons in ambient air samples without removing the moisture. To obtain a reasonable separation of volatile hydrocarbons in the C_2–C_5 range, a methylsilicone column was connected in series with a megabore methylphenylsilicone column. Better resolution of C_2–C_6 hydrocarbons was achieved on the latter system using an Al_2O_3–KCl porous-layer open tubular (PLOT) column. A dry-ice-cooled water trap was devised to remove moisture from ambient air samples. The PLOT column was not suitable for α- and β-pinene owing to decomposition. The reproducibility of GC performance (*i.e.*, retention time and peak-area integration) is discussed in detail. Both systems showed good long-term stability and gave good results for the determination of hydrocarbon concentrations ranging from several ppt to 100 ppb by volume in air samples from the Toronto urban area.

INTRODUCTION

Many non-methane hydrocarbons (NMHCs) present in the urban atmosphere are known to be toxic and certain hydrocarbons are known to participate in photochemical reactions resulting in the formation of oxidants such as ozone and peroxyacetyl nitrate (PAN) [1]. Large amounts of volatile organic substances are emitted from mobile and stationary sources [2]. With regard to anthropogenic hydrocarbons, motor vehicles make a significant contribution to ground-level concentrations of NMHCs [3,4] at urban sites. The median profile of hydrocarbon concentrations in many USA cities is predominantly made up of alkanes, aromatics, alkenes and acetylene, whereas in many European cities the relative abundances of aromatics are dominant over alkanes [5]. There is relatively little information in the literature on the distribution of species within each type of NMHC emission or the spatial distribution of NMHC emissions in urban air. This information is necessary for assessing air quality and implementing pollution control measures. A reliable analytical system for determining the hydrocarbon concentration in air is a prerequisite for this purpose [6].

Commonly employed techniques for measuring atmospheric NMHCs include a gas chromatogra-

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phy (GC) with flame ionization detection (FID) [5,7–12] and/or photoionization detection (PID) [7,8,12] and flame photometric detection (FPD) [10]. GC combined with mass spectrometry (MS) has also been employed [8,13,14]. Columns with non-polar silicone stationary phases have been commonly employed for the analysis of multi-component hydrocarbon mixtures [15]. Their application to analyses of volatile hydrocarbons in ambient air has been reported [5,7,8,10,11,13,14,16–18]. Columns with chemically bonded phases and columns with a porous layer of adsorbents made of Al_2O_3–KCl [9,12,17] and Porapak Q [10,11,17] have also been used in recent years.

Concentrations of individual NMHCs in urban air are often in the low parts per billion range and below. It is therefore necessary to employ a technique that enriches a sufficient amount of the species for analytical detection [17]. Cryogenic sample preconcentration is often employed for this purpose. The sample volume typically ranges from 100 to 1000 cm^3. Air sample collection using stainless-steel containers with metal bellows valves combined with subsequent GC separation after preconcentration is in common use for analysing low- and medium-molecular-mass trace gases of moderate or low polarity and reasonable stability, such as hydrocarbons (see, *e.g.*, ref. 19). On-line preconcentration with GC involves cryogenic preconcentration of the hydrocarbons onto a solid substrate (solid or porous glass beads [7–11]), whereby a cooled adsorption tube retains all the atmospheric trace components except nitrogen, oxygen, carbon dioxide and methane. The trapped NMHC components are thermally desorbed quantitatively. Moisture in ambient air samples presents problems, as it can extinguish the FID hydrogen flame, plug columns at sub-zero temperatures and cause serious damage to the solid Al_2O_3–KCl phase of porous-layer open-tubular (PLOT) columns [17]. Thus, the air samples are commonly dried with Nafion tubing [3], potassium carbonate [7,9,10] or magnesium perchlorate [8,18] water traps. Although these techniques remove the moisture, losses of some NMHC components {on K_2CO_3 [20] , $Mg(ClO_4)2$ [11,20]}, and contamination have been observed with the use of a Nafion dryer [20].

Adsorptive sampling of hydrocarbons in industrialized and remote areas on non-polar polymers and carbon adsorption traps has been widely used [17,19,20]. In recent years multi-layer adsorbent cartridges have been employed [5,12,14]. This mostly solves the problem of moisture affecting the chromatography, but this enrichment process is at present not adequate for all hydrocarbons in the C_2–C_{12} range. According to Rudolf *et al.* [19] and Ciccioli *et al.* [14], most affected are the C_2–C_3 hydrocarbons. Lancesdorfer and Puxbaum [5], however, claimed a recovery of $>90\%$ for C_2 compounds and $>95\%$ for $\geqslant C_3$ compounds.

This paper describes techniques employed by our group for NMHC measurements in ambient air samples from the Toronto urban area using a preconcentration system (made in-house) combined with capillary GC–FID. Problems associated with moisture in humid samples are discussed and solutions proposed. Different columns (chemically bonded non-polar dimethylsilicone phase and Al_2O_3–KCl PLOT columns) were evaluated with regard to their capacity to handle moisture, the resolution of species of interest and the reproducibility of the GC performance (*i.e.*, retention time and peak integration).

EXPERIMENTAL

Preconcentration unit combined with GC

A schematic diagram of the cryogenic system combined with a gas chromatograph is shown in Fig. 1. All the gas handling lines, except the water trap, were made of chromatographic-grade stainless-steel (SS) tubing [1/16-in. SS 304 and 1/8- and 1/4-in. SS 316 (1 in. = 2.54 cm)] and connections were made by either SS 316 Swagelok or SS Valco fittings. A Valco ten-port switching valve (Model 10UWT) was used for switching both the preconcentration loop and gas flow. The preconcentration loop (16 cm in length) was constructed of 1/8-in. SS tubing packed with 60–80-mesh untreated solid glass beads. Frits (1 μm) and silanized glass-wool (Chromatographic Specialties) were placed at both ends of the tube to keep the glass beads in place. Liquid argon (-186°C) was used to cool the loop. A length of 20 cm × 1/4-in. O.D. × 1/8-in. I.D. FEP Teflon tubing cooled with dry-ice to -30°C was used as the water trap and was placed after the sample canister and before the preconcentration U-trap. Air samples from electropolished pressurized

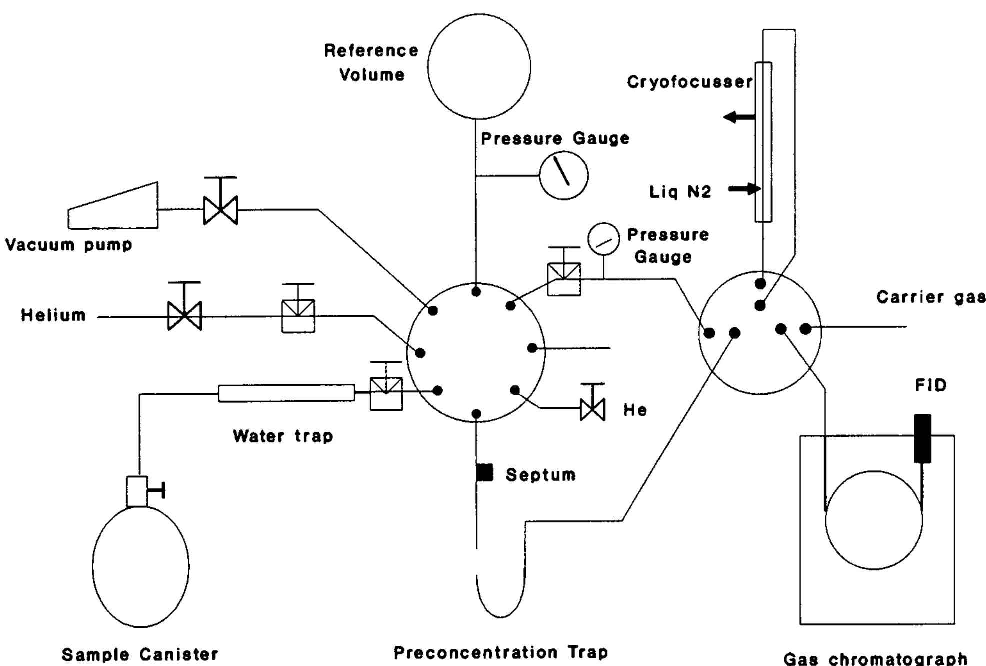

Fig. 1. Two-stage cryotrapping unit combined with capillary GC.

[*ca.* 40 p.s.i. (1 p.s.i. = 6894.76 Pa)] SS canisters (Biospherics) were introduced at atmospheric pressure (flow-rate 30 cm^3/min) into the cryogenic U-trap. The pressure was measured by an Omega pressure gauge (Model PGS-25L-30 V/30) and adjusted by the use of a Nupro SS-4BMW needle valve placed immediately after the water trap and a second Nupro needle valve (SS-SS2) placed between the preconcentration loop and the reference volume.

In addition to the cryogenic preconcentration loop, a second cryogenic system (Cryotherm Model 100; Tekran), which can be ballistically heated, was mounted at the head of the GC column. The Cryotherm was a cryo-prefocus device (cryo-loop) employed to generate narrower hydrocarbon bands.

When the required volume of air sample (100–1000 cm^3, depending on the NMHC content of the sample) was loaded, the preconcentration loop was heated to 100°C with an aluminium heating block and the sample was swept with helium (flow-rate 20 cm^3/min) into the cryo-prefocusing device cooled to −180°C with liquid nitrogen. After all the NMHC had been transferred, the prefocusing device was heated for 11 s (up to 150–200°C) and helium was used to sweep the sample on to the GC column. The reference volume (1600 cm^3), made of stainless steel, along with a Barocell pressure gauge (Datametrics, Model 600A-1000T-R12-H21X-4) was used to determine the volume of air sample that passed through the preconcentration loop.

After every cycle through the system, the reference volume was evacuated to $<10^{-2}$ Torr (1 Torr = 133.322 Pa) and ultra-high-purity (UHP) (99.995%) helium (Liquid Carbonic) was used to purge the heated lines of hydrocarbons that may have remained on the walls. Heating and back-flushing with UHP helium were also employed to purge the dry-ice water trap. During sample loading and sample purging, all the lines and valves in the system were heated at 100°C to minimize the surface adsorption of NMHC.

Two preconcentration–GC systems were used in the analysis of NMHCs. The first system employed a tandem megabore capillary arrangement [SPB-1 + DB-5; SPB-1 (Supelco), 60 m × 0.53 mm I.D. with a 5-μm bonded film thickness of dimethylsilicone phase; DB-5 (J&W Scientific) 30 m × 0.53 mm I.D. with a 1.50 μm bonded film of methyl-

phenylsilicone]. The second system used a 50 m × 0.32μm I.D. Al_2O_3–KCl PLOT column (Chrompack) with a 5-μm adsorbent layer. Moisture removal from this system was achieved by passing the sample through the dry-ice water trap. A conventional-bore dimethylsilicone column (DB-1, J&W Scientific; 30 m × 0.25 mm I.D., with a film thickness of 0.25 μm) was also tested in this system. A Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector was connected to a personal computer. All GC gases (helium, hydrogen and air) were passed through a hydrocarbon trap (Supelco, Model 2-2445). The sample (after preconcentration, cryofocusing, and ballistic heating) was swept directly on to the GC column using UHP helium. All GC measurements were performed with temperature programming. Hewlett-Packard GC software (HPCHEM) was used for data collection, data display and integration purposes.

Preparation of standards for calibration

Calibration of the GC preconcentration system was accomplished by the use of multi-component gas mixtures (10–20 ppm, Scotty Can mix 2, 3, 4, 5, 6, 7, 8, 30, 54, 55 and 243) prepared by Scott Specialty Gases. Certain gas mixtures (*i.e.*, benzene, toluene, ethylbenzene, xylenes, isoprene and pinenes) were prepared in-house from pure samples. The NMHC samples were diluted with UHP helium to the level of 1 ppb by volume (ppbv). A hydrocarbon standard of nominal 10 ppbv concentration of 103 species in the C_2–C_{12} range was prepared gravimetrically (D. Wang). The diluted NMHC mixtures were employed as calibration gas mixtures for the preconcentration–GC system. A canister filled with UHP helium was used for blank tests on a daily basis.

RESULTS AND DISCUSSION

Two systems were evaluated and compared: (1) a megabore capillary column with a thick film of non-polar silicone; this column can be operated without removing the moisture from humid samples; and (2) a conventional methylphenylsilicone column and a Al_2O_3–KCl column PLOT; water was removed from ambient air samples before analysis with these columns.

Megabore column system

Urban air samples contain hydrocarbons at concentration levels that can be determined using sample volumes in the range 150–500 cm^3 at atmospheric pressure. It has been demonstrated in this study that a thick-film megabore column with a non-polar chemically bonded silicone stationary phase is suitable for hydrocarbon analysis of ambient air without removing the moisture from the sample. In order to prevent moisture from freezing on the column and obstructing the gas flow, the GC oven was operated above 0°C. Good separation of the volatile hydrocarbons (C_2–C_5 range) was obtained using the tandem column (SPB-1 + DB-5) and the column performance was not affected by problems caused by moisture.

Particular attention was given to the resolution of isoprene from the neighbouring components. The isoprene concentration in the Toronto urban area is not usually very high as biogenic sources are not in the immediate vicinity of the sampling sites and the lifetime of isoprene can be as short as a few h. The resolution of isoprene and *n*-pentane at the optimum carrier gas flow-rate increased from 1.27 on the SPB-1 column to 1.47 on the tandem column. Satisfactory resolution of several C_3–C_5 hydrocarbons was also obtained. The increase in resolution, however, also resulted in an increase in the overall retention times. By implementing a multi-ramp temperature programme, we achieved reasonable separations of individual species of atmospheric interest in the entire C_2–C_{10} range in addition to shortening the overall analysis time (60 min). The chromatograms of a standard calibration mixture and a real air sample are illustrated in Fig. 2.

The reproducibility tests of absolute retention times and area count measurements were performed using a commercial gas mixture (Scotty Mix 243) of C_1–C_7 *n*-alkanes with a mixing ratio of nominal 10 ppbv (*e.g.*, for *n*-butane, 100 μl injected represent 4.74 ng) with and without (direct injections into the cryofocuser) the first preconcentration device. Good reproducibility of absolute retention times of individual species (number of measurements $n = 3$) was observed regardless of whether the sample was passed through the first preconcentration stage or was injected directly on to the cryofocuser (Table I). The relative standard deviation (R.S.D.) was between 0.01 and 0.04%. The reproducibility of rela-

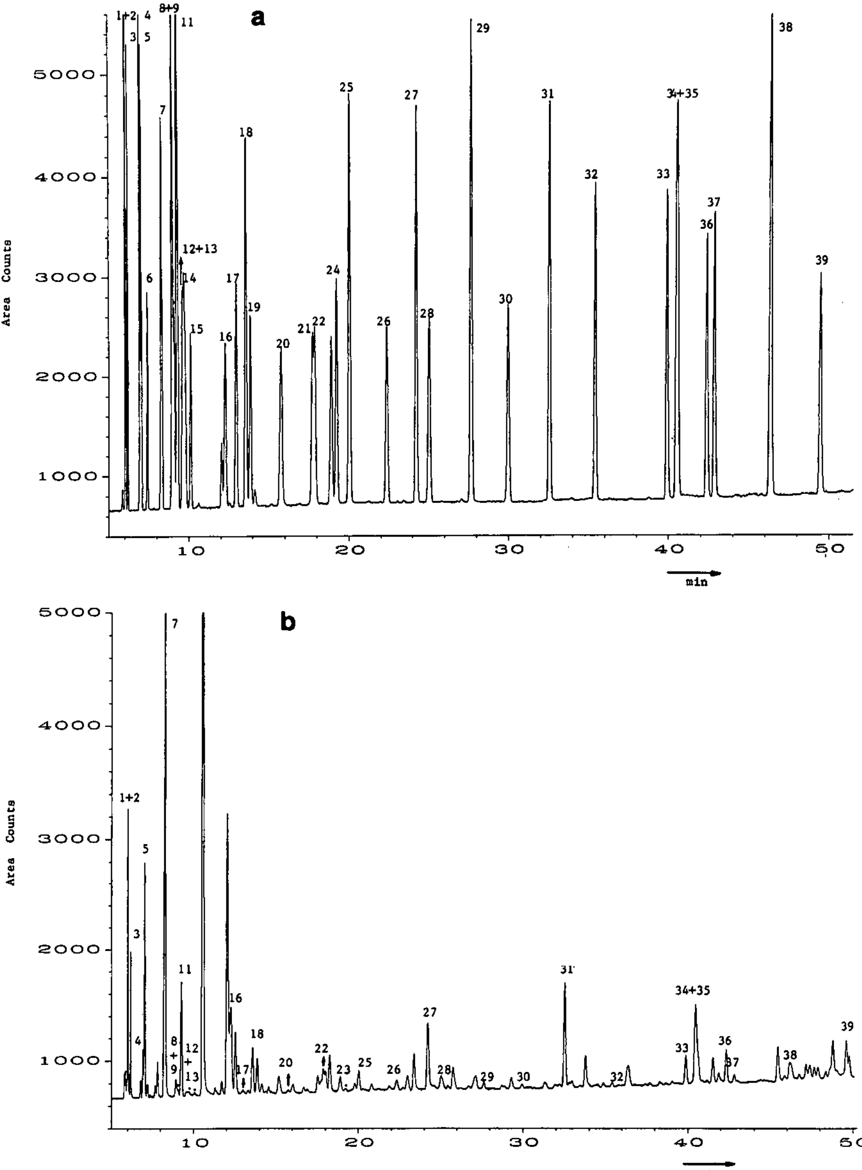

Fig. 2. (a) Separation of speciated hydrocarbons from a multi-component mixture using the tandem column (SPB-1 60 m × 0.53 mm I.D. with 5-μm chemically bonded non-polar dimethylsilicone plus DB-5, 30 m × 0.53 mm I.D. with 1.5-μm film of bonded phenylmethylsilicone) with temperture programming: initial temperature 30°C, increased at 3°C/min to 180°C, then at 25°C/min to 240°C, 15 min isothermal; carrier gas, He (linear velocity 26.65 cm/s); FID. Peak assignments: 1 = ethylene; 2 = acetylene; 3 = ethane; 4 = propene; 5 = propane; 6 = propyne; 7 = isobutane; 8 = 1-butene; 9 = isobutene; 10 = 1,3-butadiene; 11 = *n*-butane; 12 = *trans*-2-butene; 13 = butyne; 14 = 2,2-dimethylpropane; 15 = *cis*-2-butene; 16 = isopentane; 17 = 1-pentene; 18 = *n*-pentane; 19 = isoprene; 20 = 2,2-dimethylbutane; 21 = cyclopentane; 22 = 2-methylpentane; 23 = 3-methylpentane; 24 = 1-hexene; 25 = *n*-hexane, 26 = methylcyclopentane; 27 = benzene; 28 = cyclohexane; 29 = *n*-heptane; 30 = methylcyclohexane; 31 = toluene; 32 = *n*-octane; 33 = ethylbenzene; 34 = *m*-xylene; 35 = *p*-xylene, 36 = *o*-xylene; 37 = *n*-nonane; 38 = α-pinene; 39 = β-pinene. (b) Separation of speciated hydrocarbon pollutants in an urban air sample. Sampling site, Toronto, CN (Canadian National) Tower, 380 m level; collection date, September 10, 1991; sample volume, 466.7 cm^3; 90% humidity. Experimental conditions and peaks assignments as in (a).

TABLE I

COMPARISON OF RETENTION TIMES AND PEAK-AREA INTEGRATIONS OF SELECTED HYDROCARBONS (ppbv) LEVEL CALIBRATION MIXTURE) USING CRYOTRAPPING FOLLOWED BY CRYOFOCUSING AND CRYOFOCUSING ALONE

Compound	Direct syringe injection with cryofocusing[a]			Cryotrapping and cryofocusing[a]		
	Peak area		Absolute retention times:	Peak area		Absolute retention times:
	Mean	R.S.D. (%)	R.S.D. (%)	Mean	R.S.D. (%)	R.S.D. (%)
Ethane	4362	2.1	0.031	4281	1.4	0.044
Propane	6254	2.7	0.038	6167	1.3	0.044
n-Butane	7975	4.4	0.020	7832	1.4	0.005
n-Pentane	9413	7.3	0.021	9261	1.4	0.023
n-Hexane	10 391	10.2	0.015	10 265	1.6	0.016
n-Heptane	10 754	12.4	0.006	10 469	1.8	0.006

[a] $n = 3$.

tive retention times was checked over a period of 30 days and the results obtained are shown in Fig. 3. The reproducibility of area count measurements was considerably worse for syringe injections than with large-volume sample preconcentration. Typical R.S.D.s with sample preconcentration and direct syringe injection were 1.5% and 6.5%, respectively. The reproducibility of the system in analysing a multi-component standard mixture (ppbv concentration) is given in Table II.

The reproducibility of retention time measurements and determination of hydrocarbons concentrations in an urban sample is given in Table III. For ambient air samples, the R.S.D. for absolute retention time of individual species is better than 0.15% and for quantitative measurements the R.S.D. is less than 6% at low ppbv levels. With hundreds of sample runs as a database (the column was in continuous use for the analysis of air hydrocarbons over 1 year), this technique has proved to

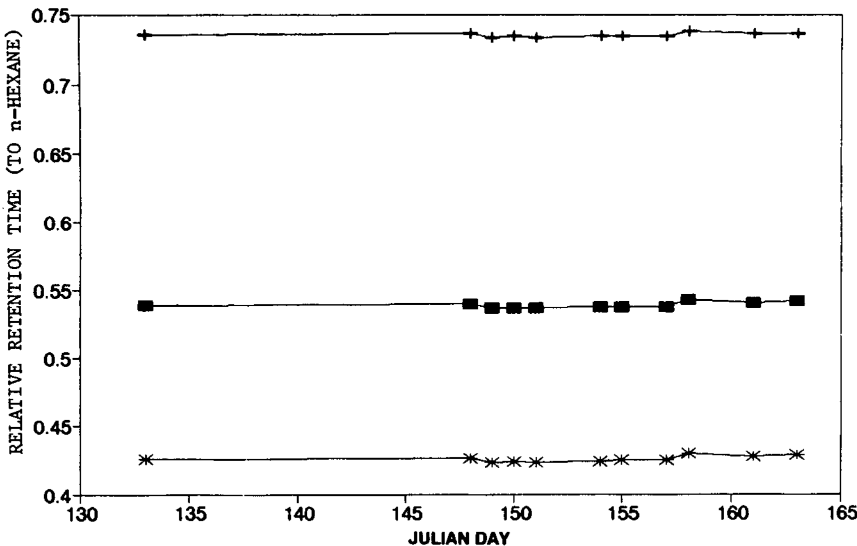

Fig. 3. Reproducibility of relative retention times of *n*-alkane standards (to *n*-hexane) measured over a period of 30 days after preconcentration and GC analysis using the tandem column (SPB-1 + DB-5). * = *n*-Propane; ■ = *n*-butane; + = *n*-pentane.

TABLE II

REPRODUCIBILITY OF CONCENTRATION DETERMINATION IN ppbv USING PRECONCENTRATION–GC AND VARYING VOLUME OF STANDARD IN THE RANGE 200–350 cm^3

Compound	Mean concentration (ppbv)[a]	R.S.D. (%)[a]
Alkanes		
Propane	10.27	2.62
n-Butane	20.14	0.85
n-Pentane	11.89	2.23
Isopentane	10.52	2.70
Cyclopentane	2.47	2.01
n-Hexane	13.41	0.37
Cyclohexane	1.95	0.36
Methylcyclopentane	3.55	2.58
2,2-Dimethylbutane	10.81	1.05
2-Methylpentane	13.95	0.51
3-Methylpentane	10.36	0.44
n-Heptane	12.88	0.13
Methylcyclohexane	1.60	0.44
n-Octane	3.00	0.77
n-Nonane	1.47	3.76
Alkyne and alkanes		
Acetylene	19.84	2.24
Propene	10.79	1.79
1-Butene	30.04	3.37
trans-2-Butene	12.99	1.74
cis-2-Butene	10.72	2.67
1,3-Butadiene	10.79	0.76
1-Pentene	11.06	1.49
1-Hexene	15.97	1.46
Aromatics		
Benzene	6.21	0.91
Toluene	5.25	0.94
o-Xylene	2.53	0.84
m,p-Xylene	4.87	1.60
Ethylbenzene	2.35	3.61
Natural		
Isoprene	3.18	1.33

[a] $n = 3$.

be reliable in producing qualitative and quantitative data for both calibration standards and real air samples.

Recently, a review on the performance of preconcentration units connected on-line to a GC system was published by Schaeffer [17]. He concluded that the unit constructed by Schmidbauer and Oehme [9] was the best system built up to 1989. The latter workers reported a typical R.S.D. of absolute retention time measurement to be about 1%, with a quantitative reproducibility of about 2% for hydrocarbon concentrations in the C_2–C_6 range with a mixing ratio of 1–6 ppbv. Although the system displayed excellent precision, one drawback was the sample handling. The system was constructed with three cryo-trapping devices and required 20 min for the sample to be transferred from the second trap to the cryogenic prefocusing device in addition to the time required to transfer the sample into the first part of the preconcentration unit. The sample transfer required a long time and a correspondingly high consumption of liquid nitrogen.

Calibration of the GC–preconcentration system was accomplished using three sources of standards. The literature reveals large discrepancies using different sources of calibration standards. Hov *et al.* [21] stated a difference of up to 51% for individual species between the calibration gases from two commercial suppliers. In our laboratory the calibration gas standards from different sources (Scott Specialty Gases, C&P Gas Standards and laboratory-made standards from neat liquids) were consistent for many species, with the largest discrepancy being 12%.

Good linearity of the FID response to sample amount was found for syringe injections of standard hydrocarbon mixtures with a mixing ratio in the ppmv range. A linear dependence of FID response on sample volume (100–650 cm^3) was also observed. Good reproducibility (sample volumes of 200 cm^3 and higher) was found for measurements of the mixing ratios of speciated NMHCs in ambient air in the pptv to ppbv range. The detection limit was *ca.* 5 ppbv for all species. The capacity to handle moisture from humid samples and the lack of a requirement for a moisture-removal process are major advantages of using a megabore column. For highly polluted air samples, the use of a dry-ice-cooled trap is still desirable for removing high-boiling components ($>$C10), so that peak co-elution of species from the previous run with the C_2–C_{10} hydrocarbon analysis can be prevented. Also the removal of the heavy components extends the lifetime of the column in addition to reducing column bleeding and reducing the total analysis time. The tandem column system was checked for possible losses of species in the C_2–C_{10} range with and without the dry-ice-cooled trap in operation. No losses were

TABLE III

REPRODUCIBILITY OF AREA COUNT AND RETENTION TIME MEASUREMENTS FOR ANALYSIS OF URBAN AIR SAMPLE USING PRECONCENTRATION–GC

Compound	Area counts[a]		Absolute retention time: R.S.D. (%)[a]	Concentration (ppbv)
	Mean	R.S.D. (%)		
Alkanes				
Propane	*81 328*	*4.65*	*0.09*	*13.104*
n-Butane	45 770	1.05	0.16	5.613
n-Pentane	26 693	1.61	0.07	2.871
i-Pentane	53 843	2.73	0.11	4.991
n-Hexane	34 459	1.90	0.01	3.461
Methylcyclopentane	12 737	3.42	0.01	1.052
2-Methylpentane	28 060	1.18	0.02	2.278
3-Methylpentane	15 418	3.39	0.02	1.272
n-Heptane	14 005	1.98	0.01	1.375
Methylcyclo-hexane	15 329	1.14	0.01	1.051
n-Octane	9148	4.57	0.01	0.647
Alkyne and alkenes				
Acetylene + ethylene	36 137	3.31	0.09	7.627
Propene	5245	4.15	0.09	0.878
1-Butene	3220	4.01	0.13	0.405
cis-2-Butene	930	3.79	0.15	0.123
1,3-Butadiene	876	4.88	0.12	0.126
1-Pentene	1731	2.66	0.07	0.133
Aromatics				
Benzene	12 788	3.51	0.03	0.984
Toluene	266 735	0.60	0.01	21.344
o-Xylene	16 879	3.41	0.01	1.202
m,p-Xylene	40 627	3.33	0.01	2.474
Ethylbenzene	14 410	2.14	0.01	0.909
Natural				
Isoprene	2385	1.53	0.06	0.278

[a] $n = 3$.

found for trap temperatures above −30°C.

Hydrocarbon data (obtained using the tandem column system) from an urban Toronto site are shown in Fig. 4a and b. The NMHC distributions observed at the CN Tower are shown in Fig. 4a for two different height levels (ground and 380 m) on a day when the wind was blowing across Lake Ontario from the south. The hydrocarbon concentrations are seen to be generally lower by a factor of >2 at the 380 m level, but are markedly reduced for highly reactive olefinic compounds such as 1-butene (a factor of 5.5), isobutene (8.0), 1-pentene (10) and 1,3-butadiene (12). These observations are indicative of long-range transport and the corresponding ageing of the air mass aloft. Fig. 4b shows the temporal distribution of hydrocarbon concentrations at an elevated level (380 m) for the CN Tower. More detailed results of hydrocarbon distributions in Toronto urban and suburban sites will be presented elsewhere [22].

Performance of narrow-bore DB-1 and Al_2O_3–KCl PLOT columns

Capillary columns with a non-polar stationary phase (depending on the column length, inside diameter, film thickness and operating conditions) resolve many of the hydrocarbons of atmospheric importance in the C_2–C_{10} range [14,16–18]. In our laboratory , a 30 m × 0.25 mm I.D. column with a 0.25-μm film thickness was used to analyse of ambi-

ent air samples for hydrocarbons. In order to obtain good resolution of low-boiling compounds (*e.g.* baseline separation of *n*-pentane and isoprene in ambient air samples), it was necessary to programme the GC oven to start from − 50°C. Even at this low initial temperature it was not possible to separate completely the C_2 hydrocarbons (*i.e.* C_2H_2, C_2H_4 and C_2H_6). Fig. 5 shows a chromatogram of a hydrocarbon standard that was obtained using this column. With this column, moisture had to be remove from humid air samples prior to GC analysis.

PLOT columns (with an Al_2O_3–KCl layer) are excellent at separating volatile hydrocarbons of atmospheric importance in the C_1–C_6 range [23]. Without operating the GC oven at sub-ambient temperatures, the resolution is often better than that achievable by a non-polar stationary phase column. Another advantage of PLOT columns is that they can be operated [under optimum conditions with regard to HETP (height equivalent to a theoretical plate) at high linear carrier gas velocities [24], resulting in shorter analysis times. Hence their employment for the analysis of ambient air samples is an obvious choice. As the column can be operated at temperatures above 0°C, clogging of the column due to moisture is not a problem. However, it has been reported that water can change the adsorption properties of the solid phase and cause unpredictable changes in the retention times [9] and, in some instances, irreparable damage to the column [17].

Moisture was removed from our systems by passing the air sample through a length of FEP Teflon tubing cooled with dry-ice. After the sample had passed through, the entire tube was heated with a resistive-wire wrapping to 80°C and back-flushed with UHP helium, so that the trapped water was removed. The FEP Teflon tubing was then cooled and cycled for the next sample. Losses of aromatic components (from an ambient air sample) due to the dry-ice water trap are displayed in Fig. 6. In the temperature range +70 to −42°C, no significant loss of the sample species was observed. Below −42°C, however, ethylbenzene and the xylenes

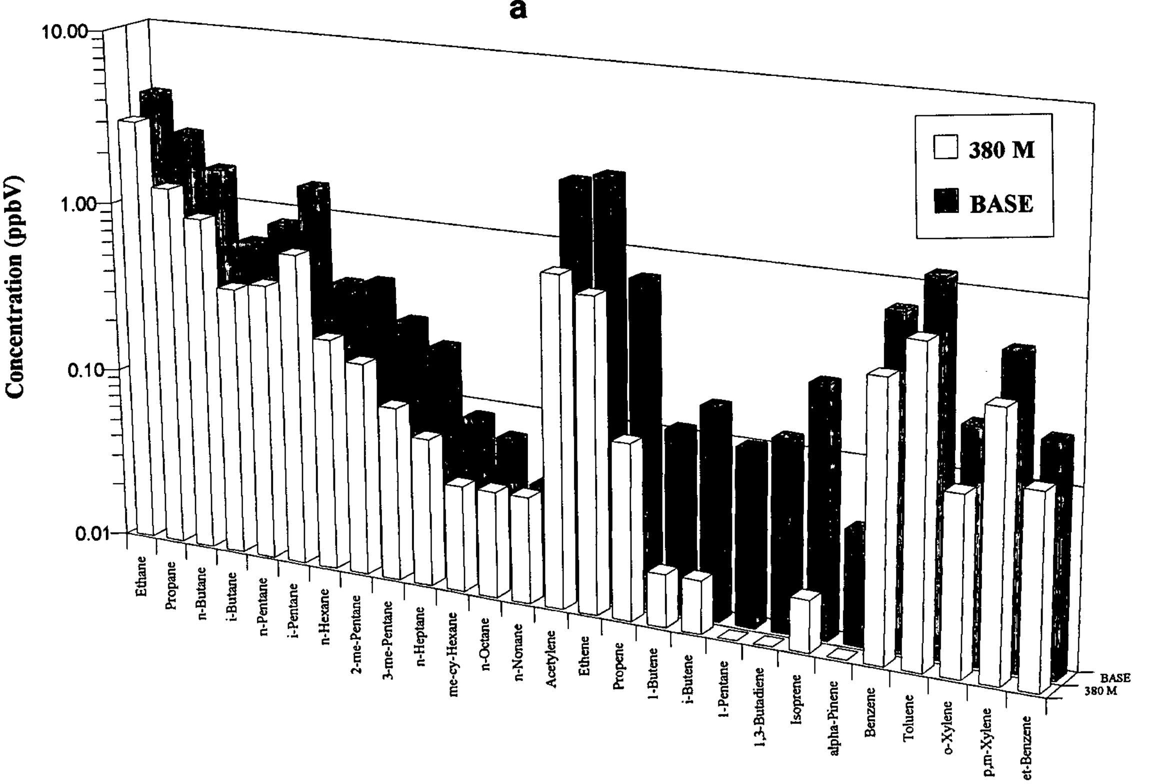

Fig. 4.

(*Continued on p. 86*)

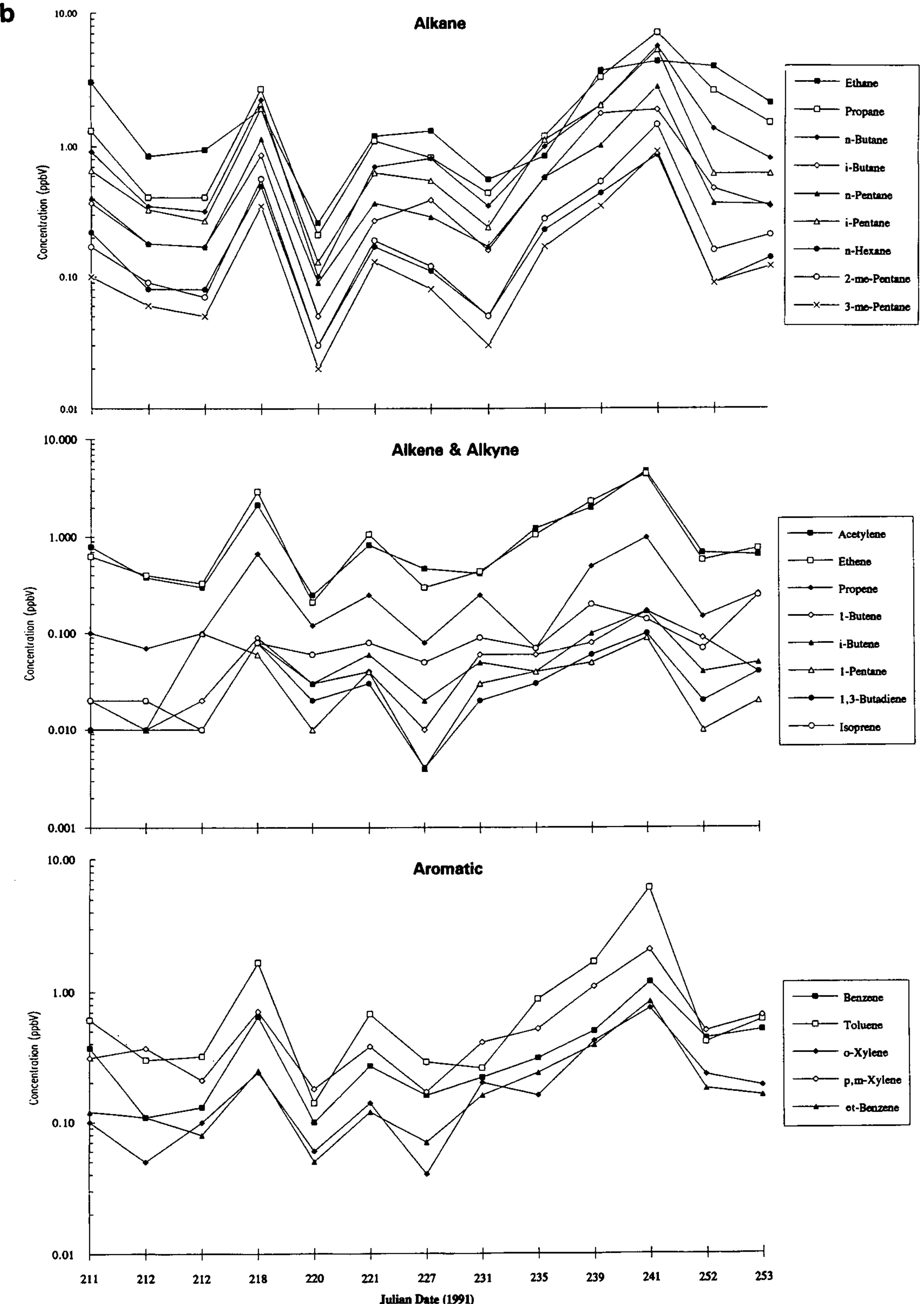

Fig. 4. (a) Hydrocarbon distribution at two height levels (ground and 380 m). Sampling site: Toronto, CN Tower, July 30, 1991. (b) Distribution of hydrocarbons (alkanes, alkenes, alkynes and aromatics) over several days (1991). Sampling site: Toronto, CN Tower, 380-m level.

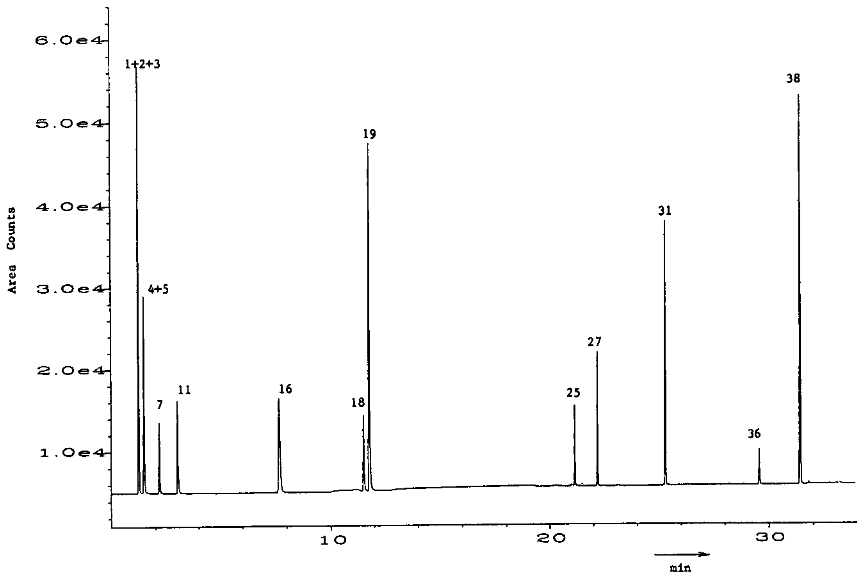

Fig. 5. Separation of hydrocarbon species of a calibration standard (using preconcentration–GC) on the DB-1 column (30 m × 0.25 mm I.D. 0.25-μm film thickness) with chemically bonded dimethylsilicone phase with temperature programming: initial temperature, −50°C (8.00 min), increased at 5°C/min to 115°C, then at 30°C/min to 250°C, 10 min isothermal; carrier gas, He; FID.

were observed to be partially trapped in the cooled length of Teflon tubing. Benzene and toluene passed through the water trap at −60°C without being affected. It is apparent from the graph that different components exhibit different losses with decreased temperatures. It appears that −30°C is an optimum temperature to use for removing moisture from air samples without significant losses of

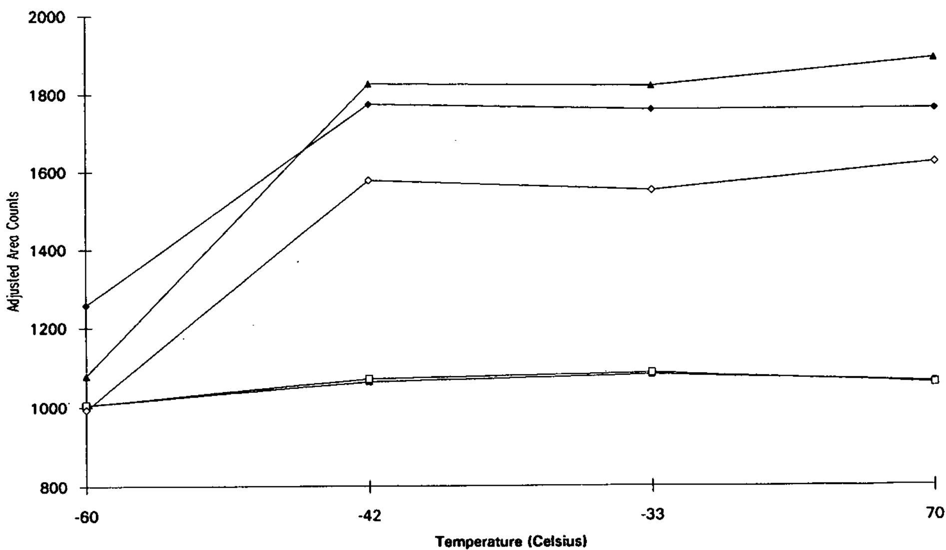

Fig. 6. Dependence of FID response (adjusted area counts) of aromatic hydrocarbons on the temperature of the dry-ice-cooled water trap. ■ = benzene; □ = toluene; ◆ = ethylbenzene; ◇ = *p,m*-xylene; ▲ = *o*-xylene.

analysed components. Ambient air samples with xylene concentrations ranging from 10 to 300 pptv were passed through the Teflon water trap at −30°C without significant loss. Linear behaviour was observed for the dependence of area count on sample volume in the range 100–650 cm^3 at 1 atm (1 atm = 101 325 Pa).

Both the literature and our experience indicate that CO_2 trapped from the preconcentration of ambient air samples is a potential interferent in the trace analysis of hydrocarbons [9,10,17,18,25,26]. In the GC analysis of urban air samples, the sample volume needed for preconcentration is small (usually <300 cm^3) because of the relatively high concentrations of hydrocarbon species. For this reason, the amount of CO_2 preconcentrated is sufficiently

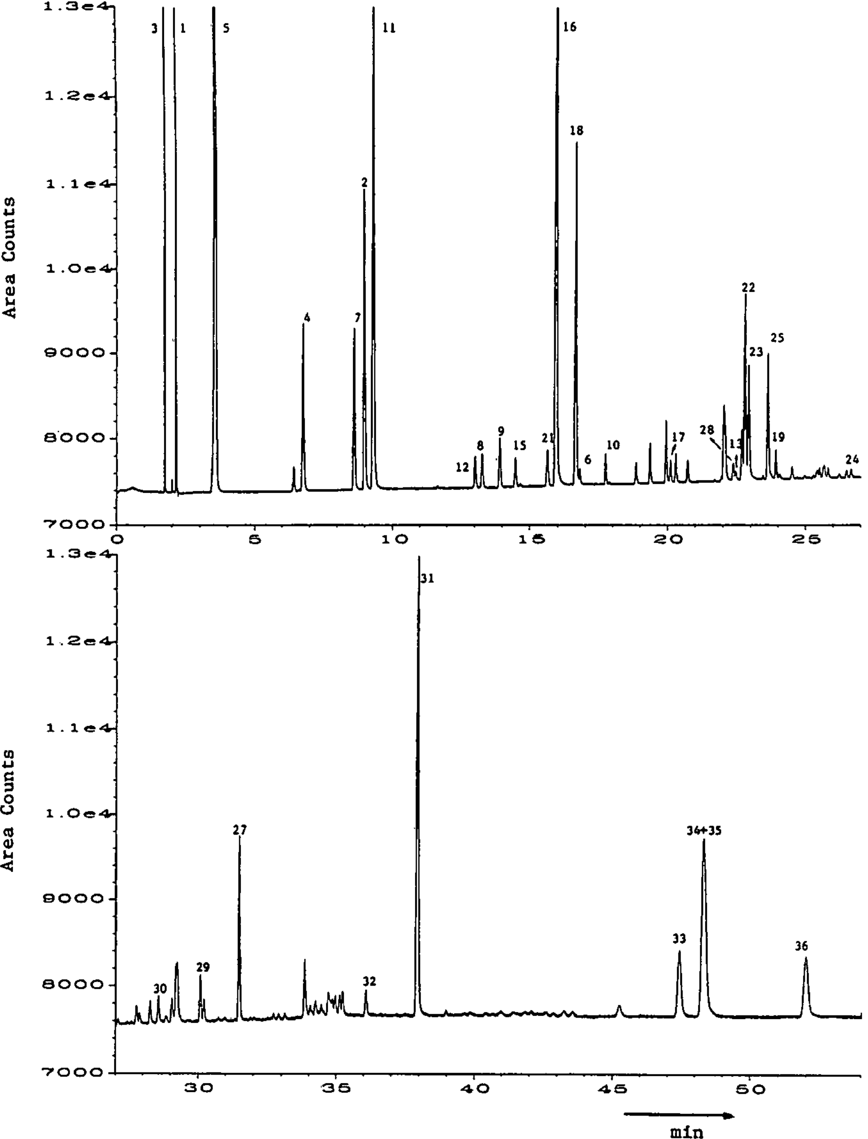

Fig. 7. Separation of urban air pollutants on Al_2O_3–KCl PLOT capillary column (50 m × 0.32 mm I.D. 5-μm film thickness) with temperature programming: initial temperature 35°C (2 min), then increased at 5°C/min to 200°C, 22 min isothermal; carrier gas, He (linear velocity 56.84 cm/s); sampling site, Toronto, Bay Street; sampling volume, 640 cm^3; Collection date: July 9, 1992. Peak assignments same as in Fig. 2a.

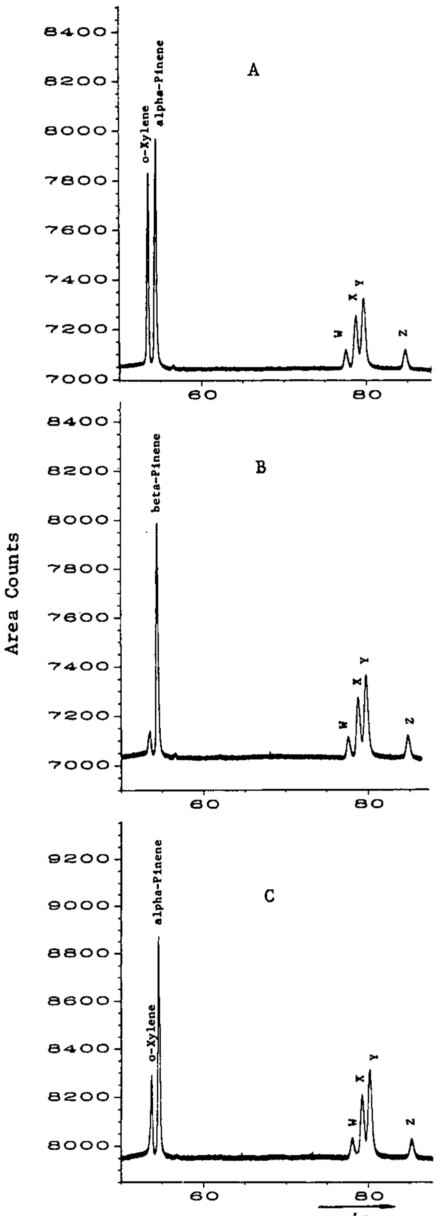

Fig. 8. Part of a chromatogram from hydrocarbons standard analysis on Al_2O_3–KCl PLOT column. Experimental conditions as in Fig. 7 (except final isothermal period, 52 min). (A) α-pinene + *o*-xylene; (B) β-pinene; (C) NCAR (National Centre for Atmospheric Research, USA) sample containing α-pinene plus *o*-xylene; W, X, Y, Z, = assumed degradation products.

small and we did not encounter problems associated with CO_2. A chromatogram of an air sample obtained with a PLOT column is shown in Fig. 7. Degradation of the pinene peak was evident with this column, as peaks corresponding to decomposition products were observed with α- and β-pinene samples (Fig. 8). Degradation of the pinene peak was not observed using the non-polar silicone column and hence this became the GC column of choice for terpene analyses.

The reproducibility of the PLOT column analysis (qualitative and quantitative) of calibration standards and urban air samples has been found to be excellent. One such a column has been in continuous use for the analysis of ambient air samples for hydrocarbons for over 5 months. The relative retention times of individual components were monitored for a period of 50 days and were found to be constant. The reproducibility of peak-area integration (repeated runs) for NMHCs in ambient air samples was better for the PLOT column than for the non-polar tandem column system. The R.S.D.s were usually $<4\%$ and $<6\%$, respectively. The lower R.S.D. with the PLOT column can be attributed to better resolution of the individual components, resulting in a smaller computer integration error of area counts.

CONCLUSIONS

The analysis of urban air samples were carried out using a in-house developed preconcentration system combined with a GC capillary column and FID. For most urban measurements of C_2–C_{10} hydrocarbons, 500-cm^3 samples provided an ample signal for analyses. Two GC columns were used for hydrocarbon separation. A megabore capillary column (DB-1) with a chemically bonded non-polar stationary phase of film thickness 5 μm was used successfully without removal of moisture from humid samples. For increased resolution of the low-molecular-mass hydrocarbons (C_2-C_5) the megabore column was connected with a 30-m DB-5 column to make a 90-m long tandem column system. A multi-step temperature programme was used to separate as many of the hydrocarbon components as possible in a reasonably short time (60 min) without sub-ambient oven temperatures. The PLOT column provided the better separation of low-molecular-mass hydrocarbons without the need to go to sub-ambient oven temperatures. The PLOT columns tested, however, are not suitable for α- and β-pine-

nes as these compounds were observed to degrade in the column. Because the column is sensitive to water, moisture had to be removed from the air sample using a FEP Teflon water trap cooled with dry-ice prior to GC analysis. Tests indicated that no observable amount of aromatics (up to the xylenes) was lost in the water trap when operated at -30°C.

The use of the preconcentration–GC system gave excellent reproducibility of both retention times and integration results for both calibration standards and ambient air samples. The use of the PLOT column gave better area count reproducibility than the tandem column. This can be attributed to the fact that the PLOT column was better at resolving individual components leading to a smaller computer integration error. Hundreds of urban air samples with concentrations ranging from 5 pptv to 100 ppbv have been analysed in the Toronto urban and suburban area using the two different columns over a period of 2 years.

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Review

Study of polychlorinated dibenzodioxins and furans from municipal waste incinerator emissions in the Netherlands: analytical methods and levels in the environment and human food chain

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ABSTRACT

An overview is given of the methods that have been used in the study of polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans in agriculture and the human food chain in a national survey and monitoring programme, including sampling stategies, sampling in the field and clean-up and analysis in various biological and environmental samples by high-resolution gas chromatography–high resolution mass spectrometry. The quality of data was evaluated as a result of internal quality control protocols and participation in interlaboratory comparison studies. Statistical analysis techniques and modelling were applied in order to compare and relate congener profiles in various matrices and to evaluate levels found in field studies for their use for regulatory purposes.

CONTENTS

1. Introduction . . . 92
2. Experimental . . . 92
  2.1. Sampling strategy . . . 92
    2.1.1. Stack gas . . . 93
    2.1.2. Cow's milk . . . 93
    2.1.3. Soil . . . 93
    2.1.4. Food stuffs . . . 93
  2.2. Analysis . . . 93
    2.2.1. Sample pretreatment and extraction . . . 93
    2.2.2. Clean-up . . . 94
    2.2.3. Gas chromatography–mass spectrometry (GC–MS) . . . 94
      2.2.3.1. Gas chromatography . . . 94
      2.2.3.2. Mass spectrometry . . . 94
      2.2.3.3. Quantification . . . 95

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3. Results and discussion . . . 95
3.1. Sample preparation . . . 95
3.2. GC–MS . . . 95
3.2.1. Gas chromatography . . . 95
3.2.2. Mass spectrometry . . . 96
3.2.3. Quality of data . . . 97
3.3. Results from field measurements . . . 98
3.3.1. PCDD/F emissions . . . 98
3.3.2. Regulatory analysis of farm animal samples . . . 101
3.3.2.1. Pattern comparison and modelling . . . 102
3.3.3. Environmental analysis . . . 104
3.3.4. Dietary intake of PCDDs and PCDFs . . . 104
4. Conclusions . . . 105
5. Acknowledgement . . . 105
References . . . 105

1. INTRODUCTION

Until recently, relatively little was known about the role that disposal waste combustion plays in the contamination of the animal and human food chain by polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs). In 1977, Olie *et al.* [1] identified for the first time these compounds in emissions from municipal incinerators. Soon after this initial report, the presence of PCDD/Fs was demonstrated in flue gas and fly ash of municipal solid waste incinerators (MWIs) in Europe, North America and Japan [2–6], followed by numerous studies on their formation mechanism [7,8] and the relevant parameters in the combustion process. More recent studies have attempted to quantify the emission rates in relation to amounts and composition of the incinerated waste [9]. It was only recently that airborne PCDD/Fs were identified in the food chain of farm animals. In 1987, Rappe *et al.* [10] reported increased levels in milk of cows grazing in the vicinity of incinerators. Two years later, Olie [11] reported elevated levels of PCDD/Fs in cows' milk in the vicinity of a large capacity municipal and hazardous waste incinerator facility in Netherlands. This finding led to a great public health concern about the possible significant contamination of animal feeds in several parts of the country and human foods such as milk, meat and vegetables. These preliminary findings were interpreted that local consumption of such products could easily increase the daily exposure of humans to PCDD/Fs above the tolerable daily intake (TDI). Consequently, PCDD/F levels in cows' milk for human consumption were restricted to a limit of 6 pg of 2,3,7,8-tetrachloro-CDD equivalents (TEQ) per gram of milk fat. This value was based on previous estimates in Germany of human exposure to PCDD/Fs by consumption of foods [12,13], the average milk consumption and a TDI of 240 pg of TEQ per person per day. Regulatory measures near incinerators included either the banning from the market of cows' milk containing PCDD/F levels above the 6-pg limit or the closure of the neighbouring source. In this framework, a national investigation and monitoring programme was initiated, aimed at the identification and quantification of sources, the environmental distribution, the pathway of airborne PCDD/Fs in the farm animal food chain and the exposure of the general Dutch population by consumption of food.

In this paper, attention will be primarily focused on the analytical aspects of PCDD/F in various matrices and the quality of analytical data. In addition, results from our studies on the environmental occurrence of dioxins are discussed.

2. EXPERIMENTAL

2.1. Sampling strategy

Representative sampling is of major importance for obtaining good analytical data in accordance with the objectives of the study or the processes under investigation. Analysis in this study included (1) measurements in emissions from incinerators (conducted by TNO, Delft and Apeldoorn, Netherlands), (2) monitoring and regulatory analysis of cows' milk, (3) study of levels in soil and (4) survey of food stuffs for PCDDs and PCDFs.

2.1.1. Stack gas

Sampling and analysis were carried out by TNO Institute of Environmental Sciences, Delft and Apeldoorn, Netherlands. Stack gas sampling was performed using a dilution-type sampler (Ströhlein) during three consecutive days at each facility. Representativeness of samples was checked by a comparison of other parameters of the combustion process such as the fed load and composition, CO, SO_2, NO_x, HCl, HF, dust concentration and E-filter temperature during the sampling period compared with normal levels at that particular facility.

2.1.2. Cows' milk

Several factors such as emission rates and weather conditions can affect levels in milk [14]. As these factors may vary substantially in field measurements, time compositional samples were obtained at individual dairy farms by mixing subsamples of 25 ml, collected at 2–3-day intervals, over a period of 1 month. These subsamples were taken from the milk containers just before these storage containers were emptied. Criteria for the selection of dairy farms included: (1) a significant part of their pastures should be located inside the expected deposition area (5 × 5 km north-east of the source); (2) their winter forage must be harvested in this region; and (3) supplementary feeding may not be excessive.

2.1.3. Soil

The purpose of soil analysis in this work was twofold: (1) determination of levels in the top layer in order to assess exposure of cows by ingestion of soil and (2) determination of the remaining previous accumulation of PCDD/Fs in soil due to former depositions as a measure for previous emissions. Criteria for the selection of fields were (1) their present and former use (pasture, agricultural), (2) applied mechanical treatment in the past (*e.g.*, tillage) and (3) application of sewage sludge or other products including fertilizers.

At each area, 40 samples were taken diagonally to the depth of interest (0–2, 2–5, 5–10, 10–50 and 50–100 cm below the surface), and cores were combined for each layer separately. Additional parameters determined in soil samples were: water content (at 40 and 105°C), glow losses (550°C) and the total organic carbon content.

2.1.4. Food stuffs

A wide range of lipid-rich food products were investigated in the national food survey [15]. The foods selected for investigation were fats and oils (from the food industry), cows' milk (bottled and cartoned), animal fat, butter and cheese, meat products, nuts, eggs and fish. This selection was based on the relative contribution of each category to the total fat intake by the Dutch population. Data were obtained from a database from the Dutch Food Consumption Study, performed in 1987–88. Samples of (refined) fats and oils were obtained from the food industry and included both vegetable and fish oils. Other samples were randomly collected from food stores and slaughter houses in four different regions in the Netherlands.

2.2. Analysis

Analysis of abiotic and biotic samples followed a similar procedure, including spiking of samples with a mixture of sixteen carbon-13 labelled analogues ($^{13}C_{12}$-labelled standards from Cambridge Isotope Laboratories, Woburn, MA, USA), followed by sample digestion (optional), sample extraction, clean-up and analysis by gas chromatography–mass spectrometry.

2.2.1. Sample pretreatment and extraction

Particulate samples such as fly ash, flue gas and soil were treated with concentrated hydrochloric acid (9%, v/v) to improve the permeability of the surface for extraction. Next, distilled water was added and the resulting solids were extracted for 20 h with toluene in a Soxhlet apparatus.

Fats and oils were dissolved in dichloromethane. Milk samples were mixed with methanol and sodium oxalate prior to extraction with diethyl ether and light petroleum (b.p. 40–60°C). Meat samples were pretreated with anhydrous sodium sulphate (1:10, w/w) and after homogenization refluxed for 16 h with dichloromethane. Adipose fat was heated in an oven and the resulting fat was dissolved in dichloromethane. Butter was first heated in an oven to remove water, dissolved in dichloromethane and dried over anhydrous sodium sulphate. Samples of cheese and nuts were extracted with hexane in a Waring blender and dried over sodium sulphate. Homogenized (boiled) egg and fish samples were

freeze-dried and refluxed for 16 h with dichloromethane.

2.2.2. Clean-up

Clean-up methods consisted of consecutive column chromatographic separations on active carbon (Carbosphere activated carbon, 80–100 mesh, surface area 1000 m^2/g, from Chrompack, Middelburg, Netherlands) and alumina (basic, activity super I, from ICN Biomedicals, Eschwege, Germany), initially developed for the determination of PCDDs and PCDFs in milk samples [16]. The method is a modified version of the procedure according to Smith *et al.* [17] and makes use of glass columns filled with active carbon, placed inside a conventional-type reflux unit. This combination allows the rapid and efficient purification of sample extracts with small amounts (30–40 ml) of either dichloromethane or toluene to separate the PCDD/Fs from residual fat and non-planar interferences and to recover the PCDD/Fs by back-elution with toluene. The resulting extracts were then transferred on to a basic alumina column for the separation of PCDD/Fs from residual amounts of other planar compounds. For soil samples, an additional clean-up on multi-layer silica was needed prior to the carbon step [18]. This multi-layer column contained silica impregnated with H_2SO_4, silica impregnated with NaOH and silica impregnated with $AgNO_3$. *n*-Hexane was used as the eluent. Precleaned extracts then followed the standard procedure.

2.2.3. Gas chromatography–mass spectrometry (GC–MS)

2.2.3.1. Gas chromatography. During the programme, several types of columns and conditions were used. In general, GC separations were carried out on an apolar fused-silica capillary column for analysis biotic samples and on a polar column for the analysis of abiotic samples (environmental, fly ash) and aquatic samples.

The usual gas chromatographic conditions were as follows. Non-apolar columns were 50–60 m × 0.25 mm I.D. with a 0.20-μm film thickness, either CP-Sil 5 (Chrompack), HP-Ultra 2 (Hewlett-Packard, Palo Alto, CA, USA), or DB-5 (J&W Scientific, Rancho Cordova, CA, USA). The temperature programme was initially 70°C for 2 min, increased at 25°C/min to 200°C, then at 3°C/min to 300°C and held isothermally for 10 min at 300°C. Polar columns were (A) 50 m × 0.25 mm I.D. with a 0.20-μm film thickness of CP-Sil 88 (Chrompack), the temperature programme being initially temperature 70°C for 2 min, increased at 25°C/min to 200°C, then at 3°C/min to 240°C and held isothermally for 40 min at 240°C and (B) 30 m × 0.25 mm I.D. with a 0.15-μm film thickness of Rtx-2330 (Restek, Bellefonte, PA, USA), the temperature programme being initially temperature 70°C for 2 min, increased at 25°C/min to 200°C, then at 3°C/min to 275°C, and held isothermally for 1 min at 275°C.

In all instances helium was used as the carrier gas at a linear velocity of 30 cm/s. Samples were injected either (initially) by the use of a solid all-glass falling needle injector (Koppen, Best, Netherlands) or using an autosampler (Hewlett-Packard HP 7673A). In the latter instance the injector glass liner (275°C) was filled with deactivated and precleaned glass-wool to prevent severe discrimination. Using polar columns, a piece of non-polar column was connected to the front and back-end of the column for the connection with the injector (275°C) and the mass spectrometer source. The GC–MS interface was maintained at 275°C in all instances.

2.2.3.2. Mass spectrometry. Analyses were carried out on VG 70SQ and VG AutoSpec mass spectrometers (Fisons Instruments, Manchester, UK). Ionization of samples was performed in the electron impact (EI) mode with 30–70-eV electrons. The instruments were operated at increased resolution. The resolving power (RP) was usually between (static) 3000 and 5000 for biotic samples and between 5000 and 10 000 for environmental samples.

Detection was performed by simultaneous recording of the two most abundant ions of the chlorine isotope cluster of molecular ions of analytes, the syringe standard ($[^{13}C_6]$-1,2,3,4-T_4CDD) and the $^{13}C_{12}$-labelled internal standards. The total number of approximately 50 ions, including lock mass ions, were divided over five (apolar column) and six groups, respectively, with 10–15 ions per group. During the analysis, consecutive groups were selected during a certain time interval that matched the congener elution profile. Each group contained a lock mass ion for fine setting of the magnet current in accordance with pre-performed mass callibration of the electrostatic field for each group.

Typical sampling and settling times were 50 and 10 ms and the interchange times between groups were 1 s, resulting in cycle times of less than 1 s.

2.2.3.3. Quantification. Quantification of the analytes was based on the response ratio of analytes and the corresponding internal standards obtained for the unknown sample compared with the ratio for a standard mixture containing known amounts of native PCDD/Fs and the same amount of $^{13}C_{12}$-labelled standards as used in samples. Congeners were identified and quantified when the following criteria were met: (1) signal to noise ratio >3; (2) the ratio between the two isotopic ions monitored should be within 15% of the theoretical value; (3) for native PCDD/Fs having an internal standard, the retention time should be within 1 s of that of the internal standard, the labelled compound eluting earlier; otherwise, the relative retention time must be within 0.1% as determined for standards, and (4) recovery of internal standards used may not exceed 120%.

Other perfomance checks that are regularly included (1) procedural (before a series of samples) and instrument blank (before and within series), (2) check of isomer specificity of GC separation, (3) sensitivity check of MS using the syringe standard and (4) check of MS resolution under dynamic conditions (RP 10 000 only).

3. RESULTS AND DISCUSSION

3.1. Sample preparation

So far, more than 1000 samples have been successfully processed with the Carbosphere methodology, previously described in detail [16]. Most samples were of animal origin (milk, adipose tissue), but when applied to abiotic samples similarly good results were obtained. Carbosphere combines a high efficiency for the separation of planar from non-planar compounds and possesses an extremely low or no affinity for lipids, which allows the processing of large amounts of extracted fat. Moreover, the use of conventional glassware and heating baths to reflux the carbon columns at elevated temperature allows the use of relatively small solvents volumes, which reduces unwanted side-effects of possible impurities in the solvent used. PCDD/Fs are quantitatively recovered from the carbon column by backrefluxing, which typically requires 16 h and 30–40 ml of toluene. In addition, the use of parallel units for each sample reduces the risk of cross contamination, which often occurs with single-loop equipped automated LC or gel permeation chromatographic systems.

3.2. GC–MS

3.2.1. Gas chromatography

High-resolution GC is required for the separation of the large number (210) of compounds of the PCDD/F family. The tetra- to octa-substituted congeners are the most interesting group and include 49 PCDDs and 85 PCDFs. Seventeen of them have the 2,3,7,8-substitution configuration (seven and ten PCDDs and PCDFs, respectively) and are considered to be the most toxic. Generally, analysis is concerned with congener group separation or isomer-specific determination of the toxic congeners, or both. For general survey of sources and environmental distribution, the analysis of total congener groups may be appropriate, whereas risk assessment and toxicological applications need congener-specific determination of the 2,3,7,8-substituted congeners. Total toxicity levels in samples expressed in 2,3,7,8-TCDD equivalents are obtained from the sum of indiviual congeners multiplied by their toxicity factors using the international model for toxicity of PCDD/Fs (i-TEF values) [19].

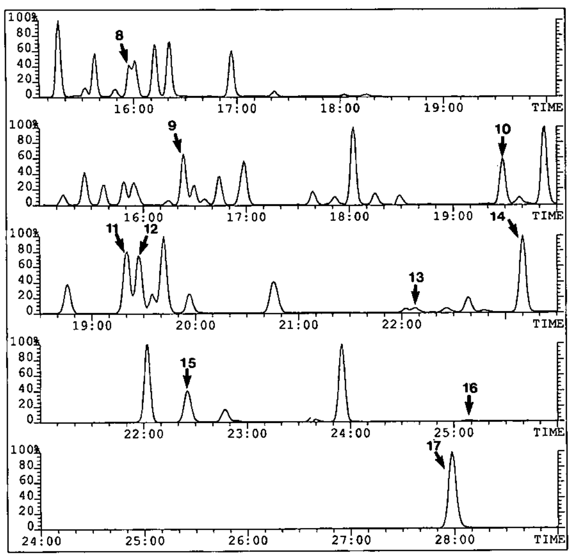
Non-polar columns (CP-Sil 5, DB-5, HP-Ultra 2 and equivalents) can separate chlorine homologous groups and all toxic congeners from each other but not from all non-toxic congeners. In contrast, polar columns (DB-DIOX, CP-Sil 88, SP 2331, Silar 10C and equivalents) can almost uniquely resolve the toxic congeners in the presence of all other congeners. Incomplete separation is achieved, however, for 2,3,7,8-T_4CDF, 1,2,3,7,8-P_5CDF and 1,2,3,4,7,8-H_6CDF. A not well understood phenomenon that occurs with the use of polar columns is the partial or complete dechlorination of octa-substituted congeners, particularly for OCDF. This occurred both after heated splitless injections and after cold on column injection. In contrast, these compounds travel through the column unaffected when injected with an all-glass solid injector (so-called falling needle injector). The phenomenon of dechlorination is difficult to understand. It can be rationalized that

the process must take takes place inside the stationary phase at the very beginning of the column owing to presence of hot vapours from the injection solvent or possibly by traces of moisture introduced during the injection. This is because dechlorination does not take place either with solvent injections on apolar stationary phases or with solid injections on-column with a non-polar stationary phase. Apolar columns are frequently used in analyses of biological samples from higher terrestrial vertebrates such as farm animals and man. These organisms normally have the ability to metabolize and subsequently excrete the non-2,3,7,8-substituted isomers effectively, which results in a selective accumululation of the planar, toxic isomers. Advantages of the use of apolar columns are that they require shorter analysis times, have longer lifetimes and no decomposition occurs for highly chlorinated PCDD/Fs. Polar columns are required for 2,3,7,8-isomer-specific analysis in samples that may contain non-laterally substituted isomers, such as in environmental samples, fly ash, ambient air and aquatic organisms, and in PCDD/F-contaminated products of different origins.

Recently, we obtained good results with the use of an Rtx 2330 cross-bound polar (90% biscyanopripyl–10% phenylcyanopropyl) stationary phase capillary column. Rapid analyses are facilitated by elevated temperature programming (up to 275°C) with satisfactory isomer-specificity. Fig. 1 shows an example of an isomer specific analysis of a stack gas sample. Concerning dechlorination, Fig. 1 shows that octa-CDD/F (peaks 7 and 17, respectively) travellled through this polar column unaffected, also when injected in the splitless mode, so as far as this problem is concerned there is no need for reanalysis on a second column.

3.2.2. Mass spectrometry

High-sensitivity EI sources developed recently for magnetic sector instruments permit improved sensitivity down to the low femtogram range on col-



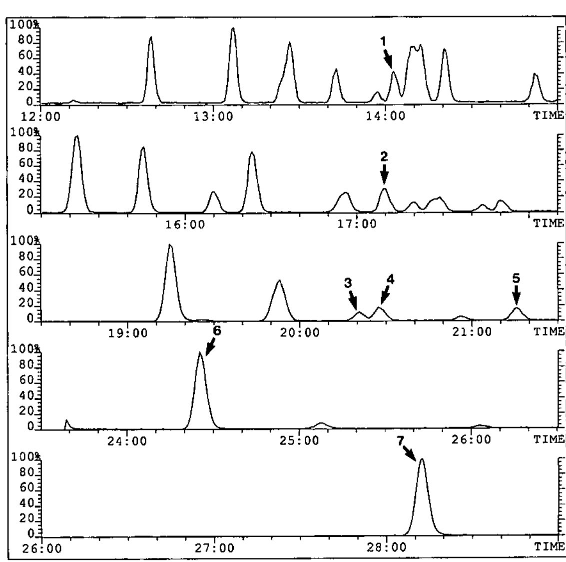


Fig. 1. GC–HRMS analysis of PCDDs (top) and PCDFs (bottom) in a municipal waste incinerator emission sample. Traces are multi-group selected-ion recordings, normalized in each group, of one ion of the molecular mass chlorine isotope cluster of each congener. GC separation was accomplished on a 30-m Rtx-2330 column. Monitoring time windows were selected so that all 2,3,7,8-substituted congeners were measured. Time windows do not match the entire congener group elution region. 1 = 2,3,7,8-T_4CDD; 2 = 1,2,3,7,8-P_5CDD; 3 = 1,2,3,4,7,8-H_6CDD; 4 = 1,2,3,6,7,8-H_6CDD; 5 = 1,2,3,7,8,9-H_6CDD; 6 = 1,2,3,4,6,7,8-H_7CDD; 7 = octa-CDD; 8 = 2,3,7,8-T_4CDF; 9 = 1,2,3,7,8-P_5CDF; 10 = 2,3,4,7,8-P_5CDF; 11 = 1,2,3,4,7,8-H_6CDF; 12 = 1,2,3,6,7,8-H_6CDF; 13 = 1,2,3,7,8,9-H_6CDF; 14 = 2,3,4,7,8,9-H_6CDF; 15 = 1,2,3,4,6,7,8-H_7CDF; 16 = 1,2,3,4,7,8,9-H_7CDF; 17 = octa-CDF. Analysis was performed at a mass resolution of 10 000:1. The calculated TCDD toxic equivalent level in the sample was *ca.* 0.5 ng TEQ/m^3 ind (in normal state dry) (sample size *ca.* 4 m^3 ind). Time in min.

umn with the mass spectrometer operated at increased resolution [high-resolution selected-ion monitoring (HRSIM)]. High-resolution MS (HRMS) [20] and tandem MS (MS–MS) techniques have been shown to be superior to low-resolution MS (LRMS) in trace analysis [21–23]. Although the sensitivity in HRMS and MS–MS are typically a few percent of that of LRMS analysis, the reduced noise levels compensate for this lower sensitivity and superior determination levels may be obtained. MS–MS analysis is considered to be particularly meaningful when using low resolution in the first mass spectrometer or in combination with reduced clean-up. The required resolution in single HRMS depends mainly on the nature and amounts of coextractants that have passed the clean-up procedure [22]. Moderate resolution in combination with effective clean-up methods will usually be appropriate for the analysis of relatively clean samples such as cows' milk. This is demonstrated in Fig. 2, which compares low (RP 900) and elevated resolution (RP 3000) analyses of TCDD at a level of 0.3 pg/g of milk fat in a cows' milk sample.

3.2.3. Quality of data

For generating good analytical data, samples and analysis should meet the following requirements: (1) representative sampling; (2) high sensitivity, selectivity and specificity; and (3) reliable quantification, good reproducibilty and identity confirmation [24]. Sampling strategies in the different studies in this work are described in brief in Section 2.1.

The recovery and accuracy of the method were tested in spiking experiments and in round-robin studies. Determination of compounds added to milk yielded recoveries between 92 and 112% for individual congeners, corresponding to 101% on TEQ basis. In a recent round robin study [25], unknown spikes were determined at *ca.* 80% of the values added, on average. The reliability of determi-

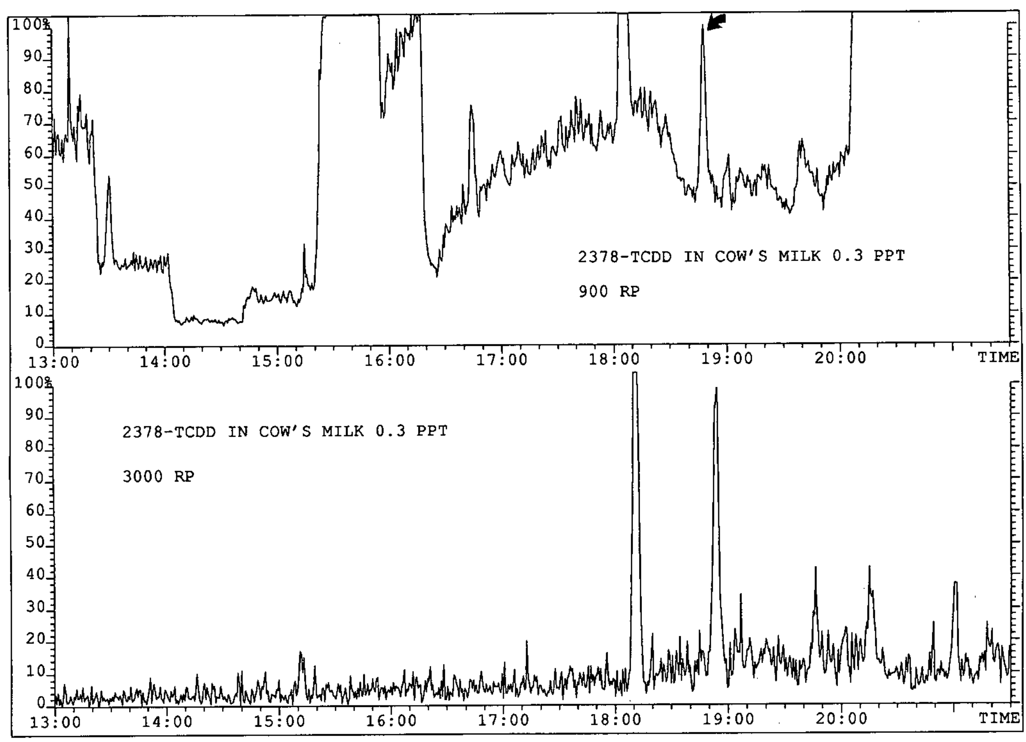


Fig. 2. Comparison of PCDD/F analyses in cows' milk at 900 (top) and 3000 (bottom) resolving power. Shown are the ion trace at *m/z* 319.8965 of 2,3,7,8-TCDD (arrow) with a level of *ca.* 0.3 pg/g milk fat, corresponding to an amount injected into the GC column of *ca.* 60 fg.

nation was improved by the use of sixteen carbon-13-labelled internal standards (one for each 2,3,7,8-substituted PCDD/F, except OCDF) instead of one in each congener group. By this method temporal sensitivity changes within such groups will be compensated for adequately.

Parallel analysis of control samples is used to verify the between-series reproducibility and the quality of quantitative results. Results of frequent analysis of such a quality control (QC) sample demonstrate a long-term reproducibility of about 7% [relative standard deviation (R.S.D.)] at a level of 3.0 pg TEQ/g milk fat (n = 35; not shown). Additional validation of the method and quality of information was obtained from participation in round-robin studies, such as that recently organised by the WHO/EURO for human milk and blood, cows' milk and fish, the Bureau Communautaire de Référence (BCR) in 1992 for cows' milk and by our laboratory also for cows' milk [26]. The last study showed that analytical results for laboratories having wide experience in PCDD/F analysis were comparable to within 10% on a TEQ basis at levels between 3 and 10 pg TEQ/g milk fat. Within-laboratory reproducibilities on a TEQ basis in this study ranged between 2 and 17%. Limits of determination for individual congeners in cows' milk were on average between 0.1 and 0.5 pg/g milk fat. The determination limit of the TEO value is dependent on the congener distribution in samples owing to the different TEF values. For example, extreme values for determination limits will correspond to 0.1 and 0.0001 pg TEQ/g milk fat when the only toxic congener present is 2,3,7,8-T_4CDD (TEF = 1) and OCDD (TEF = 0.001), respectively. For normal congener distributions TEQ determination limits in cows' milk were estimated to be *ca.* 0.6 pg TEQ/g milk fat [26]. Normal congener distrubutions in cows' milk in terms of congener TEQ values consist of three major congeners (average% contribution to total TEQ): 2,3,4,7,8-P_5CDF (40 ± 10%), 1,2,3,4,7,8-P_5CDD (25 ± 5%) and 2,3,7,8-T_4CDD (10 ± 5%). The contribution to the total TEQ value of five of the seven hexa-CDD/Fs ranges between 0 and 10% with a sum of about 25 ± 5%. 1,2,3,7,8,9-H_6CDD and 1,2,3,7,8,9-H_6CDF are mostly undetectable and hepta- and octa-DD/Fs are toxicologically minor congeners.

3.3. Results from field measurements

3.3.1. PCDD/F emissions

It has been estimated that the current solid municipal solid waste production amounts over 30 mil-

TABLE 1

SUMMARY OF EMISSION DATA AND CORRESPONDING LEVELS IN COWS' MILK AND SOIL IN THE VICINITY OF THE MAJOR MUNICIPAL WASTE INCINERATORS AND A METAL RECLAMATION PLANT IDENTIFIED IN THE NETHERLANDS

Figures concern the situation in 1989–90.

Source	Capacity (· 10^6 kg/year)	PCDD/F			
		Stack gas (ng TEQ/m^3 ind)	Estimated emission (g TEQ/year)	Cows' milk (range, pg TEQ/g fat)	Soil (0–2 cm) (ng TEQ/kg dry matter)
MWI-A	970	53	250	2.8–12.2	18–55
MWI-B	135	240	178	3.1–13.5	13–252
MWI-C	75	100	38	1.0–2.4	3–23[a]
MWI-D	115	31	21	1.6–8.1	NA[b]
MWI-E	75	4	2	3.3–10.0	NA
MWI-F	510	5	14	1.6–3.3	NA
Other MWIs	35–385	8.3–92	1.4–107	NA	NA
MRP	ND[b]	ND	ND	4.0–8.6	NA

[a] 0–5 cm.
[b] ND = Not determined; NA = not analysed.

TABLE 2

RESULTS OF INTERLABORATORY COMPARISON OF ANALYSIS OF PCDD/F COMPOUNDS IN MUNICIPAL INCINERATOR EMISSIONS

The relative composition denotes the mean relative contribution of individual congeners to the total TEQ level per m^3 ind in fly ash, calculated from results for all samples analysed by both laboratories. Crosses in the systematic difference columns indicate which of the congeners are included for a given uncetainity interval for TEQ values. The table also indicates which and the fraction of TEQ of the total TEQ. For example, the systematic difference between the two laboratories was less than 10% for six congeners, which account for 61.3% of the total TEQ, and so on.

Compound	Relative composition (% of TEQ)	Relative standard deviation (%)	Ratio of means, Lab. 1/Lab. 2	Systematic differences between Lab. 1 and Lab. 2 within given ranges (%) of the highest							
				10	20	30	40	50	60	70	80
Dioxins											
2,3,7,8-	2	34	1.09	×	×	×	×	×	×	×	×
1,2,3,7,8-	16.1	22	0.97	×	×	×	×	×	×	×	×
1,2,3,4,7,8-	4.7	40	1.70					×	×	×	×
1,2,3,6,7,8-	4.1	33	1.21		×	×	×	×	×	×	×
1,2,3,7,8,9-	3.5	24	0.99	×	×	×	×	×	×	×	×
1,2,3,4,6,7,8-	2.4	18	1.00	×	×	×	×	×	×	×	×
Octa-	0.3	64	0.85			×	×	×	×	×	×
Furans											
2,3,7,8-	1.3	64	2.79							×	×
1,2,3,7,8-	1.6	46	0.64				×	×	×	×	×
2,3,4,7,8-	29.4	12	0.97		×	×	×	×	×	×	×
1,2,3,4,7,8-	8.4	47	0.71			×	×	×	×	×	×
1,2,3,6,7,8-	7.9	34	1.02		×	×	×	×	×	×	×
1,2,3,7,8,9-	1.3	89	3.82								×
2,3,4,6,7,8-	12.9	45	0.74			×	×	×	×	×	×
1,2,3,4,6,7,8-	3.6	30	1.28			×	×	×	×	×	×
1,2,3,4,7,8,9-	0.3	59	0.82		×	×	×	×	×	×	×
Octa-	0	77	0.63				×	×	×	×	×
TEQ	100	15	0.99	×	×	×	×	×	×	×	×
Number of congeners within the range				6	8	12	14	15	15	16	17
Percentage of TEQ covered				61.3	65.8	91	92.6	97.4	97.4	98.7	100

lion tons ($30 \cdot 10^9$ kg) each year in the Netherlands [27], of which about $3 \cdot 10^9$ kg were combusted (1989) in twelve facilities. Most of these facilities stem from the 1970s and some of them did not comply with the Dutch guidelines (1985) for combustion. Recently, a few facilities have been upgraded for reduced emissions including PCDD/Fs. The capacity of the incinerators varies between $33 \cdot 10^6$ and $970 \cdot 10^6$ kg of waste per year (Table 1). PCDD/F stack gas concentrations ranged between 4 and 240 ng TEQ/m^3 ind (in normal state dry). Anual PCDD/F emissions by individual stationary sources ranged between 2 and 250 g TEQ/year, resulting in an estimated total of about 800 g TEQ/year. In general, the lowest emission rates were found with the modern incinerators. The high range value was found in an old-fashioned incinerator having an improperly functioning electrostatic dust filter (E-filter) at the time of sampling. Extrapolation of emission data to an annual basis must be treated with caution. It has been demonstrated that PCDD/F emissions may vary considerably and in an unpredictable manner [28]. At one facility emission rates differed by a factor of about 15 from one day to another.

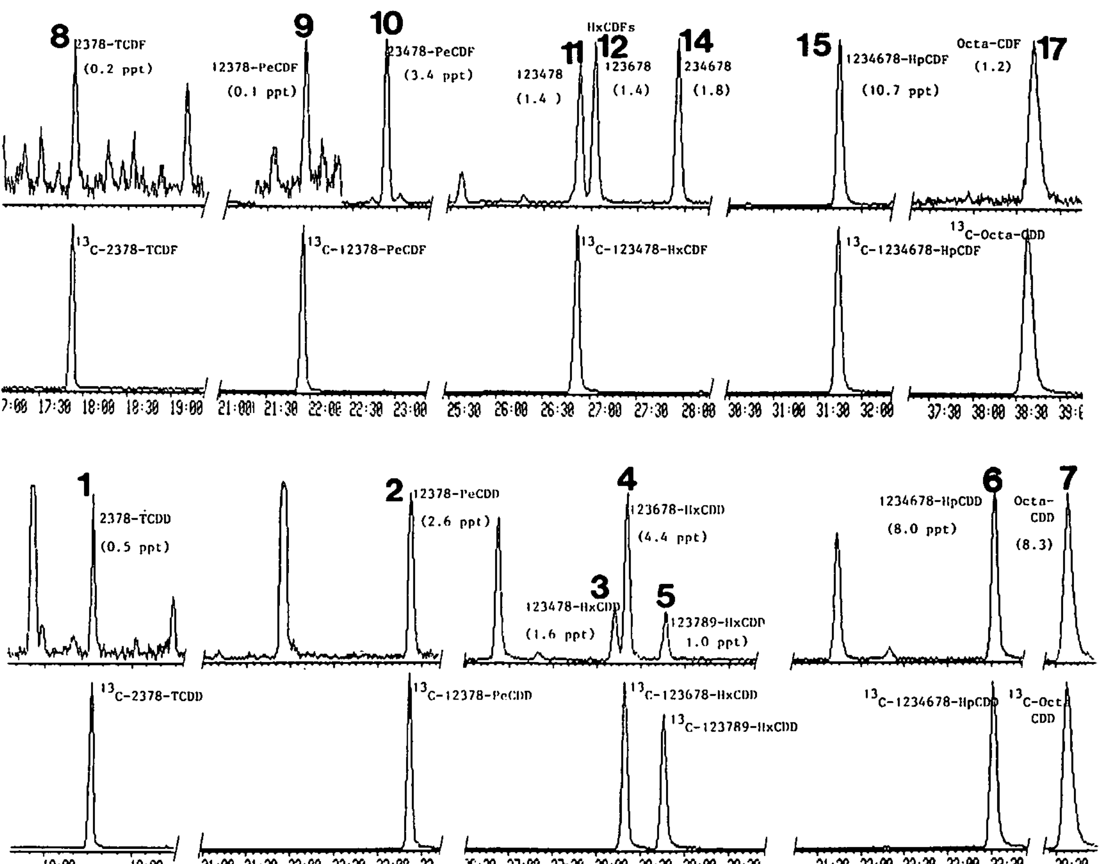


Fig. 3. Example of GC–MS analysis of 2,3,7,8-substituted PCDFs (top) and PCDDs (bottom) in cows' milk. Traces were obtained by multi-group (five) selected-ion recordings of the most abundant ions of the molecular chlorine cluster of native PCDD/Fs (top) and carbon-13-labelled internal standards (bottom). GC separation was carried out on a 50-m non-polar column (HP-Ultra 2). Peaks and levels (in parentheses in pg/g milk fat) of individual congeners were identified and detemined as follows: 1 = 2,3,7,8-T_4CDD (0.5); 2 = 1,2,3,7,8-P_5CDD (2.6); 3 = 1,2,3,4,7,8-H_6CDD (1.6); 4 = 1,2,3,6,7,8-H_6CDD (24.4); 5 = 1,2,3,7,8,9-H_6CDD (1.0); 6 = 1,2,3,4,6,7,8-H_7CDD (8.0); 7 = octa-CDD (8.3); 8 = 2,3,7,8-T_4CDF (0.2); 9 = 1,2,3,7,8-P_5CDF (0.1); 10 = 2,3,4,7,8-P_5CDF (3.4); 11 = 1,2,3,4,7,8-H_6CDF (1.4); 12 = 1,2,3,6,7,8-H_6CDF (1.4); 14 = 2,3,4,7,8,9-H_6CDF (1.8); 15 = 1,2,3,4,6,7,8-H_7CDF (10.7); 17 = octa-CDF (1.2).

In the course of the survey programme, an interlaboratory comparison was performed, involving clean-up and analysis of ten stack gas samples. The results are summarized in Table 2. It can be seen that systematic differences were within 20–30% for most of the individual congeners, resulting in an overall standard deviation of 15% on a TEQ basis. Large differences were found for 2,3,7,8-T_4CDF and 1,2,3,7,8,9-H_6CDF. For the latter, differences between the two laboratories could be ascribed to an insufficient separation between 1,2,3,7,8,9-H_6CDF and 1,2,3,4,6,7,8-H_7CDF by one laboratory, causing interferences with the molecular ion chlorine cluster of the hexa-congener (M) by a relatively low-abundant M-HCl fragment ion cluster of the otherwise prominent hepta-congener in stack gases.

3.3.2. Regulatory analysis of farm animal samples

The main objectives of the cows' milk survey were (1) to identify areas where increased levels in milk could occur and (2) to determine the area boundaries where levels exceed the limit of 6 pg TEQ/g milk fat. Such an investigation is challenged by the availability of representative samples. As an example, levels in milk may vary considerably depending on the actual and earlier exposure of animals to PCDD/F emissions in conjunction with (1) fluctuating emissions and associated deposition rates depending on meteorological conditions and half-lives of PCDD/Fs deposited on grass, (2) the pharmokinetics of PCDD/F in the lactating cow, (3) the movement of herds across the polluted area and (4) animal feeding and management systems [29–31].

During the course of the programme, several hundred biological samples were analysed for PCDD/Fs, including cows' milk and sheep milk, meat and other tissue samples from cows, sheep and horses. Fig. 3 shows a typical example of the determination of PCDDs and PCDFs in a cows' milk sample containing fifteen detectable (>0.1 pg/g fat) 2,3,7,8-substituted congeners. The TEQ level was determined as 4.9 ± 0.3 pg/g milk fat. In most milk samples, *i.e.*, in background and in MWI exposed cows, 2,3,4,7,8-P_5CDF, 1,2,3,7,8-P_5CDD and 2,3,7,8 T_4CDD were the most prominent compounds when expressed in toxicity terms. These isomers represent typically 40 ± 10, 25 ± 5 and 10 ± 5%, respectively, of the total TEQ level in normal milk. The chromatogram shows that a near-baseline separation on a non-polar column was obtained for all toxic congeners.

Results from analyses of cows' milk and soil are summarized in Table 1. In general, the levels in cows' milk correlate fairly well with estimated emission rates from the nearby source. Interesting data were obtained after the closure of a facility. The TEQ levels in cows' milk declined with a half-life of about 40–50 days, which is comparable to the half-

TABLE 3

COMPARISON PF PCDD/F DETERMINATION IN SOIL IN A ROW OF 0–8 km IN THE PREVALENT DIRECTION OF MUNICIPAL WASTE INCINERATOR EMISSIONS AND DEPOSITIONS WITH PREDICTED VALUES USING MODEL CALCULATIONS FOR PARTICLE DEPOSITIONS FROM A STATIONARY SOURCE

Levels expressed in ng TEQ/dm³ were measured in the top layer of pastures the surfaces of which have not been mechanically treated for several years.

Location	Distance to source (km)	PCDD/F (ng TEQ/dm³)		
		Measured	Predicted	Predicted/measured
1	0.9	27.1	20.9	0.77
2	2.4	22.1	13.7	0.62
3	2.7	16.8	12.1	0.72
4	3	15.1	11.0	0.73
5	4.6	9.2	7.7	0.84
6	5.7	6.7	6.9	1.03
7	8.1	3.2	4.8	1.51

life found from the study with lactating cows orally administered carbon-13 labelled PCDD/Fs. Near-steady-state levels after 6 months differed slightly for the various dairy farms, all being higher than the background level of 0.8–2.5 pg TEQ/g milk fat. Reasons for these differences may be the different initial values and probably also because of the variations in PCDD/F levels in soil (see below). Other interesting data were obtained from analyses of cheese from earlier years (Table 3). Although the cheese samples have an incident character (produced from milk of one or two days) and therefore are not necessarily representative, then analyses may be a valuable means for obtaining information on contaminations in earlier years.

Fig. 4 shows two year time courses of PCDD/F levels in milk from dairy farms in the deposition area of an MWI with high PCDD/F emissions (MWI-A in Table 1). It is seen that the levels fluctuate considerably. Apparently, the levels tend to increase in the autumn. The reason for this is difficult to give, but it might be that during this season the lower growth dilution rates [14] and higher wet deposition rates lead to higher concentrations on grass. In contrast, weathering processes such as increased leaf wash-off by increased wet precipitation would counteract such increased grass contamination.

3.3.2.1. Pattern comparison and modelling. Principal component analysis (PCA) [32,33] was found to be a very useful technique for the recognition of related samples from their PCDD/F patterns. The data set used contained several hundred cows' milk samples ranging from background locations and dairy farms in the neighbourhood of MWIs and a metal reclamation plant. Briefly, data treatment consisted of scaling to the most abundant congener in individual samples followed by the determination of the principal components of the entire set. In the present application, the first two principal components (PC-1 and PC-2) represented 43.7 and 17.0% of the total variance in the dataset, respectively. Fig. 5 shows the projection of the sample pattern on PC-1 and PC-2. Clustering of samples having similar patterns using 95% confidence intervals yielded a distinct separation between cows' milk samples from different locations. From this it may be con-

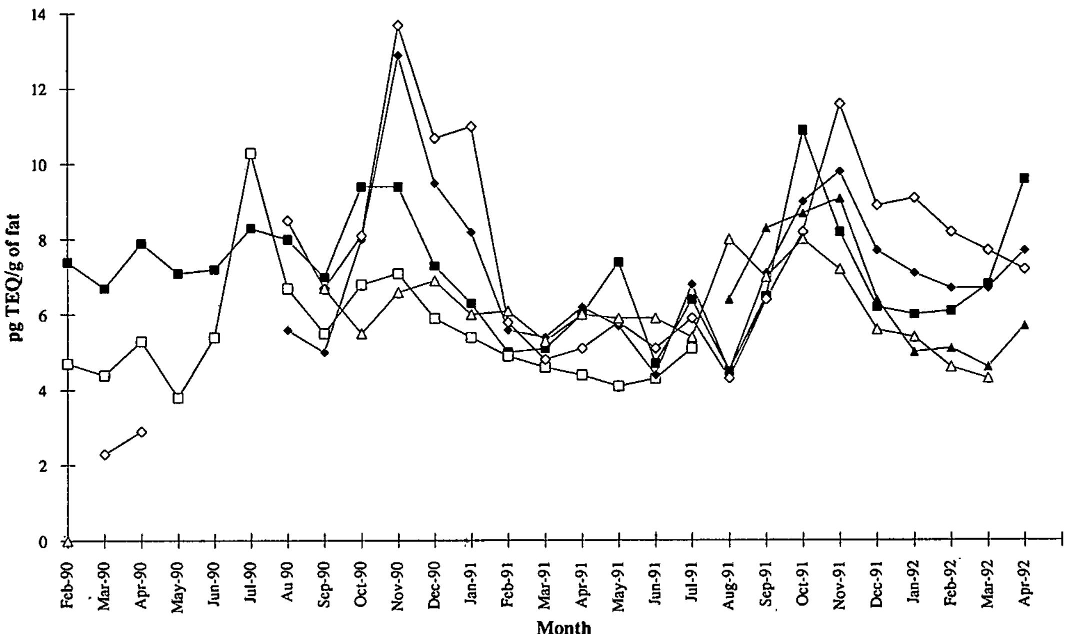


Fig. 4. Time courses for a period of 2 years for measured dioxin levels (in pg TEQ/g milk fat) in cows' milk from dairy farms in the vicinity of a municipal waste incinerator (MWI-A in Table 1).

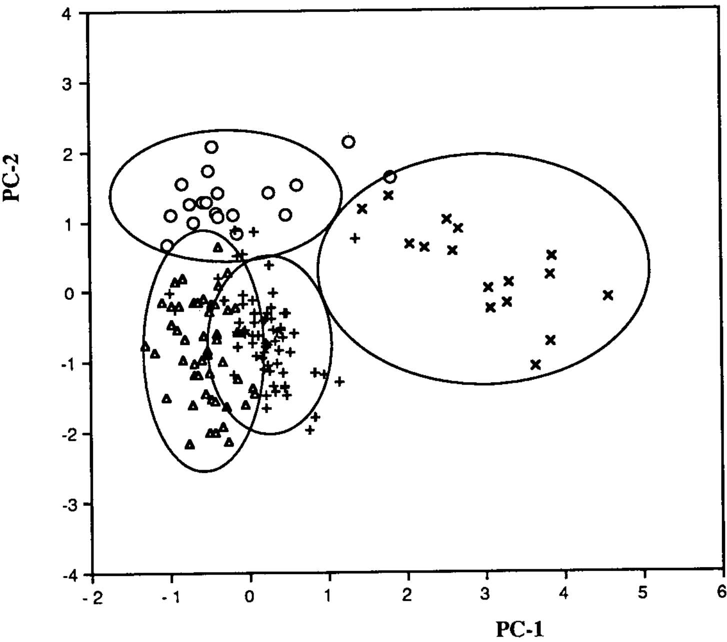


Fig. 5. Result of principal component analysis (PCA) of PCDD/F pattern in cows' milk in the vicinity of municipal waste incinerators (MWI) and a metal reclamation plant (MRP). Shown are the sample projections on the first and second principal components (PC-1 and PC-2). Ellipses are the 95% confidence contours for identified sample subgroupings. From Liem *et al.* [33]. + = MWI-A; △ = MWI-B; ○ = MWI-C; × = MRP.

cluded that the patterns are consistently related to the exposure pattern. A most striking example was the difference between cows exposed to MWI and metal reclamation emissions; the latter were found to contain relatively more dibenzofurans [34]. Analysis of the principle components showed that PC-1 was related to the relative fractions of PCDDs and PCDFs in samples and PC-2 to the chlorination level of both PCDDs and PCDFs, respectively.

With this large number of milk data we have developed a model to predict TEQ levels in milk near stationary sources [35]. The main parameters in the model are the source characteristics and emissions, weather conditions and the geographical location of pastures relative to the source.

The model uses the assumption of a constant background deposition level (A) and a variable influence by the source, the weather and other variables:

$$\mathrm{TEQ}(r,\phi)_{ij} = A + B_i \frac{f(\phi - \phi_j)r}{(r + 3R)^4}$$

where R = distance between maximum deposition and source, r = estimated distance between the (centre of) pasture(s) and the source, ϕ = direction of the centre of pastures of a dairy farm relative to the source and ϕ_j = direction of maximum deposition in period j. The variable f describes the average weather conditions in a period of 1 month, *i.e.*, the direction of deposition . The distribution of the dependent deposition is given by

$$f(\phi - \phi_j) = c + cos^2(\phi - \phi_j) \text{ when } \cos(\phi - \phi_j) > 0 \text{ and } f(\phi - \phi_j) = c \text{ when } \cos(\phi - \phi_j) < 0$$

The term B_i in the model represents the source parameters, such as the emission rate, stack height and other parameters for a particular source relative to the so-called standard source.

Parameters in the model were estimated by minimizing the sum of differences between the calculated and measured TEQ values in a training set (109 samples with TEQ levels between 0.7 and 13.5 pg TEQ/g milk fat). The following values were obtained:

Background TEQ level in milk:
A = 1.6 ± 0.3 pg/TEQ g milk fat
Distance of maximum deposition:
R = 1.6 ± 0.2 and 0.3 ± 0.1 km for MWIs and RMPs, respectively
Angular parameter:
c = 0.6 ± 0.2

These parameter values will probably be closely related to the local conditions and may not be valid for other countries with different weather profiles, landscapes, etc. Fig. 6 shows the predicted distribution of TEQ levels in milk in the vicinity of MWI-B (Table 1). The model was found to be useful for (i) the determination of area boundaries in which TEQ levels could exceed certain limit values and (ii) for the selection of sampling sites.

The model has also been used to estimate of the uncertainty interval for the TEQ value in milk samples. Variations depending on different variables in the contamination pathway were estimated to be of the order of 1.3 pg/g milk fat. This means that the representativeness of milk samples for a certain area may be approximated within this range. Combination with the analytical uncertainty of 10% (result of an intercomparison study) yields an overall uncertainty interval of *ca.* 1.5 pg TEQ/g at a level of 6 pg TEQ/g milk fat.

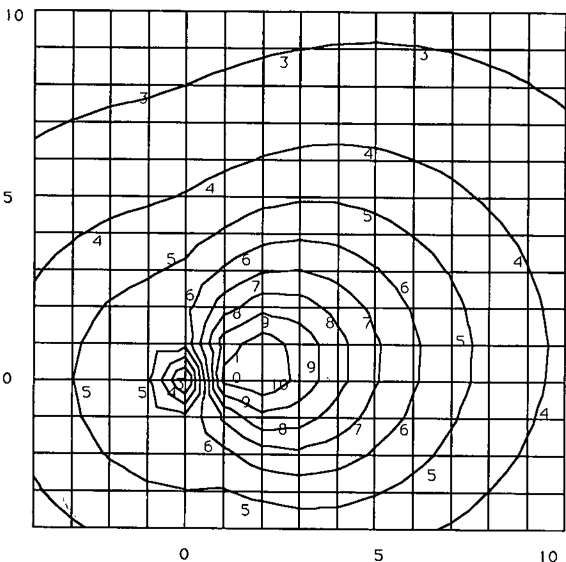


Fig. 6. Estimation of PCDD/F iso-concentration levels in cows' milk (in pg TEQ/g of milk fat) in the vicinity of MWI-B for october 1989 according to an emprical model (see text). Distances in km. The source is situated in the origin (0,0). Reprinted from Hoogerbrugge *et al.* [35].

3.3.3. Environmental analysis

Environmental analysis in this study was applied mainly to soil samples. The origin of PCDD/Fs in soil may vary greatly. They may originate from the former use of herbicides containing PCDD/Fs, spills of pentachlorophenols, airborne depositions and others. Soil analysis was primarily directed to determinations in top layer levels, as these PCDD/Fs may cause an additional exposure of cows by soil ingestion. Deposition-related steady-state levels in top soil layers will be dependent on the dilution caused by mixing to various depths and the persistence of PCDD/Fs in soil. Leaching and volatilization are not considered to be important factors affecting the movement and dissipation of PCDD/Fs in soil [36]. A second objective of soil analysis was to use the accumulated PCDD/F mass in soils as an indicative measure of former emissions. Data were used together with estimates of depositions and deposition profiles near sources in attempts to differentiate between the relative importance of wet and dry depositions near stationary sources [37].

Table 3 shows results of PCDD/F determinations in soil in a row between 0 and 8 km from an MSW and one compared with calculated values. The differences between the measured and predicted values may be due to lower background depositions than expected (assumption: 4 $ng/m^2 \cdot year$) and a possible underestimation of wet deposition rates in the model used [37]. The congener patterns in the top layers were consistent with that found in fly-ash. In the lower layers (< 10 cm) the hepta- and octa-PCDDs were the most prominent congeners, a pattern similar to that for pentachlorophenol.

3.3.4. Dietary intake of PCDDs and PCDFs

In order to assess the dietary intake of PCDDs and PCDFs by the Dutch population, 63 different food products were analysed [15]. A detailed description of the study design, including sampling strategy, analytical methods, levels and the statistical model used to calculate dietary intakes, will be published elsewhere. The results are summarised in Table 4, expressing the relative contributions of dif-

TABLE 4

RELATIVE CONTRIBUTION OF PCDD/F IN FOODS TO THE AVERAGE DAILY INTAKE OF TOXIC EQUIVALENTS (TEQ) IN THE NETHERLANDS

Category	Food	Contribution to daily TEQ intake (%)
Dairy foods	Milk	23.3
	Cheese	14.5
	Butter	7.1
Meat	Beef	7.1
	Horse and lamb	0.5
	Pork	5.8
Poultry	Eggs	4.5
	Meat	2.0
Micellaneous	Sliced cold meat	9.0
	Oils and nuts	1.1
	Industrial fats	21.8
Fish	Fish	3.4

ferent food categories to the total dietary intake of PCDDs and PCDFs by the average Dutch person during a lifetime of 70 years. It can be seen that the consumption of milk, butter, cheese and associated bovine fats accounts for almost half of the total exposure by foods. Another 20% of the total dietary intake appeared to be associated with the consumption of fats in food products from the food industry (so called hidden fat). A median daily intake by food was calculated on 35–70 pg TEQ for children below 20 years and on 70 pg TEQ for adults (20–70 years). These intake figures compare well with previous reports from the UK [38], Germany [12,13] and Canada [39], in which intakes ranged between 92 and 203 pg TEQ per person per day. This may again illustrate the diffuse global distribution of persistent compounds such as PCDDs and PCDFs responsible for general contamination of the human food chain.

4. CONCLUSIONS

Chromatographic separation and mass spectrometric detection techniques currently used in analyses for PCDD/Fs are capable of the determination of ultra-trace levels in different environmental and biological samples. Difficulties still exist with the unique determination of some 2,3,7,8-substituted congeners in complex samples, which may lead to overestimation to some extent of reported TEQ values in risk assesment studies, particularly for environmental and fly-ash samples. Problems with the determination of OCDF and OCDD on polar stationary phases due to dechlorination were solved by the use of a new type slightly modified polar stationary phase, which avoids the need for costly and laborious re-analysis of environmental samples on a non-polar column. Mass spectrometric resolutions of 3000–5000 were found to be adequate for analyses of most biological samples. For environmental samples slightly higher resolutions of 5000–10 000 were sometimes found to be beneficial, depending on the nature and concentration of co-extractants.

Extensive studies of emissions of municipal and some other combustion processes have yielded a much better insight into the behaviour and distribution in the environment of PCDD/Fs and the subsequent contamination of farm animals and the human food chain.

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Detection of airborne cocaine and heroin by high-throughput liquid-absorption preconcentration and liquid chromatography–electrochemical detection

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ABSTRACT

A high-throughput liquid-absorption preconcentrator (HTLAP) for rapid and/or ultrasensitive detection and analysis of trace contaminants samples air at a rate of 600–700 l/min and collects analytes from vapors or aerosols at an efficiency of 40–60% into a small volume of liquid absorbent dripping at a rate of 0.1–2 ml/min. These features combine to reduce the lower detection limit (LDL) of available analytical instrumentation by a factor of > 1000 and/or to permit faster sampling and far more rapid on-site air monitoring than were previously practicable. LDLs of *ca.* $1{:}10^{13}$ (v/v) of alkaloids have been achieved with LC and electrochemical detection. The HTLAP is directly adaptable to most liquid-phase analyzers. The small rate of liquid collection is also compatible with available interfaces to mass spectrometers. Moreover, the HTLAP permits detection and quantitation of polar or highly reactive compounds that cannot be readily analyzed by conventional preconcentration and GC.

INTRODUCTION

The purpose of this work was to develop and evaluate a high-throughput liquid-absorption preconcentrator (HTLAP) that could facilitate detection of very low concentrations of trace air contaminants, such as those emanating from concealed low-vapor-pressure drugs or explosives. The focus was on the preconcentration of airborne cocaine and heroin, whose equilibrium vapor pressures at 20°C are about $1.0 \cdot 10^{-10}$ bar and $4 \cdot 10^{-13}$ bar [1], corresponding to 100 ppt (v/v) (pptv) and 0.4 pptv, respectively. The availability of an effective HTLAP might facilitate detection of drug contraband, especially in enclosed cargoes, or of concealed explosives, especially in an aircraft.

Various liquid-absorption-type air scrubbers are available [2–4] that preconcentrate air contaminants and provide an interface between air samples and liquid-phase analyzers, such as liquid chromatographs or colorimeters. However, few of these devices are designed for a throughput (*i.e.*, air-sampling rate) of > 1 l/min.

While this preconcentrator was being developed and challenged with cocaine and heroin vapors, a somewhat different HTLAP, developed independently for vapors of explosives, was being challenged by 0.1–0.5 pptv of trinitrotoluene [5]. This device uses much larger liquid volumes and achieves

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several times lower collection efficiencies than those reported here. Also being developed are dry high-throughput preconcentrators in which the analyte from a large volume of air is first adsorbed onto wall surfaces, then thermally desorbed into a small volume of carrier gas. Such preconcentrators may operate fast enough in conjunction with a mass spectrometer or ion mobility spectrometer to permit rapid screening for explosives that may be concealed on persons or in baggage. However, utmost sensitivity would be assured by operating an ultra-high-throughput preconcentrator for a longer time, *e.g.*, for 10 min rather than 6 s so as to extract 100 times more analyte at a given sampling rate, *e.g.*, while an aircraft is preparing for takeoff. In such applications, the adsorption sites at the surfaces of the dry high-throughput preconcentrators might get saturated within the first min, especially if interfering adsorbates are present in relatively high concentrations.

Although this study was restricted to the detection of cocaine and heroin, the results may be applicable to the detection and monitoring of other compounds, many of which are of environmental concern.

EXPERIMENTAL

Vapor generation

Measuring the collection efficiency for vapors of semivolatile compounds required an efficient and reliable vapor generator that could permit easy replenishment of test material between experiments and preparation of known concentrations of air-vapor mixtures (Fig. 1). A Neslab thermostat bath was set to the desired vapor pressure of the test material in accordance with a phase diagram that was derived from published data [1]. A stream of temperature-equilibrated carrier gas (nitrogen), flowing at an adjustable rate, served to control the rate of sublimation of solid test material in a disposable sample tube (4 cm long × 4 mm I.D.) containing a plug of silanized glass wool, and thereby yielded the desired concentrations of the tested vapors. Before

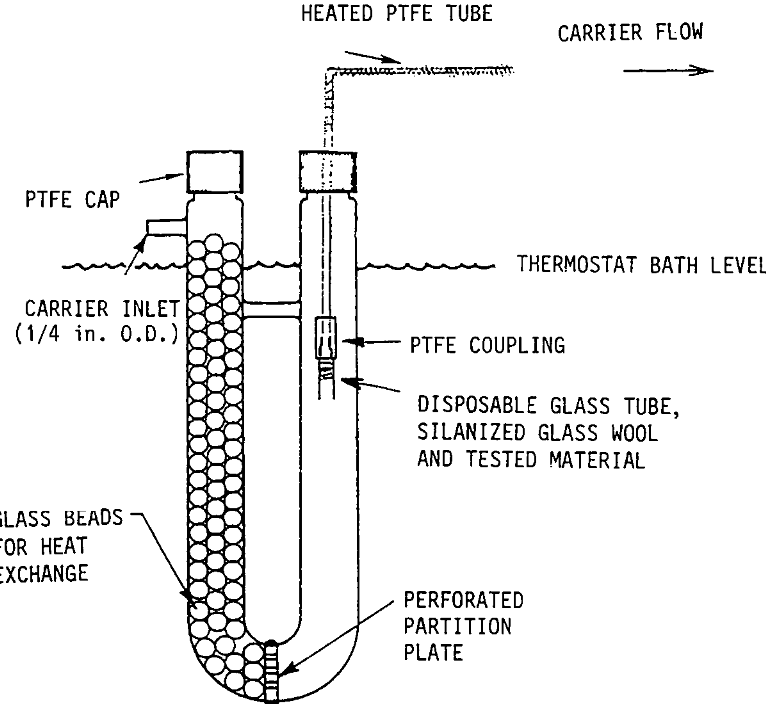

Fig. 1. Cross-sectional view of the U-tube contents of the vapor-generation apparatus. The carrier gas (nitrogen) passes at a rate of 0.5 l/min through a disposable glass tube containing a silanized glass wool plug impregnated with 0.1–1 mg of cocaine or heroin. 1 in. = 2.54 cm.

insertion into the U-tube, the glass wool was impregnated with a dichloromethane solution of 0.1–1.0 mg of the tested substance. The dried sample tube was then inserted into a PTFE coupling which connected the tube to an external 6 mm O.D. 4 mm I.D. PTFE perfluoroalkoxyl tubing (Catalog No. 6375-02, Cole Palmer Instrument Co., Chicago, IL, USA) that delivered the vapor-containing carrier to the preconcentrator inlet. To prevent analyte adsorption onto surfaces of the external tubing, the latter was uniformly wrapped with nichrome wire whose ends were plugged into a Variac transformer. The Variac was adjusted so as to yield a steady-state thermocouple reading on the inside of the tube that was at least 20°C above the temperature of the bath. Furthermore, the flow-rate of the carrier gas (0.5 l/min) was found to be 2–3 times higher than that yielding nearly saturated vapor, which further minimized any tendency for the analyte to condense or be adsorbed at the heated walls.

Analytical method

The analyses were performed by liquid chromatography (LC) with electrochemical detection (ED) [6]. The following general conditions applied to all the analyses: mobile phase, potassium phosphate buffer (pH 7–7.4, 0.02 *M* total phosphate)–acetonitrile (40:60, v/v); flow-rate, 1.0 ml/min, isocratic; solvent-delivery system, Waters (Milford, MA, USA) Model 6000 pump and Rheodyne Adsorbosphere HS C_{18} 25 cm × 4.6 mm cartridge (Alltech, Deerfield, IL, USA); electrochemical cell, Bioanalytical Systems (West Lafayette, IN, USA) LC/4B amperometric detector with a glassy carbon working electrode set at a potential of 1.0 V *versus* a Ag/AgCl reference electrode.

Collection efficiency measurements

To verify the vapor generation rate, the U-tube effluent was fed into a solid sorbent tube [Supelpak 204 (particle size, 0.06–0.12 cm), ORBO-40, Supelco, Bellefonte, PA, USA] for 30–60 min. The analyte was eluted from the sorbent tube with acetonitrile and quantitated by LC–ED. Independent tests confirmed that the sorbent tubes collected cocaine and heroin vapors at nearly 100% efficiency.

Analyses of sorbent tubes before and after each test were used to calculate the upper, lower, and average values of the HTLAP's collection efficiency. The absorbates collected from the HTLAP were usually pretreated to eliminate or minimize interferences picked up from the sampled air and to gather the collected analyte into a smaller (0.5-ml) volume of liquid, ready for injection into the LC–ED system. The collected absorbate was first passed through a Sep-Pak C_{18} cartridge (Cat. No. 23501, Waters). The analyte was extracted from the cartridge with 3 ml of diethyl ether and, after evaporation of the ether, was redissolved in 0.5 ml of acetonitrile. In each case, chromatographic peak areas for collected samples were compared with those obtained in the same manner with tested solutions of known concentrations.

Preconcentrator materials and dimensions. An earlier preconcentrator design [7] was modified as shown in Fig. 2. A Pyrex glass tube, 56 cm long, 60 mm I.D., had an elliptical air inlet hole centered at 10 cm from its lower edge, a liquid distributor wheel located 6 cm below its upper edge, and a polyvinyl chloride plug with a 2.5 cm deep funnel-shaped cavity at the lower edge. A 6-mm-diameter hole at the center of the cavity abutted an external nipple that could be either closed off, to permit accumulation of liquid in the cavity during a sampling test, or left open, to permit collection of the liquid in different vials at selected time intervals.

Other components and accessories. The liquid absorbent was supplied by an adjustable metering pump (Model RPG-20, Fluid Metering, Oyster Bay, NY, USA). Adequate air suction was provided by a ring compressor (Model VFC301P-5T, Fuji Electric Corp. of America, New York, NY, USA). The air-sampling rate was measured by a rotameter (Model K72-10-0111, King Instrument Co., Huntington Beach, CA, USA) that was connected to the outlet of the ring compressor.

Liquid absorbent. The liquid absorbent was an aqueous solution of 0.1 g/l of Triton X-100 (Catalog No. 3555-4*NY, Baxter Healthcare, McGaw Park, IL, USA) either acidified with 0.01 *M* H_3PO_4 or kept neutral with a 0.01 *M* sodium phosphate pH 7 buffer.

Operation of the preconcentrator. The air sampling rate was varied over the range of 550–700 l/min, the preferred range being 620–680 l/min. Higher flow-rates resulted in observable entrainment of liquid absorbent, whereas lower rates yielded reduced collection efficiencies (presumably associated

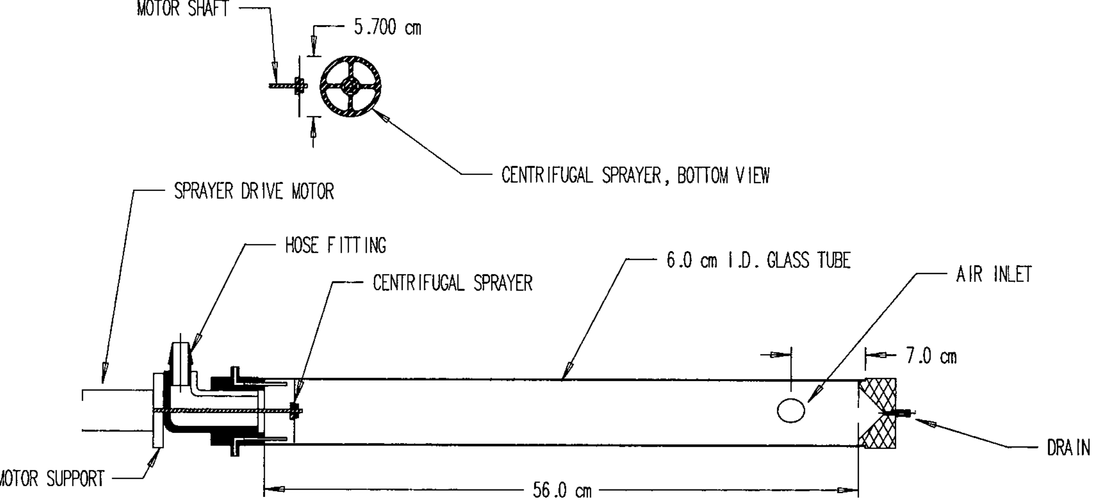

Fig. 2. Experimental high-volume liquid-absorption-type preconcentrator sampler. Air, at a rate of 700 l/min, passes through the 6.0 cm I.D. tube in a swirling, highly turbulent motion, which assures rapid transfer of trace constituents to a liquid film which covers the inner walls of the tube and is drained from the bottom at a rate of 0.1–1 ml/min.

with decreased air turbulence). The air flow in the HTLAP was highly turbulent and swirling, assuring rapid interchange of trace constituents between the air and the liquid film that covered the inner wall of the preconcentrator tube. The suction of the ring compressor generated a partial vacuum at the tube inlet that interfered with the drainage of absorbent from the bottom of the tube. To effect drainage, collection vials were enclosed in a tight container that was connected to the suction of the compressor. Use of Triton X-100 as wetting agent permitted reduction of the input rate of the absorbent to only about 1.0 ml/min while still maintaining acceptable wetting of the inner walls of the tube. Evaporation of water from the liquid film helped minimize the volume of collected absorbent, which was usually in the range of 3–5 ml for sampling times of 10–60 min.

Flow dynamics and optimization of air intake. The flow dynamics and collection efficiency of the preconcentrator are greatly influenced by the size and geometry of the air inlet. To assure that the entering air follows a swirling path (which results in swirling of the extractant, full wetting of the inner walls, and efficient analyte transfer from the air to the absorbent), a 0.08-cm-thick sheet of PTFE having a 2.7-cm-diameter hole was taped over the air inlet hole in the Pyrex tube so that the holes partly overlapped. By trial and error, the most pronounced swirling was obtained when the hole centers were displaced as shown in Fig. 3. This air inlet configuration was used in the latest tests and yielded improved collection efficiencies (see Fig. 4).

Also measured was the minimum drip rate at which the absorbent could be continuously collected, while maintaining the inner walls at least 80% wetted. The minimum drip rate was as low as 0.06 ml/min. The drip rate can be increased at will, preferably to 0.1–2 ml/min, by increasing the liquid input rate.

RESULTS AND DISCUSSION

Collection efficiencies

The results of the collection efficiency measurements are shown in Fig. 4 for analyte concentrations of 0.1–150 pptv. There is no evident tendency for the collection efficiency to change with analyte concentration, type of analyte (cocaine or heroin) or acidity of the absorbent. For measurements performed before optimization of the air intake (Fig. 4) the root-mean-square value of the collection efficiency is nearly 40% (lower dashed line). After optimization, that value apparently increased to about 60% (upper dashed line, Fig. 4), but the apparent increase needs to be confirmed by more experimen-

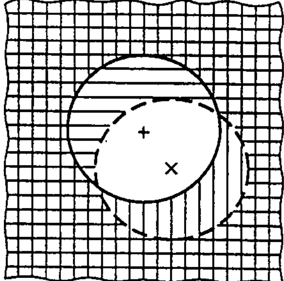

Fig. 3. Optimized air intake of the preconcentrator. Shading: vertical lines = PTFE sheet; horizontal lines = Pyrex wall. Symbols: × = center of elliptical hole in Pyrex (horizontal 2.7-cm major axis; vertical 2.5-cm minor axis); + = Center of circular 2.7-cm-diameter hole in PTFE (displaced relative to the ×-marked center by 0.7 cm upward and 0.5 cm to the left).

tal data. The wide scatter of the experimental points may be due to analytical errors associated with background interferences, to unexplored variables (variations in air and liquid flow-rates, absorption of analyte by the plastic plug, etc.), and to a non-optimal configuration of the air intake.

The background interferences arose from two sources —contaminated laboratory air and impurities in the sorbent tubes. To correct for these contaminants, blank absorbent samples (obtained by sampling the laboratory air without injection of analyte) were spiked with known concentrations of the tested analyte, and the resulting chromatographic peaks were matched with those obtained in a preceding or following collection efficiency test. Such matching assumed that the contaminants in the ambient air remained the same during a 3-h period that included the collection of: (a) a first blank sample (60 min), (b) an analyte-enriched absorbent (60 min) and (c) a second blank sample (60 min). Variations in contaminants during the 3-h period could have resulted in either high or low values of the measured collection efficiencies, especially for the lower analyte concentrations (< 1 pptv).

Short-duration experiments. The two experimental points of Fig. 4 obtained for an analyte concentration of 114 pptv represent the results of short-duration measurements at two different drip rates, as detailed in Table I. Under the heading "Initial drip" (3rd to 5th columns) are the data for the first 0.5 or 1.0 ml of extractant collected during the first 30 or 15 s after introduction of the generated cocaine vapor into the air intake of the preconcentrator. The collection efficiencies measured for these initial samples are comparable to those for the extractant

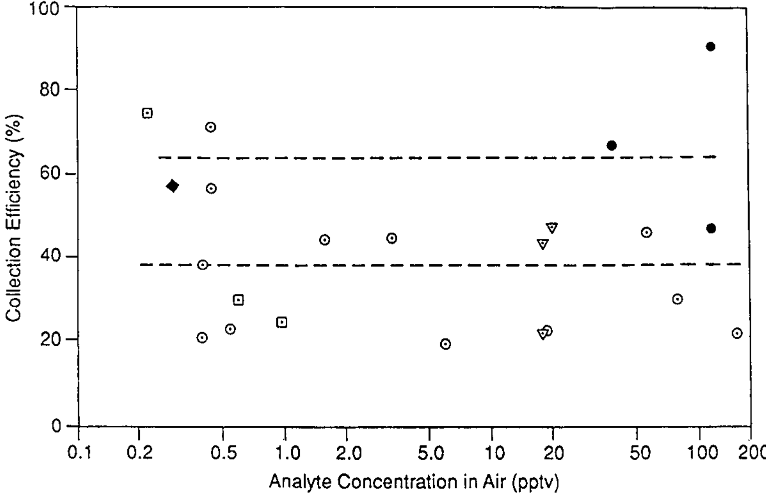

Fig. 4. The efficiency of collection of cocaine or heroin vapors using 0.1 g/l Triton X-100 in the extractant. □ = Heroin vapor collected by acidic extractant (0.01 *M* phosphoric acid); ▽ = cocaine vapor, same acidic extractant; ○ = cocaine vapor, neutral extractant (0.01 *M* pH 7 sodium buffer). ◆ = heroin vapor, same neutral extractant, after optimizing the air intake according to Fig. 3; ● = cocaine vapor, same neutral extractant, with optimized air intake. Lower dashed line: least root-mean-square (rms) collection efficiency for results obtained before intake optimization (open symbols). upper dashed line = least rms collection efficiency for results obtained after optimization of the air intake (filled in symbols).

TABLE I

RESULTS OF SHORT-DURATION COLLECTION EFFICIENCY MEASUREMENTS AT TWO DIFFERENT DRIP RATES

Drip rate (ml/min)	Cocaine vapor generation rate (μg/min)	Analyte collected from:											
		Initial drip			Continuous drip			Preconcentrator wall surface			All sources		
		Time (min)	Weight (μg)	Collection efficiency (%)	Time (min)	Weight (μg)	Collection efficiency (%)	Time (min)	Weight (μg)	Collection efficiency (%)	Time (min)	Weight (μg)	Overall collection efficiency (%)
1.0	0.98	0.5	0.11	35	8.5	6.3	76	9.0	1.54	17	9.0	8.0	91
4.0	0.98	0.25	0.25	103	4.75	1.8	39	5.0	0.23	5	5.0	2.3	47

collected immediately afterward under the heading "Continuous drip" (6th to 8th columns), *i.e.*, 35–103% as compared with 39–76%. The high value of 103% is probably due to a timing error, as a time difference of 5 s, *i.e.*, 20 s in lieu of 15 s, could have reduced the computed collection efficiency by 25%, *i.e.*, from 103 to 77%. The comparable collection efficiency values for the initial drip and continuous drip measurements imply that a whiff of analyte collected by the preconcentrator over a brief time interval (*ca.* 10 s) should show up in the drip sample immediately after collection. This suggests that this preconcentrator may also be useful for rapid screening of passengers or baggage at airports and other ports of entry.

The tenth and eleventh columns of Table I indicate that, after a sampling run of 5–9 min, only a small fraction of the analyte collected by the liquid extractant is retained in the liquid film along the preconcentrator surface for drip rates of 1–4 ml/min. As expected, this fraction decreases with increasing drip rate, amounting to about 17%/91% = 19% and 5%/47% = 11% for drip rates of 1.0 and 4.0 ml/min, respectively.

Aerosol collection. To measure the HTLAP's ability to pick up aerosols, a corn oil mist (0.3–3-μm droplets) was produced by a Laskin nozzle, set at a pressure of 0.83 bar. The relative concentrations of these droplets at the preconcentrator inlet and outlet were measured with a Model TDA-2EL light-scattering photometer (Air Techniques, Baltimore, MD, USA). The photometer readings yielded an aerosol collection efficiency of 42–48%. This result was obtained before the optimization of the air intake and falls within the range of values of Fig. 4.

Potential applications

Estimated sensitivity enhancement. The mass W_i of analyte collected for various analyte concentrations in sampled air, should vary with sampling time approximately as shown in Fig. 5. For example, air containing 0.1 pptv of analyte, corresponding to about 0.1% of the equilibrium vapor pressure of cocaine or 20% of that of heroin at room temperature when sampled for 10 min, yields 4 ng of cocaine or 5 ng of heroin in the liquid extractant according to Fig. 5. Since the 10-min sampling time at the rate of 800 l/min corresponds to a sampled volume of 8000 l of air, the preconcentrator could not be applicable to containers of <8000 l. The 4-ng or 5-ng analyte yield from a 10-min sampling time is well above the lower detection limit (LDL) of avail-

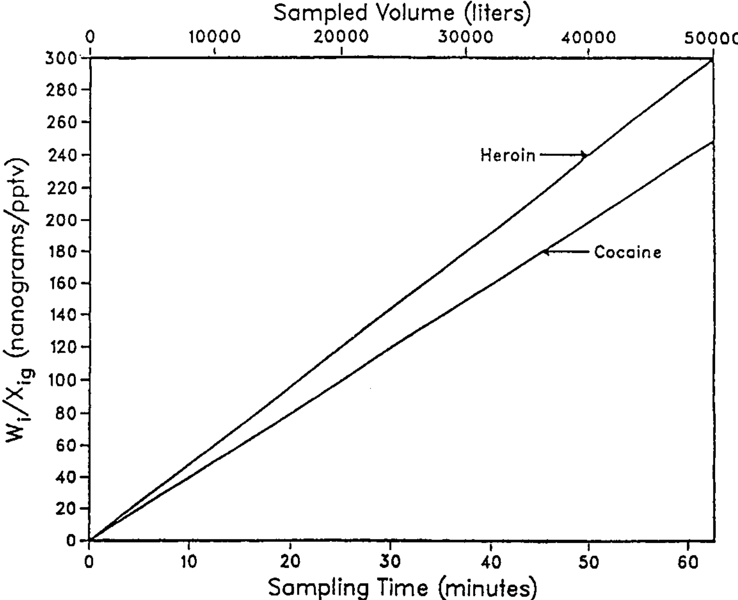

Fig. 5. Dependence of the ratio of collected analyte mass, W_i, to the analyte mole fraction, X_{ig}, on the sampling time or volume of sampled air, assuming a collection efficiency of 40% and an air flow-rate of 800 l/min.

able analyzers. The LDLs of our LC–ED procedures were 0.5 ng for heroin and 2 ng for cocaine.

Adaptability to most analyzers. The analyte-enriched absorbent should require few or no preparatory steps before introduction into most liquid-phase analyzers, such as a liquid chromatograph, a colorimeter, electrochemical systems, or immunoassay detectors. For gas-phase analyzers, such as a gas chromatograph or mass spectrometer, special interfaces may be required. The HTLAP's preferred absorbent drip rate of 0.1–2 ml/min coincides with the liquid flow-rate used in existing LC–MS interfaces [8].

Applicability to many compounds. Comparison with results obtained from various types of liquid-absorption samplers indicate that the HTLAP should be applicable to many analytes of interest, including trinitrotoluene [5], primary aromatic amines [4], nitrogen dioxide [9], hydrogen peroxide [10], formaldehyde [10], sulfur dioxide [10] and several inorganic halogen compounds (HCl, HF, F_2, and other hydrolyzable fluorides) [11].

The HTLAP should be especially useful in detecting and quantitating compounds that are not readily analyzed by conventional preconcentration with GC, *e.g.*, polar or highly reactive analytes, such as primary aromatic amines or hydrazines. Through a proper choice of liquid absorbent it should be possible to achieve high selectivity and/or stabilization of labile or reactive analytes and/or entrapment of volatile analytes. For instance, to trap N_2H_4 vapor, a non-volatile aldehyde may be included in the absorbent. Conversely, to trap formaldehyde, the absorbent may contain a non-volatile hydrazine compound.

CONCLUSIONS

With the collection efficiencies of 40–60% (Fig. 4) and a throughput of 600–700 l/min, the sensitivity enhancement achievable by the HTLAP will depend on the analyte, the analyzer, and the sampling time. For most existing analyzers that operate at sampling rates of <200 ml/min, the HTLAP's much higher throughput will yield a >1000-fold higher rate of analyte collection, with a corresponding increase in sensitivity (reduction in the lower detection limit for the HTLAP-analyzer combination). Alternatively, the required sampling time could be greatly shortened. Also, with certain portable and fast-responding analyzers, such as ion-selective electrodes, the HTLAP should permit far more rapid on-site air monitoring than was previously practicable.

More work is needed to (a) further optimize the geometry of the preconcentrator tube and the air and liquid flow rates, (b) narrow the range of collection efficiency values under optimized operating conditions and (c) establish the basic design parameters for upscaling or downscaling the HTLAP to higher or lower throughputs.

ACKNOWLEDGEMENTS

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Review

Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water

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ABSTRACT

The different priority lists of pesticides in water from the European Economic Community (EEC) and the US Environmental Protection Agency (EPA) in water are listed and discussed. The chromatographic protocols of the EPA employed in the National Pesticide Survey for a total of 101 pesticides and 25 transformation products are reviewed. A comparison with the official methods of the United Kingdom Standing Committee of Analysts (SCA) is shown. Critical comments aimed at improving the present Official methods are made. Special emphasis is devoted to the development of new analytical techniques based on solid-phase extraction combined either off-line or on-line with chromatographic separations. The main aims of the different approaches are the development of screening methods for pesticides and their transformation products in water, the achievement of low limits of detection, especially in the case of the EEC Drinking Water Directive which sets a limit of 0.1 μg/l for individual pesticides, and the use of confirmation methods based on mass spectrometric approaches.

CONTENTS

1. Introduction: priority lists of pesticides and general analytical considerations 117
2. EPA methods of analysis 124
3. SCA methods of analysis 129
4. Other GC methods 135
5. LC techniques 137
6. Conclusions 140
7. Acknowledgements 141
References 141

1. INTRODUCTION: PRIORITY LISTS OF PESTICIDES AND GENERAL ANALYTICAL CONSIDERATIONS

Several hundred pesticides of diverse chemical nature are currently widely used in the USA and Europe for agricultural and non-agricultural purposes. Some are substitutes for the organohlorine pesticides, which were banned after evidence of their toxicity, persistence and bioaccumulation in environmental matrices was found. According to a report published by the US Environmental Protection Agency (EPA), a total of $5 \cdot 10^8$ kg of pesticides was used in 1985 [1]. Pesticide consumption in European countries such as the UK was in the

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region of $14 \cdot 10^6$ kg per year during the period 1980–83 [2]. As far as specific pesticides are concerned, world-wide consumption of malathion and atrazine in 1980 amounted of $24 \cdot 10^6$ kg and $90 \cdot 10^6$ kg, respectively [3,4]. In the Mediterranean countries $2.1 \cdot 10^6$ kg of malathion (active ingredient) were sprayed during the same period *versus* $9.7 \cdot 10^6$ kg in Asia [3].

A recent report published by the Commission of the European Communities (CEC) indicated the total turnover of the major pesticides used in Denmark, France, Germany, the UK, Greece, Netherlands, Italy, Spain and Sweden. The report included non-agricultural uses [5]. Atrazine, one of the herbicides most widely used in the USA and European countries over the last 30 years, is employed for pre- and post-emergence weed control of corn, wheat, barley and sorghum, and on railways and roadside verges. In this respect, in England and Wales the non-agricultural use of this herbicide represented 140 000 kg of active ingredient, whereas for France it was 43 000 kg during 1989 [5]. Not surprisingly it has been detected in ground and surface waters through the world (*e.g.*, in some USA ground waters at concentrations in the range 0.1–3 μg/l [1]), and in ground waters in various European countries [5] and in estuarine areas such as the Rhône river in France [6] and the Ebro delta in Tarràgona, Spain [7]. An example of the level of contamination by herbicides in the Ebro delta area is shown in Table 1 with the different contamination levels of the river and the canals. A higher level of pollution (*ca.* ten times higher) was found in the canals owing both to their proximity to the fields where pesticides are being applied and to their low water flow as compared with the Ebro river.

Owing to the environmental impact of pesticides, several priority lists, also called "red" and/or "black lists" have been published to protect the quality of drinking and surface waters. In Table 2, the different pesticides listed in the 76/464/EEC Directive (the so-called black list) are indicated [8]. Following the three general parameters (toxicity, persistence and input) for selecting the priority list of pollutants [9] in the UK, a red list of substances that include several pesticides, most of them common to the EEC list, was established [9].

In order to prevent the contamination of ground and drinking water by pesticides in Europe, a priority list [5], which considers pesticides used over 50 000 kg per annum (and over 500 are indicated) and their capacity for probable or transient leaching, was recently published. This is shown in Table 3. There are a few other pesticides, such as demeton-S-methyl, fentin acetate, mancozeb, propineb, thiobencarb and zineb, for which, although they are used in amounts over 50 000 kg per annum, at present there are insufficient data to evaluate the

TABLE 1

CONCENTRATIONS OF HERBICIDES IN TWO SAMPLING STATIONS (RIVER AND DRAINAGE CANAL) OF THE EBRO DELTA AREA (TARRAGONA, SPAIN) DURING 1991

Compound	Concentration (ng/l)					
	April		May		June	
	River	Canal	River	Canal	River	Canal
Atrazine	36	308	17	58	190	190
Simazine	28	302	35	87	138	440
Molinate	n.d.[a]	1400	38	254	n.d.	3187
Alachlor	206	50	31	862	n.d.	n.d.
Metolachlor	132	68	32	n.d.	69	35
Bentazone	n.d.	n.d.	n.d.	n.d.	n.d.	5520
Trifluralin	14	n.d.	n.d.	n.d.	n.d.	n.d.

[a] n.d. = Not detected (below 0.1 ng/l, except for bentazone, for which it means 10 ng/l).

TABLE 2

PESTICIDES LISTED IN 76/464/EEC COUNCIL DIRECTIVE ON POLLUTION CAUSED BY CERTAIN DANGEROUS SUBSTANCES DISCHARGED INTO THE AQUATIC ENVIRONMENT OF THE COMMUNITY (BLACK LIST)

Aldrin	Disulphoton	Monolinuron
Atrazine	Endosulphan	Omethoate
Azinphos-ethyl	Endrin	Oxydemeton-methyl
Azinphos-methyl	Fenitrothion	Parathion-ethyl
Chlordane	Fenthion	Parathion-methyl
Coumaphos	Heptachlor	Phoxim
2,4-D	Hexachlorobenzene	Propanil
DDT	Linuron	Pyrazon
Demeton	Malathion	Simazine
Dichlorprop	MCPA	2,4,5-T
Dichlorvos	Mecoprop	Triazophos
Dieldrin	Metamidophos	Trichlorfon
Dimethoate	Mevinphos	Trifluralin

probability of leaching. Consequently these are not included in Table 3. In addition, glyphosate and thiram were not included in Table 2 because large differences in the ground water ubiquity score (GUS) index were found. This index is a measure of the leaching capacity of a pesticide through soil [5].

Another important point regarding the different pesticides reported in Table 2 is that although no transformation products (TPs) are included, in the report published by the CEC [5], it was indicated that there is much interest in the determination of such TPs for triazine, organophosphorus, carbamate and chlorinated phenoxy acid herbicides. In this respect, although the EEC Directive on the Quality of Water Intended for Human Consumption sets a maximum admissible concentration (MAC) of 0.1 μg/l for individual pesticides and related products and 0.5 μg/l for total pesticides, it is unclear what can be considered as "related products". It has been indicated that these "related products" refer to TPs that are toxic, which in the context of ground water contamination could be interpreted as exceeding a water quality standard derived from toxicological considerations [5]. In this respect, it is clear that some specific TPs, *e.g.*, fenitrooxon (from fenitrothion) and 1-naphthol (from carbaryl), are more toxic to aquatic organisms than the parent compounds. This also applies to ethylenebisthiourea (ETU), which is a well known TP of maneb and related pesticides and is more toxic than the parent pesticides [5].

Following considerations based on usage information, physico-chemical properties and persistence, a priority list of herbicides was established for the Mediterranean countries France, Italy, Greece and Spain. The list, which is shown in Table 4, considers selected herbicides that can cause contamination of estuarine and coastal environments. The selection of pollutants was based on the availability of usage data and the consideration of half-lives so that pesticides that do not exceed a total of 10 000 kg after 90 days of application have been omitted [10]. Note that some of these pesticides are common to Table 3. We should emphasize that pesticides in drinking water derived from ground water should be considered in a different way to pesticide that reach estuarine waters. The transport of pesticides from river waters to estuarine areas and coastal environments will be dependent on several parameters, *e.g.*, how they are absorbed into the suspended particulates and how they are affected by the higher salinity and pH. An example of such contamination corre-

TABLE 3

PESTICIDES USED IN EUROPE IN AMOUNTS OVER 50 000 kg PER ANNUM WHICH WERE CLASSIFIED AS PROBABLE OR TRANSIENT LEACHERS

Pesticides used in amounts over 500 000 kg are in italics.

Alachlor	Dinoseb	*Methabenzthiazuron*
Aldicarb	*Diuron*	Methiocarb
Amitrole	DNOC	Oxydemeton methyl
Atrazine	EPTC	Phenmedipham
Benazolin	Ethofumesate	*Prochloraz*
Bentazone	Ethoprophos	Propham
Bromofenoxim	Fenamiphos	Prometryn
Carbaryl	Fluoroxypyr	*Propiconazole*
Carbendazim	Iprodione	Propyzamide
Carbetamide	*Isoproturon*	*Pyrethrin*
Chloridazon	Linuron	Simazine
Chlorpyrifos	*Maneb*	Terbutylazine
Chlortoluron	*MCPA*	Terbutryn
Cyanazine	*MCPP*	Triademinol
2,4-D	Metamitron	Trichlorfon
Dalapon	*Metazachlor*	*Trichloroacetic acid*
Diazinon	*Metham-sodium*	Vinclozolin
Dichlobenil	*Metolachlor*	Ziram
Dimethoate		

TABLE 4

HERBICIDES OF POTENTIAL CONCERN IN THE MEDITERRANEAN REGION

Alachlor	EPTC	Metribuzin
Amitrole	Ethalfluralin	Molinate
Atrazine	Ethofumesate	Napropamide
Bentazone	Flamprop-M-isopropyl	Neburon
Bromoxynil	Glyphosate	Paraquat
Butylate	Isoproturon	Pendimethalin
Carbetamide	Linuron	Phenmediphan
Chlortoluron	MCPA	Prometryn
2,4-D	Mecoporp	Simazine
Di-allate	Metamitron	Trichloroacetic acid
Dichlobenil	Metazachlor	Terbumeton
Dichlofop-methyl	Metabenzthiazuron	Terbutylazine
Dinoterb	Metobromuron	Terbutryn
Diquat	Metolachlor	Tri-allate
Diuron	Metoxuron	Trifluralin
DNOC		

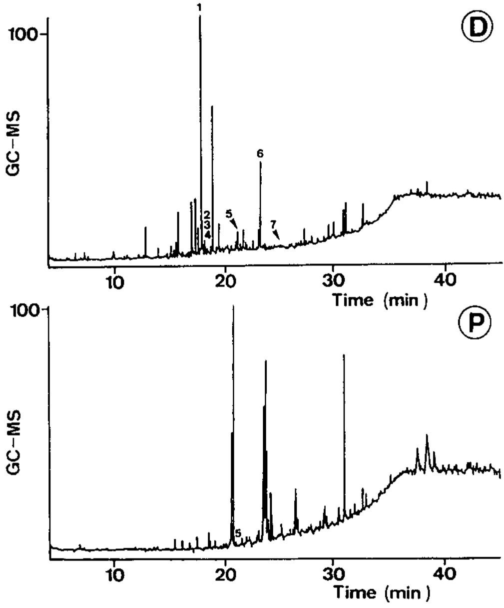

Fig. 1. Total ion current GC–MS of dissolved (D) and particulate (P) matter of river water extract from one of the stations located at the Rhône river estuary. Sampling was carried out during November 1990. Extraction of 5 l of river water sample was carried out using dichloromethane [6]. Compounds identified corresponded to: 1 = tributyl phosphate; 2 = deethylatrazine; 3 = simazine; 4 = deisopropylatrazine; 5 = atrazine; 6 = caffeine; 7 = propanil. Concentration levels for deethylatrazine, simazine, deisopropylatrazine, atrazine and propanil were 4, 10, 3, 17 and 2 ng/l, respectively. A DB-1701 capillary GC column was used.

sponds to the Rhône estuary in the Camargue region, as indicated in Fig. 1, which shows the total ion current chromatogram obtained after extraction of 5 l of river water extract with dichloromethane of the dissolved (D) and particulate (P) organic matter. The levels of the different chlorotriazine herbicides varied from 1 to 17 ng/l in the dissolved phase, whereas in the particulate matter the levels found were below 1 ng/l [6]. This indicates that for this particular group of herbicides transport from river water to the sea occurs mainly in the dissolved phase.

It is estimated that ground water is the source of drinking water for 90% of rural households and three quarters of USA cities. In total, more than half of the USA citizens rely on ground water for their everyday needs. Owing to the amount of information indicating the presence of pesticides in ground water in the different USA States [1], a joint research project between the EPAs Office of Drinking Water (ODW) and the Office of Pesticide Programs (OPP) involved a statistically based survey of pesticide contamination of drinking water wells. During the National Pesticide Survey (NPS), 1349 drinking water wells were sampled and analysed for 101 pesticides, 25 pesticide TPs and nitrate, with a total of 127 analytes. The results of the NPS were released in November 1990 (Phase I) and January 1992 (Phase II) [11,12]. The selection of the different analytes was based on the use of at least 10^6 lbs. (1 lb. = 7000 g) in 1992, a water solubility greater than 30 mg/l and a hydrolysis half-life longer than 25 weeks. Pesticides and pesticide degradation products previously detected in ground water and pesticides regulated under the Safe Drinking Water Act were automatically included in this priority list [13]. The compounds were grouped according to their analysis; seven methods were used that covered all the 126 analytes and are indicated in Table 5 [14]. Of the pesticides listed in Table 5, stability was checked for 147 analytes, 121 being stable for at least 14 days when stored in well water at 4°C. Among the 26 unstable pesticides (with a loss of 100% after storage under the conditions mentioned above) were many organophosphorus pesticides such as azinphos-methyl, disulphoton, fenitrothion, fenthion, malathion and parathion-methyl. It should be noted that these organophosphorus pesticides are included in the priority list of compounds of the EEC (see Table 2). Although they are on this list, their proven degradation when stored at 4°C and for 14 days in well water suggests that they are not so harmful, and their incorporation in a priority list is questionable. Other pesticides, such as ETU and heptachlor, suffered slight degradation (between 15 and 22%) under identical storage conditions, whereas the sample extract generally remained stable [14].

The list shown in Table 5 is so far the most comprehensive list used to conduct a monitoring programme on pesticides. It should be noted that in the last few years (since 1990), an early-warning system for the on-line screening and liquid chromatographic detection of 50 polar pesticides and other pollutants in Rhine river water has been developed in Europe. It involves the Rhine basin, with research groups for Switzerland, Germany and the Netherlands. The first results on the analytical method development have been published recently

TABLE 5

PESTICIDES AND TPs INCLUDED IN THE NATIONAL PESTICIDE SURVEY (USA)

Method 1 (EPA 507)	
Alachlor	Methyl paraoxon
Ametryn	Metolachlor
Ametraton	Metribuzin
Atrazine	Mevinphos
Bromacil	MGK 264
Butachlor	Molinate
Butylate	Napropamide
Carboxin	Norflurazon
Chlorpropham	Perbulate
Cycloate	Prometon
Diazinon	Prometryn
Dichlorvos	Pronamide
Diphenamid	Propazine
Disulfoton	Simazine
Disulfoton sulphone	Simetryn
Disulfoton sulphoxide	Stirofos (tetrachlorvinphos)
EPTC	Tebuthiuron
Ethoprop	Terbacil
Fenamiphos	Terbufos
Fenamirol	Terbutryn
Fluridone	Triademefon
Hexazinone	Tricyclazole
Merphos	Vernolate
Method 2 (EPA 508)	
Aldrin	Endrin aldehyde
α-Chlordane	Etridiazole
γ-Chlordane	α-HCH
Chlorneb	β-HCH
Chlorobenzilate	δ-HCH
Chlorothalonil	γ-HCH
DCPA	Heptachlor
4,4′-DDD	Heptachlor-epoxide
4,4′-DDE	Hexachlorobenzene
4,4′-DDT	Metoxychlor
Dieldrin	*cis*-Permethrin
Endosulphan I	*trans*-Permethrin
Endosulphan II	Propachlor
Endosulphan sulphate	Trifluralin
Endrin	
Method 3 (EPA 515.1)	
Acifluorfen	Dichlorprop
2,4-DB	Dinoseb
Bentazone	5-Hydroxy dicamba
Chloramben	4-Nitrophenol
2,4-D	PCP
Dalapon	Picloram
DCPA acid metabolites	2,4,5-T
Dicamba	2,4,5-TP
3,5-Dichlorobenzoic acid	
Method 4	
Atrazine dealkylated	Linuron
Barban	Metribuzin DA
Carbofuran phenol	Metribuzin DADK
Cyanazine	Neburon
Diuron	Pronamide metabolite
Fenamiphos sulphone	Propanil
Fenamiphos sulphoxide	Propham
Fluometuron	Swep
3-Ketocarbofuran phenol	
Method 5 (EPA 531.1)	
Aldicarb	Carbofuran
Aldicarb sulphone	3-Hydroxycarbofuran
Aldicarb sulphoxide	Methiocarb
Baygon (propoxur)	Methomyl
Carbaryl	Oxamyl
Method 6	
ETU	
Method 7 (EPA 504)	
EDB	*cis*-1,3-Dichloropropene
DBCP	*trans*-1,3-Dichloropropene
1,2-Dichloropropane	

[15,16]. These results deal only the analytical development stage.

One of the main differences between the USA and European regulations on pesticide programmes is that in Europe each country uses its own analytical method, whereas in the USA the EPA methods are widely implemented. The different approaches used by European governmental laboratories, which prefer to use conventional liquid–liquid extraction (LLE) procedures, and research or other laboratories that prefer to use solid-phase extraction (SPE) techniques, mean that within Europe there are no consensus methods for the determination of pesticides in water. Consequently, it is difficult to employ an NPS monitoring programme approach within the different countries, and intercomparisons and validation of results have not been conducted. This aspect will be discussed in detail in another section of this paper, but is one of the major problems within the EEC, since an agency similar to the EPA does not exist in Europe, although the Council of Ministers agreed in 1990 to create a European Environmental Agency.

Some general comments can be made regarding the different priority lists presented in Tables 2–5. Although in some instances there is agreement on the priority pesticides to be monitored, *e.g.*, atrazine, 2,4-D, linuron and dimethoate, in others there is complete disagreement. This is the case for, *e.g.*, the carbamates, which have been of relatively high importance in the USA monitoring programmes (see Table 5), and the EPA has developed an excellent method of analysis for these pesticides in water with a very low limit of detection (LOD), which will be discussed later. In contrast, in Europe, in the first black list of pesticides they were no carbamates at all (see Table 1). As they were not included in this first list of dangerous substances (Table 2), no tradition of monitoring carbamates has been established, although their use in several countries such as Netherlands, Spain, the UK and Italy has been reported. In addition, the official EPA method for monitoring carbamate pesticides (Method 531.1) has seldom been used in Europe, although it is a highly sensitive and robust method. Another aspect that should be considered is the leachability of these carbamates to ground and well waters, and in this sense they have been studied in different USA well waters through the NPS. However, in Europe, although ground waters are also an important source of drinking water, no such investigation has been undertaken. The percentage of ground water used for drinking water purposes in Europe is close to 100% for Denmark and between 60 and 85% for Italy, Germany, France and the UK, whereas in Spain it is about 30%.

Finally, another remark concerning the different priority lists is that the NPS list (Table 5) is the only one that especifically mentions the TPs of pesticides. This is a very significant aspect, because although in the EEC regulations the importance of TPs of pesticides is indicated [5], no specific TPs are named. This makes it more difficult for laboratories currently involved in monitoring programmes to monitor and select the different TPs. It should also be mentioned that many of the TPs need specific methods of analysis, and are poorly recovered using conventional screening methods. Therefore, in this sense, the efforts made through the NPS with specific methods of analysis and the list of priority pesticides and TPs provided in Table 4 are of great importance and can be implemented, allowing for the different circumstances of each country, worldwide.

As pointed out previously [9], in the selection of priority lists one of the relevant parameters to be taken into consideration is the toxicity of the compound. Such toxicity evaluations depend on the compound and its concentration in water, and should take into account both human toxicity and toxicity to aquatic organisms. For drinking water, the CEC has fixed a level of 0.1 μg/l for individual pesticides and 0.5 μg/l for total pesticides. This is a very strict measure, and analytical methods still need to be developed for a variety of pesticides to comply with this Directive.

The Office of Water of the EPA has established drinking water regulations and health advisory levels for individual pesticides. A selection of the different health advisory levels, also referred to by the EPA as maximum contaminant level goals, are indicated in Table 6. Values given in this table were selected from refs. 12 and 17. Such levels are more correct than the EEC levels, which have been fixed for all the individual pesticides without making any distinction between pesticides of different toxicities. Regarding the levels of the TPs, it can be argued that their levels should follow toxicity values [9].

Establishing the maximum concentration levels for individual pesticides is very important to demonstrate compliance with the different Directives. In the EEC, the strict Directive has the disadvantage that some ubiquitous pesticides, such as atrazine, which is not especially toxic to humans, is found in many instances at levels higher than 0.1 μg/l, and it can be seen from the literature that the levels found in some EEC countries exceed the EEC regulations. However, as the levels set in the EEC Directive were not based on toxicological data, in some instances higher levels are permissible. This restrictive regulation for pesticides in Europe has resulted in the development of analytical methods that can detect pesticides at levels of 0.02 μg/l in order to determine the pesticides at 0.1 μg/l. However, in addition to the lack of information on which pesticides to monitor, it will also be impossible to determine all the pesticides approved for use within the EEC at this level of sensitivity. Because it is difficult to know which pesticides require monitoring, one approach is to focus efforts on those pesticides which are (a) likely to reach water resources, (b) are used in

TABLE 6

HEALTH ADVISORY LEVELS FOR SELECTED PESTICIDES IN DRINKING WATER (EPA OFFICE OF GROUND WATER AND DRINKING WATER)

Compound	Health advisory level (μg/l)	Compound	Health advisory level (μg/l)
Alachlor	2	Diquat	20
Aldicarb	10	Endothall	100
Aldicarb sulphoxide	10	Endrin	2
Aldicarb sulphone	10	Glyphosate	700
Atrazine	3	Methomyl	200
Bromacil	80	Methyl parathion	2
Carbofuran	40	Metolachlor	10
Chlorthalonil	2	Oxamyl	200
Cyanazine	9	Picloram	500
2,4-D	70	Simazine	4
Dalapon	200	Trifluralin	2
Dinoseb	7		

sufficient amounts and (c) have a tendency to be persistent and mobile (see Table 3). The EEC Drinking Water Directive also sets a limit of 0.5 μg/l for total pesticides. It is difficult to carry out proper monitoring of such a parameter, particularly in relation to defining required detection limits and accuracy, unless an arbitrary maximum number of total pesticides is assumed. In the most recent report of the EEC [5] it was stated that analytical methods need a detection limit of 0.02 μg/l or less (ideally 0.01 μg/l) and need to provide data of sufficient accuracy. In the latter respect, around 20% total errors (random and systematic) should be aimed for.

The pesticides of highest priority to the EEC are listed in Table 3. It has also been recommended [5] that significant analytical results need to be confirmed by an additional technique, preferably involving some form of mass spectrometry, because of the likelihood of false positives with the commonly applied methods such as gas chromatography with electron capture (GC–ECD) or nitrogen–phosphorus detection (GC–NPD) and liquid chromatography (LC) with UV detection. For some difficult pesticides such as maneb, ziram and trichloroacetic acid, analytical methods need to be developed in order to reach the LOD indicated in the EEC regulations for water. In the USA, most of the EPA methods in use comply with the Health Guidance levels indicated in Table 6.

In this review, the development of methods of analysis and confirmation for most of the "conventional" organochlorine pesticides will not be considered, as most have been withdrawn and replaced with organophosphorus and carbamate pesticides. However, organochlorine pesticides are covered by method 2 (see Table 5), with a few exceptions such as chlorneb, chlorbenzilate, chlorothalonil, etridiazole, metoxychlor, *cis*- and *trans*-permethrin, propachlor and trifluralin.

In this review, the official methods of analysis for pesticides in drinking water used in the USA (EPA–NPS) and in the UK will be discussed. References will be made to examples of developed methods involving GC using NPD, ECD and flame photometric detection (FPD) and LC using UV, electrochemical and fluorescence detection. As stated previously, it is necessary to use confirmation methods to avoid false positives. In this sense the use of mass spectrometric techniques or two different GC or LC columns of different polarity, which is the common EPA procedure, will be mentioned. Some examples and comments on the different approaches will be given, emphasizing the main advantages and disadvantages.

One of the main decisions to be taken at the beginning of an analysis for pesticides is whether to use GC or LC. In some instances the choice can be very clear and for sufficiently volatile compounds,

such as most of the organochlorine pesticides and some organonitrogen pesticides such as atrazine, or organophosphorus pesticides such as fenitrothion, GC will be preferred. Problems arise when pesticides that are thermally labile and/or polar need to be analysed. The use of derivatization techniques and further GC analysis with a selective detector generally allows good LOD. Other workers prefer to use LC techniques, without prior derivatization, which simplifies the method. There are some specific cases of polar and thermally labile pesticides which, when analysed by GC, need careful attention, because although peaks can be detected in the GC traces, such peaks do not correspond to the compound itself but to a degradation product that is generally formed in the injection port. One such group is the carbamates, compounds with proved thermal instability under conventional GC conditions. It has been pointed out that some of them can be determined by GC with careful selection of the instrumental conditions of analysis [18]. For the determination of carbaryl and other carbamates such as carbofuran, the use of cold on-column injection [19] has been reported to give good recoveries after isolation from water samples. Aldicarb sulphoxide and aldicarb sulphone have also been studied and it has been shown that aldicarb (the parent herbicide of aldicarb sulphoxide and sulphone), degrades at injection port temperatures of 130°C and that longer GC capillary columns do not allow elution in a reasonable time. Consequently, thermal degradation is observed [20]. A previous EPA method oxidized aldicarb to aldicarb sulphone by treatment with peracetic acid and then the aldicarb sulphone was thermally degraded in the injection port, producing the volatile species 2-methyl-2-(methylsulphonyl)propionitrile (EPA, 1981) [21]. It has been also recommended, if GC methods are still to be used, that the problems of decomposition of carbamates giving phenols and isocyanates should be overcome by prior derivatization with appropriate reagents such as acetic anhydride [22]. Similar considerations could be applied to oxamyl and benomyl.

With phenylurea herbicides although linuron can be determined by GC [19,23] with cold on-column injection, monuron and diuron are too thermally unstable and degrade under the GC conditions [23]. To avoid these problems, derivatization with reagents such as heptafluorobutyric anhydride [24] can be applied.

It is difficult, in some instances, to make a choice between GC or LC techniques. In general, a method that offers less manipulation of the sample and which provides good sensitivity is to be preferred. This review will focus on different examples, demonstrating the procedures of the official methods such as those of the EPA, and other methods that are being developed. In many instances the choice of one method over the others depends on the experience of the laboratory, and is dependent on the facilities and know-how available.

2. EPA METHODS OF ANALYSIS

Two reports concerning the revision of methods for the determination of organic compounds in drinking water have recently been published [25,26]. Revision and comments on the different EPA methods for water analyses have been also discussed in two recent papers, which recommended dropping some of the 600 series methods, the encouragement of the use of capillary columns in GC and of microextraction methods and the increasing use of GC–MS methods [27,28]. Of the different methods for determining purgeable organics, it has been recommended that EPA Method 524.2 should be kept, and all others (524.1 and 624) dropped [27].

The philosophy behind EPA methods is clearly stated in their objectives, *viz.*, developing and evaluating analytical methods for organic contaminants in water, determining the response of aquatic organisms to water quality and the development of a quality assurance programme to support the achievement of data quality objectives. The different EPA methods used for pesticide determinations in water can be divided into three groups: (i) those which use GC with selective detection (ECD or NPD), (ii) those which use GC–MS and (iii) those which use LC. The numbering of the different methods is based on the groups of pesticides as given in Table 5. Most of the EPA methods for pesticides in water use LLE procedures, with the exception of Method 525.1, which uses SPE procedures either with C_{18} cartridges or Empore extraction disks. Although in this review it is not the intention to discuss aspects of sample extraction, SPE is gaining in importance as it avoids problems with emulsions and those associated with the consumption and disposal of large volumes of toxic and flammable solvents [29].

The general characteristics of most of the EPA methods are as follows: (i) the acceptance of recoveries in the range from 70 up to 130%, with a maximum relative standard deviation of 30% each; (ii) preservation and storage of the samples is carried out at 4°C, and the recommendations made as to whether a sample should be analysed within a few days of storage or can be kept for a maximum of 14 days [14]; (iii) the description of apparatus and equipment (with safety considerations), reagents, standards and consumable materials; (iv) the use of two columns of different polarity, the so-called primary column, generally a DB-5 or similar, and a second column, called a confirmatory column, such as DB-1701 or equivalent, both in GC determinations; in LC, the primary column is a standard C_{18}-bonded silica and the second column is a cyano type; and (v) the way to proceed with blank samples, internal standards and surrogate solutions, interferences, calibration, standardization and quality control. As the use of surrogates in internal standards can lead to some confusion, definitions of both terms are given. The internal standard is added to measure the relative responses of other analytes and surrogates that are components of the solution. It is a requirement that the internal standard must be an analyte that is not a sample component. In contrast, a surrogate standard is a compound that is extremely unlikely to be found in any sample, is added to a sample aliquot before extraction and is measured with the same procedures as used to measure other sample components. The purpose of a surrogate is to monitor method performance with each sample. The use of a surrogate and internal standard is not common in other official methods as they generally use only an internal standard. A final general comment on the different EPA methods is that although sometimes minimum detection limits (MDLs) are used, estimated detection limit (EDL) and limit of detection (LOD) are used to indicate the same idea, but with a different terminology. It is recommended that in future these criteria be unified, and a single term used to indicate the limit of detection of a method.

A summary of each method used for the determination of pesticides and their corresponding TPs is given in Table 7. In this table, first a microextraction method, using GC–ECD, is mentioned. Although only a small water volume is used (35 ml), the MDLs are acceptable. Conventional analytical methods for organics in drinking water use sample volumes of at least 1 l.

Another EPA method for organonitrogen- and organophosphorus-containing pesticides is shown. In this instance, special attention is given to the storage of the water samples. As mentioned previously [14], it has been shown that 26 organophosphorus pesticides were unstable, with a 100% loss when stored under the usual conditions, at 4°C for 14 days. Among those, disulphuton sulphoxide, diazinon, fenitrothion, pronamide and terbufos deserve special attention as their determination should be carried out immediately after extraction. Other analytes, such as carboxin, EPTC, fluridone, metolachlor, napropamide, tebuthiuron and terbacil, exhibited recoveries of less than 60% after storage for 14 days. For such compounds it was also pointed out that although sample extracts, stored under identical conditions, were stable for 28 days, storage for only 14 days was recommended. Fig. 2 shows two examples of the use of LLE with dichloromethane for the determination of various organonitrogen pesticides in Ebro Delta water. By extracting 5 l of water with a final extract volume of 0.5 ml, an LOD down to 0.1 ng/l can be obtained, as is shown in Fig. 2 [7].

The EPA method for organochlorine pesticides is briefly described in Table 7. Careful attention should be paid to certain pesticides such as chlorthalonil, *cis*-permethrin, *trans*-permethrin and trifluralin, as preservation data are non-definitive, and therefore it is recommended that samples should be analysed immediately. For the other modern pesticides the samples are stable for 7 days at 4°C. For organochlorine pesticides, the method gives very low LODs (in the range 0.01–0.5 μg/l), with the exception of chlorobenzilate. Trifluralin can also be determined by GC–NPD with a low LOD [7].

The method for N-methylcarbamates is also reported in Table 7. No surrogate is used in this instance, as the method involves a direct analysis of water samples, without sample pretreatment. Sample preservation of carbamates is very important, and it has been observed that oxamyl, 3-hydroxycarbofuran, aldicarb sulphoxide and carbaryl can all degrade quickly in neutral and basic waters at room temperature. Therefore, samples should be kept at pH 3 and preserved at −10°C. These compounds

are much more easily degraded than other pesticides reported in EPA methods. An example of the determination of several carbamates using the detection principle of this EPA method is given in Fig. 3, where the LC–postcolumn fluorescence trace for a 10-ml drinking water sample spiked with 0.2 μg/l of a carbamate mixture, and a blank sample, are shown. The water sample was preconcentrated using SPE disks coupled on-line with the LC–postcolumn fluorescence detection system as used by the EPA. LODs in the range 5–40 ng/l are achieved, which are far below those achieved using direct injection of the water sample into the same system.

Analyses for the more volatile pesticides are also indicated in Table 7. A purge-and-trap method with subsequent GC–MS determinations and a microextraction method with n-hexane are described. In one instance, the use of MS ensures unequivocal identification of the compounds and the use of a microextraction method avoids volatility problems, giving an acceptable estimated detection limit. The so-called methods for unusual pesticides are also described in Table 7. Such methods have a peculiarity in that each is specific for the pesticide to be determined, *e.g.*, the method for glyphosate can only be used for this compound. These methods are not multi-residue methods and were developed for individual pesticides because of their occurrence in the different waters. The use of specific methods of analysis for one or two pesticides is not advantageous, as this approach increases the analysis time for a laboratory involved in the analysis of a variety of pesticides in water.

One of the most recent methods described in Table 7 is based on SPE techniques. Few EPA methods have been changed during the last few years to incorporate SPE instead LLE. In this method only one GC column is needed as MS confirmation is provided by the fragment ions. Special attention should be paid to possible sources of contamination of the cartridges or disks, which often contain phthalate esters, silicon compounds and other con-

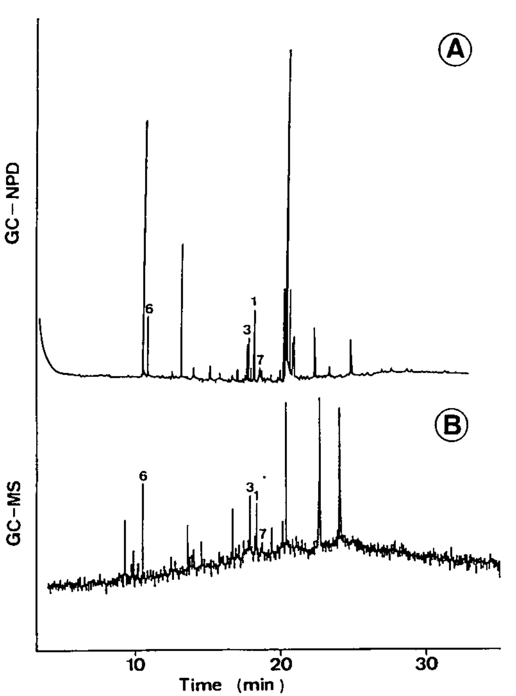

Fig. 2. (A) GC–NPD and (B) GC–MS of an extract of water sample from the Ebro delta containing: (6) molinate (0.050 μg/l), (3) atrazine (0.010 μg/l), (1) simazine (0.012 μg/l) and (7) alachlor (0.005 μg/l). A DB-225 capillary GC column was used.

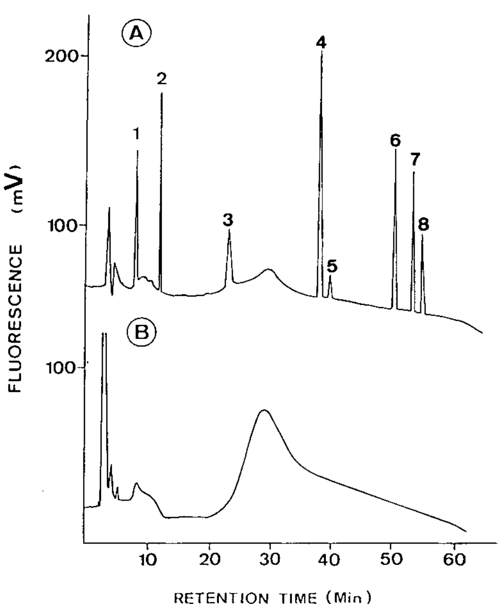

Fig. 3. LC–postcolumn fluorescence detection after preconcentration on C_{18} Empore disks of 10 ml of drinking water spiked with a pesticide mixture at 0. 2 μg/l (A) and a drinking water blank sample (B). Compounds analysed: 1 = aldicarb sulphoxide; 2 = aldicarb sulphone; 3 = hydroxycarbofuran; 4 = aldicarb; 5 = 3-ketocarbofuran; 6 = carbofuran; 7 = carbaryl; 8 = 1-naphthol. A 4-μm Superspher 60 RP-8 LC column (Merck) was used.

TABLE 7

SUMMARY OF EPA AND NPS METHODS

EPA Method 505

Determination of organohalide pesticides and commercial polychlorinated biphenyl (PCB) products in water by microextraction and GC–ECD

Dichloromethane extraction

MDLS (only for commonly used pesticides) (μg/l):

Alachlor	0.075	Metoxychlor	0.96	Simazine	6.8
Atrazine	2.4				

EPA Method 507 (NPS Method 1, see Table 5)

Determination of nitrogen- and phosphorus-containing pesticides in water by GC–NPD

Dichloromethane extraction

EDLS (μg/l):

Alachlor	0.4	EPTC	0.2	Perbulate	0.1
Ametryn	2.0	Ethoprop	0.2	Prometon	0.3
Ametraton	0.6	Fenamiphos	1.0	Prometryn	0.2
Atrazine	0.1	Fenamirol	0.4	Pronamide	0.8
Bromacil	2.5	Fluridone	3.8	Propazine	0.1
Butachlor	0.4	Hexazinone	0.8	Simazine	0.1
Butylate	0.1	Merphos	0.2	Simetryn	0.2
Carboxin	0.6	Methyl paraoxon	2.5	Stirofos	0.8
Chlorpropham	0.5	Metolachlor	0.7	Tebuthiuron	1.3
Cycloate	0.2	Metribuzin	0.1	Terbacil	4.5
Diazinon	0.2	Mevinphos	5.0	Terbufos	0.5
Dichlorvos	2.5	MGK 264	0.6	Terbutryn	0.2
Diphenamid	0.6	Molinate	0.1	Triademefon	0.6
Disulfoton	0.3	Napropamide	0.2	Tricyclazole	1.0
Disulfoton sulphone	3.8	Norflurazon	0.5	Vernolate	0.1
Disulfoton sulphoxide	0.4				

EPA Method 508 (NPS Method 2, see Table 5)

Determination of chlorinated pesticides in ground water by GC–ECD

Dichloromethane extraction

EDLS (only for commonly used pesticides) (μg/l):

Chlorneb	0.5	Etridiazole	0.02	*trans*-Permethrin	0.5
Chlorobenzilate	5	Metoxychlor	0.05	Propachlor	0.5
Chlorothalonil	0.02	*cis*-Permethrin	0.5	Trifluralin	0.02
DCPA	0.02				

EPA Method 515.1 (NPS Method 3, see Table 5)

Determination of chlorinated acids in ground water by GC–ECD

Diethyl ether extraction and derivatization (with diazomethane)

EDLS (μg/l):

Acifluorfen	0.1	DCPA acid metabolites	0.02	4-Nitrophenol	0.1
Bentazone	0.2	Dicamba	0.08	PCP	0.08
Chloramben	0.1	3,5-Dichlorobenzoic acid	0.06	Picloram	0.14
2,4-D	0.2	Dichlorprop	0.3	2,4,5-T	0.08
Dalapon	1.3	Dinoseb	0.2	2,4,5-TP	0.07
2,4-DB	0.8	5-Hydroxy dicamba	0.04		

EPA Method 531.1 (NPS Method 5, see Table 5)

Determination of N-methylcarbamoxyloximes and N-methylcarbamates in ground water by direct aqueous injection HPLC with postcolumn derivatization

Direct injection of water samples. After elution, hydrolysis with 0.05 *M* (NaOH at 95°C, reaction with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol to form a highly fluorescent derivative

EDLS (μg/l):

Aldicarb	1.0	Carbaryl	2.0	Methiocarb	4.0
Aldicarb sulphone	2.0	Carbofuran	1.5	Methomyl	0.5
Aldicarab sulphoxide	2.0	3-Hydroxycarbofuran	2.0	Oxamyl	2.0
Baygon	1.0				

(Continued on p. 128)

TABLE 7 *(continued)*

EPA Method 524.2 (NPS Method 7, see Table 5)
Measurement of purgable organic compounds in water by capillary column GC–MS
Trapping of volatile compounds in a tube, heating and desorption
MDL (μg/l):

1,2-Dibromethane (EDB)	0.02	*cis*-1,3-Dichloropropene	Not determined
1,2-Dibromo-3-chloropropane (DBCP)	0.05	*trans*-1,3-Dichloropropene	Not determined
1,2-Dichloropropane	0.02		

EPA Method 504 (NPS Method 7, see Table 5)
1,2-Dibromethane (EDB) and 1,2-dibromo-3-chloropropane (DBCP) in water by microextraction and GC–ECD
n-Hexane extraction
EDLS (μg/l):

EDB	0.01	DBCP	0.01

EPA METHODS FOR UNUSUAL PESTICIDES

EPA Method 547
Determination of glyphosate in drinking water by direct aqueous injection LC, postcolumn derivatization and fluorescence detection
Filtration into a cation-exchange LC column, oxidation with calcium hypochlorite. The product (glycine) is then coupled with OPA–2-mercaptoethanol complex at 38°C to give a fluorophor
MDL (μg/l):

Glyphosate	6 (for drinking water)
	9 (for ground water)

EPA Method 548
Determination of Endothall in drinking water by aqueous derivatization, liquid–solid extraction and GC–ECD
Derivatization with pentafluorophenylhydrazine (PFPH)
MDL (μg/l): 11.5

EPA Method 549
Determination of diquat and paraquat in drinking water by liquid–solid extraction and LC with UV detection
Extraction with C_8 solid sorbent cartridge in the ion-pair mode
MDL (μg/l):

Diquat	0.4	Paraquat	0.8

EPA Method 525
Determination of organic compounds in drinking water by liquid–solid extraction and capillary column GC–MS
Extraction into C_{18} Empore or cartridge. Elution with dichloromethane
EDLS (only for currently used pesticides) (μg/l):

Alachlor	0.09	Methoxychlor	0.08	Simazine	0.12
Atrazine	0.14				

EPA Method 552
Determination of haloacetic acids in drinking water by liquid–liquid extraction, derivatization and GC–ECD
Extraction with MTBE and diazomethane derivatization
MDL (μg/l):
Trichloroacetic acid 0.08

NPS Method 4 (see Table 5)
Determination of pesticides in ground water by LC with UV detection
Dichloromethane extraction
MQL (μg/l):

Atrazine dealkylated	4.4	Fenamiphos sulphoxide	9.4	Neburon	0.6
Barban	3.8	Fluometuron	0.9	Pronamide metablite	6.3
Carbofuran phenol	42	3-Ketocarbofuran phenol	1.9	Propanil	0.6
Cyanazine	4.7	Linuron	0.9	Propham	11
Diuron	0.6	Metribuzin DA	1.2	Swep	0.3
Fenamiphos sulphone	57				

NPS Method 6 (see Table 5)
Determination of ethylene tiourea (ETU) in ground water by GC with NPD
Use of Extralut column, ETU and elution with dichloromethane
MQL (μg/l): 9.0

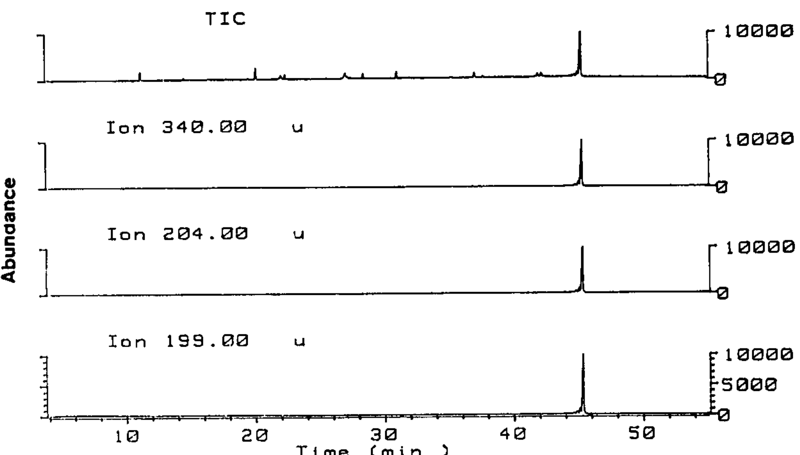

Fig. 4. Total ion current (TIC) and selected ion chromatograms obtained using GC–EI-MS of an estuarine water sample from the Ebro delta which contained 4 μg/l of pyridafenthion. Ions were monitored at *m/z* 340, 204 and 199. An FSQT RSL-300 capillary GC column was used.

taminants [30,31]. Requirements for MS include: (i) scanning should be performed between 45 and 450 u and (ii) the calibrant, bis(perfluorophenyl)phenylphosphine (decafluorotriphenylphosphine, DFTPP) meets all the criteria specified in the method with *m/z* ions varying from 51 up to 443, the most important peaks being at *m/z* 198 and 442. Other relevant ions are at *m/z* 51, 127 and 275. This MS method allows for three different types of MS analysers: magnetic sector, quadrupole and ion trap. The LODs reported in Table 7 correspond to the use of ion trap MS. As an example of the use of Empore extraction discs of C_{18}-bonded silica in combination with GC–MS, Fig. 4 shows the total ion current chromatogram and selected ions of the organophosphorus pesticide pyridafenthion identified in real environmental waters from the Ebro delta area.

The different haloacetic acids determined by EPA methods are also listed. Trichloracetic acid (TCA), which is also very important in the EEC list of pesticides (Table 3), is included. The method reported in Table 7 is also valid for other haloacetic acids and several chlorophenols.

Owing to the need to monitor a variety of pesticides, some of which are not included in the EPA methods for organics in waters, two other methods were developed within the NPS. Method 4 includes most of the TPs of organonitrogen and organophosphorus pesticides, which have shown, in general, good recoveries, varying from 79 up to 97% [13]. For both methods the minimum quantification limit is indicated instead the estimated detection limit (EDL). The value of EDL depends on the degree of interferences to which the method is subjected. So, for methods 4 and 6, the EDL is five and three times lower, respectively [13]. An example of the use of the method indicated in Table 7, although using a water volume of 4 l, is shown on the LC–diode-array detection (DAD) trace in Fig. 5. This corresponds to an extract from a Ebro delta water sample containing low levels of herbicides at 0.1 μg/l, very close to the LOD of LC–DAD. Atrazine (peak 3) could be unequivocally identified from its UV spectrum [7].

3. SCA METHODS OF ANALYSIS

These methods are the Official Methods of the Department of the Environment Drinking Water Inspectorate Standing Committee of Analysts (SCA). Many of these methods, also known as SCA methods, were discussed in two recent reviews [29,32]. Although there are also official methods of analysis for pesticides in water in several EEC countries, these methods will not be discussed here. Differences exist between such official methods of analysis, based on LLE procedures, and other multi-residue methods based on SPE techniques. The number of laboratories that use SPE techniques for the isola-

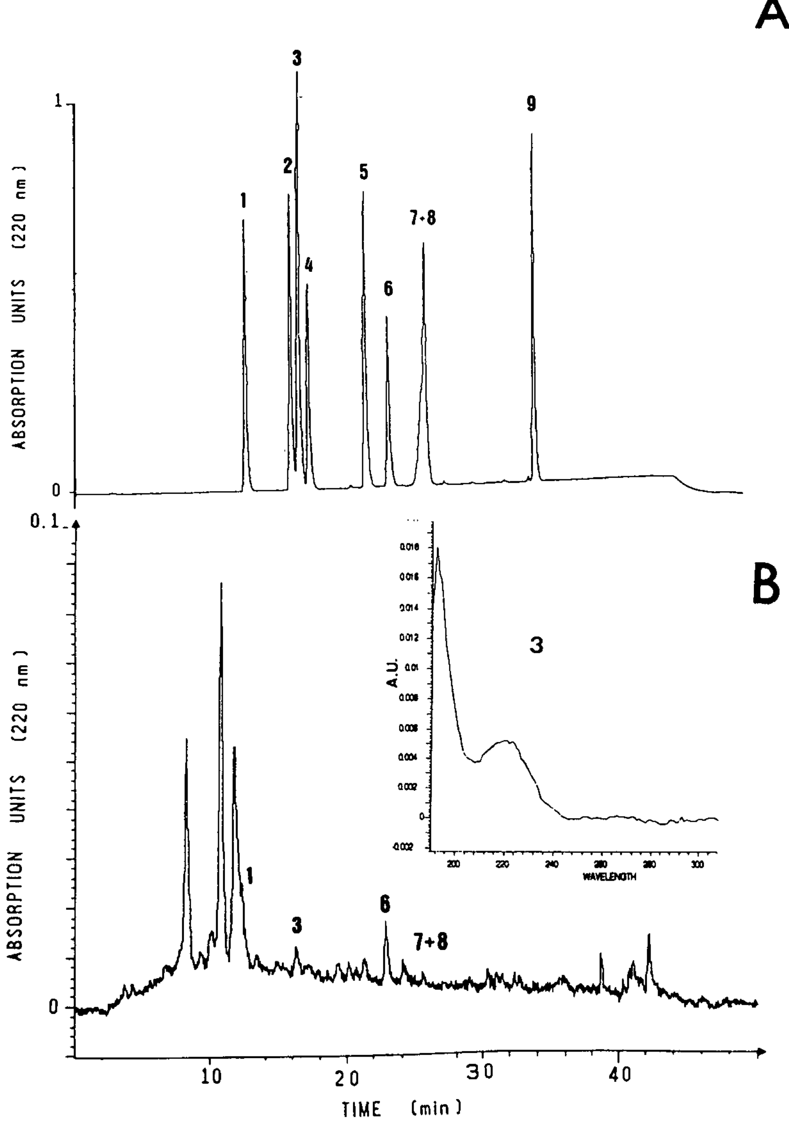

Fig. 5. (A) LC–DAD of a standard sample containing the dichloromethane extract after liquid–liquid extraction of: 1 = simazine; 2 = chlortoluron; 3 = atrazine; 4 = isoproturon; 5 = linuron; 6 = molinate; 7 = alachlor; 8 = metolachlor; 9 = trifluralin. Amount of each pesticide injected, 2 μg. (B) LC–DAD of an extract of an Ebro delta water containing simazine (0.040 μg/l), atrazine (0.010 μg/l), molinate (0.080 μg/l) and alachlor (0.025 μg/l). A Serva LC column packed with 4-μm octadecyl-Daltosil 100 was used.

tion and analysis of pesticides in water in Netherlands [15], Italy [33,34] and Germany [16,35] is increasing. Only selected SCA methods for pesticides in water will be compared with those used by the EPA. One of the current shortcomings in EEC countries is that there is no body similar to the EPA in Europe, so official methods for the whole of Europe have not yet been developed. Similarities and differences between the UK and the USA methods will be shown, with the hope that in the future common EEC methods of pesticides in drinking waters can be discussed.

When comparing the UK and the USA, two aspects need to be mentioned. From the point of

view of pesticides of interest (see Tables 2–5) there are many pesticides which are common. The second important aspect to be mentioned is that in Europe, the levels for any pesticide, as mentioned earlier, have a limit of 0.1 μg/l for drinking water requirements, which is a value much lower than most of the maximum concentration values fixed by the EPA, which are based on health advisory levels (see Table 6). Therefore, in this sense, method development in Europe has been required to produce methods with LODs approximately one order of magnitude lower than the EPA methods, thus causing more difficulties in monitoring a large number of pesticides. The efforts of different research groups working in this area are directed towards achieving the detection limits required by the EEC (which should be at least 0.02 μg/l) in order to determine analytes at 0.1 μg/l. However, many of the UK methods of analysis still do not have LODs as low as the EEC requirements, and only during the last few years has method development been carried out in different European laboratories to achieve such a goal [15,16,33,34].

For the organophosphorus pesticides dichlorvos, dimethoate, malathion, parathion, fenitrothion, chlorfenvinphos, carbophenothion, pirimiphos-methyl and chlorpyrifos, an extraction method involving 25 ml of *n*-hexane and 50 ml of dichloromethane with 1 l of river or drinking water has been employed. The extracts are concentrated to 1 ml in acetone, after evaporation of the dichloromethane extract. Subsequently, 1 μl is injected on to a GC column with flame thermionic detection or FPD [36]. Although the first version of this method used packed GC columns (as in the EPA methods), the SCA method now recommends the use of 25–50-m OV-1 or SE-54 capillary columns [37]. However, the method is less specific than the EPA method and does not include the use of a confirmatory column. The EPA also recommends determining the organophosphorus pesticides as soon as possible, as they can degrade rapidly. The method is based on LLE but uses two extraction solvents, hexane and dichloromethane, instead of only dichloromethane in the EPA method (see Table 7). The use of a mixture of *n*-hexane and dichloromethane allows a better recovery of the less polar organophosphorus pesticides, such as chlorpyrifos, fenitrothion, carbophenothion and pirimiphos-methyl. It is worth mentioning that the SCA method does not result in significant differences in the recoveries between water with high and with low suspended solids. The LOD varies between 0.04 and 0.8 μg/l. In order to compare these results with those of the EPA method (Table 7), it should be mentioned that there are very few organophosphorus pesticides in the EPA method, as degradation of the water solutions kept in a refrigerator occurred rapidly, as reported [14]. One of the compounds, dichlorvos, had an LOD of 0.04 μg/l in the SCA method, which can be attributed to the different way of determining the LOD (by baseline fluctuation and using FPD, which is usually more sensitive to P, as reported [38]).

The SCA method for the determination of triazine herbicides in drinking waters is based on an alkaline extraction (2 ml of ammonia) into dichloromethane (100 and 50 ml), followed by concentration and dissolution in 2 ml of methanol, with injection of 5 μl into the GC–NPD system. A 50-m Carbowax 20M wall-coated open-tubular (WCOT) column is recommended. A detection limit of 0.015 μg/l is estimated for atrazine, simazine, prometryne, propazine and terbutryne [39]. The method does not differ substantially from the EPA method reported in Table 7 for the determination of different organonitrogen and organophosphorus pesticides. It is not necessary to use a 50-m column, as with the EPA method the separation can be achieved using a 30-m column.

A modification of this method has been recently published [40]. This was developed for the determination of the chlorotriazine metabolites deethylatrazine, deisopropylatrazine and hydroxyatrazine. With the use of a mixture of ethyl acetate and dichloromethane with 0.2 *M* ammonium formate, it was possible to increase the extraction recovery of the different chlorotriazine TPs. The final determination was carried out by LC–DAD, which permitted the direct determination of polar metabolites from water samples. Fig. 6 shows the LC–DAD traces for a spiked drinking water sample with 10 μg/l of chlorotriazines extracted with (A) dichloromethane and (B) dichloromethane–ethyl acetate containing 0.2 *M* ammonium formate. The better recovery obtained from the LC–DAD traces is evident, especially for the TPs.

The most extensive list of alternative methods provided by the SCA is for the determination of

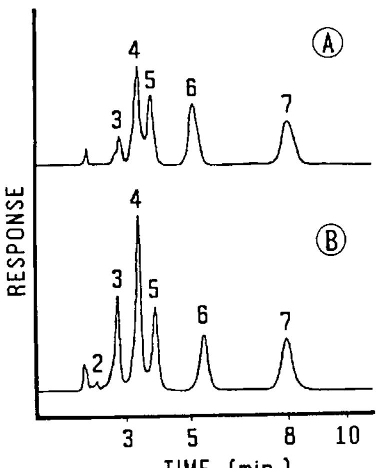

Fig. 6. LC–DAD of a spiked drinking water sample with 10 μg/l of chlorotriazine herbicides extracted with (A) dichloromethane and (B) dichloromethane–ethyl acetate containing 0.2 *M* ammonium formate. Compounds analysed: 2 = chlorodiamino-*s*-triazine; 3 = deisopropylatrazine; 4 = deethylatrazine; 5 = cyanazine; 6 = simazine; 7 = atrazine. DAD detection at 220 nm. A Brownlee cartridge column packed with 5-μm Spherisorb ODS was used.

chlorinated acids [39]. The different methods, as for the EPA (see Table 7), are based on the formation of derivatives followed by GC–ECD. As the SCA method recommends a different derivatization for each of the chlorinated acid herbicides, it is interesting to present a summary of all the alternatives (Table 8). The different methods indicated in this table generally use 1 l of water, with an acidic extraction with diethyl ether, followed by hydrolysis and derivatization and final concentration to 1 ml. Volumes of 5 μl of the sample are injected on to the GC column using a 25-m fused-silica WCOT column containing a methylsilicone stationary phase. The methylation, indicated in Table 8 under Method B, is the most similar to the EPA method. The method for the acidic herbicides it is also valid for other compounds such as polychlorinated phenols.

It should be noted that DCPA (Dacthal or Chlorthal) and DCPA acid metabolites are not included in the SCA methods of analysis, probably because the parent compound is not used in Europe. No evidence of its use was found when ground waters from different European countries were monitored [5]. In contrast, such compounds, as we have seen previously, are the most relevant herbicides detected in the NPS. DCPA acid metabolites can be analysed by Method B in Table 8, which is similar to the EPA method (Table 7). This compound and its TPs are fairly stable in soil, with half-lives of 100 and 365 days, respectively [12]. The incidence of this acidic herbicide is a notable difference between the USA and Europe, as it can affect the water supply of more than 10 million people

TABLE 8

SUMMARY OF SCA METHODS

SCA methods foe the determination of chlorinated phenoxy acids in water

Method A. Extraction, hydrolysis, butylation and GC–ECD
Preferred for: 2,4-D, 2,4,5-T and dalaphon
LOD (μg/l): 2,4-D 0.024; 2,4,5-T 0.004

Method B. Extraction, hydrolysis, methylation and GC–ECD
Preferred for: 2,3,6-trichlorobenzoic acid (TBA), dicamba, polychlorophenols
Also suitable for: 2,4-D, 2,4,5-T and dichlorophenols
LOD (μg/l): 2,4,6-trichlorophenol 0.07; 2,4,5-trichlorophenol 0.2; 2,3,4,6-tetrachlorophenol 0.02; pentachlorophenol 0.02; 2,3,6-TBA 0.0005

Method C. Extraction, perflurorobenzylation and GC–ECD
Preferred for: MCPA, MCPB and MCPP (mecoprop)
Also suitable for: dicamba and TBA
LOD (μg/l): MCPP 0.11; dicamba 0.10; MCPA 0.08; 2,3,6-TBA 0.08; 2,4-D 0.14; 2,3,5-T 0.11; MCPB 0.10

Method D. Extraction, hydrolysis, methylation and GC–MS
Preferred for: MCPA, MCPB, MCPP, 2,4-D and 2,4,5-T
LOD (μg/l): 1 (two suitable ions for each analyte are used in the multiple ion detection)

Method E. Extraction, hydrolysis, nitration, methylation and GC–ECD
Preferrd for: MCPA, MCPB and MCPP
LOD (μg/l): MCPA 0.004

SCA method for the determination of synthetic pyrethroid insecticides in waters by gas–liquid chromatography

Extraction of 1 l of water with *n*-hexane with GC–ECD analysis and confirmation by GC–MS with negative chemical ionization (NCI). A DB-5 column is used
LOD (μg/l): 0.01
Suitable ions for GC–NCI-MS confirmation:

Pyrethroid	Ion (*m*/*z*)
Cyhalothrin	205, 241
Permethrin	207, 209
Cyfluthrin	207, 209
Cypermethrin	207, 209
Deltamethrin	79, 81

within the USA, whereas in Europe it has not been detected.

The determination of glyphosate does not differ substantially from the EPA method (Table 7) [39]. In the SCA method the sample is concentrated by evaporation and passed through an ion-exchange column. After further concentration the glyphosate (and its major TP aminomethylphosphonic acid) is separated by reversed-phase LC and fluorogenically labelled using OPA and mercaptoethanol, before fluorimetric detection. The LOD is 0.08 μg/l when concentrating 1 l of water sample to 5 ml with injection of 20 μl into the LC system. The difference in the EPA method is that no concentration of the sample is carried out as a large injection loop of 200 μl enhances the detection limit. In the SCA method more manipulation of the sample takes place, thus making possible a better LOD which closely meets the requirements of the EEC Drinking Water Directive.

Carbamates are determined by LC, with either normal- or reversed-phase systems. Here, the method involving reversed-phase systems will be discussed, as it is more frequently used. It allows the determination of most carbamates and urea herbicides in river and drinking waters and in addition allows the determination of soluble dithiocarbamates. A 1-l volume of water sample is concentrated by extraction using 50 + 25 ml of dichloromethane, with prior acidification of the solution to pH 3. After evaporation, the sample is dissolved in 500 μl of acetonitrile or methanol and injected on to the LC column using a 20-μl loop. The method is valid for all the carbamates with the exception of benomyl, which needs adjustment of the pH to 11 with sodium hydroxide prior to extraction. The LODs (μg/l) were 0.08, 0.05, 0.05, 0.02 0.04, 0.04, 0.02 and 0.04 for perbulate, EPTC, triallate, propham, carbaryl, methiocarb, benomyl and dinocap, respectively. The wavelength recommended for the analysis is 220 nm, with the exception of benomyl and dinocap, which are monitored at 364 nm. The method is, evidently, less selective than the EPA method (see Table 7) but is apparently more sensitive with lower LODs. It should be borne in mind that in the EPA method the water samples are directly injected using a 400-μl loop, and considering this fact, the LODs are excellent. When a few millilitres of the sample are concentrated (see Fig. 3), then the EPA method is much more sensitive. A drawback of the SCA method is that it does not specify the column type and, in contrast to EPA, does not indicate the use of a second column for confirmation purposes. This should be recommended as detection at 220 nm is not very selective.

For the determination of dithiocarbamates and related compounds such as maneb, mancozeb, nabam, zineb, ferbam and thiram, the water sample is heated with acid in the presence of tin(II) chloride and 2,2,4-trimethylpentane (isooctane). The carbon disulphide formed dissolves in the isooctane and is determined by GC–FPD [41]. The LOD is 0.48 μg/l (0.84 μg/l as maneb). A great disadvantage of this method is that the result corresponds to the total of the compounds listed, together with any others that undergo the same reaction. During the last few years an elegant LC method based on postcolumn complexation of the dithiocarbamates with finely divided copper to form a coloured complex has been developed [42]. Fig. 7 shows the analysis of a surface water sample spiked with 10 mg/l of thiram.

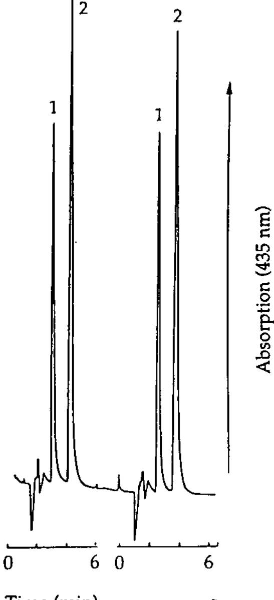

Fig. 7. Chromatograms for the duplicate injection of surface water spiked with 10 mg/l of thiram and 20 mg/l of Cu(dimethyldithiocarbamate)$_2$. LC conditions: column packed with 5-μm Hypersil ODS; copper reactor, 2.0 × 2.1 mm; eluent, acetonitrile–10 mM aqueous acetate buffer (pH 5.0) (70:30, v/v); flow-rate, 0.3 ml/min; wavelength, 435 nm.

Synthetic pyrethroids such as permethrin, cypermethrin, α-cypermethrin, fenvalerate and deltamethrin are determined using solvent extraction of 1 l of water with 100 ml of hexane, with clean-up methods involving Florisil (or aminopropylsilica or alumina) and with analysis by GC–ECD with confirmation by GC–MS with negative chemical ionization (NCI) [43]. The method allows an LOD of 0.005 μg/l for each of the studied compounds, which complies with EEC Directives. The columns used are 30 m × 0.33 mm I.D. DB-5 or SE-54. This method is the first that recommends the use of NCI in the selected ion monitoring mode and is in contrast to the EPA methods, which only use GC–MS in the conventional EI mode. In this case, the SCA method also indicates that when MS facilities are not available, another capillary column coated with a different stationary phase should be used for confirmation purposes. This method is clearly more specific and advanced than the EPA method, as (i) more pyrethroids are analysed, (ii) there are three options of clean-up steps and (iii) the use of NCI is recommended. A summary of the method is given in Table 8.

The SCA method for diquat and paraquat involves concentration by ion exchange, reduction with alkaline sodium dithionite and determination of the reduced compound by visible light spectrophotometry by direct or second derivative measurement. The LOD for a 5-l water sample is 0.4 μg/l (direct) or using the second derivative 0.02 μg/l [44]. The problems with this method are the interferences, as any component remaining after the procedure which absorbs light in the relevant region of the visible spectrum will interfere. The maximum wavelengths for measurement are: 396 and 379 nm for paraquat and diquat, respectively. The EPA method has advantages as compounds are separated by LC, with a better elimination of interferences, and further confirmation by DAD.

To summarize the general similarities and differences between the EPA and SCA methods, for SCA methods (i) less emphasis is placed on the use of confirmatory columns (to avoid false positives), surrogates and internal standards, (ii) the number of compounds to be monitored is smaller than the 126 in the NPS–EPA list, so fewer screening methods are available, (iii) DCPA, the most important herbicide within the USA, and its TPs are not monitored although there are generally similarities in compounds and methods between the EPA and SCA, (iv) virtually no information is offered for the analysis of TPs whereas the NPS has already introduced a method that monitors up to 25 TPs, (v) they are based on GC (changing from packed to capillary columns as in EPA methods), whereas few LC methods are used, (vi) two of the methods (for pyrethroids and phenoxy acids) are superior to the EPA methods, as confirmation by GC–MS with NCI with an extended list of pyrethroids is shown and for phenoxy acids they offer three different alternatives of derivatization, depending on the compound to be analysed and also GC–MS confirmation, (vii) less selectivity and sensitivity for the analysis of quats and carbamates compared with the EPA (when concentrating 10 ml of water, the EPA method for carbamates can go as low as 5–10 ng/l) and (viii) for triazines and organophosphorus pesticides there are not many differences compared with the EPA methods.

To conclude this comparison, we can state that, critically, all the official methods of analysis can be improved. In general, it can be commented that within Europe, method development for the determination of pesticides in drinking water is more of a requirement and more needed than in the USA because of the more stringent limits relating to the quality of drinking water. Another general remark concerning all of the methods is that too many official methods are still based on LLE, with the associated problems of solvent disposal. The future within Europe will certainly be the development of screening methods for a wide range of pesticides based on SPE principles, either off-line [33] or on-line [34] with LOD of at least 0.02 μg/l, thus permitting the determination of 0.1 μg/l of each individual pesticide.

An example of a such a way to proceed is shown in Fig. 8 with an on-line LC–DAD analysis obtained after preconcentration on C_{18} Empore extraction disks of 350, 500 and 1000 ml of tap water sample spiked at 0.2 μg/l levels with a pesticide mixture that includes carbamates and TPs. The water volume that needs to be preconcentrated for achieving an LOD that will satisfy the EEC Drinking Water Directive can vary between 150–350 ml.

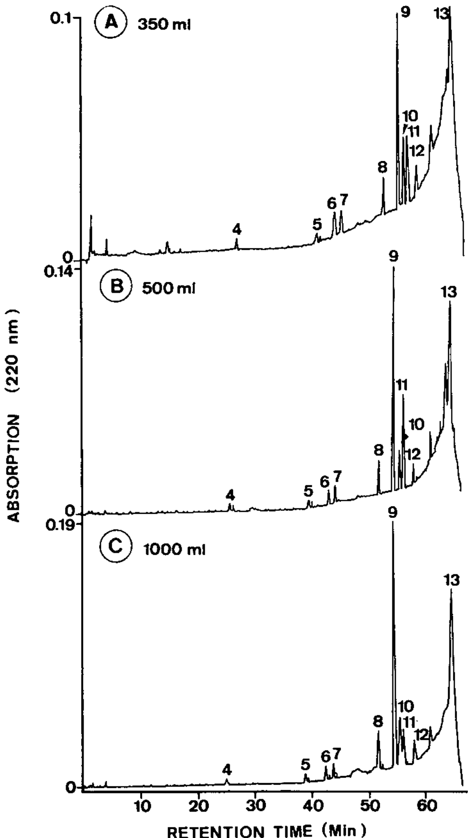

Fig. 8. LC–UV detection after preconcentration on C_{18} Empore extraction disks of (A) 350, (B) 500 and (C) 1000 ml of drinking water spiked at 0.2 μg/l with (1) aldicarb sulphoxide (2) aldicarb sulphone, (3) 3-hydroxy-7-phenol carbofuran, (4) 3-hydroxycarbofuran, (5) 3-ketocarbofuran phenol, (6) aldicarb, (7) 3-ketocarbofuran, (8) carbofuran, (9) carbaryl, (10) chlortoluron, (11) 1-naphthol, (12) isoproturon and (13) metolachlor. A 4-μm Supersphere 60 RP-8 LC column was used.

4. OTHER GC METHODS

Capillary gas chromatography (GC) in combination with selective detection methods, mainly nitrogen–phosphorus (NPD), electron-capture (ECD), flame photometric (FPD) and mass spectrometric (MS), is still the most common technique for the determination of environmental pesticide residues in water, as shown in the above discussion of official methods of analysis. The low LOD, high selectivity and affordability of GC instrumentation is appealing to most laboratories involved in pesticide residue analysis. Several reviews on the use of GC–NPD, GC–ECD and GC–MS have been published [45,46]. Recently, a book presenting various GC and LC approaches, either for multi-residue analysis and for specific groups of compounds, such as carbamates and organophosphorus and organonitrogen compounds, has been published [47].

Examples of the use of GC–NPD for the routine determination of organophosphorus and organonitrogen pesticides in water samples following a multi-screening method similar to the EPA method 507 (Table 7) have been reported [7,48,49]. Examples of organonitrogen and organophosphorus pesticides determined were ametryne, atrazine, atraton, prometryne, metolachlor, fenitrothion, fenthion and parathion-methyl. However, in recent years, as already mentioned, methods based on SPE, instead of the conventional dichloromethane LLE, have been developed. Examples of the use of SPE, using C_{18} silica cartridges followed by either GC–ECD (for atrazine, alachlor, metribuzin and metolachlor [50]), GC–NPD (for carbaryl, carbofuran, fonofos, parathion, alachlor, cyanazine and metribuzin [51, 52]) or GC–alkali flame ionization detection (for organophosphorus pesticides such as pyridafenthion and tetrachlorvinphos or triazines such as atrazine and prometryne [53]) have been reported.

The use of SPE methods has been of increasing interest in the last few years for the isolation of pesticides from water and will probably replace conventional LLE not only in research laboratories (where it is already fairly common), but also in government laboratories, where conventional LLE procedures are still very much in use. The application of SPE has been expanded recently by the use of a novel product, *viz.*, Empore extraction disks containing either C_{18} or polystyrene–divinylbenzene material. These can be used in a similar way to cartridges but with major advantages such as faster extraction owing to the lack of channelling and faster mass transfer owing to smaller pore sizes (8 μm *versus* 40–60 μm). It has been applied to the determination of various pesticides in water matrices, followed by GC–ECD [54]. Recently Empore disks have been coupled on-line with GC–NPD for the direct analysis of 2.5-ml water samples containing various organophosphorus pesticides with LODs of 0.1 μg/l being achieved [55].

Electron-capture detectors (the most commonly

used for classical chlorinated pesticides such as DDT and endrin, which are not discussed here) are resorted to when the molecule contains chlorinated groups (*e.g.*, atrazine, chlorpyrifos, metoxychlor and trifluralin). GC–ECD is the method of choice for the identification of several unstable pesticides which need derivatization prior to GC–ECD determination. Examples are carbamates (trichloroacetyl), chlorinated phenoxy acids (pentafluorobenzyl, methyl esters) and urea pesticides (heptafluorobutyric esters) [22,24,56–58]. Some of these methods include a confirmation procedure, using the derivatives formed, by GC–MS [22,24,58]. Most of the derivatization methods developed are for the acidic herbicides and usually refer to EPA methods (see Table 7). These methods are usually more rapid [56] or introduce refinements related to sampling, clean-up, confirmation of compound identity and quality assurance [58]. For instance, for the detection of acidic herbicides at the 0.02–0.05 μg/l level, the pentafluorbenzyl derivatives are recommended in preference to the methyl esters formed by the classical diazomethane reagent [58], as the latter method lacks sensitivity at the low level of detection required for the monitoring of pesticides in drinking water samples within the EEC.

In order to avoid "false positives" in the determination of pesticides in water samples, confirmatory techniques are needed. As we have seen with the EPA methods, such confirmation is usually achieved by injecting the sample extract on to a second column of different polarity. However, such comparisons do not constitute a foolproof means of confirmation. Another way to carry out confirmation by using a second column is the application of so-called two-dimensional capillary GC, where two columns of different selectivity are combined in such a way that a fraction of the eluate can be directly transferred from one column to another. The different aspects, involving valve switching, pneumatic switching, pneumatic effluent transfer, the different modes of operation (cut, straight and backflush), etc., have been discussed in a review [59]. Examples of the use of linked response data from parallel PFD and ECD instruments with retention data from linear temperature programming [60], and even with the use of three selective detectors (FPD, NPD and ECD types), have recently been published [61]. A third approach is the use of chemical derivatization, which is a technique that has found substantial use in pesticide residue analysis when other means of confirmation were not available. Examples of the use of reagents and chemical reactions for organophosphorus pesticides have been reported recently [38]. The formation of a derivative, *e.g.*, after trifluoroacetylation, means that the original pesticide peak disappears and the derivative, with a different retention time, appears, thus providing confirmation.

GC–MS is the most widely used confirmation technique. The increasing importance of this approach in the determination and confirmation of pesticides in water is linked to the fact that the EPA and SCA methods previously discussed have already implemented GC–MS in some of their protocols, with a tendency to include MS confirmation in the future or in new modified methods. EPA method 525 (see Table 7), based on SPE with either cartridges or disks, has also been evaluated [31], and showed low interferences from the disks in the background mass spectra. A screening method based on the use of SPE with various SPE materials (C_{18}-, amino- and phenyl-bonded) and GC–MS determination for 50 pesticides at sub-μg/l levels, *e.g.*, atrazine, propanil, trifluralin, chlorpyrifos and tetradifon, was developed by the Mario Negri Institute [33]. The isolation of several triazines was evaluated using Sep-Pak C_{18} SPE cartridges [62] and by using a styrene–divinylbenzene copolymer such as PLRP-S [63] in combination with GC–MS with various ionization modes such as EI and positive and negative chemical ionization (PCI and NCI). The use of XAD-2 and XAD-7 in combination with GC–MS with an ion-trap analyser has been reported for several pesticides, *e.g.*, alachlor, diazinon and metribuzin [64]. C_{18} cartridges in combination with isotope dilution GC–MS has been reported for maize herbicides with an LOD of 0.05 μg/l [65].

Applications of the use of LLE based on dichloromethane extraction, similar to the EPA method (Table 7), for oganonitrogen compounds in combination with quadrupole GC–MS [7] and ion-trap GC–MS [66] have been reported for common maize herbicides, such as atrazine, alachlor, metolachlor and simazine.

Although most of the confirmation is carried out by using GC–MS with EI, NCI is increasingly recommended, as has already observed in the SCA

method for pyrethroids. Recent work has also demonstrated the use of this technique for the determination and confirmation of acidic herbicides, *e.g.*, MCPA and dicamba, in natural waters at levels of 0.02 μg/l [67].

In this review, methods for determination of organometallic compounds used as pesticides have not been mentioned. In a recent review references relating to methods for the determination of these compounds are given [45]. It is worth indicating that a common method for the determination of organometallic compounds, *e.g.*, butyltins, involves dichloromethane and tropolone extraction. An LOD of 5 ng/l can be achieved for tributyltin in sea water using GC–MS with an ion-trap detector [68].

5. LC TECHNIQUES

LC systems used for environmental pesticide analyses have been extensively reviewed in two recent papers [45,69]. The increasing use of LC methods for pesticides is chiefly the result of their suitability for thermally labile and polar herbicides, including their TPs, which require derivatization prior to GC analysis. LC methods of analysis also have a major advantage over GC methods in that on-line pre- and postcolumn reaction systems are compatible with LC. LC is therefore becoming an important tool for analysing modern pesticides and their TPs in monitoring programmes, *e.g.*, the different EPA and NPS methods, for the determination of carbamates, unusual pesticides and TPs which are shown in Table 7. This is also due to the development, during the last few years, of UV diode-array detectors with better sensitivity than similar detectors used a few years ago, so making their use in environmental analysis attractive.

The use of a UV detector in LC in conjunction with off-line LLE or SPE is still the most common choice in environmental pesticide analyses of water samples. Information on specific wavelengths, LC eluents and columns for over 200 pesticides is available in the literature. UV detectors are no doubt the most commonly available in laboratories and also traditionally the most frequently used in LC. In Table 9 the UV characteristics of relevant pesticides are shown. These data were summarized from the literature [7,15,16,35,69,70].

Several off-line LLE methods using dichloromethane have been reported covering several groups of pesticides [7,29,71,72]. These methods do not differ substantially from the NPS method reported in Table 7, followed by LC–UV or DAD. In two instances [7,72], further acidification of the water sample to pH <2 allows the extraction of acidic herbicides. Many of the compounds are difficult to determine using GC methods, *e.g.*, isoproturon, linuron and bentazone. Off-line SPE procedures involving packing materials which may contain functional groups of different polarity such as C_8- or C_{18}-bonded silica phases, graphitized carbon black or Amberlite XAD resins have been reported. C_8- and C_{18}-bonded phase cartridges have been used for the development of screening methods for various organonitrogen pesticides such as urons and triazines [35,71] and carbamates and their TPs such as carbofuran, 3-hydroxycarbofuran-7-phenol and 3-ketocarbofuran [73,74]. The use of SPE with acidified water followed by ion-pair LC was developed for the determination of various chlorinated herbicides, *e.g.*, 2,4-D and dicamba in waters [75]. The use of off-line Empore extraction disks in combination with LC–DAD has been developed for the determination of a variety of pesticides, including carbaryl, linuron and fenamiphos in different water matrices, *e.g.*, surface river and simulated sea-water samples. The method, much faster than using C_{18} cartridges, could easily handle 4-l water samples, allowing an LOD of 0.02 μg/l, which is appropriate for the determination of pesticides in drinking waters within the EEC [76]. Graphitized carbon black has been shown to offer effective trapping possibilities for polar pesticides such as aldicarb, diuron and bentazone, and by using flow-rates of 150 ml/min for preconcentrating up to 2 l of river and drinking water [77]. Eighty-nine pesticides of environmental interest were analysed using two different columns, a primary C_{18} and a confirmation cyano column, in a similar way as recommended in the NPS method in Table 7. The good recoveries obtained when using this adsorbent allowed LODs of less than 0.1 μg/l, and consequently it is recommended for demonstrating compliance with the EEC Drinking Water Directives.

SPE methods can be easily converted into fully automated on-line systems coupled to LC. Such systems, also referred to as "precolumn technology", show additional advantages such as lower

TABLE 9

UV CHARACTERIZATON OF PRIORITY PESTICIDES AND TRANSFORMATION PRODUCTS [7,15,16,35,69,70]

Pesticide	UV absorption (nm)	EEC [5]	NPS [14] (nm)
Alachlor	200	×	×
Aldicarb	207, 220, 247	×	×
Aldicarb sulphone	<200		×
Aldicarb sulphoxide	<200		×
Ametryn	220		×
Atrazine	222, 263	×	×
Barban	205, 237		×
Baygon (propoxur)	200, 220		×
Bentazone	219, 232, 316	×	×
Bromacil	210, 277		×
Carbaryl	220, 270	×	×
Carbendazim	223, 280	×	
Carbofuran	200, 225, 279		×
Chloridazon	229, 284		×
Chlorpropham	210, 237		×
Chlorpyrifos	230, 289	×	
Chlortoluron	211, 243	×	
Cyanazine	220	×	×
2,4-D	208, 224	×	×
2,4-DB	208		×
Dalapon	No UV	×	×
Desethylatrazine	214		×
Desisopropylatrazine	214		×
Diazinon	248, 288	×	×
Dicamba	277		×
Dichlorprop	208, 228, 285		×
Dichlorvos	No UV		×
Dinoseb	211, 269	×	×
Disulfoton	No UV		×
Diuron	211, 252	×	×
ETU	231		×
Fenamiphos	200, 248	×	×
Fenamiphos sulphone	200, 226		×
Fenamiphos sulphoxide	200, 236		×

Pesticide	UV absorption	EEC [5]	NPS [14]
Hexazinone	254		×
3-Hydroxycarbofuran	206		×
3-Hydroxy-7-phenol carbofuran	<200, 208		×
Isoproturon	201, 243	×	
3-Ketocarboforan phenol	<200, 215		×
Linuron	211, 249	×	×
MCPA	200, 230	×	
Metazachlor	<200, 220	×	
Methabenzthiazuron	223, 269	×	
Metham-sodium	208, 232	×	×
Methiocarb	225, 254, 265	×	×
Methomyl	220, 232		×
Metolachlor	202	×	×
Metribuzin	295		×
Mevinphos	218		×
Molinate	<200, 208		×
1-Naphthol	210, 232		×
Napropamide	214		×
Norflurazon	239		×
Oxamyl	216		×
Permethrin	271	×	×
Prometon	219		×
Prometryn	223, 254	×	×
Propachlor	<200		×
Propazine	254		×
Simazine	223, 244, 263	×	×
Stirofos (tetrachlorvinphos)	210, 250		×
2,4,5-T	214		×
Terbutylazine	225	×	
Terbutryn	225	×	×
Trichlorfon	<205	×	
Trifluralin	211, 233		×
Vinclozolin	200	×	

detection limits (analysis of an eluate instead of a sample aliquot), no evaporation losses, no contamination and easy automation. Similarly to off-line techniques, different packing materials have been employed in the precolumn, the most common so far being C_8- or C_{18}-bonded silica [16,78–86]. Coupling of various precolumns of different chemical composition, whether isolated or serially connected, usually packed with C_{18} and PRP-1 (styrene–divinylbenzene copolymer), has been demonstrated to exhibit better clean-up possibilities, as interfering compounds are trapped on the C_{18} precolumn which acts as a filter [78,84]. In other instances the C_{18} precolumn was coupled to a short concentration column containing an "aniline" filter, in order to separate in the same chromatographic run phenylurea herbicides and their corresponding anilines [80]. For the determination of acidic herbicides, *e.g.*, bentazone, the C_{18} precolumn was flushed with phosphate buffer in order to trap these herbicides [82]. The combination of two column-switching devices using longer C_{18} precolumns increases the selectivity by applying a "cutting" technique, and the sensitivity by using large injection volumes. This

technique has allowed the determination of ethylenbisthiourea (ETU) in water samples, providing an elegant way of decreasing the LOD to 0.1 μg/l, which is much lower than that achieved with the EPA method reported in Table 7 [85]. It has also been used for the determination of chloroallyl alcohol, a metabolite of the soil sterilant 1,3-dichloropropene (see Table 7) [83].

In the last few years another styrene–divinylbenzene copolymer, PLRP-S, has become popular. This has been employed in an on-line early-warning system for the monitoring of 50 pesticides in river water [15] and also in combination with the Prospekt apparatus, a fully automated device with a cartridge-exchange system that permits the separation of a variety of compounds with automation of the relevant parameters of the preconcentration step such as pH, volume and ionic strength of the sample. The system combined with UV detection [87] or DAD [88] provided a powerful approach for the automated on-line determination of a broad range of pesticides in water matrices. On-line preconcentration using a two-step approach with PRP-1 in combination with an ion-exchange precolumn has allowed the determination of various chlorotriazines and urons in water at the 10 ng/l level. PRP-1 acts as a powerful filter to remove many neutral interferents present in drinking water samples [89,90]. The on-line coupling of Empore extraction disks with LC–UV [91,92] and LC–postcolumn fluorescence detection and DAD [93] has allowed the determination of various groups of pesticides, *e.g.*, triazines, carbamates and their polar TPs. Empore disks have higher breakthrough volumes and their small particle size (8 μm) eliminates channelling.

Most of the examples of the use of on-line precolumn systems in LC use UV detectors; they are set at different wavelengths according to the pesticides to be determined (*e.g.*, 247 nm for phenylurea pesticides [78–80], 220 nm for carbamates [81,86], 230 nm for phenoxy acids [86] and 233 nm for ETU [85]). Other detectors used include electrochemical and fluorescence [78,84] and DAD instruments, which permit structural information to be obtained; DAD is being increasingly used in monitoring programmes for screening for a variety of non-polar and medium-polarity pesticides in river water samples, *e.g.*, Rhine Basin Programme [15,16,88].

On-line precolumn technology with selective detectors can provide another powerful means of determining pesticides in water, *e.g.*, N-methylcarbamates and O-(methylcarbonyl)oxime pesticides have been determined by employing the same reaction as proposed in the EPA method reported in Table 7. This has allowed low-level determinations of carbamates and their polar TPs in drinking water matrices at 5–40 ng/l [93]. Other examples include the use of oxidation and derivatization reactions with OPA for the determination of glyphosate, a highly polar herbicide, with detection by fluorogenic labelling [94]. One of most complete multi-screening methods developed for the NPS includes the use of postcolumn photolysis followed by fluorescence, electrochemical or conductivity detection, and permitted the detection of over 100 of the pesticides included in this programme (see Table 7) [95].

The combination of LC with MS is the most powerful approach for the detection and confirmation of pesticides in water matrices. It is certainly the preferred approach to avoid false positives. Of the different LC–MS methodologies, thermospray (TSP) and particle beam interfacing (PB) systems are probably the most widely used in water analysis. References to the use of both TSP [96–98] and PB [99–101] have been reported. The most complete screening study based on positive ion LC–MS determination used off-line LLE and SPE [96]. The method permitted the simultaneous determination of 29 pesticides in water samples, with MDLs in the μg/l range, and relative standard deviations of 11–17%. A similar approach to that reported in ref. 96, but with the combined use of positive and negative ionization (PI and NI, respectively) thermospray LC–MS is shown in Fig. 9, which shows chromatograms of a well water extract obtained after LLE with dichloromethane. Another complete multi-screening method which has recently been published uses particle beam LC–MS for the identification and determination of 43 of the 126 NPS pesticides (see Table 5). For these analytes it was feasible to confirm their presence at 0.1 μg/l in water [100].

Buffers and ion-pairing agents present in the LC mobile phases which may interfere with detection can be removed by using a postcolumn extraction system to transfer the organic phase to the detector while the inorganic ions remain in the aqueous layer. This procedure has been used for the ion-suppressed

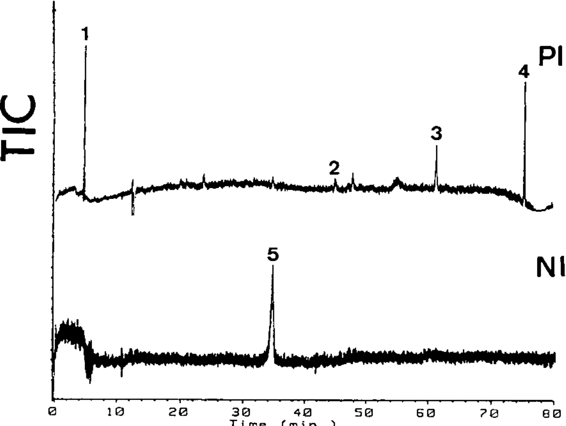

Fig. 9. LC–TSP-MS with PI and NI of a well water extract obtained after LLE using dichloromethane. Compounds determined: 1 = methomyl (13 ng/l); 2 = butocarboxim (18 ng/l); 3 = carbaryl (30 ng/l); 4 = methiocarb (250 ng/l); 5 = methiocarb sulphone (320 ng/l). Ions monitored in PI were $[M+H]^+$ for methomyl and methiocarb at *m/z* 163 and 226, respectively, and $[M+NH_4]^+$ for butocarboxim and carbaryl at *m/z* 208 and 219, respectively. In the NI mode the $[M-H-CH_3COONH]^-$ ion at *m/z* 199 was monitored for methiocarb sulphone.

extraction of chlorinated phenoxy acid pesticides with on-line MS detection [98].

A final remark on the use of LC–MS interfaces for quantitative purposes concerns the problems associated with interlaboratory comparisons of data and validation of results. These aspects have been recently pointed out in a comparison of TSP and PB interfaces [102] and various atmospheric pressure ionization (API) techniques [103] for the determination of a variety of pesticides, including chlorinated phenoxy acids and N-methylcarbamates. From these studies it was shown that the TSP interface gave better sensitivity in the NI mode for the chlorinated phenoxy acid herbicides than did PB. Statistically significant differences in quantification at 50 μg/ml were shown with average relative standard deviations of 36% and 49% for PB and TSP interfaces, respectively [102]. Another interesting study showed more problems with the PB interface, *e.g.*, nonlinearity for the determination of carbamates. API with a heated nebulizer interface offered good sensitivity and linearity, providing protonated molecular mass information and abundant fragment ions for structural information [103]. It was concluded that API and TSP performed comparably for the determination of carbamate pesticides, and either was preferable to the PB interface.

6. CONCLUSIONS

From the methods of analysis reported in this review, it is clear that considerable differences exist between the official methods of analysis used in different countries and the newest techniques used in research and other laboratories. The progress in incorporating modern analytical methods, *e.g.*, the use of SPE techniques, capillary GC columns or MS confirmation, into official methods is slow. However, advances are taking place, especially within the EPA.

From the results reported from the National Pesticide Survey in collaboration with the EPA, several general comments can be made concerning the determination of pesticides in drinking water samples. (i) These methods described in detail all the parameters necessary for a good monitoring programme for pesticides, and consist of the most complete study published to date, *e.g.*, storage of samples, with preservation by adding $HgCl_2$ or monochloroacetic buffer; the use of replicate analysis after storage for 0, 14 and 28 days in a refrigerator at 4°C; the use of two different columns (at least), one primary column and a secondary column of different polarity which is used for confirmation purposes. When MS is used, then only one GC column is employed, as confirmation is achieved by MS. (ii) The NPS has for the first time monitored many TPs of pesticides. (iii) The development of microextraction LLE methods, SPE and GC–MS methods is being encouraged and is one of the strongest recommendations of the EPA.

From the final report of the NPS for different drinking water wells, it was shown that DCPA acid (and its metabolites) was the pesticide that occurred in the greatest proportion of community water wells and rural domestic wells, and it has been estimated that over 10 million people are exposed to this pesticide. However, very few are expected to be exposed to levels above the health advisory level. Other pesticides found in 0.1–6.4% of wells were atrazine, simazine, prometon, lindane, ETU, bentazone and alachlor; hexachlorobenzene and dibromochloropropane, ethylene dibromide and dinoseb were also found but their registration has been cancelled by the EPA [12].

Future recommendations for work are as follows. (i) There is a need to develop off-line SPE techniques based on new adsorbent types (*e.g.*, styrene–divinylbenzene copolymers, carbon types or Empore disks) in combination with GC–MS using selective ionization methods, *e.g.*, NCI. In the same way as NCI has been used for pyrethroids in the SCA methods of analysis, it could be a technique recommended for confirmation of organophosphorus pesticides exhibiting electron-withdrawing properties, *e.g.*, the parathion group. (ii) The on-line combination of SPE with LC–(UV)–MS, using either TSP, PB and/or API, will be welcome for screening the more polar pesticides and their polar TPs. Certainly such an approach will allow the determination of pesticides by on-line LC–UV and confirmation by MS, without the need to use derivatization steps. It is worth mentioning that there are still no official methods of analysis involving LC–MS for confirmation. (iii) The development of specific methods of analysis for particular pesticides will be of interest, *e.g.*, the EPA methods for unusual pesticides (*e.g.*, quats or glyphosate). The EEC has defined the need for developing analytical methods with low LODs for difficult pesticides such as maneb, ziram and metham-sodium, among others. (iv) The development of immunochemical methods, radioimmunoassay or enzyme-linked immunoassays will be of interest in the future, especially when linked to chromatographic techniques. Until now most of these techniques have been used for the detection of pesticides in waters and checking the selectivity and sensitivity with conventional chromatographic methods, and in some instances do show a good correlation for quantification purposes. The use of the immunoassay principle for binding specific compounds, *e.g.*, antibodies to the silica surface of the LC precolumn could be an useful method for the isolation of specific pesticides, as it would be a more selective way of using SPE techniques in water analysis. (v) There is a need for validation studies when modern techniques are incorporated into the official and/or routine methods of analysis. This will be the case when using LC–MS for quantitative purposes. From the few studies reported, the intercomparison of results between well established interfaces, such as TSP and PB, is still a problem.

From the above it is clear that much more work still needs to be done on the determination of pesticides in water samples. This particular field of research is also changing each year, as new pesticides are being developed to replace the more toxic ones or those which cause widespread contamination. This is the case for, *e.g.*, atrazine, which is being slowly replaced in some countries by terbutylazine or propazine. In this sense analytical developments need to be continually carried out to determine the new pesticides and the toxic TPs that are being released into the different types of environmental waters.

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Pollutants in drinking water and waste water

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ABSTRACT

Extracts of drinking water and effluents from municipal and industrial sewage treatment plants were analysed by gas chromatography–mass spectrometry and by high-performance liquid chromatography combined with ultraviolet and/or mass spectrometric detection. After column chromatography or flow-injection analysis bypassing the analytical column, ionization was performed by a thermospray interface. Identification of the pollutants was carried out by tandem mass spectrometry, generating daughter-ion spectra by collision-induced dissociation. Most pollutants in drinking water and in the effluents of waste water treatment plants are surface-active compounds of anthropogenic origin or their biochemical degradation products. Difficulties encountered during separation, detection and identification are presented and discussed and techniques for solving these problems are proposed.

INTRODUCTION

Although the number of biological waste water treatment plants in Germany has doubled within the last 20 years [1], the pollution of the river Rhine as the most important drinking water reservoir did not decrease correspondingly [2]. Using this water for the drinking water treatment process, some pollutants cannot be completely eliminated from the water, even by large-scale treatment processes such as soil filtration, ozone or hydrogen peroxide combined with UV radiation, activated carbon filtration and others. These so-called drinking-water-relevant compounds (poorly degradable polar pesticides [3], detergents [4,5] and their metabolites [6,7] or non-biodegradable, non-target compounds from chemical synthesis [8]) can be found in high concentrations in the raw river water and drinking water produced from it [4,5].

Important changes concerning the range of pollutants spectrum can be observed in the last decade. Legislation has regimented the use of certain substances, and the efficiency of biological waste water treatment has increased. Especially polar, non-volatile, persistent compounds [9,10] can be found in the waste water, owing to the substitution of soaps by detergents in washing and cleaning agents. Although so-called "biologically degradable" detergents are widely applied, the portion of the original compounds and their primary degradation products in the pollution of these surface waters may be high. So far there has been a lack of data in this field, because surface and waste water analysis dealing with polar compounds is relatively new. Substance-class-specific methods have been applied, which, however, turned out to be inefficient in many instance because of the extremely complex matrix. Only the use of substance-specific methods such as HPLC combined with UV or fluorescence [11,12], refraction [13] or conductivity detection [14] or HPLC–flow-injection analysis (FIA) coupled by thermospray (TSP) with MS–MS [4,5,9,15–17] or fast atom bombardment (FAB) MS [6,7] improved the detection of anthropogenic and biogenic pollutants in water. In spite of the introduction and improvement of these methods in recent years, great difficulties remain.

We have attempted to develop substance-specific analytical methods, not only by using conventional separation techniques coupled with MS-detection but also by mixture analysis, using the FIA technique and selective MS–MS separation and detec-

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tion (FIA–TSP-MS–MS) to determine and characterize these pollutants from a complex matrix. These techniques should enable us to state which pollutants, especially detergents are eliminated from the water phase, *i.e.*, adsorbed, primarily degraded or even mineralized during soil filtration in the drinking water treatment process or during biological waste water treatment.

EXPERIMENTAL

Materials

Drinking water samples were taken from a drinking water treatment plant of a German city located on the river Rhine. Waste water samples were taken from two different waste water treatment plants in Aachen or from a treatment plant of a German chemical company located on the Rhine. The waste water for laboratory experiments was taken from one of Aachen's treatment plants (Aachen-Soers).

Water pollutants were extracted using either continuous liquid–liquid extraction or solid-phase extraction cartridges from Baker (Deventer, Netherlands). Solid-phase extraction materials were conditioned as prescribed by the manufacturer. Glass-fibre and membrane filters used for the pretreatment of the water samples were obtained from Schleicher & Schüll (Dassel, Germany). Before use, the glass-fibre and membrane filters were heated to 400°C or were treated with ultra-pure water obtained with a Milli-Q system (Waters, Milford, MA, USA) for 24 h and then washed with 100 ml of the same water. Hexane, diethyl ether and methanol used for the liquid–liquid extraction or desorption of water pollutants from the solid-phase material were Nanograde solvents from Promochem (Wesel, Germany). Acetonitrile, chloroform, dimethyl sulphoxide and methanol used for column-cleaning purposes were of analytical-reagent grade from Merck (Darmstadt, Germany). Nitrogen for drying of solid-phase cartridges was of 99.999% purity (Linde). All surfactant standards for the identification via daughter-ion spectra library and for waste water spiking purposes were gifts from the producers (Hüls, Marl; Hoechst, Frankfurt; and BASF, Ludwigshafen, Germany) and were of technical grade.

GC analyses were performed with a DB 1701 fused-silica column (J&W Scientific, Folsom, CA, USA) and helium of 99.999% purity (Linde) was used as the carrier gas. HPLC separations were carried out with a μBondapak C_{18} (5 μm) column (30 cm × 3.9 mm I.D.) (Waters) or on a Hyperchrome NC NH_2 (5 μm) column (25 cm × 4.6 mm I.D.) (Bischoff). The mobile phase was methanol (HPLC grade) from Promochem and Milli-Q-purified water or hexane and 2-propanol (HPLC grade), respectively. Ammonium acetate for TSP ionization was of analytical-reagent grade from Merck.

Sampling and sample preparation

All samples from the waste water and the drinking water treatment plant were taken as grab samples in glass bottles. The bottles were rinsed carefully with several portions of the same water that was subsequently stored in them. The storage temperature was 4°C.

Depending on the degree of pollution, different amounts of water were used for liquid–liquid and solid-phase extraction. Water samples for LC–MS analysis were forced through the solid-phase extraction cartridges after passage through a glass-fibre filter. To ensure complete adsorption, the water samples were forced through two cartridges in series. The adsorbed pollutants were desorbed separately. Solvents of different polarities (hexane, hexane–diethyl ether, diethyl ether, water–methanol and methanol) were used for this purpose. All eluates except those with methanol and methanol–water were evaporated to dryness with a stream of nitrogen, and the residue was dissolved in methanol. The samples were rinsed into glass bottles after solid-phase extraction, and freeze-drying was applied to enrich non-C_{18}-adsorbable compounds. After freeze-drying, the samples were dissolved in methanol and used for LC–MS investigations. Liquid–liquid extracts were dried with anhydrous sodium sulphate, filtered and concentrated by rotatory evaporation.

Gas chromatographic system

A Varian (Darmstadt, Germany) Model 3400 GC system with a fused-silica capillary column was used. The conditions were as follows: carrier gas, helium; linear gas velocity, 15 cm/s; injector temperature, 250°C; transfer line temperature, 250°C; column, DB-1701, film thickness 0.25 μm, 30 m × 0.32 I.D.

Combined with GC, electron impact (EI) ionization was applied with an ionization energy of 70 eV. Under these conditions the pressure in the ion source was $8 \cdot 10^{-6}$ Torr (1 Torr = 133.322 Pa) and in the manifold $3 \cdot 10^{-2}$ Torr. The electron multiplier was operated at 1200 V with a dynode voltage of 5 kV. The temperature in the ion source was 150°C.

Liquid chromatographic (LC) system

LC separations coupled with MS, MS–MS and UV detection were achieved with a Waters Model 60 MS system. A Waters Model 510 pump was used for postcolumn addition of 0.1 *M* ammonium acetate solution in the TSP mode. A Waters Model 490 MS UV detector was connected in-line with the TSP interface. The conditions in FIA bypassing the analytical column were as follows: mobile phase I, methanol–water (60:40); mobile phase II, 0.1 *M* ammonium acetate in water; overall flow-rate 1.5 ml/min, with a ratio of 0.8 ml/min of mobile phase I and 0.7 ml/min of mobile phase II.

The chromatographic separations on the analytical columns were carried out after optimization of the conditions by a standardized method, shown in Table I; if they differ from this gradient, they are specified in the legends of the figures.

The flow-rate for column separation was 1.0 ml/min of mobile phase I. After passing the UV detector, 0.5 ml/min of mobile phase II was added, which resulted in an overall flowe-rate of 1.5 ml/min.

The reversed-phase column was cleaned with acetonitrile–chloroform–methanol–dimethyl sulphoxide (3:3:3:1, v/v) after finishing analysis and before equilibration for a new separation.

TABLE I

GRADIENT ELUTION SCHEME AND COMPOSITION OF MOBILE PHASE I

A = acetonitrile; B = water–methanol (80/20, v/v).

Time (min)	Solvent A (%)	Solvent B (%)
0	10	90
10	30	70
25	60	40
35	90	10

MS and MS–MS systems

The mass spectrometer was a TSQ 70 combined with a PDP 11/73 data station. The TSP interface was obtained from Finnigan MAT. For coupling the HPLC system with the mass spectrometer, the conditions for TSP ionization using ammonium acetate were vaporizer temperature 90°C and jet block temperature 250°C. The conditions varied during the analytical separations. Under the above conditions the ion source pressure was 0.5 Torr and the pressure in the manifold was $2 \cdot 10^{-5}$ Torr.

Using discharge ionization and hexane–2-propanol as isocratic eluent, the conditions were changed to vaporizer temperature 70°C and jet block temperature 250°C. The discharge electrode was operated at 700 V, the electron multiplier at 1200 V and the dynode at 5 kV. In the MS–MS mode the ion source pressure was 0.5 Torr. Under collision-induced dissociation (CID) conditions the pressure in quadrupole 2 (collision cell) normally was 1.3 Torr or is specified in the legends of the figures. The collison energy was adjusted to −15 eV. The electron multiplier voltage in quadrupole 3 was 1500 V with a dynode voltage of 5 kV.

FIA and column separation analysis were applied, recording TSP mass spectra beginning at m/z 150 and ending at m/z 1200.

RESULTS AND DISCUSSION

The liquid chromatographic separation of drinking, surface or waste water extracts is one of the most difficult applications of HPLC if no clean-up procedures as in pesticide, polychlorinated biphenyl (PCB) or polychlorinated dioxin and furan (PCDD/PCDF) analysis have been applied in advance. Using the same analytical column, the difficulties normally increase dramatically on going from drinking water to waste water. Drinking water has passed through several clean-up steps during the treatment process and contains only non-degradable drinking-water-relevant compounds at concentrations worth mentioning. In our experience [4], these compounds often cannot be determined using UV or fluorescence detectors because of a missing chromophore in the molecule. If they are registered as a signal by these detectors, however, they cannot be identified.

Using a mass spectrometer as detector coupled

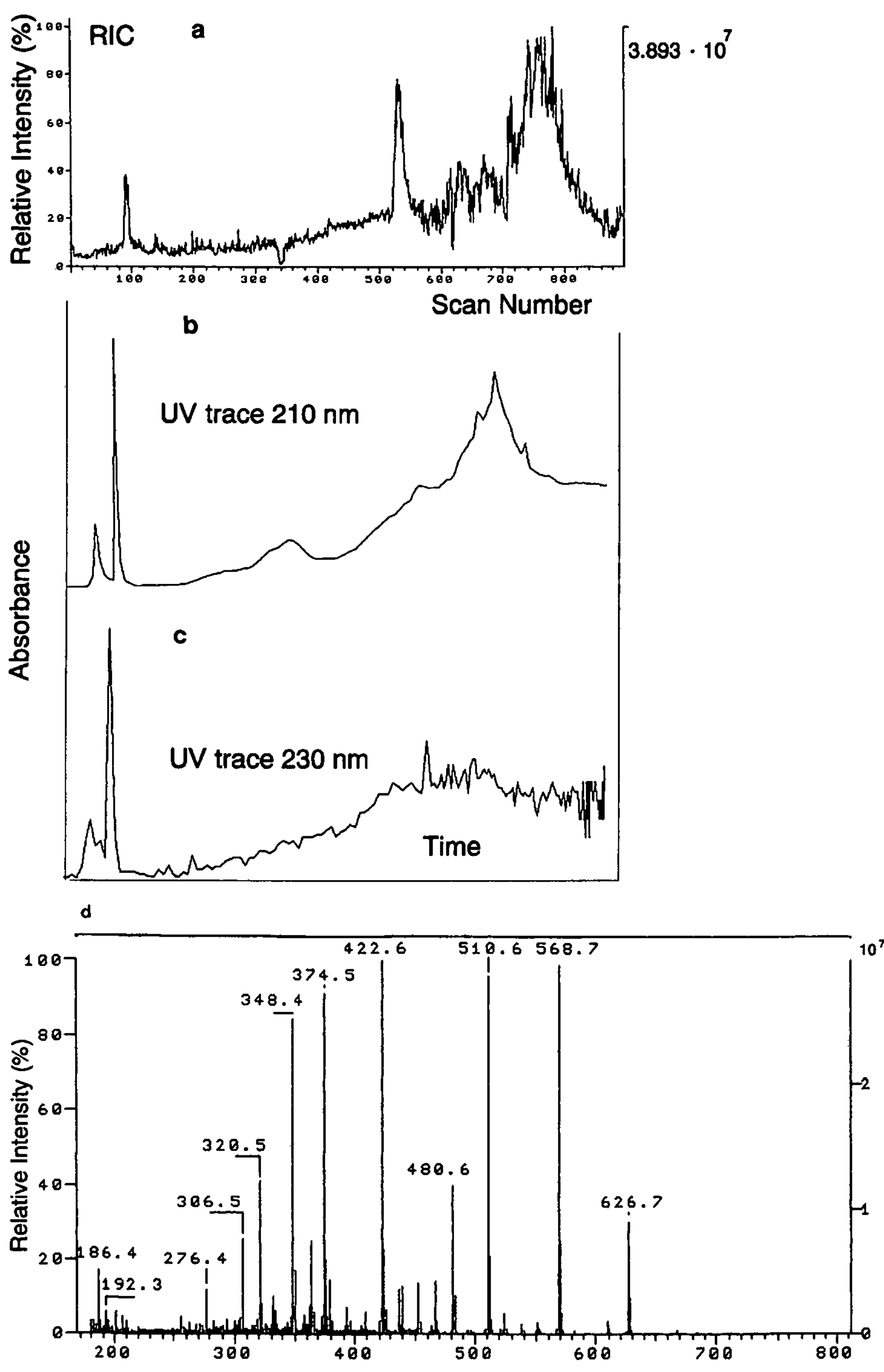


Fig. 1. (a) LC–TSP–MS total ion current trace from an activated carbon filtrate (drinking water). C_{18} solid-phase extract, eluent methanol; C_{18} column, mobile phase methanol–water, gradient from 100% water to 100% methanol in 30 min at 1 ml/min; 0.5 ml/min 0.1 *M* ammonium acetate in water was added after column separation and UV detection. (b) UV (210 nm) and (c) UV (230 nm) traces of drinking water extract in (a). LC conditions as in (a). (d) Selected mass spectra (scans 752–763) from (a).

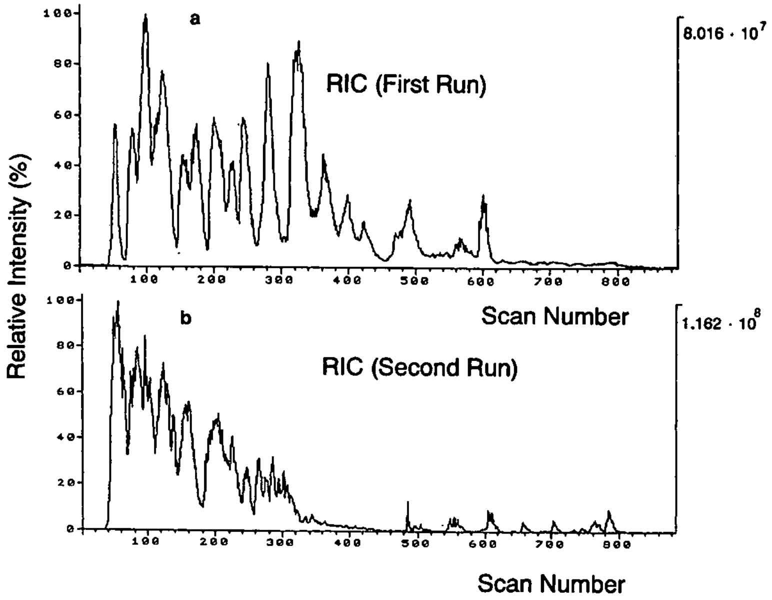
with a soft ionizing interface to generate only molecular or cluster ions but no fragments, it can be recognized that drinking water analysis by FIA will lead to many ions in the region of 150–500 u, *i.e.*, every mass in this region is occupied by the signal of at least one ion type [4]. In spite of great efforts to obtain an optimized separation by varying the chromatographic conditions, the result was a chromatographic separation shown with the reconstructed ion current (RIC) being recorded in Fig. 1a. The chromatogram consists of unresolved peaks which often contain a mixture of several eluates, as the mass spectrum of a selected peak of this total ion current (TIC) proves (Fig. 1d). The UV traces in Fig. 1b and c, recorded at 210 and 230 nm, respectively, in parallel to the ion current, show only very poor separation and either no or no distinct absorption.

During the separation of this slightly polluted water (drinking water) on an analytical column we observed that the retention times of the eluting substances after two injections of the same sample have changed markedly, so that identification by standard retention time comparison would not have been possible.

This showed that even such small portions of surface-active compounds (non-ionic detergents of the alkanol polypropylene glycol ether type [4] which had been in the drinking water extract) were adsorbed irreversibly under these chromatographic conditions, interfering with the chromatographic potential of the reversed-phase material on the analytical column. Reproducible retention times of the pollutants on this column could only be observed after a time-consuming clean-up procedure with an organic solvent mixture described under Experimental. The same problems, but on a larger scale, will arise if waste water samples which normally contain detergents are to be separated by column chromatography. The reason is that even after the described "selective elution" [4] the number of waste water compounds in these fractionated samples is still extremely high. Retention time shifts of two identical samples analysed one after the other, as shown in the RICs in Fig. 2a and b, cannot be recognized without a selective detector such as a mass spectrometer. Analysing UV or fluorescence traces by standard retention time comparison of the signals after a chromatographic separation would lead to considereable misinterpretations of the results. This is demonstrated in Fig. 2c and d, comparing selected mass traces (*m/z* 256 and 476) of the RICs in Fig. 2a and b. Large retention time shifts of the same compounds in the same sample could be observed if no procedure to clean up the analytical column had been applied before starting the second run.

Taking this behaviour of waste water extracts into account, a chromatographic separation is possible without problems [18] even in the presence of non-ionic detergents of the alkanol polypropylene glycol ether type, which had been detected by FIA–MS and identified by FIA–MS–MS. Separation occurs depending on the length of the polypropylene glycol ether chain (Fig. 3). Only TSP–MS detection was possible; UV detection recording traces at 190 or 220 nm failed because of a missing chromophore in the molecules.

In the biological waste water treatment process this type of detergent molecule may be biochemically degraded if the waste water biocoenosis has been adapted. No mineralization occurs but only a small change in the molecular structure takes place: the terminal hydroxyle function of the polypropylene glycol ether chain will be converted into an aldehyde function by biochemical oxidation, as shown in Fig. 4. The presence of this biochemical oxidation product in addition to the precursor compound "detergent" can be recognized in the FIA–MS trace bypassing the analytical column (overview spectrum) by the cluster ions at *m/z* 248, 306, 364, 422, etc., for the metabolite ions and at *m/z* 250, 308, 366, 424, etc., for the precursor compound ions (Fig. 5). This mixture of pollutants (metabolites and precursor compounds in addition to matrix compounds) can also be separated by time-consuming chromatographic methods. Under optimized conditions the TIC in Fig. 6a after chromatographic separation on a C_{18} column could be recorded. The mass spectra of the two selected peaks [*m/z* 308 (detergent), *m/z* 306 (metabolite)] of this RIC together with their characteristic CID spectra (see Fig. 6b and c) demonstrate that an excellent separation under these conditions has taken place, although the structural differences of the detergent and metabolite molecules caused by biochemical oxidation are not very impressive (compare structures in Fig. 4).



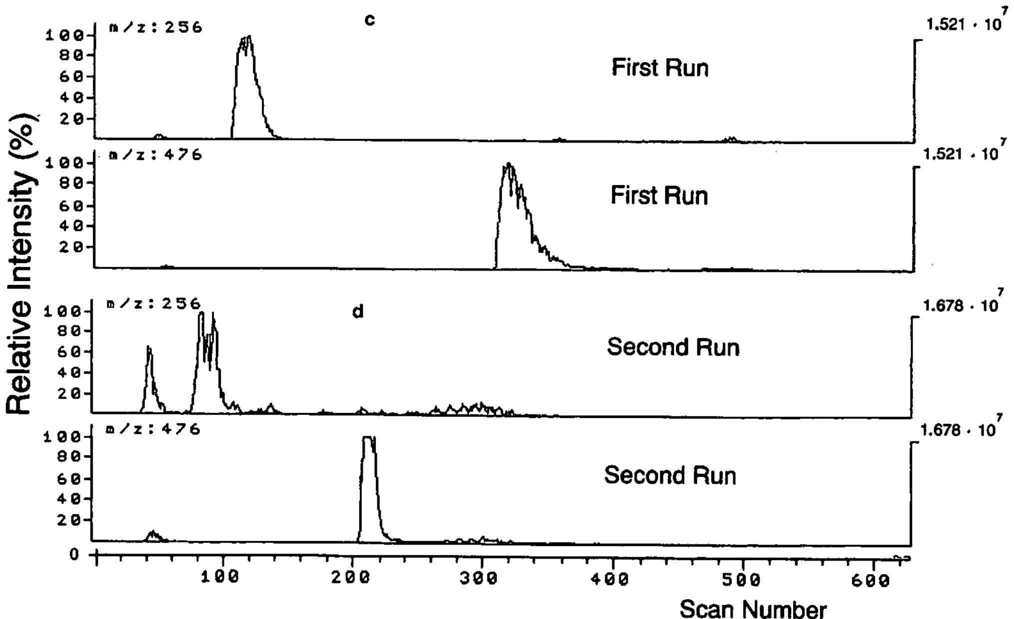


Fig. 2. (a) LC–TSP-MS total ion current trace from waste water extract containing a non-ionic detergent. C_{18} solid-phase extract, eluent methanol; C_{18} column, chromatographic conditions as under Experimental and in Table I. (b) The same extract separated under the same LC conditions as in (a). (c) Selected mass traces of (a). (d) Selected mass traces of (b).

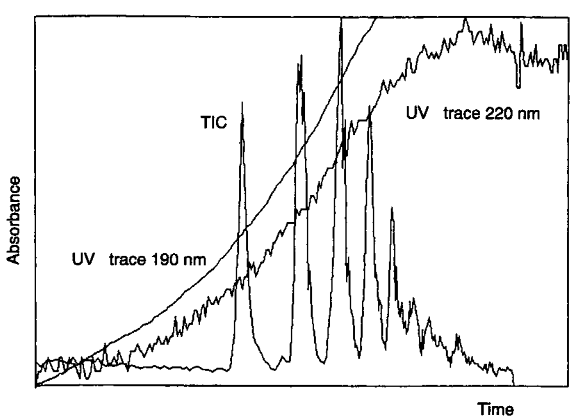


Fig. 3. LC–TSP-MS total ion current trace and UV traces (190 and 220 nm) from waste water extract containing a non-ionic detergent of the alkanol polypropylene glycol ether type shown in Fig. 4a. C_{18} solid-phase extract, eluent methanol; C_{18} column, chromatographic conditions as in Fig. 2a.

a

$C_7H_{15}-O-[CH(CH_3)-CH_2-O]-CH(CH_3)-CH_2-OH$

Biochemical Oxidation
(waste water treatment process)

b

$C_7H_{15}-O-[CH(CH_3)-CH_2-O]-CH(CH_3)-CHO$

Fig. 4. (a) Non-ionic detergent (alkanol polypropylene glycol ether). (b) Biochemical oxidation product of a waste water treatment process (metabolite).

While the described biochemical oxidation of the alkanol polypropylene glycol ether led to a terminal aldhyde function in the glycol ether chain, another non-ionic detergent of the alkanol polyethylene glycol ether type was biochemically degraded to a carboxylic compounds. This biochemical oxidation process could be observed in the waste water of a treatment plant in Aachen. Starting from this non-ionic detergent, which had been detected by FIA–MS in the overview spectrum of the treatment plant influent (see Fig. 7) and identified by the equally spaced signals (*Δm/z* 44) and its CID mass-spectrum, the biochemically hardly degradable metabolite molecule, shown in Fig. 8a, was formed by mi-

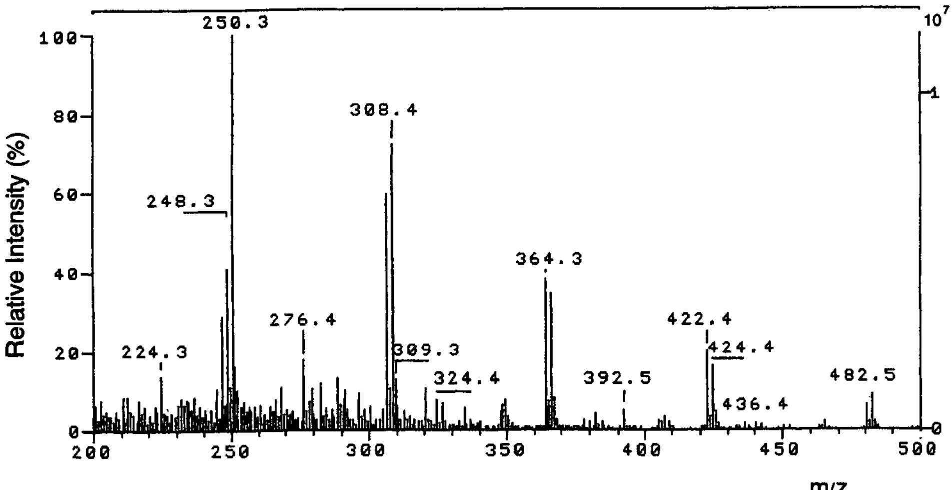


Fig. 5. FIA–LC–MS of waste water extract containing non-ionic detergents (*m/z* 250, 308, 366, 424, 482) and their metabolites (*m/z* 248, 306, 364, 422, 480). C_{18} solid-phase extract, eluent diethyl ether. For FIA conditions, see Experimental.

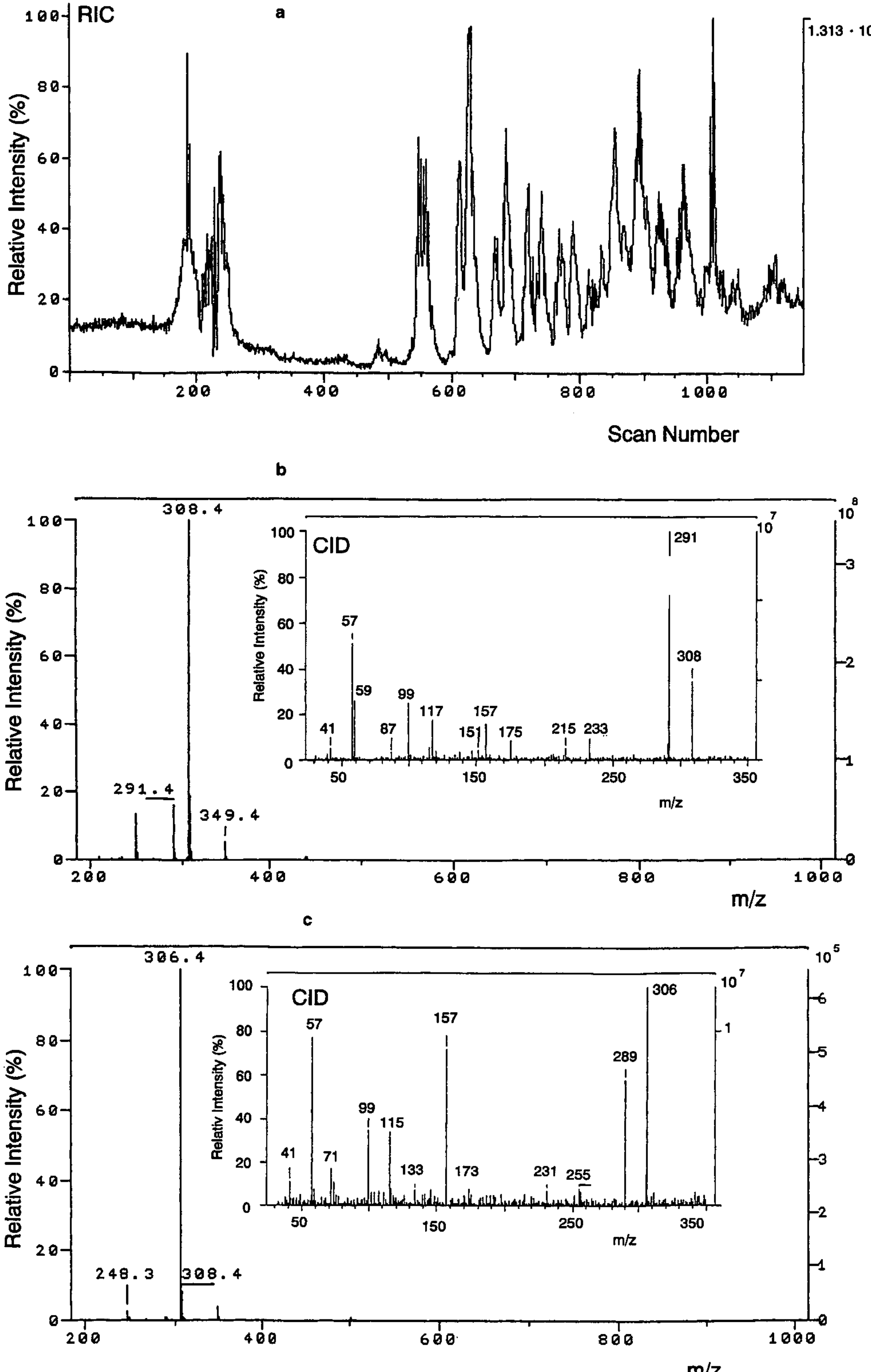


Fig. 6. (a) LC–TSP-MS total ion current trace from waste water extract in Fig. 5. C_{18} column, chromatographic conditions as under Experimental and in Table I. (b) Mass spectrum and daughter-ion mass spectrum (FIA–LC–TSP-MS) of detergent after column separation [scans 605–616 of TIC in (a)]. (c) Mass spectrum and CID spectrum (FIA–LC–TSP-MS–MS) of metabolite (scans 624–632); compare with (b).

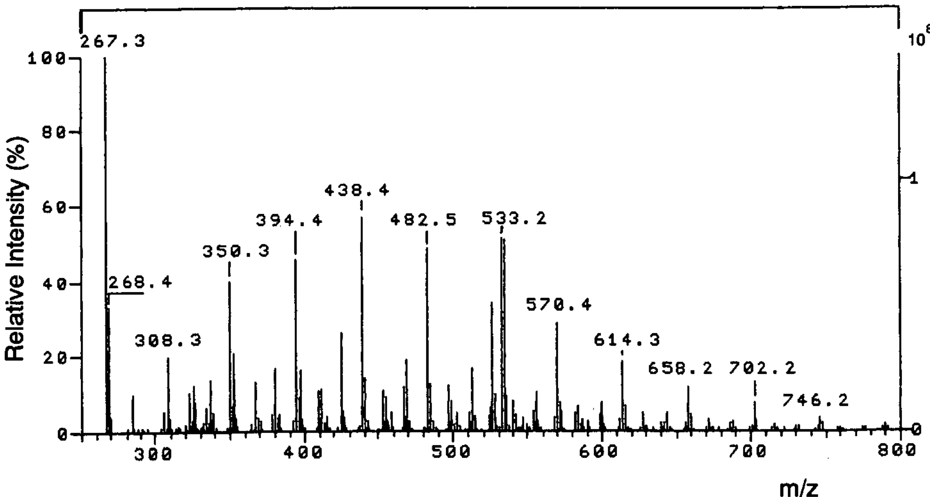


Fig. 7. FIA–LC–MS of waste water extract containing non-ionic detergents. C_{18} solid-phase extract, eluent methanol. FIA conditions as in Fig. 5.

$$C_{13}H_{27}-O\left[CH_2-CH_2-O\right]_n CH_2-C(=O)OH \quad * \; NH_4^+$$

n = 1; m/z = 320
n = 2; m/z = 364
n = 3; m/z = 408

b

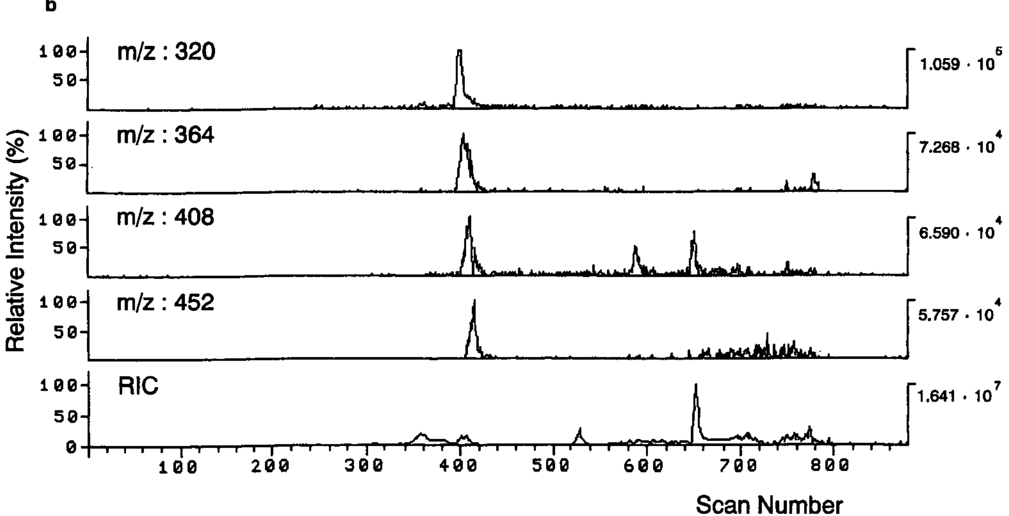


Fig. 8. (a) Structural formula of primary degradation products (metabolites) of detergent mixture in Fig. 7. (b) LC–TSP-MS traces of metabolite in (a) and RIC of waste water extract. C_{18} solid-phase extract, eluent diethyl ether; C_{18} column, chromatographic conditions as in Fig. 2a.

crobiological primary degradation. This metabolite molecule can be separated from the waste water matrix and the precursor compounds on an analytical column, as the mass traces at *m/z* 320, 364, 408 and 452 in the scan region 393–422 in Fig. 8b demonstrate. The same behaviour in the biological waste water treatment process could be observed when a fluorine-containing, non-ionic detergent of the polyethylene glycol type (Fig. 9a) was biochemically degraded [17]. This detergent, partially fluorinated in the alkanol chain, resists chemical and physico-chemical treatments such as hydrolysis, strongly oxidizing acids or mineralization in a hydrogen–oxygen flame. However, biochemical degradation could be observed in the waste water treatment process resulting in the metabolite shown in Fig. 9b. The polyethylene glycol ether chain in this molecule is shortened in parallel. This primary degradation product resists further biochemical degradation. In a batch reactor there is no significant degradation within a period of 10 days.

The precursor compound of this biochemical oxidation and the primary degradation product can be identified using their typically different daughter-ion spectra in Fig. 9a and b. Separation of the non-ionic detergents and the metabolites on a C_{18} column, depending on the different lengths of the fluorine-containing alkyl chains, is successful as the

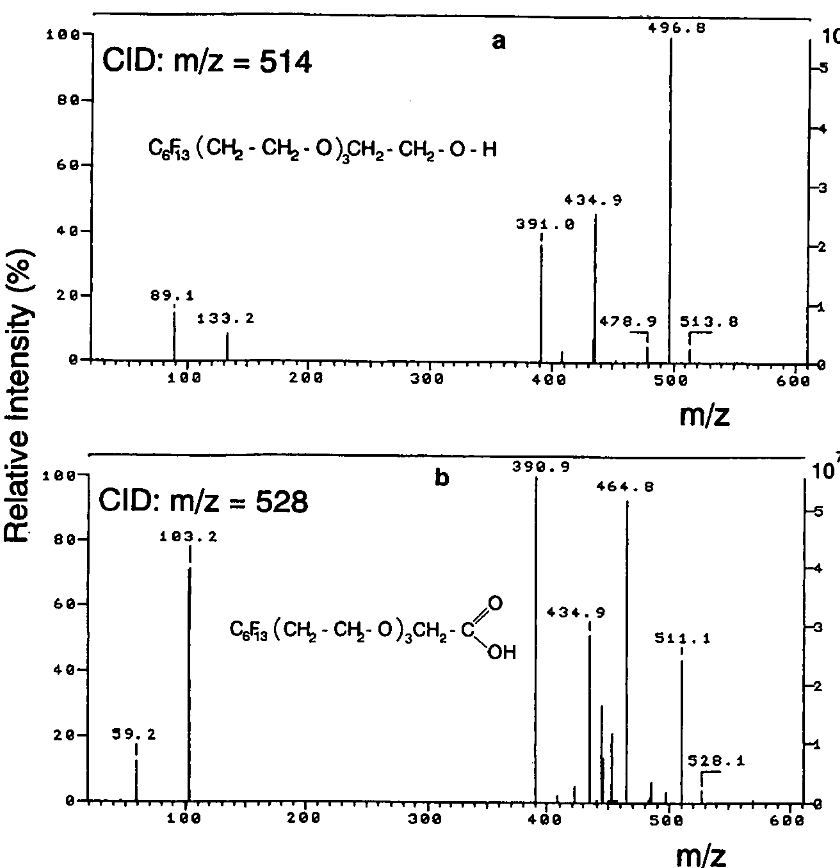


Fig. 9. (a) Daughter-ion mass spectrum (FIA–LC–TSP-MS–MS) and formula of fluorine-containing detergent from waste water extract. C_{18} solid-phase extract, eluent diethyl ether. FIA conditions as in Fig. 5. For CID conditions, see Experimental. (b) Daughter-ion mass spectrum as in (a) and formula of metabolite of detergent in (a) from waste water extract. C_{18} solid-phase extract, eluent hexane–diethyl ether (6:4, v/v); FIA and CID conditions as in (a).

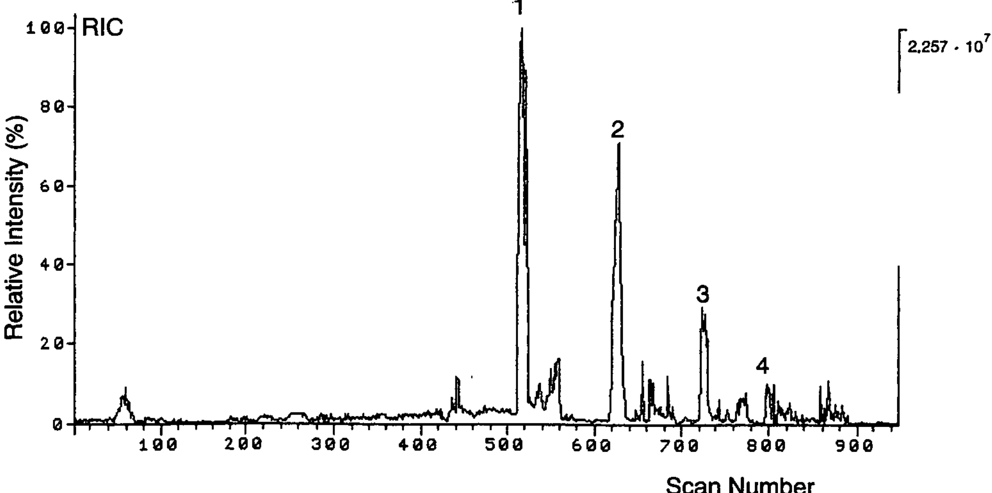


Fig. 10. LC–TSP–MS total-ion current of waste water extract containing the fluorine detergent in Fig. 9a and its metabolite (Fig. 9b). C_{18} solid-phase extract, eluent methanol; C_{18} column, chromatographic conditions as in Fig. 2a. 1 = Metabolite; 2 = detergent [Perfluoro alkyl group (R_f) = C_6F_{13}]; 3 = detergent (R_f = C_8F_{17}); 4 = detergent (R_f = $C_{10}F_{21}$).

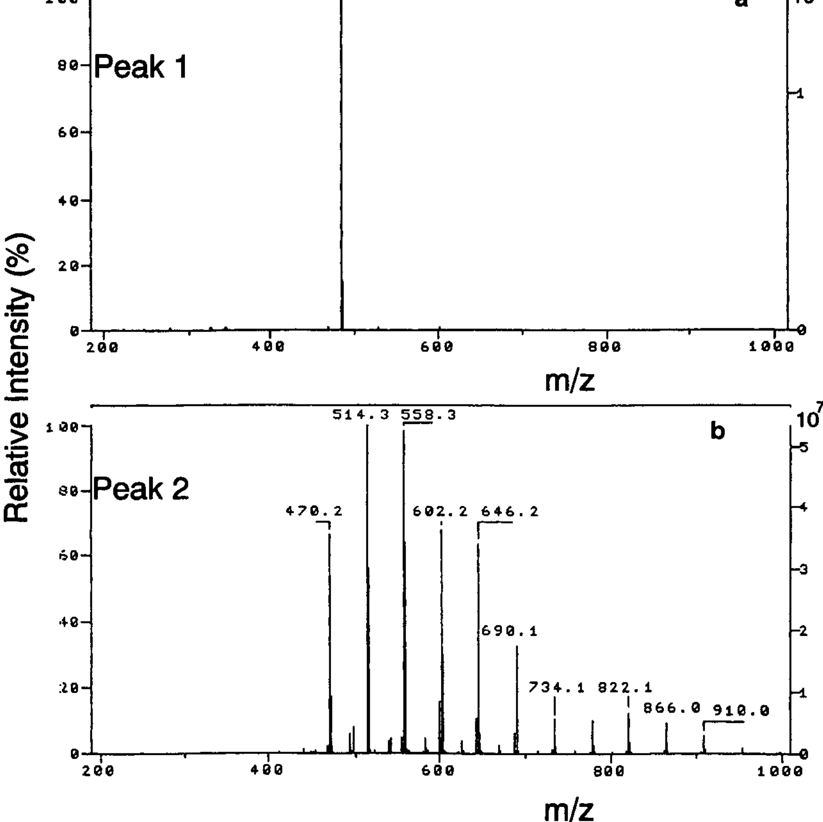


Fig. 11. LC–TSP mass spectra of (a) peak 1 and (b) peak 2 in Fig. 10.

LC–MS-TIC of the waste water extract shows (see Fig. 10). Mass spectra of selected peaks in the RIC (Fig. 10) demonstrate the separation efficiency for the metabolite (Fig. 11a) and the detergents (Fig. 11b) from this mixture. UV detection of these compounds was not possible because they did not show any absorbance in the region >210 nm.

Extracts from influents of waste water treatment plants nowadays contain non-ionic detergents of the fatty acid diethanolamide type in high concentrations. Analysis of effluent samples from these plants demonstrated the elimination of these pollutants, *i.e.*, complete degradation or adsorption on the sludge seems to occur very quickly without the formation of hardly or non-biodegradable metabolites. However, high concentrations of these pollutants in the influent make the elimination capacity insufficient [9]. Even after an intensified biological waste water treatment process, waste water extracts from the effluent of a laboratory-scale treatment plant spiked with 2 mg of detergent per litre of waste water in the influent [3] contain the single isomers of the fatty acid diethanolamide. These compounds could be easily identified in the overview spectrum by their characteristic molecular ion patterns, which appear depending on the length of the alkyl chain at *m/z* 232 (C_7H_{15}), 260 (C_9H_{19}), 288 ($C_{11}H_{23}$) and 316 ($C_{13}H_{27}$) and by their daughter-ion specra (CID) obtained by FIA–LC–MS or FIA–LC–MS–MS (Fig. 12).

Chromatographic separation of these compounds in the effluent from a laboratory-scale plant is possibly on a C_{18} column. Even the separation of a much more problematic waste water extract from the influent of a municiple sewage treatment plant in Aachen was successful, as the TIC in Fig. 13b shows. The peaks in this RIC marked 1, 2 and 3 are the ion-current signals for the molecular ions at *m/z* 232, 260 and 288. The excellent separation from all other compounds in the mixture can be recognized in the mass spectrum of peak 3 in Fig. 13c. The molecular structure of this surfactant type in Fig. 13c shows the reason why this molecule could be ionized without forming an ammonia cluster ion. The nitrogen atom in the molecule is able to carry the positive charge necessary for ionization.

The signal in the RIC (Fig. 13b) in the scan region 345–389 could be characterized as a non-ionic detergent of the alkanol polyethylene glycol ether type because of its equidistant masses of $\Delta m/z$ 44 and its daughter-ion spectrum of the selected ion with *m/z* 336 (Fig. 13d).

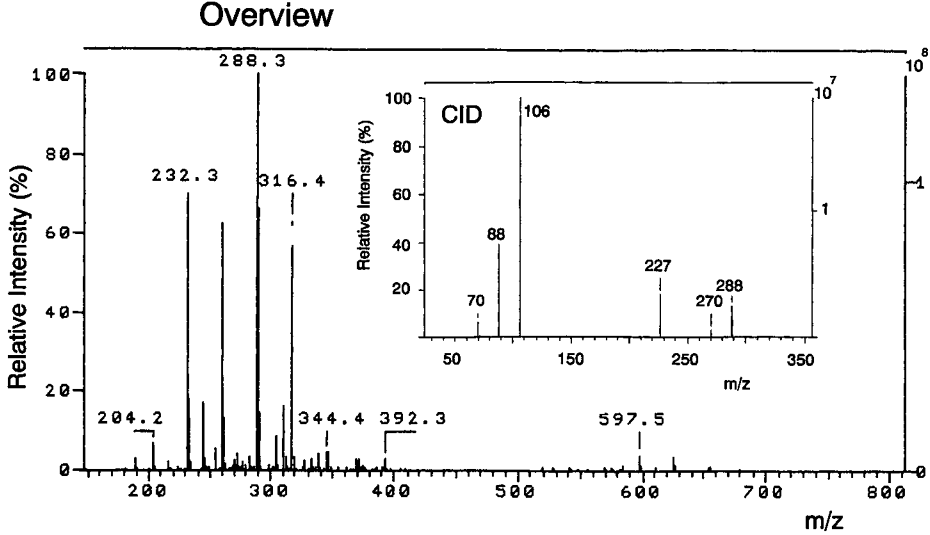


Fig. 12. FIA–LC–MS and FIA–LC–MS–MS (*m/z* 288) of waste water extract containing fatty acid diethanolamide; C_{18} solid phase extract, eluent diethyl ether; FIA and CID conditions as in Fig. 9a.

The 210-nm UV trace plotted in Fig. 13a recorded in parallel to the LC–TSP–MS TIC proves that this non-ionic detergent of the fatty acid diethanolamide type shows an absorbance at 210 nm. Many other pollutants contained in the waste water extract which cannot be ionized by TSP ionization can be detected because of their strong UV absorbance at 210 nm. The identification of these pollutants was not possible.

Another important topic, in spite of all the diffi-

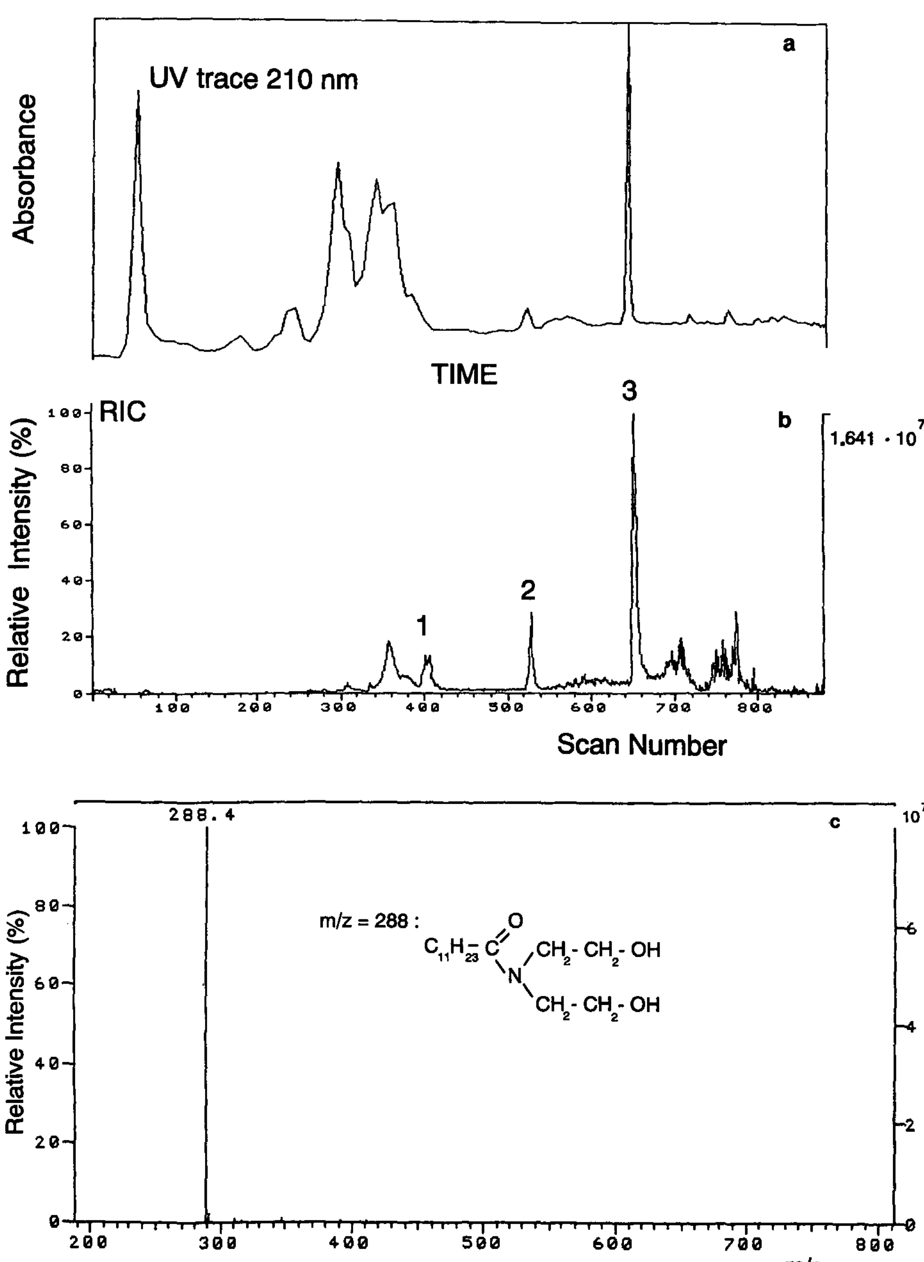


Fig. 13.

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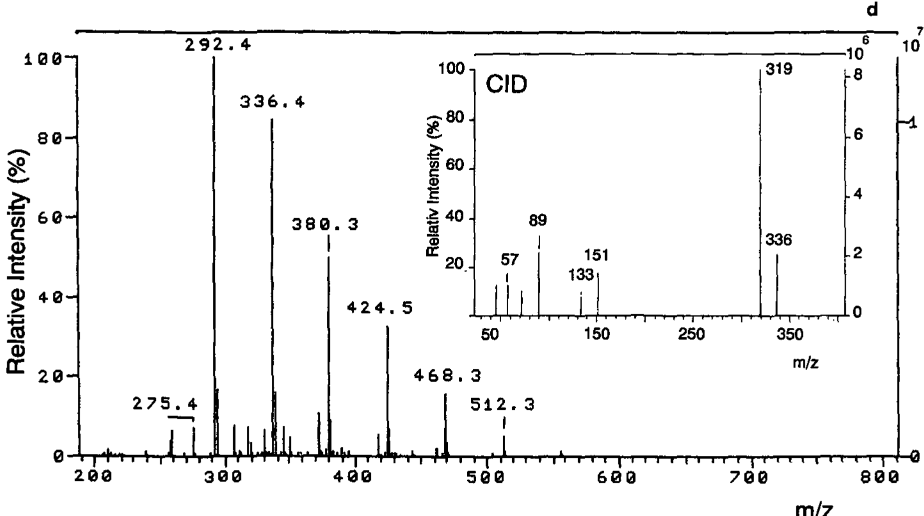
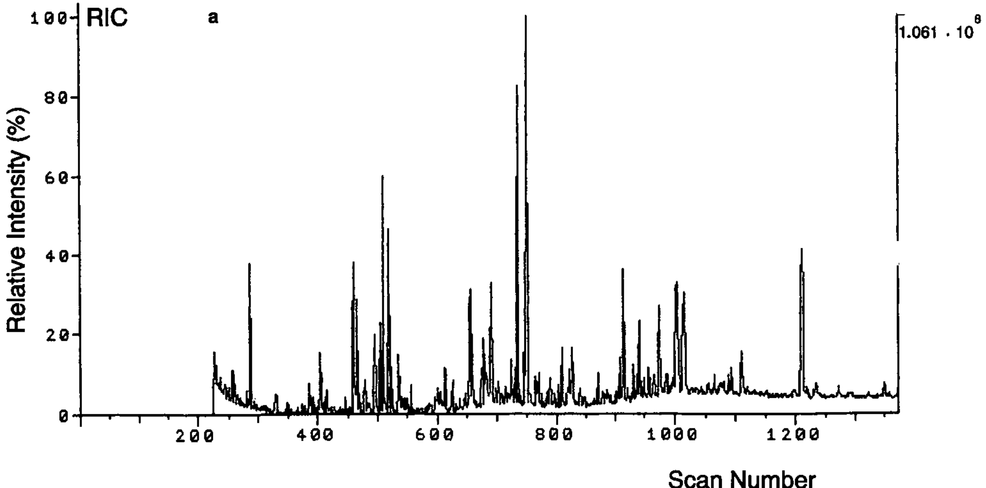


Fig. 13. (a) UV trace (210 nm) of waste water extract containing fatty acid diethanolamide. C_{18} solid-phase extract, eluent methanol; C_{18} column, chromatographic conditions as in Fig. 2a. (b) RIC of waste water extract of (a). LC conditions as in (a). (c) Mass spectrum of peak 3 in (b) and structural formula of coconut fatty acid diethanolamide (*m/z* 288). (d) LC–TSP-MS and CID spectrum of non-ionic detergent of RIC in (b) (scans 345–389).

culties, is the analysis of waste waters in the chemical industry containing large amounts of such pollutants. The complexicity of municipal waste water even in the influent is exceeded substantially by waste water in the effluent from these sewage treatment plants. This becomes obvious by GC–MS analysis of extracts of municipal and industrial waste water treatment plant effluents after an optimized biological treatment process. Very low concentrations of volatile compounds can be detected in municipal sewage treatment plant effluents [5]. In contrast, we found many volatile organic compounds in the hexane extract of chemical industry waste water discharging into the river Rhine. The TIC of this GC–MS analysis is shown in Fig. 14a. Enrichment of compounds from this waste water by C_{18} solid-phase extraction was used for examination of polar and low-volatile compounds. The methanol eluate from this extraction procedure was analysed by LC–TPS–MS on a C_{18} column using the above-described chromatographic conditions and showed a good separation (Fig. 14b). Some of the pollutants could be identified as tributyl phosphate (*m/z* 267), phthalate (*m/z* 279) and alkanol polyglycol ether (*m/z* 240, 284, 328, 372 and 416) by MS–MS. Target analysis for toxic compounds using the mass traces at *m/z* 326, 370, 414, etc., to look for the ammonia adduct ions of nonylphenol ethoxylates showed that these precursor compounds of nonylphenol were present in the effluent in low concentration. However, as the chromatographic separation on a reversed-phase column was not satisfactory (Fig. 14b) and the response of these compounds was very low with TSP ionization, normal-phase chromatography was applied to test for the presence of nonylphenol ethoxylates. Separation was obtained on a an amino-bonded packing with an organic eluent (hexane–2-propanol) [11] and recording RIC and UV traces at 254 nm in parallel. Comparison of the RIC and UV traces showed that under these chromatographic conditions nonylphenol ethoxylates could be detected much better by UV detection at 254 nm (see Fig. 15a) than by LC–TSP–MS using the discharge electrode (see Fig. 15b). However, evidence about the identity of the signals in the RIC can only be obtained from the selectivity of the mass spectrum of the selected peak 1 from the RIC in Fig. 15b. The plotted mass spectrum of *m/z* 441 in Fig. 15b belongs to the molecular ion ($[M + H]^+$) of a nonylphenol ethoxylate with five PEG units in the ether chain..

The practicability of this method using an organ-



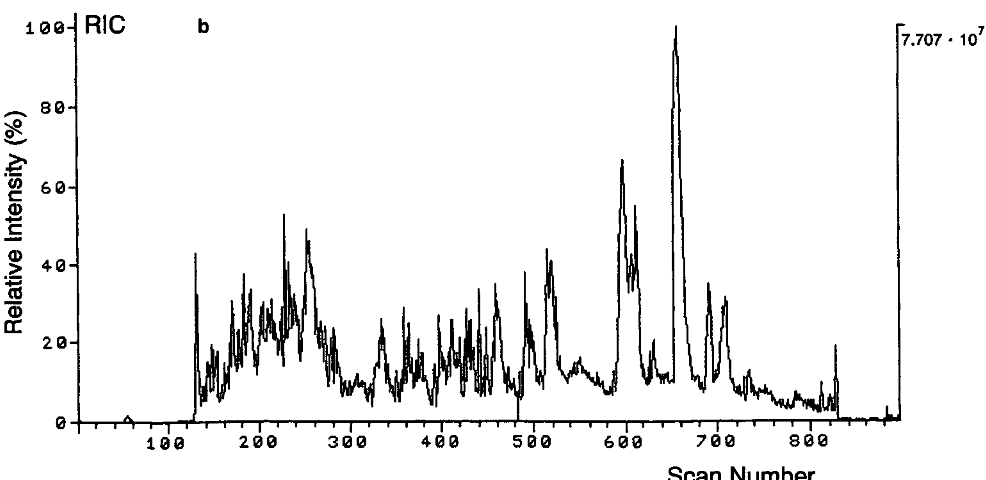


Fig. 14. (a) GC–MS total ion current trace of a waste water treatment plant effluent from a chemical company. Liquid–liquid extract, solvent hexane. (b) LC–TSP–MS total ion current trace of waste water in (a); C_{18} solid-phase extract, eluent methanol; C_{18} column, chromatographic conditions as in Fig. 2a.

ic eluent and TSP interface with discharge ionization is limited because the ionization process by the discharge electrode generates carbon by pyrolysis of hexane and 2-propanol which is deposited in the ion source, inducing a considerable decrease in sensitivity of the mass spectrometer.

The clean-up procedure after application of organic solvents as eluents is very time consuming and limits the use of this ionization technique much more than aqueous eluents.

CONCLUSIONS

Extracts of drinking water and effluents from sewage treatment plants contain a wide variety of pollutants. HPLC separations are time consuming

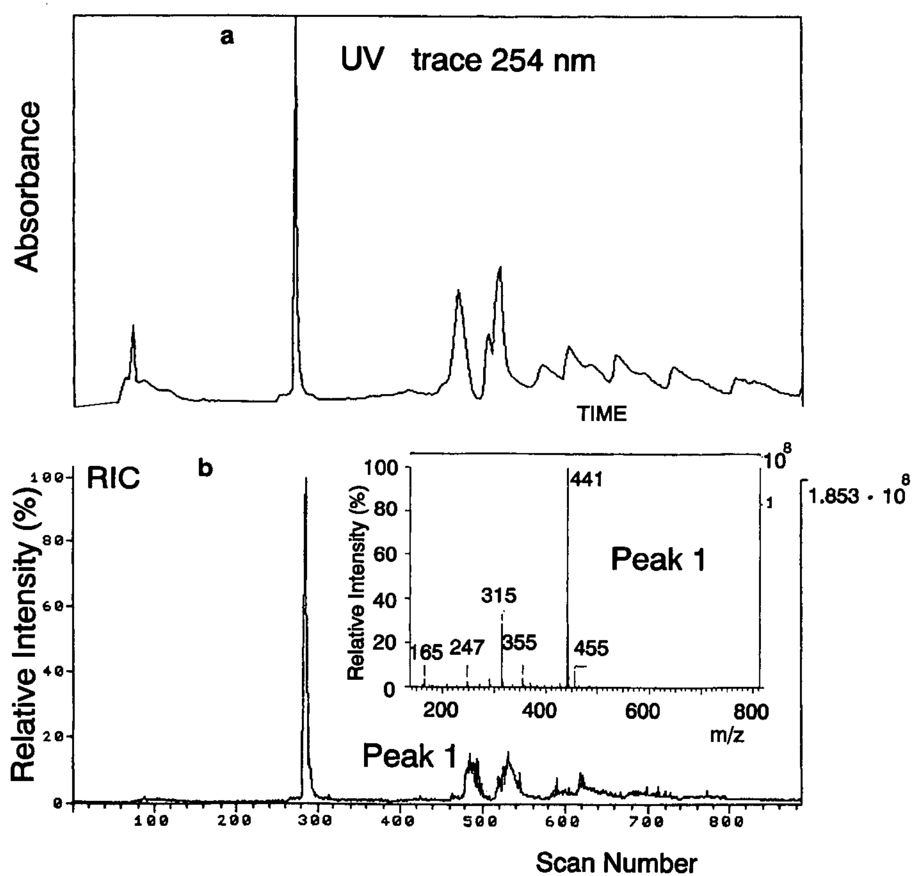


Fig. 15. (a) UV trace (254 nm) of waste water extract in Fig. 14b containing nonylphenol ethoxylates. C_{18} solid-phase extract, eluent methanol; amino-bonded normal-phase column, mobile phase hexane–2-propanol, gradient from 100% hexane to 70% 2-propanol in 60 min, flow-rate 1.5 ml/min. (b) LC–MS total ion current trace of waste water extract in (a) and LC mass spectrum of peak 1; LC conditions as in (a); discharge ionization, discharge voltage 700 V.

and not always satisfactory. Surface-active compounds in these extracts interfere and influence the elution behaviour, which makes an identification by retention time comparison impossible.

The detection and characterization of these compounds will fail if only unspecific detection systems such as UV or fluorescence detectors are used. On the one hand, many of these polar pollutants do not possess any chromophore for optical detection and on the other, it cannot be excluded that several compounds may be hidden under the signal registered by these detection systems. Soft ionization techniques such as TSP ionization in combination with MS–MS allow a definite characterization after time-consuming HPLC separation or time-saving mixture analysis using FIA. Hence polar pollutants such as non-ionic detergents of the alkanol polyethylene and propylene glycol ether types, fatty acid diethanolamides or nonylphenol ethoxylates and their possible metabolites can be detected by LC–MS and characterized by LC–MS–MS in drinking and waste waters. Many compounds of this type can be detected and identified only in this way.

Target analysis for nonlyphenol ethoxylates in waste water is more efficient using normal-phase chromatography combined with discharge ionization instead of reversed-phase chromatography and TSP ionization.

UV detection additionally performed in parallel to HPLC–TSP-MS investigations seems to be very

helpful in obtaining information about non-TSP-active compounds.

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CHROMSYMP. 2707

Determination of ethylenethiourea in water samples by gas chromatography with alkali flame ionization detection and mass spectrometric confirmation

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ABSTRACT

A gas chromatographic method with alkali flame ionization detection and mass spectrometric confirmation is described for the determination of ethylenethiourea (ETU) in water samples. The method is based on the extraction of ETU with dichloromethane in the presence of thiourea and sodium L-ascorbate. The limit of detection is less than 0.1 μg/l in water. The average recovery in groundwater is 71%. Several hundred samples of groundwater and river water were analysed over a 2-year period.

INTRODUCTION

Ethylenethiourea (ETU) is a toxicologically important metabolite of the widely used ethylenebisdithiocarbamate (EBDC) fungicides formed during biological and chemical degradation. EBDCs are frequently used for the control of diseases in seeds and crops throughout the growing season [1]. In 1987, more than 2300 tonnes EBDCs were used in Netherlands. The group includes such fungicides as maneb, mancozeb, nabam and zineb. A review of the toxicology of ETU, which may produce teratogenic, oncogenic and goiterogenic effects after being applied to laboratory animals, has been published [2]. EBDCs degrade in the presence of moisture, oxygen and/or biological systems and several degradation products are formed, including ETU. The reactions leading to ETU formation have been described previously [3]. Most of the ETU occurring in crops and environmental samples arises, however, from EBDC formulations, which contain 0.02–5% of ETU [4]. ETU is a relatively stable and very polar metabolite and in the areas where EBDC fungicides are used, its possible occurrence in groundwater and river water is a major concern for the safety of drinking water.

Residue analysis of ETU has been conducted in different matrices such as fruits and plant tissues by various methods, including high-performance liquid chromatography (HPLC) with non-selective UV absorption detection [5–8] and selective electrochemical detection [9–12] and gas chromatrography (GC) with derivatization to achieve sensitive detection [13–17]. The HPLC and GC methods share the disadvantages of laboriousness and/or insufficient sensitivity and/or specificity, except for one HPLC method [5] which was applied at the μg/l level.

However to reach the desired 0.1 μg/l water level which is required by an EEC Directive for drinking water [18], also with this method preconcentration of the water sample and extraction steps are necessary. We have developed a method for the determination of ETU without derivatization in water samples after extraction with dichloromethane and gas chromatography with alkali flame ionization detection and confirmation by mass spectrometry. This method is easily applicable to the 0.1 μg/l ETU level water in required by the EEC Directive [18]. Several hundred water samples were successfully analysed with this method.

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EXPERIMENTAL

Reagents and apparatus

ETU (99%) was obtained from Promochem (Wesel, Germany). A standard solution containing 1% (v/v) of diethylene glycol was prepared in methanol and appeared to be stable for at least 3 months if stored at 4°C in the dark.

Thiourea (99%), diethylene glycol (99%) and sodium L-ascorbate (99%) were obtained from Aldrich (Brussel, Belgium). A 0.05% (v/v) solution of diethylene glycol in methanol was used.

All other chemicals were of analytical-reagent grade and were checked for the absence of interfering impurities by means of control determinations. Evaporation of water samples was carried out at 50°C (water-bath) using a vacuum rotary evaporator.

Gas chromatography

A Carlo Erba MEGA 8000 gas chromatograph equipped with an alkali flame ionization (nitrogen–phosphorus) detector was used. The instrument was equipped with a wide-bore fused-silica capillary CP-WAX 52 CB column (10 m × 0.53 mm I.D., film thickness 2.0 μm) (Chrompack, Middelburg, Netherlands). Helium was used both as the carrier gas (constant pressure 25 kPa) and as the make-up gas for the detector (16 ml/min). The temperature of the injection port was 250°C and that of the detector 260°C. A 4-μl volume of the sample extract was injected splitless on to the column at 130°C. After 20 s the carrier gas splitting was restarted and then an oven temperature programme was started as follows: initially 130°C, increased at 10°C/min to 250°C, held for 20 min and then cooled to the initial temperature of 130°C.

Gas chromatography–mass spectrometry

The gas chromatograph–mass spectrometer (Carlo Erba MEGA 5000–QMD 1000) was equipped with a CP-WAX 52 CB fused-silica capillary column (12 m × 0.27 mm I.D., film thickness 0.22 μm) (Chrompack). Helium was used as the carrier gas (constant pressure 30 kPa). The injection port temperature was 250°C. The oven temperature programme was started at 50°C (held for 1 min), then increased at 25°C/min to 240°C and held there for 15 min. A 1-μl sample volume was injected under splitless conditions; 40 s after injection the splitting valve was opened.

The ion source temperature of the mass spectrometer was 200°C. The spectra were recorded under electron impact (EI) conditions (70 eV), with a scan range of m/z 25–130 and a scan rate of 0.5 s.

Determination

A 10-mg amount of thiourea and 1 g of sodium L-ascorbate were dissolved in 500 ml of water. The mixture was concentrated by a vacuum rotary evaporator at 50°C to *ca.* 30 ml (± 5 ml). The concentrate was transferred to a separating funnel with 2 × 5 ml of distilled water and saturated with 14 g of sodium chloride. The mixture was then extracted once with 100 ml and twice with 50 ml of dichloromethane. The extract was dried by passing it through a funnel containing *ca.* 25 g of anhydrous sodium sulphate prewetted with dichloromethane. The sodium sulphate was washed twice with 10 ml of dichloromethane. To the combined dichloromethane extract, 10 ml of a 0.05% (v/v) solution of diethylene glycol in methanol were added and the mixture was concentrated with a rotary evaporator at 40°C to *ca.* 5 ml. To the concentrate 10 ml methanol were added and the mixture was concentrated to *ca.* 5 ml. The concentrate was transferred quantitatively with 2 × 0.5 ml of methanol into a graduated test-tube and further concentrated to 0.5 ml (corresponding to 1 l of water per ml of methanol extract) by using a gentle stream of dry nitrogen at 50°C. This extract was examined by gas chromatography. Quantification was achieved by comparing the peak height of ETU with those of standard solutions of comparable concentration.

RESULTS AND DISCUSSION

Extraction procedure and recovery experiments

The extraction of ETU was based on a procedure described by Otto *et al.* [19]. To achieve a detection limit of 0.1 μg/l of ETU or less in groundwater or river water, required for water samples used as a source of drinking water [18], a concentration and extraction procedure is necessary. Because ETU is poorly soluble in organic solvents but readily soluble in water (2%, w/v) at 30°C, direct extraction of ETU with dichloromethane or a more polar organic solvent (ethyl acetate) was not feasible. Before con-

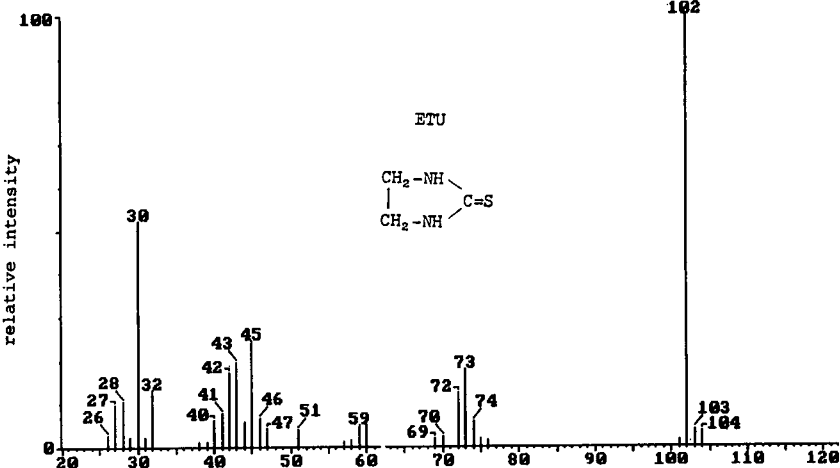

Fig. 1. EI mass spectrum of 10 ng of ETU.

centrating and extracting the water sample, thiourea and sodium L-ascorbate were added. The beneficial function of these actions cannot be fully explained, but it is assumed that thiourea and sodium L-ascorbate protect ETU against oxidation, complex formation with heavy metals [19] and adsorption on active glassware surfaces. By just extracting the concentrate with dichloromethane and concentrating the extract obtained to 0.5 ml, a considerable loss of ETU was observed, possibly caused in part by adsorption of ETU on active glassware surfaces. The recovery for spiked water samples at the residue level was 20–60%. By adding a mixture of diethylene glycol in methanol before concentrating the dichloromethane extract, the recovery of ETU in spiked water samples increased considerably and the repeatability was acceptable. Diethylene glycol is used as "keeper" and seems also to protect ETU

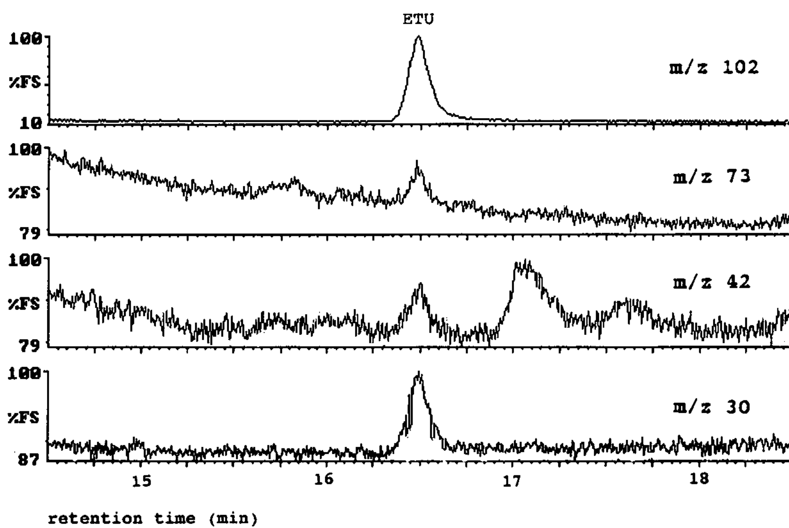

Fig. 2. SIM mass chromatogram obtained for a sample of groundwater fortified with 0.1 μg/l of ETU. Injection volume, 1 μl.

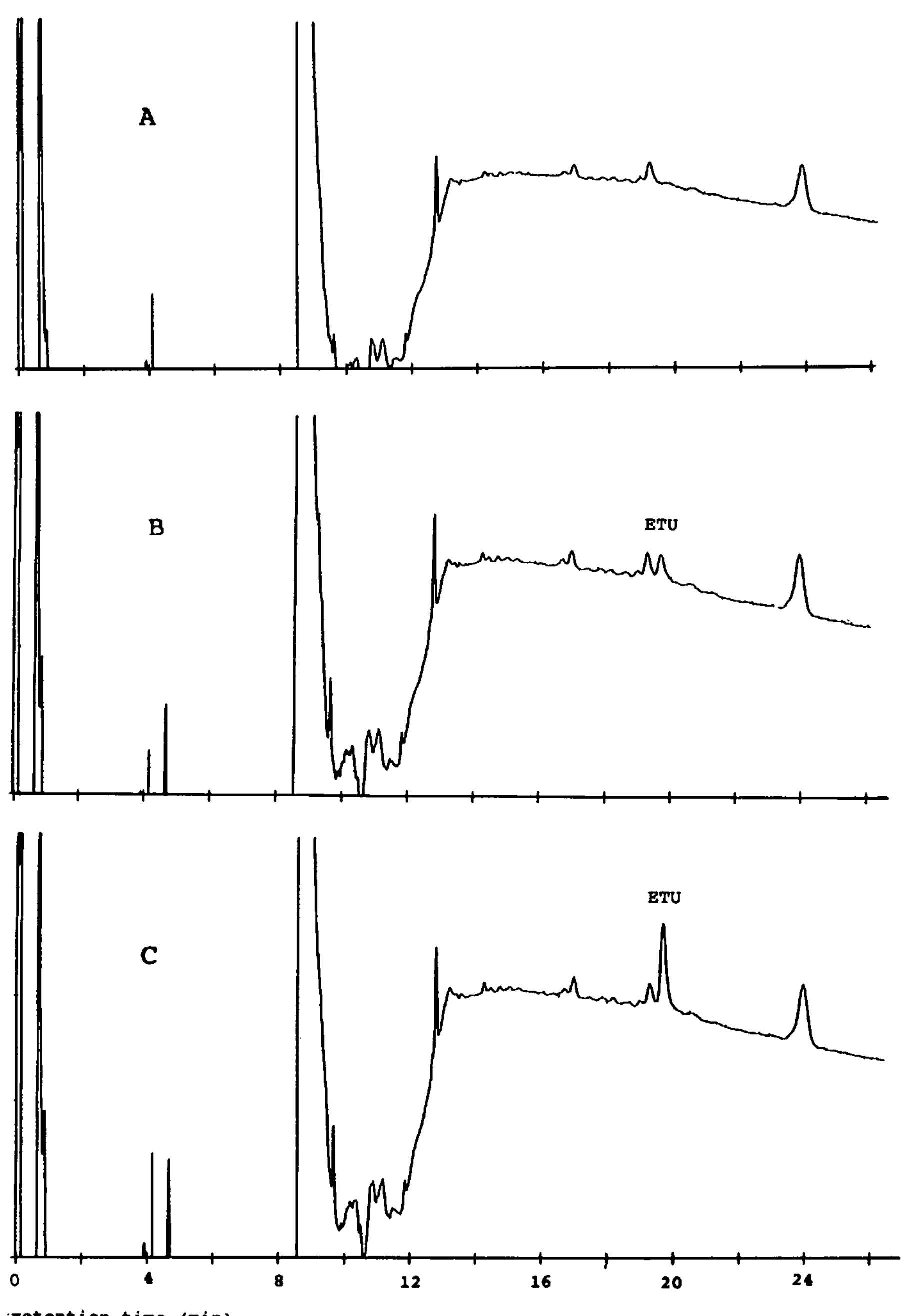

Fig. 3. Chromatograms obtained with CP-WAX 52 CB fused-silica GC column. (A) 4 ml of groundwater; (B) 4 ml of groundwater fortified with 0.2 μg/l of ETU; (C) 4 ml of groundwater fortified with 0.7 μg/l of ETU. Injection volume, 4 μl.

against adsorption on active parts of the glassware used. With diethylene glycol in the final extract also a better peak shape (no tailing) was obtained.

Recovery experiments were carried out by adding known amounts of ETU to 500 ml of groundwater. The results are given in Table I.

TABLE I

RESULTS OF RECOVERY EXPERIMENTS

ETU added to water (μg/l)	*n*	Recovery (%) (mean ± S.D.)	R.S.D. (%)
0.23	4	70 ± 15	21
1.0	13	72 ± 11	15
6.2	4	70 ± 8.2	12

Gas chromatography and gas chromatographic–mass spectrometric confirmation

The response of the alkali flame ionization detector to ETU is linear up to at least 15 ng and the minimum determinable amount is *ca.* 0.2 ng (see Fig. 3B). Generally in residue analysis, if possible positive samples should be confirmed by mass spectrometry. The identity of ETU was verified in this manner. From the EI mass spectrum the following ions were chosen for selective ion monitoring (SIM): m/z 102 (100%), m/z 73 (20%), m/z 42 (20%) and m/z 30 (55%). A typical EI mass spectrum of ETU is shown in Fig. 1. For quantification the area of the molecular ion at m/z 102 was used. The response was linear up to at least 2 ng and the minimum determinable amount was *ca.* 0.05 ng (see Fig. 2). A Typical SIM mass chromatogram of groundwater fortified with 0.1 μg/l of ETU is shown in Fig. 2.

TABLE II

INTERLABORATORY STUDY OF THE DETERMINATION OF ETU IN GROUNDWATER SAMPLES

Sample series	Sample location	ETU content (μg/l)	
		TNO	RIVM
A	1	34	53
A	2	12	17
A	3	3.1	3.2
A	4	34	38
B	5	0.1	0.1
B	6	0.1	0.1
B	7	<0.1	<0.03
B	8	0.1	0.1
B	9	0.1	0.15

Interlaboratory studies

Two series of groundwater samples were analysed both by the TNO Nutrition and Food Research Laboratory and by the RIVM Laboratory (Bilthoven, Netherlands). The latter institute used a similar extraction–concentration procedure but the determination of the ETU was performed by a column-switching RPLC procedure with UV detection at 233 nm [5]. The results are presented in Table II and show a reasonably satisfactory agreement between the two methods.

The method described has been successfully applied in the investigation of ETU in groundwater and river water samples over the past 2 years. Fig. 3 shows typical gas chromatograms of control and fortified samples of groundwater analysed by the method described.

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CHROMSYMP. 2720

Determination of alkylbenzenesulphonates in environmental water by anion-exchange chromatography

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ABSTRACT

A simple and selective method for the determination of μg/l levels of alkylbenzenesulphonates (ABSs) in environmental waters is presented. Selectivity for ABSs was obtained by using anion-exchange pre-column concentration followed by anion-exchange high-performance liquid chromatography separation and ultraviolet detection. The method is quantitative and easily applicable to the analysis of real samples. The concentrations of ABSs in tap water and river water were determined to be *ca.* 0.1 and 100 μg/l, respectively.

INTRODUCTION

Since various kinds of surfactants are synthesized and consumed in large quantities for various purposes, their qualitative and quantitative analyses are performed for different reasons. In many cases, the analyses of industrially produced surfactants such as detergents are relatively easy, because the analytes are the main constituents of the sample (in high concentration) and their compositions are usually less complicated [1,2]. On the other hand, identification and determination of surfactants in environmental samples are often difficult, because the concentration of the analytes in the sample is usually low and the matrix is very complex.

Since anionic surfactants are most widely used and discharged into the environment, their quantification methods have been studied by many workers. Anionic surfactants in waste water or in river water are often analysed by using several colorimetric methods based on ion-pair extraction with cationic colouring agents [3,4]. However, these methods are not specific for anionic surfactants such as alkylbenzenesulphonate (ABS), alkylsulphonate (ASO) and alkylsulphate (AS), and there are many interfering substances in environmental samples such as river water.

Recently, reversed-phase (RP) high-performance liquid chromatography (HPLC) methods have been developed for the specific determination of anionic surfactants [5–11]. Each class of ABS, ASO and AS is generally a mixture of homologues of various alkyl chain lengths and their positional isomers [1]. Therefore, a high-resolution RP column is required to separate and identify the individual homologues and isomers [7,9]. For the reliable RP-HPLC determination of ABSs it is necessary first to isolate the analytes from the sample matrices [7–11], because there are various compounds that show chromatographic behaviour similar to that of ABSs. From the standpoint of environmental analysis, it is more important to know the total amounts and the classes of anionic surfactants than the concentrations of the individual surfactants.

Ion-exchange HPLC methods have also been proposed for the determination of anionic surfactants [12,13]. The method is useful in identifying surfactant classes but inefficient in separating individual homologues and isomers. Since the anion-

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exchange behaviour of anionic surfactants is very different from that of inorganic and hydrophilic organic anions, an anion-exchange precolumn has been skilfully used for both concentration and prepurification of linear alkylbenzenesulphonates (LASs) in river water as described in a previous paper [11]. When a sensitive and selective detector for anionic surfactants, *e.g.*, electrochemical detection [14], is developed, the ion-exchange HPLC method will facilitate their group analysis in environmental samples.

On the other hand, field desorption [15,16] and fast atom bombardment [16–18] mass spectrometry techniques have been proposed for the analysis of anionic surfactants. Although the mass spectra have demonstrated selectivities for the detection of the analytes, the methods seem to be unsuitable for routine analyses because of their expense and the high degree of skill required for their measurement.

This paper describes a simple and selective method for the determination of ABSs at μg/l levels in environmental water, using pre-column concentration followed by anion-exchange chromatography with a low-capacity column and ultraviolet detection.

EXPERIMENTAL

Reagents and samples

Three types of alkylbenzenesulphonates (ABSs) were used. Sodium linear dodecylbenzenesulphonate (C_{12} LAS, containing phenyl-position isomers) and sodium *n*-dodecylbenzenesulphonates (LASs, laundry-analysis grade, containing homologues of C_{10}–C_{14} alkyl chain length and their phenyl-position isomers) were purchased from Wako (Osaka, Japan). Sodium alkylbenzenesulphonates (branched ABSs), and sodium 4-toluene- and 4-ethylbenzenesulphonates (short-chain ABSs) were from Tokyo Kasei (Tokyo, Japan). Sodium alkylsulphates (ASs, C_{12}–C_{15}) and sodium alkylsulphonates (ASOs, C_{13}–C_{16}) were provided by Asahi Denka (Tokyo, Japan). Sodium perchlorate and acetonitrile of guaranteed grade were purchased from Wako. Water and acetonitrile were distilled before use. The above surfactants and other reagents were used without further purification.

River water, collected in polyethylene bottles, was filtered through a 0.2-μm cellulose acetate filter and stored at −20°C before analysis.

Instrument and materials

The chromatography system consisted of a Tosoh (Tokyo, Japan) CCPM metal-free pump, a Rheodyne (Cotati, CA, USA) 7125 injector with a 100-μl sample loop, a Tosoh UV-8000 UV–VIS spectrophotometric detector and a System Instrument (Tokyo, Japan) Chromatocorder 11 integrator.

A TSKgel IC-Anion-PW (Tosoh, polymer-based anion-exchange column, 50 × 4.6 mm I.D., 30 μequiv./ml) was used for the analytical separation. A TSK precolumn IC-Conc-A (Tosoh, anion-exchange precolumn, 10 × 3 mm I.D., 2.1 μequiv. per column) was used for the preliminary concentration of diluted standard or of river water.

Chromatographic conditions

Acetonitrile–water (40/60, v/v) containing 10 m*M* sodium perchlorate was used as the mobile phase. The flow-rate was 1.0 ml/min. The detection wavelength was 220 nm. All separations were performed by isocratic elution at ambient temperature. The sample size was 100 μl for direct injections.

Concentration of ABS in water sample

The concentration and clean-up procedure was the same as described in a previous paper [11]. An aliquot of 10–100 ml of water sample was passed through the precolumn (ClO_4^- form) by using a conventional LC pump at a flow-rate of 2 ml/min. Water, 0.1 *M* sodium perchlorate, water and acetonitrile–water (50:50, v/v) were then passed through the precolumn in order.

RESULTS AND DISCUSSION

Separation column

A silica-based anion-exchange column such as Tosoh TSKgel IC-Anion-SW was not suitable for the separation, because its column life was shortened as a result of irreversible adsorption of environmental matrices. Although IC-Anion-PW is not guaranteed for the use of organic solvent, the column performance was unchanged in more than 6 months.

Chromatographic conditions

Acetonitrile–aqueous sodium perchlorate solution was used as the mobile phase for the anion-

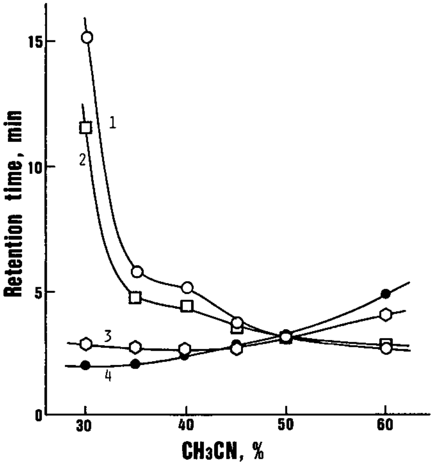

Fig. 1. Effect of acetonitrile concentration of the mobile phase (10 mM sodium perchlorate) on the retention times of LASs (1), branched ABSs (2), short-chain ABSs (3) and nitrate (4) ions.

exchange separations because of the hydrophobicity of anionic surfactants [11]. A methanol–water system was not suitable because of its high viscosity. ClO_4^- was used as the eluting ion because of its UV transparency and eluting power.

Fig. 1 shows the effect of the acetonitrile concentration of the mobile phase (10 mM sodium perchlorate) on retention of LASs, branched ABSs, short-chain ABSs and nitrate ions. The retention times of such small ions were independent of acetonitrile concentration, but those of the long-chain ABSs were greatly changed. The peak sharpness was improved by an increase in the acetonitrile concentration. At a concentration of 50% (v/v) acetonitrile, the retention times of the small and the large ions were nearly the same. Therefore, 35–40% (v/v) acetonitrile was adequate for the separation.

Fig. 2 shows the relationship between the concentration of sodium perchlorate and the capacity factor (k') of C_{12} LAS, LASs and ABSs. Both logarithmic plots were linear, and the slope for each analyte was 0.95. This indicates the ion-exchange elution of monovalent analyte ions by monovalent eluting ions [19]. Using as a criterion the separation of ABS ions and NO_3^-, 10 mM of sodium perchlorate was chosen.

Under these conditions, the analytes provided a single peak, which was somewhat broadened by the presence of many homologous compounds.

Quantitative analysis

Fig. 3 shows chromatograms of LAS standard in three different concentrations of 2 mg/l (sample size: 100 μl), 20 μg/l (10 ml) and 2 μg/l (100 ml). The relationship between the concentration and the peak area was linear from 0.1 to 10 mg/l for direct injections. The pre-column concentration followed by the analytical anion-exchange chromatography was nearly quantitative, and recoveries were nearly 100%.

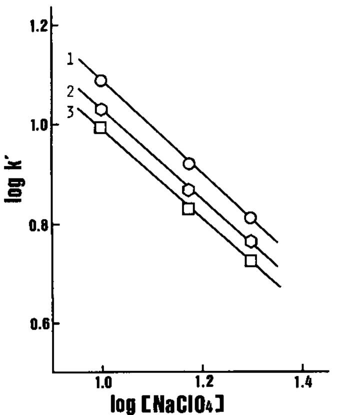

Fig. 2. Relationship between the concentration of sodium perchlorate and the capacity factor (k') of C_{12} LAS (1), LASs (2) and branched ABSs (3), in 35% (v/v) acetonitrile.

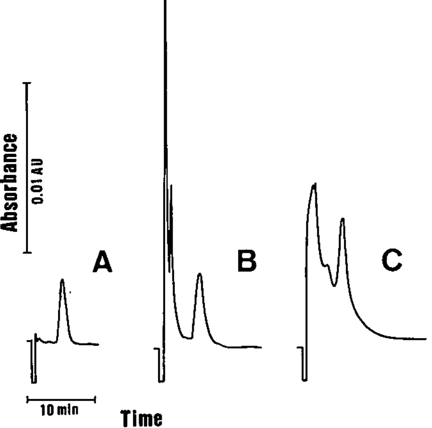

Fig. 3. Chromatograms of LAS standard in concentration of (A) 2 mg/l (× 100 μl), (B) 20 μg/l (× 10 ml) and (C) 2 μg/l (× 100 ml). For conditions, see text.

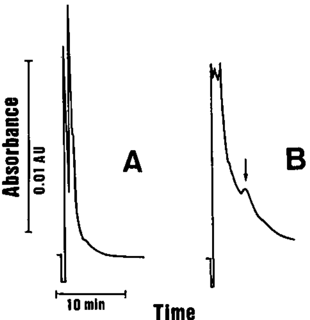

Fig. 4. Chromatograms of 100 ml of (A) Milli-Q water and (B) tap water. For conditions, see text.

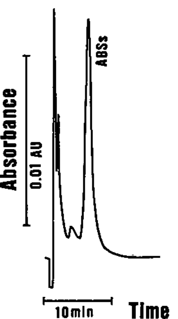

Fig. 5. Chromatogram of 2 ml of Tama River water. For conditions, see text.

Selectivity

The preliminary concentration and clean-up procedure was fairly selective for anionic surfactants. Hydrophilic neutral species and common anions and cations are removed from the analytes by passing 0.1 *M* sodium perchlorate through the precolumn. Most hydrophobic neutral and cationic species are also removed by passing acetonitrile–water (50:50). Moreover, the presence of cationic and non-ionic surfactants did not affect the determination of ABSs, because they were eluted near the solvent front. Although aliphatic anionic surfactants such as ASs and ASOs act like ABSs in the present method, they were eluted before ABSs under the proposed chromatographic conditions. Likewise their UV transparency did not affect the determination of ABSs.

Application to the analisis of river water

Fig. 4 shows the chromatograms of 100 ml of Milli-Q (Nihon Millipore) water and tap water. ABSs were not detected in the Milli-Q water but were detected in the tap water (*ca.* 0.1 μg/l).

Fig. 5 shows the chromatogram of 2 ml of river water from the Tama River in Tokyo. When the sample (50 μl) was directly injected into the analytical column, the UV-absorbing nitrate was detected as an extremely large peak. The concentration of total ABSs in the river water was about 100 μg/l.

Since the previously reported method using a reversed-phase HPLC [11] was not applicable to the determination of branched ABSs, the present anion-exchange method is useful for the quantitative evaluation of total amounts of ABSs.

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Screening methods for asulam, oxine-copper and thiram in water by high-performance liquid chromatography after enrichment with a minicolumn

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ABSTRACT

A syringe-type minicolumn containing 40 μl of C_{18} bonded silica was used for pretreatment of the samples in the determination of methyl-N-(aminophenylsulphonyl)carbamate (asulam), bis(8-quinolinolato)copper (oxine-Cu) and bis(dimethylthiocarbamoyl)disulphide (thiram) in ground, tap and river water by reversed-phase high-performance liquid chromatography (HPLC). The low levels of pesticides were adsorbed on the minicolumn, which was then connected to the injector of the HPLC system, and directly injected into the loop by eluting with a small amount of eluent. Average recoveries of asulam, oxine-Cu and thiram in water samples at concentrations of 5 μg/l were 91.5, 77.1 and 87.3%, respectively. The detection limits of asulam from 300 μl of water sample and of oxine-Cu and thiram from 1 ml were 0.2, 1.0 and 0.5 μg/l, respectively. It took only about 10 min to obtain a chromatogram on HPLC, including sample pretreatment. The syringe-type minicolumn was useful to eliminate compounds coexisting in water. This small-scale procedure enabled the sample volume required and the amount of organic solvent for elution to be reduced.

INTRODUCTION

High-performance liquid chromatographic (HPLC) determination of organics in water has generally been performed by injecting an aliquot of the extract prepared by solid-phase or liquid–liquid extraction [1–3] and by on-line pre-column sample enrichment using a column-switching valve system [4–6]. The former off-line method requires more than 100 ml of water sample to determine pesticides at the level of a few micrograms per litre. It takes time for sample preparation and requires extraction and concentration steps before analysis by HPLC. Moreover, a large volume of organic solvent waste is produced during liquid–liquid partition. As environmental pollution by chemicals including organic solvents is an object of public concern, it is preferable to be able to reduce the amount of organic solvent used in laboratories when measuring the level of a contaminant. The latter on-line method has some merits, for example it is a highly sensitive and simple technique. However, an enrichment column is usually used repeatedly for many water samples, and cross-contamination becomes a problem, especially in the case of trace levels in water, unless extensive washing between samples is carried out.

The object of this work was to develop a rapid and highly sensitive HPLC method to detect pesticides at microgram per litre levels in a small volume of water sample.

A disposable syringe-type minicolumn packed with silica gel, Extrashot-Silica, developed by Homma *et al.* [7] permits direct sample injection into an HPLC system without tedious sample preparation, and an improved version has been applied to the determination of theophylline and methylpyrazines in biological fluids such as plasma and urine [8,9].

We investigated analytical methods using a syringe-type minicolumn packed with C_{18} bonded sil-

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ica to detect methyl-N-(4-aminophenylsulphonyl)-carbamate (asulam), bis(8-quinolinolato)copper (oxine-Cu) and bis(dimethylthiocarbamoyl)disulphide (thiram). These pesticides are scattered copiously on golf courses in Japan to keep them green. Regulation levels of these pesticides were established recently in Japan (1990).

EXPERIMENTAL

Reagents and chemicals

Asulam, oxine-Cu and thiram were purchased from GL Science (Tokyo, Japan). Acetonitrile and methanol were of HPLC grade, and the other compounds obtained from Wako (Osaka, Japan) were of analytical grade. Stock solutions were prepared at 100 mg/l and stored at 4°C with acetonitrile as the solvent for asulam and thiram, and methanol for oxine-Cu.

Apparatus

A syringe-type minicolumn, Extrashot-ODS, was obtained from Kusano Kagakukikai (Tokyo, Japan). The minicolumn [8] had a 51-mm-long needle made of stainless-steel and the column portion (27 mm × 7 mm I.D.) was prepacked with 40 μl of C_{18} bonded silica (particle size 70 μm) in an internal tube made of PTFE.

The HPLC system consisted of a Model 880-PU pump (Jasco, Tokyo, Japan) a Model 870 ultraviolet detector (Jasco), a Model 7125 injector (Rheodyne, Cotati, CA, USA) with a 100-μl loop and a Model C-R4A chromatographic integrator (Shimadzu, Kyoto, Japan). An Ultrasphere-octadecylsilane (ODS) reversed-phase column (250 mm × 4.6 mm I.D., Beckman, San Ramon, CA, USA) was used to analyse asulam and thiram, and ODS-Cu (150 mm × 4.6 mm I.D., GL Science) was used for oxine-Cu.

Chromatographic conditions of HPLC

Asulam and thiram were monitored by ultraviolet detection at 270 nm, and oxine-Cu at 240 nm.

The mobile phases for asulam, thiram and oxine-Cu were acetonitrile–50 m*M* potassium phosphate buffer, pH 3.2 (30:70, v/v), acetonitrile–water (60:40, v/v) [10] and acetonitrile–100 m*M* potassium phosphate buffer pH 3.0, (10:90, v/v), respectively, as recommended by the manufacturer of the ODS-Cu column. The flow-rate of the mobile phase was 1 ml/min.

Analytical procedures

Sample pretreatment. A 10-ml aliquot of water sample was dispensed into a 13-ml glass-stoppered test tube. When the water sample contained free available chlorine, HOCl and OCl^-, they were removed by sodium sulphite. Water samples were acidified with 20 μl of 35% hydrochloric acid for asulam, and with 10 μl of phosphoric acid for thiram.

Conditioning of syringe-type minicolumn. The minicolumn, Extrashot-ODS, was connected with a glass syringe and pre-washed with 300 μl of acetonitrile and then with 500 μl of distilled water.

Sample application. An aliquot of a few millilitres of the treated water sample as above (*Sample pretreatment*) was applied onto the minicolumn through a tuberculin test glass syringe.

Direct injection of eluate into the HPLC system. After the application of water samples, the minicolumn was connected to the injection port of the HPLC system. The pesticides absorbed on the minicolumn were slowly injected into the loop of the chromatograph through a tuberculin test syringe over a period of 20 s with 130 μl of the elution solvents, which were the mixture of organic solvents and aqueous solution: acetonitrile–potassium phosphate buffer (pH 3.2) for asulam, methanol–potassium phosphate buffer (pH 3.0) for oxine-Cu and acetonitrile–water for thiram.

RESULTS AND DISCUSSION

Stability of pesticides in water

The effect of pH on the stability of asulam, oxine-Cu and thiram in water was investigated. Each pesticide was added at 50 μg/l to 50 m*M* potassium phosphate buffer at pH 3, 5, 7 and 9, and then an aliquot of the solution was injected into the HPLC system. Asulam and oxine-Cu were stable at all pH values examined. The residual amount of thiram was about 90% at pH 5, 7 and 9 after 120 min, whereas it was 98% at pH 3. Acidification of water samples, preferably to less than pH 3, is necessary to determine thiram at low levels.

Similarly, the stability of the pesticides in water samples of various origins was examined. Tap water

samples contain 0.3–0.4 mg/l free available chlorine and their pH values are 7.2–7.4. Asulam, oxine-Cu and thiram in tap water gradually disappeared without any treatment, probably by decomposition or structural transformation. The loss was overcome by the addition of sodium sulphite before spiking the pesticides. By treating sodium sulphite, more than 99% of asulam and oxine-Cu remained after 120 min, while the residual amounts of thiram after 15 and 120 min were 93.5 and 65.2%, respectively. Similar results were obtained when ascorbic acid instead of sodium sulphite was used to remove free available chlorine. Dechlorination is necessary if the water sample contains free available chlorine.

In river water samples, more than 99% of asulam and oxine-Cu were retained after 120 min. Residual amounts of thiram were 97.6 and 89.0% after 15 and 120 min, respectively.

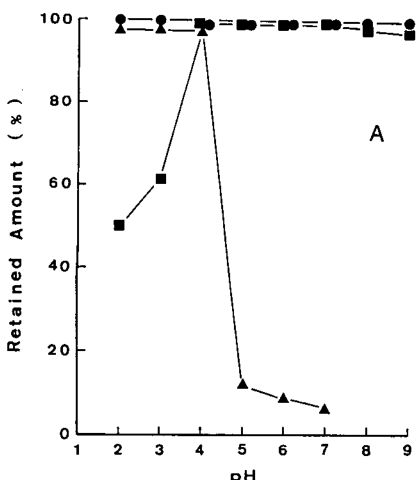
In ground water samples, asulam and oxine-Cu were stable after 120 min. Thiram was retained at 98.8% after 120 min.

It is necessary to analyse thiram as quickly as possible no matter what the sample is.

Conditions of sample adsorption on the minicolumn

The optimum pH of adsorption on the minicolumn was investigated. More than 95% of asulam was retained on the minicolumn at pH values below 4, although the amount retained was drastically reduced at pH values above 4, as shown in Fig. 1A. This result corresponds to the fact that asulam is a weakly acidic compound with $pK_a = 4.82$ [11,12]. It is necessary to acidify water samples to less than pH 4 to detect asulam in water. The amount of oxine-Cu retained was more than 95% at pH above 4, although the amount retained was reduced at pH below 4, as shown in Fig. 1A. Because the dissociation constant (pK_1) of 8-hydroxyquinoline is about 4 in 50% dioxane [13], oxine-Cu partially dissociated to give rise to 8-hydroxyquinoline at lower pH. 8-Hydroxyquinoline could not be quantitatively adsorbed on the minicolumn at pH below 4. When the pH of water sample was below 4, it was necessary to adjust the pH of water sample to neutrality by adding appropriate base. More than 95% of thiram was retained at all pH values examined, as shown in Fig. 1A.

The effect of sample volume on retention of the pesticides on the minicolumn was investigated. At least 4 ml of oxine-Cu and thiram were retained, as shown in Fig. 1B. On the other hand, asulam passed through when the sample volume was more than 300 μl. Adsorption of asulam in aqueous solution by C_{18} bonded silica was relatively weak. There-



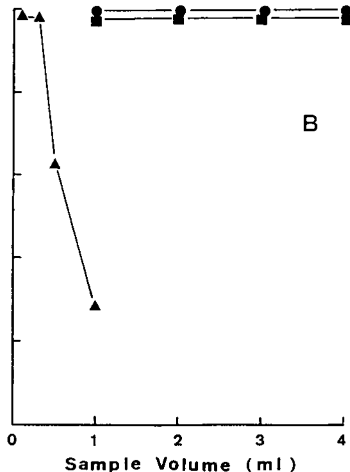


Fig. 1. Effects of pH (A) and sample volume (B) on retention of the pesticides. Each pesticide in 50 m*M* potassium phosphate buffer was spiked at 50 μg/l and the spiked solution was applied to the minicolumn. Each pesticide in the solution passed through the minicolumn was analysed by HPLC. (A) Sample volume was fixed at 200 μl for asulam (▲) and 1 ml for oxine-Cu (■) and thiram (●). The pH of potassium phosphate buffer was varied. (B) The pH of potassium phosphate buffer was fixed at pH 2 for asulam (■), at pH 7 for oxine-Cu (■) and at pH 3 for thiram (●). Sample volumes were varied.

fore, the minicolumn is suitable when the sample volume is less than 300 μl, but when a large-volume sample is to be analysed, other packing materials such as strong anion exchanger [14] have some advantages.

Conditions of eluting from the minicolumn

The elution solvent from the minicolumn was investigated. The recoveries of asulam at a concentration of 2 mg/l were more than 90% when the solvent contained more than 10% acetonitrile, as shown in Fig. 2A.

The recoveries of oxine-Cu from the minicolumn were 97.5, 65.0 and less than 5% with methanol–potassium phosphate buffer (20:80, v/v) at pH 2, 3 and 4, respectively. These results correspond to the data regarding the effect of pH on adsorption, as mentioned above. The recoveries of oxine-Cu were more than 95% when the solvent contained more than 20% methanol, as shown in Fig. 2B. When acetonitrile was used instead of methanol, the recoveries of oxine-Cu from the minicolumn were lower (data not shown).

For thiram, the recoveries were more than 95% when the solvent contained more than 50% acetonitrile, as shown in Fig. 2C. It is unnecessary to adjust the pH of eluting solvent for a neutral compound such as thiram.

In order to detect pesticides at low levels, the proportion of organic solvents in the eluting solvent from the minicolumn should be low, because the theoretical plate numbers of asulam, oxine-Cu and thiram on HPLC were reduced when the proportion of organic solvents was increased, as shown in Fig. 2. Moreover, reducing the proportion of organic solvents prevents compounds that are more hydrophobic than the pesticides from flowing into the system. From these results, the eluting solvents from the minicolumn were optimized as follows: acetonitrile–50 m*M* potassium phosphate buffer (pH 3.2) (10:90, v/v) for asulam; methanol–100 m*M* potassium phosphate buffer (pH 2) (20:80, v/v) for oxine-Cu; and acetonitrile–water (50:50, v/v) for thiram.

Recoveries of pesticides from water samples

The recoveries of asulam from ground, tap and river water samples were investigated at concentrations of 5 μg/l and 2 mg/l. The recoveries of asulam from each water sample were more than 90% at both levels, as shown in Table I. The average relative standard deviation (R.S.D.) was within 4% at

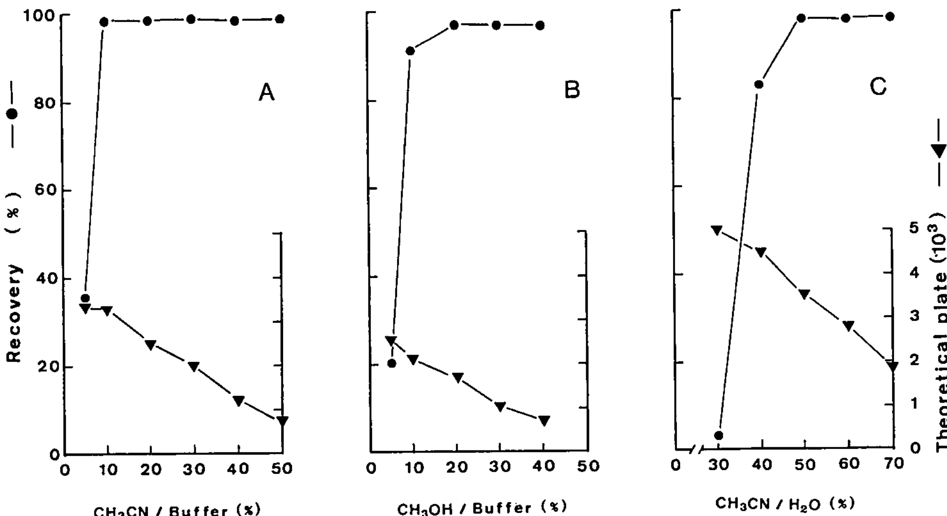


Fig. 2. Effects of the composition of the eluting solvent from the syringe-type minicolumn on the recoveries and theoretical plate numbers of the pesticides. Each pesticide was applied to the minicolumn and eluted with solvent of various compositions. (A) Asulam: spiked level, 2 mg/l; sample volume, 200 μl; buffer, 50 m*M* potassium phosphate buffer (pH 3.2). (B) Oxine-Cu: spiked level, 200 μg/l; sample volume, 1 ml; buffer, 100 m*M* potassium phosphate buffer (pH 2). (C) Thiram: spiked level, 200 μg/l; sample volume, 1 ml.

TABLE I

RECOVERIES OF ASULAM, OXINE-COPPER AND THIRAM IN SPIKED WATER

Values are mean ± S.D. (n = 5). ND = Not determined.

Pesticide	Sample[a]	Spiked level (μg/l)	Recovery (%)	
			Sodium sulphite pretreatment	No pretreatment
Asulam	G	5	92.1 ± 3.5	93.0 ± 3.0
		2000	96.6 ± 1.7	ND
	T	5	92.5 ± 2.4	<10
		2000	97.2 ± 2.0	ND
	R	5	90.0 ± 3.0	89.0 ± 2.5
		2000	92.7 ± 2.3	ND
Oxine-Cu	G	5	76.6 ± 4.0	78.0 ± 2.0
		500	95.9 ± 3.0	ND
	T	5	79.3 ± 2.7	<10
		500	99.2 ± 0.8	ND
	R	5	75.3 ± 2.9	76.2 ± 3.9
		500	97.8 ± 1.4	ND
Thiram	G	5	90.2 ± 4.0	91.2 ± 5.2
		500	99.2 ± 2.3	ND
	T	5	88.9 ± 5.0	<10
		500	97.3 ± 2.6	ND
	R	5	82.9 ± 4.8	81.5 ± 5.0
		500	99.1 ± 0.6	ND

[a] G = Ground water; T = tap water; R = river water.

both levels, and the lowest detection limit of asulam in 300 μl of water sample was 0.2 μg/l. Fig. 3A shows a chromatogram of asulam spiked at 5 μg/l in 300 μl of river water after treatment through the minicolumn.

The recoveries of oxine-Cu from ground, tap and river water samples were 75–79% at the spiked level of 5 μg/l and more than 95% at 500 μg/l, and the R.S.D. was within 4% at both levels examined. The lowest detection limit of oxine-Cu in 1 ml of water sample was 1 μg/l. Fig. 3B shows a chromatogram of oxine-Cu spiked at 5 μg/l in 1 ml of river water.

The effects of metal cations in the water samples on the recovery of oxine-Cu were examined. It is well known that 8-hydroxyquinoline is a typical chelating agent for metal cations [13]. Oxine-Cu was spiked at a concentration of 5 μg/l in 50 mM potassium phosphate buffer (pH 7) in the presence of either cadmium chloride, copper chloride, ferrous chloride, ferrous sulphate, manganese chloride, lead nitrate, aluminium chloride or nickel chloride, and extracted on the minicolumn and eluted by the eluent. In the case of nickel chloride, oxine-Cu recoveries were 81.8, 59.4 and 45.0% at nickel chloride concentrations of 0.8, 1.6 and 8 μM, respectively. The poor recoveries were improved by the addition of 27 μM disodium ethylenediaminetetraacetate. Metal cations other than nickel at a concentration of 1 mg/l had no effects on the recovery of oxine-Cu.

The recoveries of thiram from water samples were more than 90% for ground and tap water and 82% for river water at a spiked level of 5 μg/l. With fortified levels of 500 μg/l thiram, the recoveries were more than 97%. The R.S.D. values were within 5% at both levels examined, and the lowest detection limit of thiram in 1 ml of water sample was 0.5 μg/l. Fig. 3C shows a chromatogram of thiram spiked at 5 μg/l in 1 ml of river water.

Recently, Tsukioka *et al.* [14] utilized solid-phase

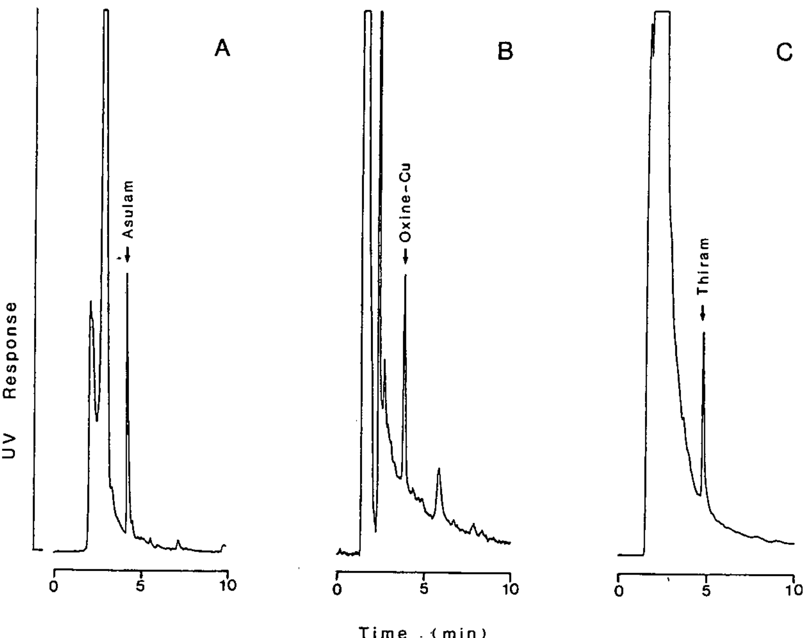


Fig. 3. Chromatograms of river water spiked with pesticides at a concentration of 5 μg/l. The water sample spiked with pesticides was applied to the syringe-type minicolumn, and adsorbed pesticides were directly injected into the HPLC system with 130 μl of eluent. (A) Asulam: sample volume, 300 μl; eluent, acetonitrile–50 m*M* potassium phosphate buffer (pH 3.2) (10:90, v/v). (B) Oxine-Cu: sample volume, 1 ml; eluent, acetonitrile–100 m*M* potassium phosphate buffer (pH 2.0) (20:80, v/v). (C) Thiram: sample volume, 1 ml; eluent, acetonitrile–water (50:50, v/v).

extraction with a strong anion exchanger (SAX) for the analysis of asulam in 100 ml of river water by HPLC. The average recovery of asulam spiked at a concentration of 10 μg/l was 80.6% and the R.S.D. was 3.0%, with the lowest detection limit of 1 μg/l. An analytical method for oxine-Cu in water by HPLC after liquid–liquid extraction with dichloromethane was presented by Ogawa *et al.* [2]. The average recovery of oxine-Cu in 1000 ml of distilled water at a concentration of 100 μg/l was 81.6% with an R.S.D. of 2.4%. Miles and Moye [15] reported on the analysis of thiram in water by HPLC, in which methylamine derived from thiram by UV photolysis was detected by fluorescence detection after reacting with *o*-phthalaldehyde–2-mercaptoethanol. In this method, the recoveries of thiram in 0.4 ml of ground water at a concentration of 10 μg/l were 86.7–96.3% with the lowest detection limit of 3.8 μg/l. In comparison with data from previously published reports, the recovery and R.S.D. of asulam, oxine-Cu and thiram in the present study utilizing syringe-type minicolumn with ODS were as good as and similar to those in large-scale analysis.

In Japan the maximum allowable contaminant levels of asulam, oxine-Cu and thiram are 200, 40 and 6 μg/l, respectively, for drinking water, and 2 mg/l, 400 and 60 μg/l, respectively, for river water. The analysis time for the whole procedure including sample pretreatment and detection on the chromatogram was only about 10 min. This method can be practically applied to estimate a low level of pesticides in water samples with only a few millilitres of samples.

Storage of minicolumn after sample application

The effects on recoveries of the pesticides of storage time of the minicolumn after sample application were evaluated. Water samples spiked with asulam, oxine-Cu and thiram at a concentration of 5 μg/l were passed through the minicolumn, and the pesticides were eluted with appropriate elution solvents

TABLE II

EFFECT OF 24 h STORAGE AT 4°C OF THE MINICOLUMN WITH SAMPLE AFTER APPLICATION

Values are mean ± S.D. (n = 5). Spiked levels of each pesticide were 5 μg/l.

Pesticide	Sample[a]	Recovery (%)
Asulam	G	91.5 ± 2.3
	T	91.5 ± 3.1
	R	95.0 ± 7.5
Oxine-Cu	G	61.5 ± 7.1
	T	67.7 ± 5.5
	R	62.4 ± 7.1
Thiram	G	6.9 ± 0.5
	T	8.2 ± 2.5
	R	4.6 ± 0.4

[a] G = Ground water; T = tap water; R = river water.

after storage for 24 h at 4°C (Table II). The recoveries of asulam and oxine-Cu were, respectively, more than 90% and 61–67% in any water sample, while those of thiram were less than 10%. When thiram was eluted with 130 μl of acetonitrile—10 mM phosphoric acid (50:50, v/v) immediately after application and analysed 24 h after storage of the eluate at 4°C, the recoveries of thiram in ground, tap and river water were 68.9 ± 9.0, 64.9 ± 6.8 and 47.0 ± 4.5%, respectively. These results suggest that the minicolumn may not be suitable for storing the pesticides on it, especially in the case of thiram. The pesticides adsorbed on the minicolumn should be eluted as soon as possible.

CONCLUSIONS

The use of the syringe-type minicolumn containing C_{18} bonded silica for the screening of asulam, oxine-Cu and thiram in water was established. The merits of this screening method with the minicolumn are: (1) a quick procedure that takes only 10 min for analysis; (2) a high sensitivity that enables detection at 1 μg/l so that only a few millilitres of water sample are required; (3) only a small amount of organic solvent is required; and (4) prepacked solid-phase minicolumns or cartridges with a versatile matrix could expand its usefulness for the analysis of chemicals in water.

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CHROMSYMP. 2691

Chromatographic methods for the analysis of size-classified and individual raindrops

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ABSTRACT

The analysis of single and size-classified raindrops is expected to give new and interesting information about anthropogenic air pollution and its consequences. A principal strategy for chromatographic apparatus based on pressure-driven and electrokinetic systems is given. Three different methods are described that are of practical use for the analysis of single and size-classified raindrops. The main cations and anions (Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^-, $HCOO^-$ and CH_3COO^-) are measured in a single raindrop with a volume of more than 500 nl.

INTRODUCTION

The atmosphere is a chemical reaction system. Reactions take place as a result of anthropogenic pollution and natural emissions of substances. These reactions lead to consequences such as a decrease in the stratospheric ozone concentration, climatological changes {*e.g.*, anthropogenic pollution with NH_3 and SO_2 is followed by the formation of $(NH_4)_2SO_4$ and NH_4HSO_4 as cloud condensation nuclei and therefore by a change in cloud formation statistics [1]}, changes in radiation balances and negative influences on biological systems. The reactions, transport and deposition of pollutants are strongly influenced by heterogeneous processes at liquid and solid particles. Wet deposition and rain in particular are an important cleaning mechanism of the atmosphere.

Owing to the large number of physical and chemical parameters that influence these processes, a simulation in the laboratory and model calculations are hardly possible. Field measurements give data that show acceptable reliability.

Measurements concerning wet deposition are often made in the bulk phase. Consequences such as clean-up of the atmosphere and influences on the ecosystem are estimated using average values of concentration.

Using a special collecting system, Georgii and Wötzel [2] and Turner [3] showed that raindrops of different size have different concentrations and these differences can often be greater than one order of magnitude. Esmen and Fergus [4] estimated pH values of single raindrops using pH paper without considering a correlation between pH and drop size.

It is impossible to give any interpretations using only average values. The aim of this work was to identify the main scavenging mechanism for each compound analysed. Typical relationships between concentration and drop radius are predicted by theoretical calculations and laboratory experiments (gas-scavenging [5] and aerosol scavenging [6,7]).

The size of raindrops has a strong influence on the scavenging coefficient [6]. Therefore, it is expected that a change in raindrop size distribution as a possible consequence of climatological changes and anthropogenic air pollution (e.g., a change in the number concentration of cloud condensation nuclei leads to a change in the cloud drop size distribution [1] and therefore to a change in raindrop size distribution), will lead to a difference in the overall scav-

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enging rate. Assuming a constant water deposition rate, a change in the composition of the atmosphere will be observed.

For calculations of these and other consequences, measurements of rain as a function of drop size and also of individual raindrops are necessary.

Analytical problem

Single raindrops have volumes ranging from 10^{-9} to 10^{-6} l. The concentrations of the main cations and anions are in the μmol/l range. As a consequence, low absolute amounts of ions have to be analysed. A further problem is the difficulty of collecting and handling single drops, owing to evaporation and contamination. Another serious task is the determination of the volumes without destroying the sample. Based on our own bulk measurements of rain, requirements for single raindrop analysis are given in Table I. The calculation is based on a drop volume of 0.5 μl. It was impossible to analyse drops of smaller volume until now. Raindrops having a volume of less than 0.5 μl (medium size raindrop) are only analysed as a size fraction.

Methods

Mainly chromatographic methods are used. Chromatographic micro systems such as micro-HPLC and capillary zone electrophoresis (CZE) usually show better absolute detection limits than larger systems such as HPLC. Hence HPLC systems are adapted to micro-HPLC and CZE. Absolute detection limits are improved by a factor of 100 or more (Table I).

Normally glass electrodes are used to measure the pH value. The volume needed for micro glass electrodes is at least 10 μl. For analysing small volumes we use a a flow-injection analysis (FIA) system with an injection volume of 0.5 μl.

Usually an increase in relative detection limit for microsystems is observed. The dilution necessary for handling the sample, however, is decreased. The observed net effect is a decrease in the relative detection limit using micro systems for this special task. Because of the sample volume required for each analysis (0.5 μl in FIA and 5 μl in micro-HPLC or alternatively 10 μl in CZE) a dilution is still necessary. As a consequence of the needed connection between capillary and electrode the required volume fraction in CZE is much larger compared to the injection volume of at least 20 nl. Additionally, the volume of the size fractions has to be distributed into three fractions (micro-HPLC/FIA or CZE/FIA). Single raindrops and size fractions are diluted with 5–10 μl of internal standard solutions. The standard consists of $LiClO_4$ of known concentration. The dilution factor of Li^+ measured together with the analysed cations is used for volume calculation. The precision of the methods employed is

TABLE I

LIMITS OF DETECTION (ng/ml) DEFINED AS 3 R.S.D.

R.S.D. = relative standard deviation of baseline noise.

Ion	Micro-HPLC	CZE
NH_4^+	1.3	25
K^+	1.3	70
Na^+	0.7	35
Ca^{2+}	3.0	40
Mg^{2+}	1.3	25
Li^+	0.2	12
Cl^-	8.0	35
SO_4^{2-}	22.0	180
NO_3^-	8.0	60
CO_3^{2-}	–	210
HCO_2^-	13.0	180
$CH_3CO_2^-$	20.0	450

TABLE II

RELATIVE STANDARD DEVIATIONS OF THE ANALYTICAL METHODS EMPLOYED AND FOR VOLUME CALCULATION ($LiClO_4$)

Species	CZE	Micro-HPLC	FIA
Cl^-	7	10	–
SO_4^{2-}	8	10	–
NO_3^-	10	15	–
$HCOO^-$	8	10	–
CH_3COO^-	10	10	–
NH_4^+	5	10	–
K^+	10	10	–
Na^+	7	10	–
Li^+	8	8	–
CA^{2+}	8	12	–
Mg^{2+}	10	12	–
pH	–	–	13

given in Table II. All the main cations and anions and the pH value are determined in size fractions. For single raindrops volume division is impossible and therefore the pH value is neglected.

The precision of the actual measurement is verified using the ion neutrality balance. Additionally, the measured concentrations weighted with the volume fractions are compared with the bulk concentrations. Volume fractions are calculated using drop size distributions measured with a dystrometer. The dystrometer is a rain spectrometer calculating drop size by impulse measurement. Usually good agreement is achieved.

Collection of rain samples

Single raindrops and size-classified raindrops are collected using the Guttalgor [8–10], which consists of a Dewar vessel filled with liquid nitrogen. Raindrops are allowed to fall into the liquid nitrogen and freeze in less than 1 s. They remain at the surface on a gas blanket until they have reached the evaporation temperature of liquid nitrogen. They then sink because of the higher density. The whole system is surrounded by an inert gas box to avoid contamination and condensation of water vapour. Depending on the rain rate, the Guttalgor is opened for several short periods. The opening time has to be limited because of the possible coalescence of two or more drops at the surface of the liquid nitrogen. Raindrops keep their spherical shape, so it is possible to separate them by seven sieves of different mesh widths in the range 0.1–1.0 mm. The collection methods allow the analysis of the bulk phase as both size fractions and individual raindrops. Experimental investigations with simulated rainwater sprayed by a scrubber system and subsequently collected show that the error based on the collection procedure is lower than the analytical detection limit.

EXPERIMENTAL

Micro-HPLC instrumentation

The analysis of alkali and alkaline earth metals was carried out with cerium(III) as eluent [11] on two laboratory-packed cation-exchange columns of different capacities [ION-210 metals column, ICT (100 × 0.5 mm I.D.) and Fast Cation 1, Dionex (150 × 0.5 mm I.D.)]. With column switching, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} were separated in one run [12]. For detection of cations a Spectroflow 980 fluorescence detector (Applied Biosystems, Weiterstadt, Germany) with a 1-μl flow cell (excitation, 251 nm; emission, 320-nm cut-off filter) was used.

The chromatographic system consisted of a metal-free IRICA Σ 871 HPLC pump (ERC, Alteglofsheim, Germany) and a Knaur metal-free injection valve. Usually 1-μl was injected for analysis.

The anions chloride, formate, acetate, nitrate and sulphate were separated on an Optisil ODS (5 μm) column (125 × 4.6 mm I.D.) (ERC) coated with dodecylamine [13] using a mixture of potassium hexacyanoferrate(II) and -(III) solutions (both 10 *μM*) as eluent with indirect UV detection. A Rheodyne Model 9125 metal-free injector (ERC) was used with a 5-μl sample loop. Usually 2.5 μl of sample were injected (Fig. 1).

CZE instrumentation

For the experimental studies two CZE systems were employed. One system was the CES I (Dionex, Sunnyville, CA, USA) with a positive and negative power supply and deuterium and tungsten lamps for UV detection. Polyimide-coated fused-silica capillaries (CS Chromatographie Service, Langerwehe, Germany) of 75 μm I.D. and 360 μm O.D. were used. The capillary dimensions were 60 cm total length with a 55-cm distance from the point of injection to the detector cell.

The other CZE system was laboratory built. The central part of this CZE system was a newly developed injection port for sample volumes between 0.5 and 5.0 μl (Fig. 2). The injection port consisted of a copper pin with a platinized funnel-shaped surface. This metal pin can be used as an electrode and as a sample vial [14,15]. The high voltage from a ±30 kV high-voltage power supply (F.u.G. Electronik, Rosenheim, Germany) was applied between both ends of the electrolyte vials and the copper pin respectively. For detection, the CES I (Dionex) was provided with a detector similar to that used in our laboratory-built system.

All electropherograms were recorded using an APEX data station (Autochrom, Milford, MA, USA).

All electrolytes were prepared fresh daily, filtered through a 0.22-μm filter (Millipore, Bedford, MA,

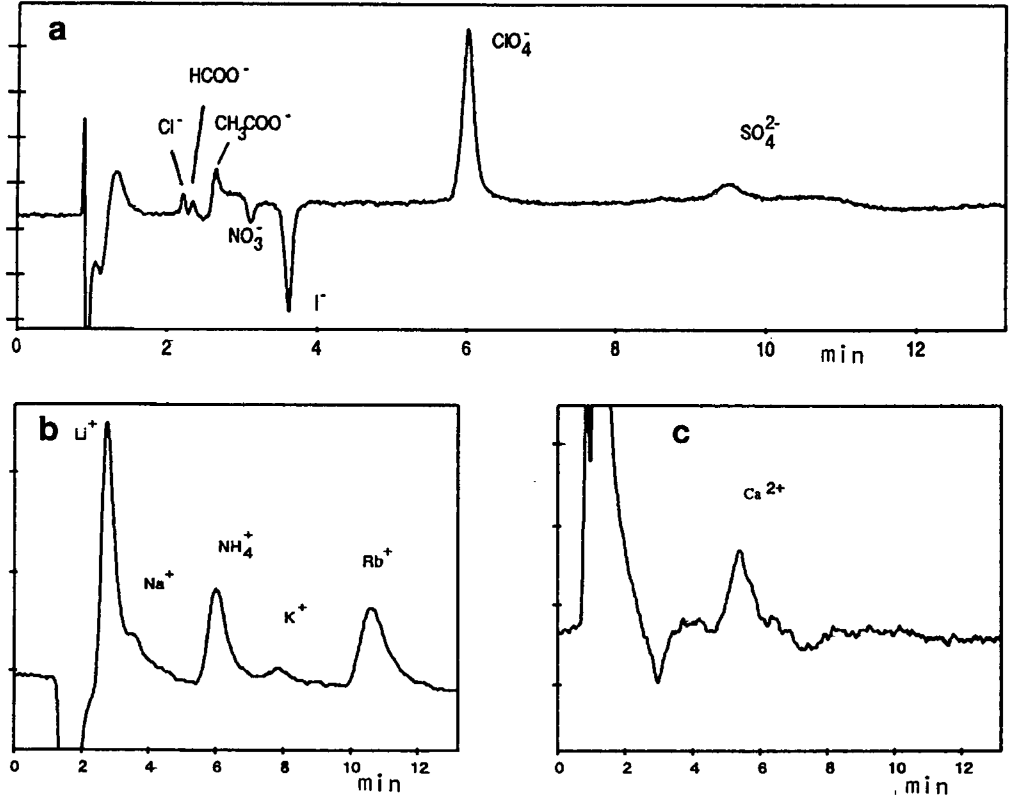

Fig. 1. Chromatograms of an individual raindrop. (a) Anions. Column, Optisil ODS (150 mm × 4.5 mm I.D.), permanently coated with dodecylamine; eluent, 2.5 μg/ml $Fe(CN)_6^{3-}$–$Fe(CN)_6^{4-}$; flow-rate, 1 ml/min; detection, indirect UV (205 nm). (b) Alkalin metal cations. Column, ION-210 metals column (120 mm × 0.75 mm I.D.); eluent, 3 μM cerium(III) nitrate; flow-rate, 43 μl/min; detection, fluorescence (254 nm). (c) Alkaline earth metal cations, column, Fast Cation 1 (150 mm × 0.50 mm I.D.); eluent, 14 μM cerium(III) nitrate; flow-rate, 36 μl/min; detection, fluorescence (254 nm).

USA) and degassed under vacuum for 5 min. For cations the electrolyte consisted of 4 m*M* 4-methylaminophenol sulphate (Fluka, Buchs, Switzerland) and 2.5 m*M* 18-crown-6 (Merck, Darmstadt, Germany) [16]. The electrolyte for anions consisted of 5 m*M* K_2CrO_4 (Merck) and 0.5 m*M* tetradecyltrimethylammonium bromide (Aldrich, Steinheim, Germany).

All standard mixtures were prepared by dilution of 10 m*M* stock solutions containing a single cation or anion. Water purified with a Milli-Q system (Millipore) was used throughout.

Before each electrophoresis the capillary was rinsed for 3 min with electrolyte solution by vacuum applied at the end of the capillary outlet. All injections during the measurement were performed in the hydrostatic mode where the capillary was immersed in the sample at a height 10 cm above the running electrolyte for 30 s. On lowering the sample to the 0 cm level the capillary was removed from the sample and the loaded capillary was immersed in the running electrolyte with a voltage of up to 30 kV.

FIA system instrumentation

Flow-injection analysis with UV detection was used for the determination of pH values [17]. A mixture of two pH indicators, Methyl Red and Methyl Orange (Merck), is used as a UV-absorbing reagent. Aqeuous solutions (40 m*M*) of the sodium salts of the pH-sensitive dyes were prepared and mixed in a ratio of 1:1. Therefore, pH values in the

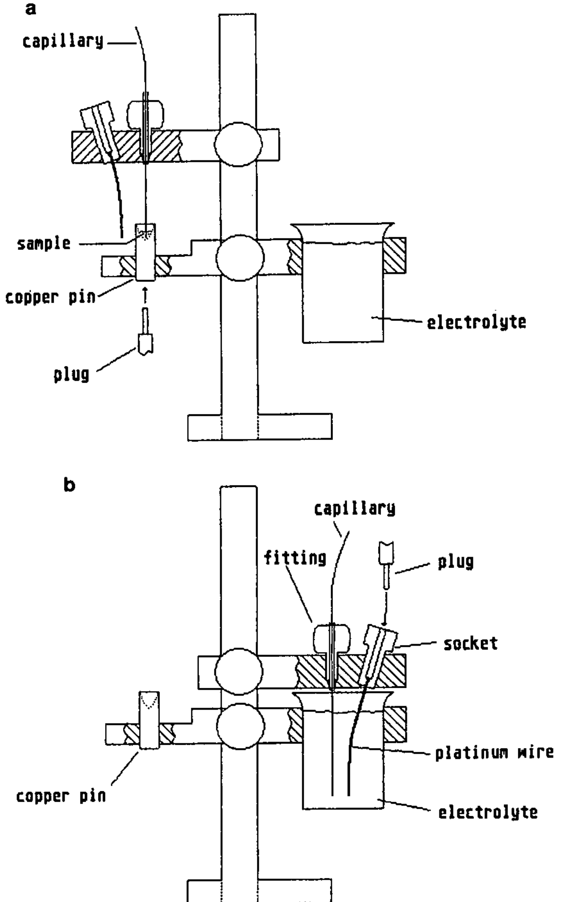

Fig. 2. CZE injection port for small volumes. (a) Injection of a sample; (b) electrophoresis.

range 3.0–7.0 appropriate to the acidity of rainwater could be measured by using this reagent [15]. The sample was injected directly into the reagent flow by a Rheodyne type 7520 valve and reacts in the connecting tube between injector and detector cell based on dispersion. The indicator solution was pumped at 0.15 ml/min with a Irica Σ 871 pump. A Spectroflow 757 UV detector (Applied Biosystems), measured the absorbance at 430 nm. The volume of the loop of the injection valve was 0.5 μl but a sample volume of 2 μl was required to guarantee a contamination-free injection. The system was calibrated with unbuffered standards prepared from 1 *M* hydrochloric acid (Merck) and 1 *M* sodium hydroxide solution (Merck) by dilution with water. These standards showed a low ionic strength comparable to that of rainwater. For each analysis it was necessary to record a calibration graph for the complete pH range.

RESULTS AND DISCUSSION

Advantages and disadvantages of the applied methods are outlined below.

CZE

The main advantage of the CZE for rain analysis is the small injection volume, usually *ca.* 10 nl. Therefore, it is possible to analyse small samples several times without using the whole sample volume. The main disadvantage is the large volume needed for the analytical procedure. For an automatic injection at least 10 μl are needed, which means a three orders of magnitude higher volume than the injection volume. This disadvantage is caused by the fact that an electrical current is needed for CZE and therefore the capillary and the electrode have to be connected by the liquid, which requires at least 10 μl. A new experimental set-up was used to overcome this problem. The pin (Fig. 2), as an alternative for sample injection, needs a volume of only 0.5–1.0 μl but no automatic version can be envisaged in the near future. CZE shows higher detection limits than Micro-HPLC, so its use for single raindrop analyses is limited at present. However, owing to the possibility of automation and the large numbers of measurements needed for rain analysis, CZE is used when possible. A typical chromatogram is shown in Fig. 3.

Micro-HPLC

Micro-HPLC is a powerful analytical method. The detection limits are usually lower than those in CZE, owing to the possible employment of off-column detection techniques. However, no automatic sample injection is possible. To avoid contamination, metals have to be excluded in the experimental set-up.

No metal-free microlitre syringe is commercially available. Therefore, the sample is sucked from the rearside through a short PEEK cappillary. Additionally, partial filling of the sample loop is necessary for cases of limited sample volume. Raindrops are limited in volume by nature and dilution has to

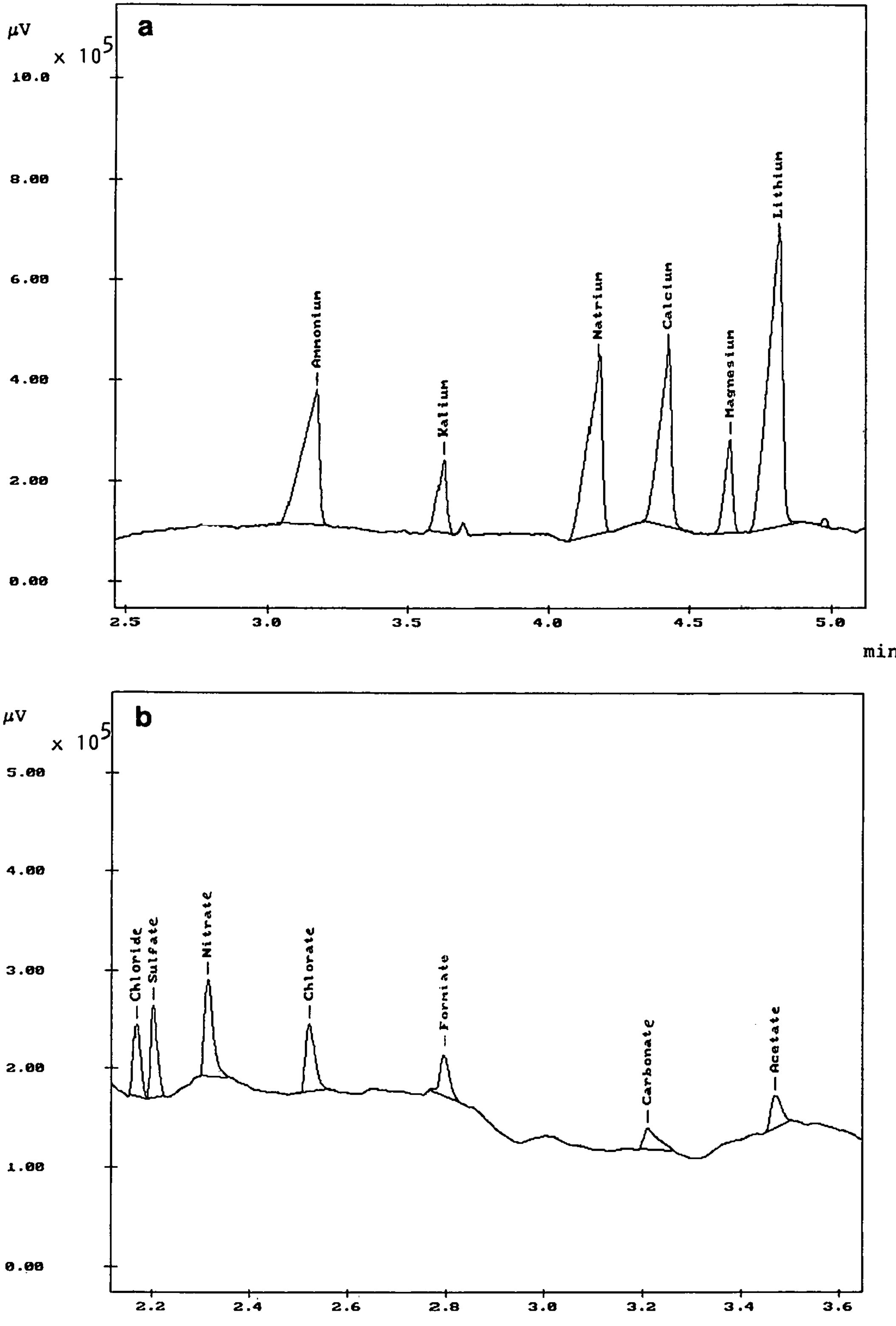

Fig. 3. Electropherogram of rain samples of (a) cations and (b) anions. (a) Electrolyte: 4 m*M* methylaminophenol sulfate and 4 m*M* 18-crown-6; 55 cm effective capillary length; injection: hydrostatic, 10 cm for 30 s; migration voltage: 22 kV; detection: indirect UV (220 nm). (b) Electrolyte: 5 m*M* potassium chromate and 0.2 m*M* tetradecyltrimethylammonium bromide; 55 cm effective capillary length; injection: hydrostatic, 10 cm for 30 s; migration voltage: 22 kV; detection: indirect UV (276 nm).

be minimized in order to avoid contamination and decreases in concentration. Often for size fractions only small volumes are collected as a consequence of the low volume of the smallest drops (< 10 nl). Therefore, the sieves with the smallest mesh width (0.2 and 0.3 mm) often showed volumes of 1 μl or less.

Micro-HPLC usually works with an analytical volume close to 1 μl. However the sample loop and all the dead volume in front of the loop are rinsed five times to obtain high reproducibility. This would lead to an unacceptable loss of sample which is not used for the analysis, so the sample plug has to be transported in the sample loop using an internal standard solution consisting of 35 μM rubidium iodide. The fraction of the volume introduced in the sample loop is calculated by measuring the decrease in internal standard concentration. The sample loop employed is compatible with the sample plug, so usually the whole sample volume is used for analysis. About 2.5 μl of the whole sample volume are injected.

For determination, not only standards of different concentrations but also with different volumes have to be measured. However, calibration is very time consuming. Therefore, micro-HPLC is used if the detection limits needed cannot be achieved by CZE.

FIA

Traditionally, a flow-injection system is distinguished by rapid sample analysis and high reproducibility. In addition, it is possible to handle small injection volumes using the applied system. The flow velocity is limited to 0.15 ml/min to achieve a maximum reaction rate by a minimum dilution factor. Therefore, about 5 min are required for each measurement. Only for size fractions with a volume larger than 2.5 μl can the pH value be determined because 2 μl of the undiluted sample are taken for the pH determination.

Analytical strategy

The following strategy is used for measurements on real rain samples. Single raindrops are always analysed by micro-HPLC, owing to the better detection limit for the available volume. It is impossible to divide such small volumes into two fractions, so it is not practicable to measure anions and cations in addition to the pH value in a single raindrop. Therefore, the pH is neglected, but for size fractions H^+ concentration, as an important cation for ion balance, is always measured. For anions and cations two different procedures are used. The decision as to whether micro-HPLC or CZE should be used is accomplished by bulk analysis. With highly concentrated rain the automatic CZE system is used. When the relative detection limits are expected to be sufficient for the manual CZE system, this experimental set-up is used because of the faster standardization. For the lowest concentrations micro-HPLC is preferred. The latter system is often used for rain events showing volumes of 500 nl–3.0 μl leading to a high dilution factor. Small volumes are observed for short rain events such as thunder storms or rain events with a low precipitation intensity.

Significance of results for the atmospheric system

Typical relationships between concentration and radius as a function of time were found. These fundamental connections were observed for several rain events independent of the meteorological conditions. Interpretations based on physical and chemical models are given elsewhere [10]. Results for ammonia concentration in single raindrops are shown in Fig. 4.

At the beginning of a rain event a continuous increase in concentration with decreasing drop radius is observed. This function changes during the rain event and maximum concentration is found for raindrops of medium size. Single raindrops also vary in concentration. These differences are significantly higher than the error of measurement.

A change in raindrop size distribution towards smaller drops as a possible consequence of anthropogenic pollution will lead to an increase in the number of highly concentrated drops with a corresponding danger potential. The bulk concentration of rain as a whole increases especially at the beginning of the rain event. A high concentration, with a decrease in pH value for instance, results in increasing environmental consequences.

A positive effect is expected for the balance of radiation. The increasing scavenging coefficient of smaller drops has the consequence that an increasing number of aerosols are scanvenged and therefore light scattering at aerosol particles decreases.

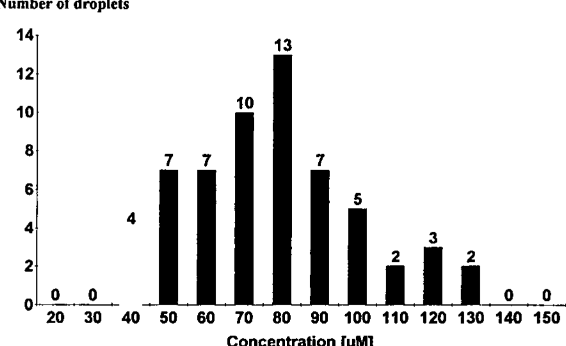

Fig. 4. Distribution of ammonia concentration for individual raindrops. Concentration intervals of 10 μM; sieve fraction, 0.4 mm; drop radii, 0.4–0.5 mm.

This phenomenon will have a positive influence on the temperature increase discussed as a consequence of CO_2 emission. Chemical reactions are a function of drop size. For example, SO_2 oxidation shows two different reaction mechanisms depending on the pH: at pH < 5.0 H_2O_2 and at pH > 5.0 ozone are the main oxidants.

Therefore, a change in dropsize distribution may lead to a change in reaction mechanism. At present it is impossible to draw any conclusions about the consequences on the change in lifetimes of atmospheric pollutants resulting from alterations to the drop size distribution. In the future it will be necessary to use these results for model calculations. Further measurements are required at different locations to give a more detailed picture.

CONCLUSIONS

Single raindrop analysis and the analysis of size-classified rain can be achieved using micro-HPLC, FIA and CZE. The main cations and anions but neglecting the pH value can be determined in a single raindrop. The drop volume is available in addition. Analysing size fractions, a volume division is possible so the pH value can be measured. The concentration variations in single drops and size fractions show a significant tendency.

In the future, enrichment systems for small volumes will be needed to obtain information about the less concentrated components in the microsystem described.

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CHROMSYMP. 2738

Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia

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ABSTRACT

Concurrent measurement of the sewage tracer coprostanol and fecal indicator bacteria were made for water and sediments collected in January 1992 from coastal waters off Sydney, Australia. The coprostanol results were compared with data from an earlier survey conducted in 1989 before the commisioning of Sydney's deepwater ocean outfalls in 1990 and 1991. Good correlations were observed for both water and sediment samples between coprostanol and the two fecal indicator organisms, fecal coliforms and *Clostridium perfringens* spores, thereby validating the use of coprostanol as a sewage signature in this environment. For sediments, most inner-shelf sites (1–10 km offshore) showed an increase in the concentration of coprostanol between the two surveys. The areas of highest concentration have been shifted further off-shore, to zones adjacent to the diffusers.

INTRODUCTION

Sewage contamination has traditionally been determined by enumeration of fecal coliform bacteria. However, the reliability of coliforms as an adequate indicator of sewage contamination has been questioned [1–3]. This is mainly due to the extreme variability found for fecal coliform survival under varying environmental conditions [4], as expected with coastal outfall systems, and a poor correlation with specific pathogens [5].

As alternatives to microbial indicators, a range of sewage specific organic compounds have been proposed. Such compounds can be readily analysed by capillary GC. One of these, coprostanol, which is produced in the digestive tract of higher animals by microbial degradation of cholesterol [6], has proven a successful and sensitive indicator of sewage pollution [7–9]. Chemical signatures also overcome many of the shortcomings of classical microbiological indicators of sewage pollution [10]. Readman *et al.* [11] proposed an analytical protocol including analysis of coprostanol to quantify sewage, oil and polycyclic aromatic hydrocarbon pollution in estuarine and coastal environments. The use of these techniques shows much potential. To date, however, only limited studies, apart from various analyses of hydrocarbons, have been undertaken in Australia.

In 1989, before the three deepwater ocean outfalls built for the disposal of the majority of Sydney's sewage were completed, a survey (twenty-six sites) was undertaken of the area deemed likely to be affected by the sewage plumes. The daily flow from the nearshore Malabar outfall, the largest of the outfalls, is approximately 640 Ml/day [12]. The Malabar discharge includes the bulk of Sydney's industrial waste that enters the sewerage sys-

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tem. The effluent is released after primary treatment together with sludge which included a median discharge of 22 tonnes day^{-1} of grease [13].

The discharge contains many industrial pollutants including heavy metals, polycyclic aromatic hydrocarbons and organochlorines [12]. The deep-water ocean outfalls were commissioned between September 1990 and August 1991 [13]. In January 1992, sites studied in the 1989 survey were reanalysed as well as additional sites bringing the total to 96.

The major objective of the program was to determine the distribution and fate of Sydney's sewage effluent using combined chemical, microbiological and physical oceanographic data. In this study, comparative water column and sedimentary results are presented for coprostanol, measured using modern capillary GC methods, and common fecal bacteria indicator groups. Results are presented for samples collected from 9 stations along a 35-km transect commencing 1 km off-shore from the old Malabar cliff-face outfall. These data were obtained to aid scientists and managers who are examining the impact of the deep ocean outfalls.

EXPERIMENTAL

Sample collection

Sampling was conducted aboard RV Franklin during November 1989 (cruise FR 13/89) and January 1992 (cruise FR1/92). Station locations are shown in Table I. The Malabar deep water ocean outfall is located immediately south of stations 29 and 30. Sediments were collected to water depths in excess of 1000 m using a Smith-McIntyre grab. Surface sediment (0–2 cm) was removed from the grab using a stainless steel or a sterile plastic spoon. Water samples were collected using a Neil Brown CTD fitted with a rosette of 10-l Niskin bottles. Water was filtered at sea through glass fibre filters (15 cm, Schleicher and Schuell No. 8, nominal pore size 0.5 μm) to obtain particulate matter samples. All samples for chemical analysis were stored immediately at −20°C and were transported to the CSIRO Marine Laboratories in Hobart for subsequent analyses.

Reagants

Nanograde solvents and reagents (Mallinkrodt) were used in the lipid procedures. All glassware was prerinsed with nanograde solvent prior to use. Concurrent analyses of laboratory blanks were undertaken during sample analysis.

Lipid extraction and fractionation

Samples (30–80 g, wet weight) were extracted quantitatively by the modified one-phase $CHCl_3$-MeOH Bligh and Dyer method [14,15]. After phase separation, the lipids were recovered in the lower $CHCl_3$ layer (solvents were removed *in vacuo*) and were made up to a known volume and stored sealed under nitrogen at −20°C. Total lipid sterols were obtained following alkaline saponification of an aliquot (10%) of the total lipids [16,17]. Products were extracted into hexane–$CHCl_3$ (4:1, v/v) and stored at −20°C. Sterols were converted to their corresponding trimethylsilyl (TMSi) ethers by treatment with bis(trimethylsilyl)trifluoroacetamide (50 μl, 60°C, 60 min).

Gas chromatography (GC) and GC-mass spectrometry (GC–MS)

GC analyses were performed with a Hewlett-Packard 5890 GC equipped with a 50 m × 0.32 mm I.D. cross-linked methyl silicone (0.17 μm film thickness) fused-silica capillary column (Hewlett-Packard), a flame ionization detector (FID) and a split/splitless injector. After addition of methyltricosanoate internal standard, samples were injected (2 μl out of typically 50–1000 μl of sample) in the splitless mode at 50°C and after 1 min the oven was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1}, and finally to 300°C at 5°C min^{-1}. Hydrogen was used as the carrier gas (inlet pressure 70 kPa, 1 ml min^{-1}). Peak areas were quantified using chromatography software (DAPA Scientific software) operated using an IBM-XT personal computor. Component identification was based on comparison of retention time data with that obtained for authentic and laboratory standards. Coprostanone eluted after both coprostanol and epicoprostanol under the GC conditions used in this study.

The FID response was found to be linear in the practical concentration range (0.5–150 ng of individual components injected) used in this study. Samples were routinely analysed by GC within 1–2 weeks of extraction. Prior to instrumental analysis samples were stored in solvent and were not allowed

TABLE I

STATION LOCATIONS, WATER COLUMN COPROSTANOL CONCENTRATIONS AND INDICATOR ORGANISM DATA

Station number	Position		Distance from shore (km)	Water depth (m)	Sampling depth (m)	Coprostanol (ng/l)	Indicator organisms (CFU/100 ml)[a]		
	Latitude	Longitude					FC	FS	CP
27	33 58.27	151 16.61	0.9	49	5	9.4	150	ND	ND
					40	19	45	ND	ND
28	33 58.14	151 17.25	1.9	70	5	5.4	3	1800	0.45
					60	625	9900	2.3×10^4	1.7
29	33 58.00	151 18.13	3.7	78	5	2.5	25	ND	ND
					70	1660	1.6×10^4	ND	ND
30	33 58.07	151 18.19	3.7	78	5	9.2	50	520	0.4
					65	3810	2.5×10^5	400	161
31	33 58.31	151 19.50	5.6	88	5	ND	2.5	39.5	2.8
					60	1500	1.4×10^4	240	53
32	33 58.23	151 20.74	7.4	91	5	2.4	<1	ND	ND
					85	55	34	ND	ND
33	33 58.03	151 22.02	9.2	95	5	2	<0.5	24.5	2
					90	11	3.5	4.6	1.2
34	33 57.90	151 27.88	18.5	141	5	0.05	1.5	41.5	0.35
					133	4	<0.5	9.8	0.15
35	33 58.18	151 33.74	27.8	169	5	0.05	1.3	320	0.85
					165	ND	<0.5	95	0.45
36	33 58.83	151 49.26		1031	–	–	ND	ND	ND

[a] CFU = Colony forming units. FC = fecal coliforms; FS = fecal streptococci; CP = *Clostridium perfringens* spores. ND = not determined.

to go to dryness. During a typical column lifetime (6–12 months), repeat analysis indicated that minimal loss of coprostanol occurred as long as routine injector maintenance was performed. Although synthetic coprostanol was not available, analysis of replicate samples indicated good agreement for within day, day to day, and longer term variation samples. The relative standard deviation for replicate samples was generally <5% or better. Duplicate GC assays of the same sample showed 1–10% standard deviation over the concentration range used.

Verification of identifications was by GC–MS analysis of samples performed on a HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and a split/splitless injector. Data were acquired and processed on an HP 59970C Workstation operated in scan aquisition mode. Operating conditions are described in detail elsewhere [17–19]. The non-polar column was similar to that described above. The mass spectra of coprostanol and coprostanone were distinguishable.

Microbiology

Fecal coliforms, fecal streptococci and spores of *Clostridium perfringens* were enumerated by membrane filtration (0.45 μm) using standard methods [20], and m-FC (Difco No. 0883-01), *m*-enterococcus (Difco No. 0746-01) and perfringens (Oxold CM543) agars, respectively. Sediments (10 g) were dispersed by sonication (180 W, 30 s) in 100 ml sterile seawater prior to filtering. Selected colonies were confirmed by standard methods and counts are reported as colony forming units (CFU) on a 100 ml or 100 g basis. Relative standard deviations for sediments were: fecal coliforms, 0.5–20%; fecal streptococci, 0.2–5%; *Clostridium perfringens* spores, <0.5%. Standard deviations were generally an order of magnitude greater for water samples.

Sedimentary organic matter

Sedimentary organic carbon content was determined by measuring weight loss on ignition (550°C, overnight).

RESULTS AND DISCUSSION

Representative gas chromatograms illustrating sterol profiles for water column particulate matter and sediments from the Malabar transect are shown in Fig. 1. A high-resolution 50-m column was chosen for the analyses in order to ensure that separation was achieved of coprostanol and epicoprostanol, in addition to other sterols and non-sterol components. Under the GC conditions employed, sterols eluted between approximately 55 and 65 min and base-line separation was observed for coprostanol and epicoprostanol. The GC program was designed to resolve other components (*e.g.* hydrocarbons, linear alkyl benzenes, alcohols; data not shown) found in the non-saponifiable neutral lipid fraction. Many of these components provide useful information on other sources of organic matter in environmental samples. If information is only being sought on coprostanol alone, then the GC analysis time can be shortened considerably through the use of a faster rate of oven temperature increase. Similarly, scope exists to further streamline the procedure through direct saponification of either filter (water column) or sediment samples, thereby eliminating the solvent extraction step used used in this study.

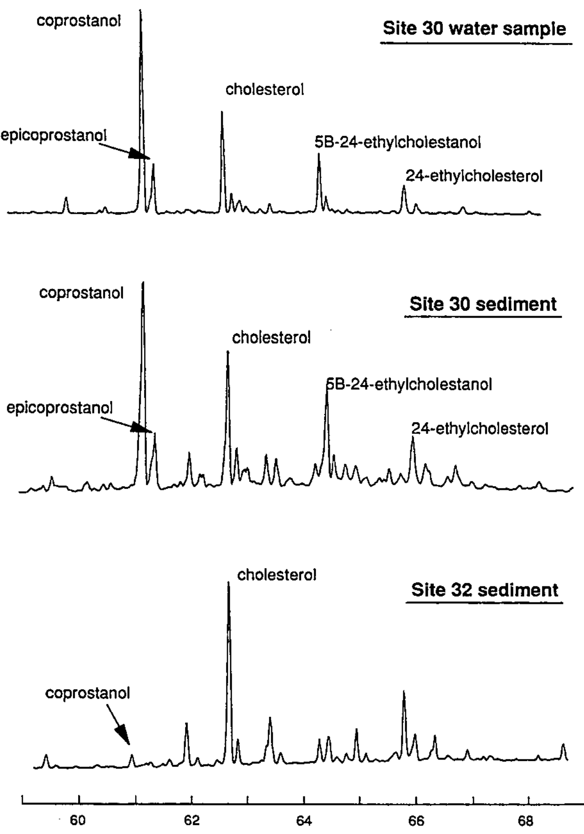

Fig. 1. Partial gas chromatograms of sterols (as TMSi ethers) in particulate matter (station 30, 65 m water sampling depth) and sediments (stations 30 and 32). Station locations refer to Table I. HP1 fused-silica capillary column.

The theoretical limit of detection for coprostanol in this study was 1 ng/l for water column particulate matter (typically 4 l analysed) and 1 ng/g for sediments (from 5–10 g). Effluent and sludge from sewage treatment plants in the Sydney region typically contain around $3 \cdot 10^5$ ng/l and $1 \cdot 10^6$ ng/g of coprostanol, respectively (unpublished data). Therefore measurement of coprostanol in field samples can theoretically estimate dilution factors approaching 10^5 and 10^6 for effluent and sludge, respectively. These detection limits could be improved through extraction of more material, performing quantitation by SIM GC–MS or other specific detectors after appropriate derivatization. Using coprostanol, in related studies we have measured dilution factors of 10^4 for sewerage effluent at distances of 100 km from the Malabar outfall. The dilution factors were verified using other chemical and microbiological parameters.

In coastal regions typical of those around Sydney and other Australian coastal cities and towns, we believe a background concentration of coprostanol in aerobic surface sediments is no greater than 5–10 ng/g. This value is based on analyses of sediments collected from pristine locations in Australian coastal waters, in central Bass Strait and near Jervis Bay. Coprostanol was below detection (*i.e.* < 1 ng/l) in all water samples from pristine locations, indicating that the background concentration in water is at least an order of magnitude lower than for sediments. Coprostanol was not present in routinely performed laboratory blank assays.

Coprostanol in marine waters

The concentrations of coprostanol in surface water samples along the Malabar transect were generally very low. Concentrations ranged from below detection at the off-shore sites (stations 34 and 35)

to 10 ng/l adjacent to the coast. In contrast, coprostanol in bottom water samples ranged from 20–3800 ng/l at stations between 1 and 7 km from the coast. Beyond 7 km from the shore, coprostanol levels were similar to those of the surface waters. The highest concentrations were at sites (stations 29 and 30) immediately north of the Malabar deep-water ocean outfall located 4 km from shore. An effluent coprostanol concentration of $330 \cdot 10^3$ ng/l was determined for effluent collected concurrently with the shipboard sampling. Using this data, effluent dilution factors of between 1/80–1/220 can be calculated for bottom waters at stations 29 to 31.

Oceanographic measurements at the time of sampling showed that the water column was well-stratified, with the bottom waters around 8°C cooler than surface waters. Although strong north to south currents can prevail for much of the year in these waters, the nearshore current at the time of this study was northward, possibly due to the passage of a coastly trapped wave [21]. An intrusion of continental slope water with a temperature of < 14°C reached in across the floor of the shelf to the 85 m contour and then withdrew. The results observed for coprostanol in waters along the Malabar transect are consistent with the oceanographic currents at time of sampling.

Coprostanol in marine sediments

The sediments reflect the cumulative effects of the different current types encountered in the Sydney region, while the water column data provides only a picture of effluent dispersion based on prevailing currents at the time of sampling. From the 1992 survey, concentrations ranged from 40–2800 ng/g in the sediments collected along the Malabar transect as shown in Table II. For 1989, coprostanol concentrations for inner shelf sediments ranged from 20–530 ng/g. The higher coprostanol concentrations (> 500 ng/g) are in the range associated with significant sewage pollution [11,22].

Nearly all inner shelf sites (1–10 km) showed large increases in the amount of coprostanol present after commissioning of the deep ocean outfalls. The increase in coprostanol concentration at most sites (1989 to 1992) ranged from 10 to 900%, with many sites showing over 100% increase. Only one site, at 1 km from the old Malabar cliff-face outfall, showed a significant decrease. The coprostanol concentration had fallen by 58%.

This study did not investigate the influence of the new outfalls on the beaches, as this has been undertaken by a number of state authorities. The results for sediments at the innermost stations and data for water at the sites closest to shore are, however, con-

TABLE II

SEDIMENT COPROSTANOL CONCENTRATIONS, INDICATOR ORGANISM DATA AND ORGANIC CARBON CONTENT

Station number	Organic carbon (mg/g)	Coprostanol (ng/g)		Indicator organisms[a] (CFU/100 g)		
		1989	1992	FC	FS	CP
27	11.6	254	106	2000	2200	$1.2 \cdot 10^4$
28	15.3	329	560	8200	2600	$3.6 \cdot 10^4$
29	26.7	531	1850	$2.4 \cdot 10^4$	3000	$1.7 \cdot 10^5$
30	42.8	531	2870	$6.4 \cdot 10^4$	10^4	$3.4 \cdot 10^5$
31	56.1	126	1260	$1.2 \cdot 10^4$	1500	$8.3 \cdot 10^4$
32	18.9	26	62	350	300	8500
33	24.9	19	38	100	<200	5000
34	52.2	23	26	1000	<100	3500
35	56.3	19	12	<200	<200	1500
36	109.9	–	13	<200	<200	<1000

[a] FC = fecal coliforms; FS = fecal streptococci; CP = *Clostridium perfringens* spores.

sistent with the early results for beaches which show a general improvement in water quality [23,24].

Relationship of coprostanol to indicator organisms

Previous reports show variability in the relationship of coprostanol to various indicator groups in water samples [1,25–27]. Some of these differences relate to the use of different indicators and their variability in die-off. Results for the three indicator organisms (fecal coliforms, fecal streptococci and *Clostridium perfringens* spores) are provided in Tables I and II). Spores of *C. perfringens* were included in the present study to largely remove the effect of variability in the die-off of the other two more commonly used indicator organisms. Nevertheless, in this study in Australian marine waters, we observed a strong linear correlation between the water column particulate matter concentration of coprostanol and faecal coliforms ($r^2 = 0.80$); it was even higher for *Clostridium perfringens* ($r^2 = 0.97$). Light is an important factor in die-off of faecal bacteria. The water depth and associated lower light penetration for the bottom water samples has, we believe, reduced fecal coliform die-off, resulting in the good correlations observed.

Good correlations were observed with all three indicator groups for sediments. We believe this is the first report for marine sediments of parallel coprostanol and standard bacterial counts (Table II). The good correlations noted in this study for sediments (coprostanol-fecal coliforms, $r^2 = 0.91$; coprostanol-fecal streptococci, $r^2 = 0.79$; coprostanol-*Clostridium perfringens* spores, $r^2 = 0.96$) emphasize the validity of using coprostanol as a tracer for sewage in the marine environment, including sediments.

Several of the coprostanol concentrations from samples near the deepwater ocean outfall were generally as high or higher than values reported in the literature, indicating that those samples are significantly polluted by sewage. Several studies have also attempted to relate coprostanol and fecal coliform concentrations [1,25–27]. For primary contact recreational waters (Canada), a water standard of 500 ng/l was proposed with secondary contact recreational water an order of magnitude higher *i.e.* 5000 ng/l.

Results from this and other studies in Sydney coastal waters and in the Derwent Estuary, Tasmania indicate that the primary contact limit used in many Australian states (median 150 fecal coliforms per 100 ml) corresponds to around 100 ng/l coprostanol.

A new method for rapid detection of sewage contamination in natural waters has been recently reported [28]. The technique involves instrumental-based fluorometric assay of β-D-galactosidase enzyme activity and the method has been found to correlate well with fecal coliform abundances enumerated by standard methods. Coprostanol concentrations were also found to correlate with enzyme assay for both surface and bottom water samples ($r^2 = 0.88$).

CONCLUSIONS

A survey of Sydney's coastal environment was conducted during January, 1992 after the commissioning of the new deepwater ocean outfalls. The study repeated sampling conducted in 1989, before the commissioning. Results for the sewage tracer coprostanol show that it has accumulated in sediments of the inner shelf (1–10 km offshore). The area of highest coprostanol concentrations has been shifted further offshore from adjacent to the old cliff face outfalls to around the zones of the deep water ocean outfalls. The dispersion of sewage appears to be confined to the inner shelf, *i.e.* no further than 10 km from the coast. Dispersion further offshore (east) appears to be minimal, rather sewage-derived material may be moving in a north-south direction.

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Determination of pesticides in river water by gas chromatography–mass spectometry–selected-ion monitoring

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ABSTRACT

A highly sensitive and specific method for the determination of trace levels of pesticides in river water has been developed. The method is based on reversed-phase (C_{18}) solid phase extraction, followed by gas chromatography–mass spectrometry–selected-ion monitoring. Recoveries at the 0.5- or 2.5 μg/l fortification level were between 79 and 98%. The detection limits were 0.05 μg/l for chlornitrofen and 0.01 μg/l for other pesticides.

INTRODUCTION

Environmental pollution caused by chemical compounds including pesticides has become a serious problem. Some pesticides are released into the environment during and/or after application to crops, and may be harmful to human beings and other species. Therefore, monitoring the residual levels of pesticides is very important for human health and environmental control.

Butachlor, pretilachlor, oxadiazon, chlornitrofen, simetryn and thiobencarb have been utilized as herbicides in rice paddy fields during the cultivation season. Determination of these pesticides has usually been carried out by extraction with solvent, clean-up on a chromatographic column and analysis by GC. Recently, solid-phase extraction (SPE) has been used to extract pesticides from water [1–7]. This extraction is a simple and rapid technique in comparison with liquid–liquid extraction. GC–MS–selected-ion monitoring (SIM) is suited for the quantification of μg/l or ng/l levels of pesticides in environmental samples. We have applied these techniques and developed a method for multiresidue analysis of these pesticides in river water.

EXPERIMENTAL

Chemicals

A Sep-Pak C_{18} cartridge (0.4 g) was obtained from Waters Assoc. Before use, the cartridge was rinsed with 5 ml of methanol, followed by 10 ml of distilled water for cleaning and conditioning. Butachlor and pretilachlor were purchased from Monsanto Japan and Ciba-Geigy Japan, respectively. Oxadiazon, chlornitrofen and simetryn were obtained from Wako. Thiobencarb was purchased from Kumiai. All standards were of $>99\%$ purity. All other reagents were of analytical reagent grade. Reagent-grade water was obtained from a Milli-Q water purification system (Millipore, Yonezawa, Japan).

Applications

Applications of butachlor, pretilachlor, oxadiazon, chlornitrofen, simetryn and thiobencarb were used for weed control in rice paddy fields. In 1987 and 1988, the amounts of active ingredient applied to the rice paddy fields during the farming period

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were 97–5612 kg in the Kokai River (K-River) watershed of Ibaraki Prefecture and 7.2–552.9 kg in the Saita River (S-River) watershed of Kagawa Prefecture. The K-River watershed comprises a flatland and an agricultural village. The S-River watershed is a mountain village, and at the middle of the watershed several small rivers join.

Sampling

Water was sampled from upper, middle and/or lower rivers at depths of 30–50 cm, at 3 to 7 day intervals during the farming periods. Samples of water of 3 l each were placed in dark containers, stored in a refrigerator at a temperature of 4°C until analysed, and analysed within 1 day of receipt. The distance from the upper river point to the lower river point in K-River is about 45 km. The length of S-River is about 30 km, and the middle river point is 15 km from the mouth of river.

Determination

A 1000-ml sample of river water was transferred into a 1000-ml separatory funnel connected to two Sep-Pak C_{18} cartridges, which was rinsed with 5 ml of methanol, followed by 10 ml of water for cleaning and conditioning. Water samples were aspirated through the Sep-Pak C_{18} cartridge. Trapped pesticides were eluted from the column with 10 ml of methanol after being rinsed with 3 ml of water–methanol (70:30, v/v), and the effluent was evaporated to dryness with a stream of nitrogen. The residue obtained was dissolved in 1 ml of acetone. 2-Aliquots (2-μl) of the solution were injected into the GC–MS system.

Monitoring ions used were as follows: butachlor, m/z 176; pretilachlor, m/z 238; oxadiazon, m/z 175; chlornitrofen, m/z 319; simetryn, m/z 213; thiobencarb, m/z 257. The peak areas of these pesticides on the chromatogram were measured, and the concentration of each pesticide was determined from its calibration curve. Duplicate water samples were analysed.

Preparation of calibration curves

A 25-mg portion of each standard was dissolved in 500 ml of acetone to give a 50-ppm stock solution. Each 50-ppm stock solution was serially diluted to prepare the standard solutions of 0.01–1.0 ng for butachlor, pretilachlor, oxadiazon, simetryn and thiobencarb, and 0.05–5.0 ng for chlornitrofen in acetone.

Gas chromatography–mass spectrometry

A Hewlett-Packard 5890 gas chromatograph was coupled to a JMS-DX 300 (JEOL) mass spectrometer. The mass spectrometer was equipped with a JMA-DA 5000 (JEOL) data system. The ionization voltage and the ion-source temperature were set at 70 eV and at 150°C, respectively. An SPB-1 fused-silica capillary column was used (15 m × 0.53 mm I.D. and 0.5 μm film thickness). Helium was used as carrier gas, and the flow-rate was 30 ml/min. The temperatures of the column, separator and injection port were maintained at 170°C, 250°C and 250°C, respectively.

RESULTS AND DISCUSSION

The structures of the pesticides monitored, their solubilities in water and their vapour pressures are given in Table I. The solubility in water of simetryn, and the vapour pressure of chlornitrofen and thiobencarb are higher than those of other pesticides. In electron impact (EI) mass spectra of the pesticides monitored, butachlor, pretilachlor, oxadiazon, chlornitrofen, simetryn and thiobencarb, molecular ions are present at m/z 311, 311, 344, 317, 213 and 257, respectively. Other structurally significant ions were found m/z 237, 176 and 160 for butachlor, m/z 238, 176 and 162 for pretilachlor, m/z 302, 258 and 175 for oxadiazon, m/z 287, 236 and 173 for chlornitrofen, m/z 170 and 155 for simetryn and m/z 125, 100 and 72 for thiobencarb. The low intensities of the molecular ions of butachlor and pretilachlor precluded their use as monitoring ions for the quantitation. In those cases, the most intense or characteristic ions for each compound were selected as the quantitation ions. These ions were m/z 176 for butachlor, m/z 238 for pretilachlor, m/z 175 for oxadiazon, m/z 319 [M + 2] for chlornitrofen, m/z 213 for simetryn m/z 257 for thiobencarb.

Solid-phase extraction reduced the analysis time and solvent consumption compared with traditional methods such as liquid–liquid extraction. No further clean-up was necessary before determination by GC–MS. The peak areas of the monitoring ions on the chromatograms were measured, and the concentration of each pesticide was determined from each calibration curve.

TABLE I

CHEMICAL STRUCTURE, SOLUBILITY AND VAPOR PRESSURE OF THE PESTICIDES EXAMINED

Pesticide	Structure	Solubility (mg/l of water)	Vapour pressure (mPa)
Butachlor	CH_2CH_3 $COCH_2Cl$ N $CH_2O(CH_2)_3CH_3$ CH_2CH_3	23	0.6
Pretilachlor	CH_2CH_3 $COCH_2Cl$ N $CH_2CH_2O(CH_2)_2CH_3$ CH_2CH_3	50	0.133
Oxadiazon	$(CH_3)_3C$ O O Cl N—N Cl $OCH(CH_3)_2$	0.7	<0.133
Chlornitrofen	Cl Cl-O-NO_2 Cl	0.25	$4.67 \cdot 10^{4}$ (109°C)
Simetryn	$NHCH_2CH_3$ N N CH_3S N $NHCH_2CH_3$	450	0.095
Thiobencarb	$(CH_3CH_2)_2NCOSCH_2$-Cl	30	2.93

The calibration curves were generated with a linear correlation coefficient of 0.999.

Selected-ion chromatograms of standard, control and recovery samples showing the ion trace for each selected mass are presented in Fig. 1. No significant interfering peaks were observed on the ion chromatograms derived from control sample extracts.

Recovery studies were performed at the 2.5-μg/l level for chlornitrofen and 0.5 μg/l for other pesticides. As summarized in Table II, the recoveries were between 79 and 98%. When chlornitrofen was eluted from the column with 5 ml of tetrahydrofuran after being eluted with 10 ml of methanol, the recovery was 85%. However, chlornitrofen was not quantitatively recovered. This phenomenon may be attributed to the adsorption of chlornitrofen in the C_{18} cartridge. The detection limits in water were 0.05 μg/l for chlornitrofen and 0.01 μg/l for other pesticides when a 2-μl aliquot of the final solution (1 ml) obtained from a 1000-ml sample was injected into the GC–MS system.

This method was applied to the analysis of the pesticides released into rivers after application to crops.

The stabilities of the six pesticides in water during transportation and storage were estimated by using distilled water. As shown in Table III, their recoveries were higher than 88% after 7 days at room temperature.

TABLE II

RECOVERY OF PESTICIDES ADDED TO DISTILLED WATER AND LIMIT OF DETECTION

Values are the mean of triplicate experiments.

Pesticide	Added (μg/l)	Recovery (%)	Limit of detection (μg/l)
Butachlor	0.5	95	0.01
Pretilachlor	0.5	96	0.01
Oxadiazon	0.5	98	0.01
chlornitrofen	2.5	79	0.05
Simetryn	0.5	90	0.01
Thiobencarb	0.5	89	0.01

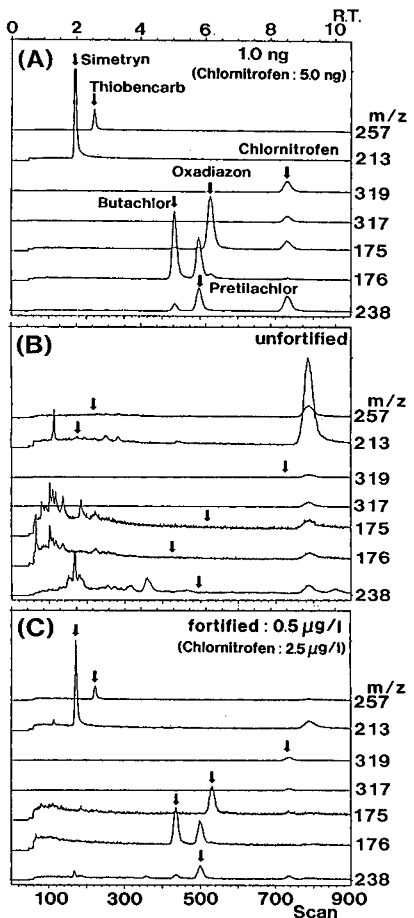

Fig. 1. Mass fragmentograms of (A) pesticides examined, (B) control sample and (C) recovery test sample.

The amounts of pesticides applied to the paddy fields are listed in Table IV as the amount of the active ingredients. All the pesticides except for thiobencarb were used in the similar amounts during 1987 and 1988, that is 97–5612 kg of active ingredient. In 1988, however, the use of thiobencarb increased by 50%. Thiobencarb and chlornitrofen were applied in amounts of 2000–5612 kg. The amounts of active ingredients used in the S-River watershed during the farming period varied from 0.2 kg of simetryn to 553 kg of thiobencarb. Pretilachlor was not used during the same period.

Representative ion monitoring chromatograms of K-River water are shown in Fig. 2. These ions were free from interfering peaks in the control water sample. Pretilachlor and oxadiazon showed similar retention times. However, as shown in this figure, these pesticides were easily separated and determined by monitoring the selected characteristic ions.

The analytical findings of surface water samples collected from rivers indicate that most of the pesticides were detected in river water at 1 to 10 µg/l levels for the first 2–4 weeks after application in the fields. They were reduced below the limits of detection at 4–6 weeks. On the whole, the levels of pesticides detected in the S-River were lower than those in the K-River. In 1987, the concentrations of butachlor and pretilachlor were found to be about 1–4 µg/l in the K-River. These pesticides were present only during the first few weeks after application and then decreased rapidly. Similar behaviour was found for oxadiazon and chlornitrofen. Simetryn

TABLE III

STABILITY OF BUTACHLOR, PRETILACHLOR, OXADIAZON, CHLORNITROFEN, SIMETRYN AND THIOBENCARB ADDED TO DISTILLED WATER STORED AT ROOM TEMPERATURE

Values are the means of triplicate experiments. Amount added: 0.5 µg/l (chlornitrofen 2.5 µg/l).

Storage period (days)	Recovery (%)					
	Butachlor	Pretilachlor	Oxadiazon	Chlornitrofen	Simetryn	Thiobencarb
0	95	96	98	79	90	88
3	101	112	100	86	96	97
7	101	100	99	88	93	89

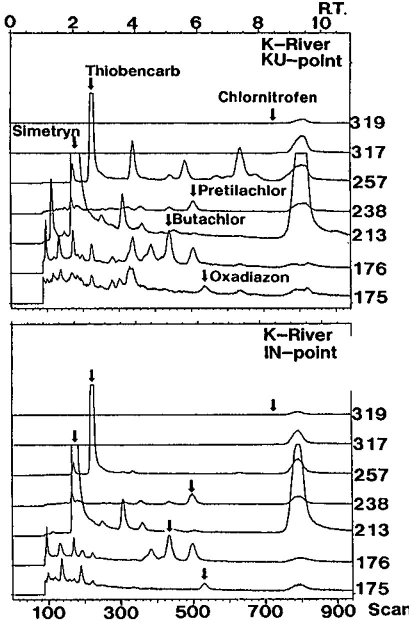

Fig. 2. Typical mass fragmentograms of butachlor, pretilachlor, oxadiazon, chlornitrofen, simetryn and thiobencarb residue in K-River water. Sampling date: June 7, 1988. Injection volume/final solution volume from 1000 ml sample: 2 μl/1 ml.

was observed at the highest residue level (3.0–13.3 μg/l) about 4 weeks after application. Except for chlornitrofen and simetryn, the detected levels of pesticides were generally consistent with the amounts applied to the rice paddy fields.

In spite of the use of a large amount of chlornitrofen, the level of chlornitrofen detected was much lower than those of other pesticides. This may be attributed to the lower solubility in water, to the higher vapour pressure and to adsorption onto soil.

In contrast to chlornitrofen, simetryn was observed at high concentration during 1987 and 1988. Simetryn is very soluble in water, and may be rapidly transported into the river when the water levels in the rice paddy fields increase after rainfall.

The amount of pesticide during sampling date X_i is $C_i f_i$, in which C_i and f_i are the concentration of pesticide detected in river water and water flow at sampling date X_i, respectively. Therefore, the amount of pesticide transported into a river over period between one sampling date and the next (W_i) can be defined as:

$$W_i = 1/2[(C_i f_i + C_{i+1} f_{i+1})(X_{i+1} - X_i)$$

The total amount of pesticides (TW) transported into a river during the farming period is thus:

$$TW = \sum_{i=1}^{n} W_i$$

TABLE IV

AMOUNTS OF PESTICIDES-ACTIVE INGREDIENTS APPLIED TO RICE PADDY FIELDS IN THE K-RIVER WATERSHED

The areas of paddy fields are 8084 ha for the upper K-River watershed, 14 700 ha for the lower K-River watershed, 124 ha for the upper S-River watershed, 713 ha for the middle S-River watershed and 557 ha for the lower S-River watershed.

Pesticide	Amount of pesticide applied (kg)									
	K-River watershed				S-River watershed					
	1987		1988		1987			1988		
	Upper	Lower	Upper	Lower	Upper	Middle	Lower	Upper	Middle	Lower
Butachlor	651	1677	540	1220	75.3	1.2	52.2	18.5	177.6	305.6
Pretilachlor	606	1543	640	1500	–	–	–	–	–	–
Oxadiazon	97	315	80	200	13.7	307.2	315.3	16.8	244.4	271.7
Chlornitrofen	2743	5612	2709	4476	7.2	475.3	166.6	3.6	295.6	256.0
Simetryn	1792	1859	1500	1614	–	–	–	–	–	0.2
Thiobencarb	2111	3473	3659	5294	8.5	552.9	191.4	4.4	368.8	344.3

Run-off (R) of pesticide transported into the river is then calculated using the following equation:

$$R = TW / TA \times 100$$

where TA is total amount of pesticide used at the river watershed. The TA used were calculated by adding the total amount of pesticide used at the upper and middle river watersheds for the middle river and at the upper, middle and lower river watersheds for the lower river.

On the basis of the calculations, the run-off of pesticides transported into the river was from 0.1% chlornitrofen to 33% simetryn for the K-River, and from 0.01% chlornitrofen to 2.4% butachlor, but 3.9% oxadiazon, for the S-River. Pesticides that are very soluble in water, such as simetryn, are efficiently transported into rivers. The influence of pesticide solubility on their mobility is an important factor in their transportation in the aquatic system [8]. In addition, it seems that the difference in the results for the K-River and the S-River can also be ascribed to the properties of soil. The soil of the K-River watershed is loam, and the pesticides used in paddy fields, expect for the chlornitrofen adsorbed on soil, were transported into the rivers. On the other hand, the soil of the S-River watershed is sand, and the pesticides in the field were transported into the river after being removed into soil.

CONCLUSIONS

A multiresidue analysis of butachlor, pretilachlor, oxadiazon, chlornitrofen, simetryn and thiobencarb in river water samples was developed. The method was based on solid-phase extraction, followed by GC–MS–SIM. By this method, the detection limits were 0.05 μg/l for chlornitrofen and 0.01 μg/l for other pesticides. The results for river water indicate that most of the pesticides were present at low levels for the first 2–4 weeks after applcation in the fields. Pesticide concentrations decreased below detectable levels at 4–6 weeks. The levels of pesticides in river water were generally consistent with the amounts of pesticides applied. In addition, pesticides that are very soluble in water are efficiently transported into river when water levels in the paddy field increase after rainfall.

The GC–MS–SIM method provides a rapid, sensitive and accurate technique for the analysis of pesticides in river water.

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CHROMSYMP. 2728

Solid-phase extraction followed by high-performance liquid chromatographic analysis for monitoring herbicides in drinking water

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ABSTRACT

A multiresidue analytical method based on C_{18} solid-phase extraction and one-run HPLC determination has been developed for the analysis of eleven acidic, neutral and weak basic herbicides in drinking water. A 1-l sample of water was preconcentrated by passage through a 500-mg C_{18} solid phase extraction column. The retained compounds were eluted from the column with 1 ml of methanol. After concentration of the extract the pesticides were separated and quantified by reversed-phase HPLC with UV detection. Bentazone, 2,4-D, MCPA, fluazifop-acid, metoxuron, monolinuron, metobromuron, diuron, linuron, atrazine and simazine were determined simultaneously in a single run on a C_{18} HPLC column. Reanalyses of the sample extracts on a second cyano column were used to confirm the identity of the neutral and basic compounds. The limit of determination, defined as four times the baseline noise, varied between 0.01 μg/l and 0.1 μg/l depending on the compound, the detection sensitivity of the instrument and the type of HPLC column used.

INTRODUCTION

The high standards for drinking water purity laid down by the European Community, maximum admisible concentrations (MACs) of 0.1 μg/l for any individual pesticide, require the development of suitable analytical methods with high sensitivity, selectivity, accuracy and reliability. Recent publications on this subject show that there is a tendency to use sophisticated techniques such as gas chromatography (GC) with mass spectrometry (MS) [1,2], liquid chromatography with particle beam mass spectrometry [3], HPLC with diode-array detection [4,5], and HPLC with column switching [6] for determining pesticide residues in water.

Pesticide monitoring of waters is also possible with rapid and simple methods that use less sophisticated instruments, and which still provide reliable identification of analytes. Reversed-phase HPLC is widely used in analyses of pesticides with high polarity, low volatility and thermal instability [4, 7–9].

Solid-phase extraction (SPE) has recently been accepted as a powerful tool for extraction of water samples prior to analysis [10]. Compared with traditional methods such as liquid–liquid extraction, SPE reduces sample handling, labour and solvent consumption. The most popular sorbent for SPE of pesticides from water is octadecyl (C_{18}) bonded silica [1,2,5]. Graphitized carbon black cartridge extraction of pesticides from water has also been reported [8,11,12].

A simple, rapid and reliable multiresidue method has been developed for the analysis of eleven herbicides in drinking water. It includes herbicides that cannot be analysed directly by GC owing to poor volatility, polarity or thermal instability. The selected compounds are widely used in agricuture and are known to be potential pollutants of natural waters. Triazine herbicides, though they can be determined by GC without preliminary derivatization, are also included because they (especially atrazine) are some of most common water pollutants. The method allows the simultaneous determi-

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nation of acidic, neutral and weak basic compounds using C_{18} SPE and one-run reversed-phase HPLC determination. Positive peak identification of basic and neutral compounds has been achieved by means of an alternative HPLC column with different polarity.

EXPERIMENTAL

Chemicals and reagents

All reagents and solvents were of reagent grade. Methanol distilled in glass and bidistilled water were used for HPLC. Octadecyl C_{18} SPE packing, Supelclean LC-18, was obtained from Supelco. Individual standard stock solutions, 1 mg/ml in methanol, were prepared from analytical-purity standards. Composite working standard solutions were prepared by mixing appropriate known volumes of each standard stock solution and diluting to 100 ml with HPLC mobile phase.

Apparatus

A Pye Unicam liquid chromatograph was equipped with a PU 4010 pump, a PU 4020 variable-wavelength UV detector and a Rheodyne Model 7125 injector with a 20-μl loop. A cartridge column, RP-18 Spheri 5 μm (100 mm × 4.6 mm I.D.) (Pye Unicam), and a LiChrosorb-CN 5 μm column (250 mm × 4.6 mm I.D.) (Merck), were used for the determination of the compounds. A guard cartridge, RP-18 5 μm (40 mm × 4.6 mm I.D.), was used with the RP-18 column.

Procedure

Aqueous samples were fortified with known volumes of standard solutions. After adjusting the pH to 2 and adjusting the ionic strength by addition of 2.5 *M* sulphuric acid and 10 g of sodium chloride, the samples were mixed well and forced to percolate through the SPE column under vacuum at a rate of *ca.* 10 ml/min. The SPE column was prepared by placing in a 5 mm I.D. glass tube a plug of quartz wool, 0.5 g of C_{18} packing material and then another plug of quartz wool to prevent clogging or crushing of the particles. The column was conditioned with 10 ml of methanol and equilibrated with 10 ml of distilled water. Just after the sample was passed through the column, it was washed with 5 ml of

TABLE I

ACCURACY AND PRECISION OF THE METHOD AT DIFFERENT LEVELS OF FORTIFICATION AND TWO SAMPLE VOLUMES

No.	Compound	Recovery (% ± S.D., $n = 5$)				
		1 l		0.5 l		1 l
		5 × LOD[a]	20 × LOD[b]	5 × LOD[a]	20 × LOD[b]	0.1 μg/l
1	Bentazone	39.2 ± 6.3	52.7 ± 4.8	75.8 ± 4.6	77.3 ± 9.0	86.7 ± 0.3[c]
2	2,4-D	72.3 ± 3.8	75.6 ± 6.2	90.9 ± 7.1	102.8 ± 6.5	77.1 ± 4.9
3	MCPA	80.1 ± 4.2	90.3 ± 5.6	92.3 ± 9.9	98.0 ± 8.4	91.9 ± 8.1
4	Metoxuron	83.5 ± 7.4	93.6 ± 4.8	95.8 ± 9.9	92.6 ± 5.8	84.6 ± 8.8
5	Fluazifop-acid	90.7 ± 5.2	96.7 ± 4.1	91.3 ± 8.1	95.8 ± 5.2	85.3 ± 5.2
6	Simazine	77.3 ± 7.9	89.9 ± 5.1	94.1 ± 10.1	98.3 ± 4.2	[d]
7	Atrazine	82.7 ± 5.2	97.5 ± 2.9	91.8 ± 3.9	94.4 ± 3.1	[d]
8	Monolinuron	84.9 ± 11.6	91.7 ± 6.9	104.6 ± 10.4	97.3 ± 6.1	92.3 ± 6.7
9	Metobromuron	86.7 ± 10.8	96.4 ± 8.3	100.6 ± 10.6	99.5 ± 4.4	80.5 ± 7.3[e]
10	Diuron	87.7 ± 11.9	95.3 ± 9.8	89.1 ± 7.4	96.1 ± 3.5	82.4 ± 9.5[e]
11	Linuron	86.5 ± 13.9	91.5 ± 10.1	82.1 ± 9.5	94.7 ± 7.3	79.2 ± 5.1[e]

[a] Fortification level five times limit of determination (μg/l).
[b] Fortification level twenty times limit of determination (μg/l).
[c] Sample volume 0.5 l.
[d] Equal to (5 × LOD) μg/l.
[e] Determinations on a CN column.

distilled water, the eluate discarded and the sorbent bed dried under vacuum for 5 min. Analytes were eluted with 1 ml of methanol. The solvent was evaporated to dryness under a stream of air. The residue was dissolved first in 0.25 ml of methanol and then in an equal volume of 0.1 *M* acetic acid–sodium acetate buffer (pH 3.8) to a final sample volume of 0.5 ml.

For the separation and quantification of acidic, neutral and basic compounds on the RP-18 cartridge column the composition of the mobile phase was 50% methanol and 50% 0.1 *M* acetic acid–sodium acetate buffer (pH 3.8). The flow-rate was 1 ml/min and UV detection at 230 nm was used. The second HPLC column containing CN packing was used for the determination of urea and triazine herbicides with a mobile phase of methanol–water (2:8, v/v) at a flow-rate of 1 ml/min and the same UV wavelength.

The concentration of the herbicides in water samples was calculated by measuring the peak heights and comparing them with those obtained with standard solutions.

RESULTS AND DISCUSSION

In the published multiresidue methods for simultaneous analysis of acidic, neutral and weak basic pesticides in waters, after SPE they are separated by stepwise elution to fractions [2,12,13]. Determination of acidic compounds is carried out separately from neutral and basic compounds by HPLC, GC or another technique.

In the method developed, conditions for the simultaneous HPLC determination in one fraction of acidic, neutral and weak basic compounds after trace enrichment with C_{18} SPE were determined. Under these conditions, great losses of phenylureas and triazines were not observed, as was reported by Di Corcia and Marchetti [8,11] using 0.5-g C_{18} cartridges. As Table I shows, recoveries of all compounds are not less than 75%. The exception to this is bentazone, whose recovery is reduced to 39% with an increase in sample volume to 1 l and 2,4-D, whose recovery is reduced to 72%.

The influence of sample volume on recovery has been studied by analysis of five replicates of 0.5 l and 1 l of drinking water spiked with known quantities of herbicides corresponding to five times the limit of determination. Table I shows that with a doubling of sample volume only bentazone and to some extent 2,4-D show loss of analyte.

It is known that HPLC methods using only one chromatographic column for identifying a large number of pesticides in waters have a high probability of false positives. Undoubtedly, mass spectrometry does provide the most definitive confirmation. Since sophisticated instruments such as liquid chromatographs coupled to mass selective detectors are not available to most routine analytical laborato-

Fig. 1. HPLC chromatograms obtained by injecting (A) a mixed working standard solution of neutral and basic herbicides and (B) a tap water sample fortified with herbicides at the same concentrations. The column was a LiChrosorb-CN column and the chromatographic conditions were as described in the Experimental section. Attenuation 0.02 a.u.f.s. Peak numbering: 1 = simazine 1 ng; 2 = atrazine 1 ng; 3 = metoxuron 2 ng; 4 = monolinuron 2 ng; 5 = metobromuron 5 ng; 6 = diuron 5 ng; 7 = linuron 5 ng.

TABLE II

RETENTION TIMES AND LIMITS OF DETERMINATION ON TWO HPLC COLUMNS OF HERBICIDES ADDED TO 1 l OF TAP WATER SAMPLE

t_R = Retention time; LOD = limit of determination defined as four times baseline noise.

No.	Compound	t_R (min)		LOD (μg/l)	
		C_{18}	CN	C_{18}	CN
1	Bentazone	1.6	1.3	0.002	–
2	2,4-D	2.7	1.3	0.01	–
3	MCPA	3.5	1.4	0.01	–
4	Metoxuron	5.2	5.5	0.03	0.02
5	Fluazifop-acid	6.0	1.7	0.05	–
6	Simazine	7.6	4.8	0.02	0.01
7	Atrazine	11.8	5.2	0.02	0.01
8	Monolinuron	12.7	6.8	0.05	0.02
9	Metobromuron	14.6	7.6	0.1	0.03
10	Diuron	19.1	8.2	0.1	0.04
11	Linuron	30	10.4	0.15	0.05

ries, it is important to find more applicable methods for peak identity confirmation. The use of an alternative HPLC column of different polarity described in the method presented gives a reliable, cheap and accessible approach to validate pesticide identification.

Reanalysis of the sample extracts on a second cyano column was used. Cyano column was excellent for triazine and urea herbicides separation (Fig. 1). The elution order of the compounds on this column was different from on the C_{18} column (Table II). This fact was favourable to confirmation reliability. The retention of acidic compounds on the cyano column was very limited. Bentazone, 2,4-D and MCPA were not separated on that column (Table II). For that reason the alternative column could not be recommended for peak identification of acidic herbicides. The retention times of all triazine and urea (except metoxuron) herbicides were shorter on the cyano than on the C_{18} column. The time of

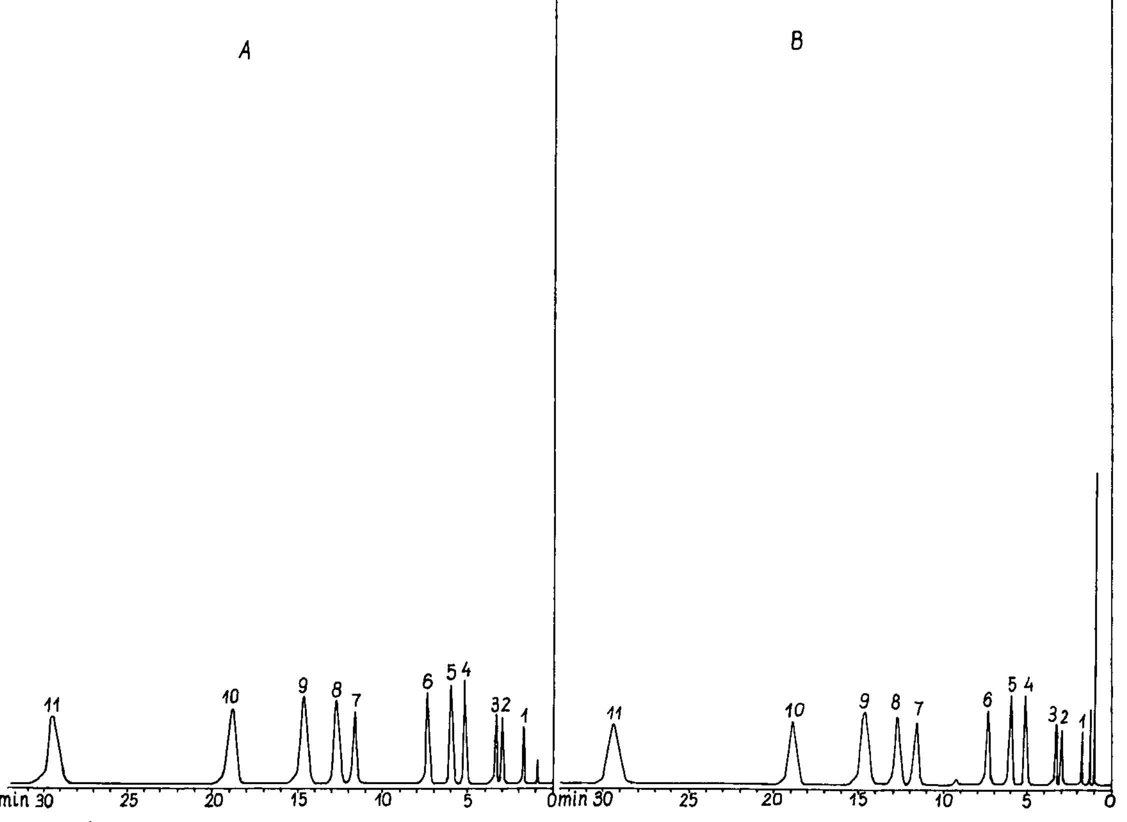

Fig. 2. HPLC chromatograms obtained by injecting (A) a mixed working standard solution of the herbicides and (B) a tap water sample fortified with herbicides at the same concentrations. The column was a Spheri-5 RP-18 cartridge column and the chromatographic conditions were as described in the Experimental section. Attenuation 0.02 a.u.f.s. Peak numbering: 1 = bentazone 0.2 ng; 2 = 2,4-D 1 ng; 3 = MCPA 1 ng; 4 = simazine 2 ng; 5 = fluazifop-acid 5 ng; 6 = metoxuron 4 ng; 7 = atrazine 2 ng; 8 = monolinuron 4 ng; 9 = metobromuron 10 ng; 10 = diuron 10 ng; 11 = linuron 10 ng.

determination was considerably reduced and the sensitivity was higher. The cyano column is to be preferred to the C_{18} column in analyses of triazine and urea herbicides residues, especially at low concentrations (Figs. 1 and 2).

The accuracy and precision of the method have been evaluated at two fortification levels —five and twenty times the limits of determination (LOD) of the herbicides. Drinking water samples of 1 l were fortified with known quantities of the standard solutions and quantitative results were obtained (Table I). Since the LODs of the analytes varied to a large extent, recovery studies at a concentration of 0.1 μg/l were carried out for all herbicides to verify the suitability of the method for monitoring compliance with the European Community drinking water directive. The results shown in Table I prove that the method can be used to detect many of the herbicides at concentrations below the maximum admissible concentration for pesticides in drinking water. For metobromuron, diuron and linuron at this concentration, better results were obtained on the cyano column.

CONCLUSIONS

A simple multiresidue method has been developed for the analysis of herbicides belonging to different classes in drinking waters. Acidic, neutral and weak basic compounds are determined simultaneously in a single HPLC run, which saves apparatus time. Application of two HPLC columns for confirmation of positive identification of neutral and basic herbicides increases the reliability of determination, avoiding the use of expensive techniques.

The enrichment factor is about 2000. The sensitivity of the method is sufficient to achieve quantitative determination at or below 0.1 μg/l for each of the herbicides.

The method is also directly applicable to sample preparation automation. For its simplicity, reliability and usage of generally applied instrumentation this method is suitable for pesticide monitoring of waters.

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CHROMSYMP. 2742

Determination of resin acids by gas chromatography and high-performance liquid chromatography in paper mill effluent, river waters and sediments from the upper Derwent Estuary, Tasmania

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ABSTRACT

Resin acids in effluent from a paper mill situated on the upper Derwent Estuary near Hobart, Tasmania (Australia) were determined by HPLC analysis of their 7-methoxycoumarin-4-yl and 7-acetoxycoumarin-4-yl methyl esters. Total concentrations ranged from 1.0 to 4.8 mg l^{-1} with a mean of 2.7 mg l^{-1} during 1991–1992. Capillary GC–flame ionization detection and GC–MS analyses of organic constituents in river waters collected in April 1992 confirmed the presence of resin acids derived from the paper mill effluent, but the concentrations were highly variable and strongly influenced by freshwater flow and tidal movements. At a site just 500 m downstream of the effluent discharge, concentrations ranged from <0.01 to 0.78 mg l^{-1} over a 6-h period. Resin acids were also found in sediments close to the discharge (up to 87 mg kg^{-1} dry mass), but amounts in sediments downriver were generally considerably less (most samples <7 mg kg^{-1}). The major resin acids in the effluent were dehydroabietic, palustric, abietic and pimaric acids. Smaller amounts of isopimaric, neoabietic, levopimaric and sandaracopimaric acids were also found. The proportions of individual resin acids in some of the water and sediment samples showed considerable differences from those in the effluent. The abundance of resin acids with conjugated double bonds such as palustric, levopimaric and neoabietic acids were particularly variable suggesting that they are more easily degraded. Resin acids of the pimarane type, such as pimaric acid, were considerably more stable. Variations in the water column distributions reflect both degradation of the more labile resin acids and redistribution of the resin acids between aqueous, colloid and sediment phases. Dehydroabietic acid was the most resistant to degradation and in some water samples it represented up to 66% of the resin acids compared with only 34% in the effluent. This result confirms earlier observations, and suggests that dehydroabietic acid could be used as a tracer for organic matter derived from the paper mill.

INTRODUCTION

Resin acids are tricyclic diterpenoids which occur naturally in conifers. The major acid is often dehydroabietic acid (DHAA), which has an aromatic C ring (Fig. 1), although which acid predominates depends on tissue type, age and species. Several of the resin acids have two conjugated double bonds, and it is these which appear to be least resistant to chemical degradation. In the manufacturing of pulp and paper, resin acids are released from the wood during chemical and mechanical pulping processes. Large amounts can be present in untreated effluent streams if the mill uses a high proportion of softwood species, such as *Pinus radiata*. This species is fast growing and has good mechanical properties and so it is widely used as a raw material in the Australian paper industry. In *P. radiata*, free resin acids may comprise up to half of the extractable

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Pimaric Acid Sandaracopimaric Acid Isopimaric Acid

Levopimaric Acid Palustric Acid Dehydroabietic Acid

Abietic Acid Neoabietic Acid

Fig. 1. Structures of the resin acids referred to in the text.

organic compounds present [1], although the amount present varies with the age of the trees and the conditions under which they are grown [2].

High concentrations of resin acids are acutely toxic to fish so it is desirable to minimise the amounts discharged to the environment. Most resin acids have similar LC_{50} values for fish (0.4–1.7 mg l^{-1}, [3–6]). This range is similar to values obtained for chlorinated guaiacols and catechols in bleached kraft effluent [7–9]. Resin acids are taken up by fish where they concentrate in the liver producing sublethal effects such as jaundice (accumulation of bilirubin in the plasma) and reduction of UDP-glucuronyl transferase enzyme activity [4,10,11]. In rainbow trout, enzyme activity changes are observed when the fish is exposed to dehydroabietic acid concentrations as low as 0.005 mg l^{-1} [10], although Oikari *et al.* [4] suggested that 0.02 mg l^{-1} is close to the "minimum effective concentration" of dehydroabietic acid to rainbow trout. At 0.4 mg l^{-1} or higher concentrations, rainbow trout develop jaundice in 2–4 days at water temperatures over 17°C, but they can recover from severe intoxication within 6 days [11].

Over recent years, there has been considerable concern about the water quality of the Derwent Estuary in Tasmania due to the impact of industry and sewage effluent. The condition of the upper estuary has been affected by the input of wood fibre and extractives into the river from the Australian Newsprint Mills over a 45-year period [12–14]. Large deposits (estimated to exceed 4 million m^3 in 1988) of organic-rich sludge have been found on the river bed downstream of the mill [14]. The study described here was carried out using samples collected in March 1990 and April 1992 to assess the concentrations of resin acids in the waters and sediments near to, and downstream of, the mill as part of a larger study to assess environmental conditions in the Derwent river and estuary.

EXPERIMENTAL

Collection and extraction of water and effluent samples

Samples of effluent from the paper mill were collected throughout 1989–1992 at approximately 14-day intervals from a sampling site in the effluent stream just prior to its discharge into the river. Each sample was a composite of effluent collected every hour over the 24-h period.

Surface river water samples were collected on April 1, 1992 in pre-cleaned Niskin bottles over a depth of 0.5–1.5 m. A 500-ml subsample from the 10 l collected by each Niskin bottle was immediately placed into glass bottles for transport to the laboratory. Resin acids have a strong affinity for glassware and plastics so it was essential that all equipment was cleaned thoroughly before use. All glassware was washed in 1% Extran solution, rinsed in Milli Q water, and then rinsed in Nanograde acetone before use. Resin acids remain in alkaline solution, so the 500-ml water samples were immediately adjusted to pH 11.0 by adding KOH. This procedure also liberates resin acids bound to particles in the water so the method gives total dissolved plus particulate resin acids.

The extraction method used here followed that of Richardson *et al.* [15]. In the laboratory, the water was filtered through a glass fibre Whatman GF/C filter to remove particulate matter such as wood fibre. The conductivity of the filtrate was adjusted to >2.0 mS cm^{-1} by adding solid NaCl and the pH was adjusted to 8.4–8.6 immediately before extraction. The water sample was then placed on a C_{18}

Bond Elut column under slight vacuum at a constant flow of approximately 1.0 ml min^{-1}. The Bond Elut column had been prepared by washing with acetone (2 × 1 ml), methanol and distilled water. Failure to maintain a constant ionic strength resulted in low and variable recoveries of the resin acids from the Bond Elut column. The vacuum was left on for 5.0 min after the sample had run through to remove any water remaining in the column. The resin acids were eluted with two 1-ml washes of acetone, dichloromethane, and methanol. This differs slightly from the procedure used previously by Richardson *et al.* [15]. Heptadecanoic acid methyl ester (17:0) was added to the combined eluents as an internal standard, and the samples were then dried under nitrogen. Resin acids were converted to methyl esters using diazomethane. Other esterification reagents, such as 14% BF_3 in methanol, were tried, but all gave low recoveries of esterified resin acids.

Sediments

Sediments were collected using a Smith-MacIntyre grab sampler at the same sites as the river water samples above, adjacent and downstream of the mill. Representative subsamples of the sediments were stored in glass jars and refrigerated until analysed. A 60-g portion of the wet sediment was freeze dried, and 10 to 15 g of the dried sediment was then extracted with 200 ml of acetone. The sediment-solvent mixture was agitated at hourly intervals for 5 h and then left to stand overnight. The acetone was then decanted and fresh solvent added (2 × 25 ml) to remove any residual resin acids. The combined extracts were filtered through a 0.2-μm Anatop disposable filter to remove any sediment particles, rotary evaporated to dryness and derivatized with diazomethane as previously described. Each sample was analysed by capillary gas chromatography after addition of the 17:0 methyl ester internal standard.

The presence of high loadings of organic matter in the sediment extracts produced very complex chromatograms so it was necessary to purify the extracts further. The total extract was dried under nitrogen, then dissolved in Milli Q water at pH 11.0 (KOH) and loaded onto a Bond Elut C_{18} column prepared as for the water extraction. The resin acids were eluted with two 1-ml portions of acetone, derivatised and then analysed as before. This procedure removed a number of compounds which interfered with the analysis, but resin acids were quantitatively recovered unchanged.

Quantification of resin acids in effluent using high-performance liquid chromatography

The concentration of total resin acids in effluent was monitored approximately every two weeks using the HPLC method described by Richardson *et al.* [15]. Briefly, the resin acids were extracted by passage through a C_{18} cartridge at pH 9 and converted to 7-methoxycoumarin-4-yl methyl esters (MMC) and 7-acetoxycoumarin-4-yl methyl esters (MAC) of the resin acids using 4-bromomethyl-7-methoxycoumarin and 4-bromomethyl-7-acetoxycoumarin respectively. The HPLC analysis used a Rainin Dynamax C_8 5-μm 25 cm × 4 mm I.D. reversed-phase column with guard column and filter. The solvent system was acetonitrile–water (70:30, v/v) at 1.5 ml min^{-1} followed by a linear gradient to acetonitrile–water (90:10, v/v) at 1.5 ml min^{-1}. The MMC esters were detected by UV absorption at 318 nm while the 7-hydroxycoumarin-4-yl methyl esters obtained by post-column alkaline hydrolysis of the MAC esters [15] were detected using fluorescence. The detection limits were 0.02 mg l^{-1} and 0.001 mg l^{-1}, respectively. The resulting chromatograms show a peak for dehydroabietic acid and a single, later-eluting peak for all other non-aromatic resin acids. Monocarboxylic fatty acids produce an additional peak or peaks later in the chromatogram (Fig. 2).

Identification of resin acids by capillary GC with flame ionization detection (FID) and GC–mass spectrometry

Resin acid methyl esters were analysed by capillary GC using a HP-1 methyl silicone non-polar capillary column (50 m × 0.32 mm I.D., 0.17 μm film thickness). The samples were injected using a cooled OCI-3 on-column injector and the constituents were detected with a flame ionization detector operated at 310°C. A column temperature program of 45 to 140° at 30°C min^{-1}, and 140 to 310°C at 4°C min^{-1} was used. Resin acids were identified by comparing retention time data with those of laboratory standards and from mass-spectral data obtained from a Hewlett-Packard 5970 MSD coupled to an HP 5890 GC by a direct capillary inlet. The

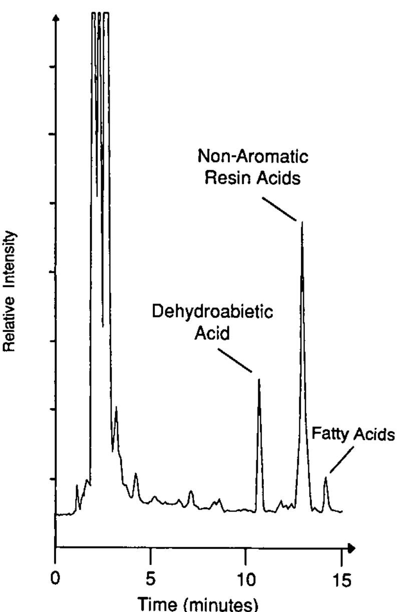

Fig. 2. Typical HPLC chromatogram showing resin and fatty acid constituents in effluent from the paper mill. The main peaks in increasing time of elution are: dehydroabietic acid, non-aromatic resin acids and monocarboxylic fatty acids, all as (methoxycoumarin-4-yl) methyl esters.

non-polar column, injector and chromatography conditions were similar to those described above with the exception that helium was used as the carrier gas. Electron impact mass spectra were acquired and processed with an HP 59970A Computer Workstation. Typical MSD operating conditions were: electron multiplier 2200 V; transfer line 310°C; electron impact energy of 70 eV; 0.8 scans s; mass range 40–650 u. Recoveries and response factors were determined by analysing known amounts of resin acid standards spiked into water and sediment samples.

RESULTS AND DISCUSSION

Comparison of the HPLC and GC–MS methods

The methods used in this study were designed to be applicable for routine monitoring and for more detailed characterization of organic constituents in effluents, process streams, river waters and sediments. The HPLC method (Fig. 2) was ideal for monitoring total concentrations of resin acids and dehydroabietic acid in effluent and in-plant aqueous streams [15]. The GC–MS method was more time consuming both in instrument time and analysis of the data, but it provided a better appreciation of changes in composition of the resin acids in the sediments and waters of the estuary. Richardson *et al.* [15] have previously shown that results from both the GC and HPLC methods show excellent agreement. The HPLC method with solid-phase extraction can be used at concentrations of resin acids in water at concentrations as low as 0.001 mg l^{-1} [15], although information about individual resin acid abundances is not obtained apart from dehydroabietic acid.

Several papers describe the analysis of resin acids in effluents [9,15,16]. Concentrations are often very high (of the order of several mg l^{-1}) and so the methods used do not require the same high sensitivity as those required for analysis of natural waters. Kal'chenko and Svitel'sky [16] reported that chloroform–diethyl ether can be used to extract a variety of constituents including resin acids, fatty acids and phenols in paper mill effluents and white waters although recovery figures were not given. Lee *et al.* [9] used methyl *tert.*-butyl ether followed by transfer to acetone which yielded recoveries generally greater than 95%, except at concentrations of resin acids <0.01 mg l^{-1}. These authors remarked that dichloromethane can cause emulsions with effluent samples, and that hexane gave recoveries of only 60% or less.

There are still surprisingly few studies of resin acids in matrices other than water such as sediments. Some early work using packed GC columns was reported by Brownlee and co-workers [17,18]. More recently, a very sensitive method based on the analysis of pentafluorobenzyl esters by capillary GC with ECD detection and negative-ion chemical ionization mass spectrometry has been developed [19,20], but we chose to base our methods on HPLC, capillary GC and GC–MS equipment that would be readily available in most analytical laboratories. Morales *et al.* [21] recently reported methods for the analysis of resin acids in water, sediments and fish bile based on extraction from a pH 5 medium with methyl *tert.*-butyl ether–dichloromethane solutions.

The non-polar GC column provides very good separation of the methyl esters of resin acids from

other components such as fatty acid methyl esters, but levopimaric and palustric acids which have very similar structures (Fig. 1) are not separated. The two have quite different mass spectra, so GC–MS data (*e.g.* selected ion monitoring [21]) can be used to quantify them if required. The major ions in methyl levopimarate in decreasing order of abundance are: m/z 91 (base), 121, 146, 316 (molecular ion), 187, 256 and 241 (weak). In contrast, methyl palustrate has a base peak at m/z 301 (not found in methyl levopimarate) and major ions at m/z 241 and 316. For example, GC–MS data confirmed that levopimaric acid was a minor constituent of this peak in chromatograms of the resin acids in the effluent, and water samples near to it, but in sediment samples the peak was almost entirely due to palustric acid methyl ester. Mass spectra of common resin acid methyl esters are given in ref. 22, and characteristic ions for resin acid methyl esters and some chlorinated products are given in ref. 21. DB-5 and DB-17 GC column phases also provide useful separations of the pentafluorobenzyl esters of most of the common resin acids [9,19].

The recovery of resin acids in effluent using the HPLC procedure was 95 ± 8% for dehydroabietic acid and 91 ± 11% for the non-aromatic resin acids [15]. Recoveries of spiked samples using the GC and GC–MS procedure for the water and sediment samples (where concentrations were much less) were greater than 75%. In contrast, Lee and Peart [19] found that soxhlet extraction of resin acids from sediments with acetone was 10–30% more efficient than either a high-speed homogenizer or ultrasonic extraction. The efficiency was further improved (by 200–300%) using acidified 12% methanol in acetone, except for palustric and neoabietic acids which isomerize under acid conditions. More recently, these same authors [20] showed that extraction with supercritical carbon dioxide from a 1:1 mixture of methanol and formic acid yielded quantitative recoveries of most resin acids, apart from palustric and neoabietic acids which were recovered in 40% yield [20]. Our experience also shows that the widely used Bligh and Dyer technique using chloroform–methanol mixtures gives very poor yields [14].

Composition and concentration of resin acids in effluent

Total concentrations of resin acids in the paper mill effluent over the 12 months from March 1991 to March 1992 determined by the HPLC method ranged from 1.0 to 4.8 mg l^{-1}, with an average of 2.7 mg ml^{-1}. Routine monitoring of the effluent by the paper mill in 1989 showed that the mean concentration of resin acids was 3.5 mg l^{-1}. These variations mainly reflect day to day changes to operations in the paper mill and differences in the amounts of paper produced each month, although some improvement in effluent water quality from 1989 to 1992 is apparent. Process waste waters within the paper mill consist of brown water (mainly dissolved and colloidal organic matter) and white water (mainly suspended fine pine fibre) streams each of which receives separate primary treatment. Alum is added to the white water clarifier at a concentration of 60 mg l^{-1} which removes a substantial fraction of the resin acids associated with fibre and particles. The concentration of dehydroabietic acid is reduced by about 70% compared with the input stream and other resin acids are reduced by about 80%. However, the addition of alum to the brown water stream is not effective in removing resin acids presumably because they are mainly associated with colloidal organic matter. Even without the addition of alum, reductions of 10 and 27% in dehydroabietic and non-aromatic resin acid abundances in the brown water clarifier are still achieved. The effluent concentrations found here are typical of older mills lacking secondary treatment (*e.g.* 3.4 mg l^{-1} [18]). Much lower effluent concentrations of 0.058, 0.17 and 0.68 mg l^{-1} were recently reported by Lee *et al.* [9] for three Canadian pulpmill effluents. Variations in effluent concentrations reflect different conifer feedstocks and the use of more efficient secondary treatment of the effluent.

The major resin acids in the effluent collected at the same time as the water and sediment samples were dehydroabietic, palustric, abietic and pimaric acids. Smaller amounts of isopimaric, neoabietic and sandaracopimaric acids were also found. Similar distributions are found throughout the year. These resin acids are all common constituents of conifers, and similar distributions have been reported for Canadian pulpmill effluents [9]. Comparisons of the relative abundances found with those in the

softwood feedstocks indicate that changes and losses of resin acids occur during the paper making process. For example, levopimaric and palustric acids together represent about 40–45% of the total resin acids immediately after pulping, but this value is reduced to less than 20% in the final effluent (unpublished data).

Resin acids in surface waters of the Derwent estuary

The mill presently discharges 70–80 million l of effluent per day into a river flow of about 4000 million l per day. The effluent forms a well-defined tannin-coloured plume for at least 1 km downstream of the mill, but the location of the plume in the river and the extent of its mixing with the freshwater flow is quite variable.

The control site for the 1990 survey was situated approximately 12 km upstream of the mill. There are no industrial inputs above this point in the river, although small chemical and nutrient inputs from agricultural activities would be expected. The bottom salt wedge often extends past the paper mill during low river flows in summer, but it does not extend past New Norfolk 5 km upstream of the mill and thus we would not expect to find resin acids indicative of the mill effluent to be present at the control site. Resin acids were not detected in the water column at the 1990 control site which is consistent with the predominance of eucalypts —which do not contain resin acids— in the catchment area and sparse cover of conifers. This result indicated that resin acids are at most trace constituents in the water used by the paper mill.

In the 1992 study we moved the control site (site A) to approximately 2 km upstream from the paper mill outfall. Saline water is carried upstream of the mill as a near-bottom salt water wedge during periods of high tide and low river flow, but judging from the very low concentrations of resin acids detected in waters and sediments at this site the transport of contaminants upstream is relatively minor. Of the resin acids detected, dehydroabietic and pimaric acids were most abundant, but the abundances of resin acids with conjugated double bonds were very low or not detectable.

Gas chromatograms of resin acids in the effluent and river water samples within 6 km of the mill are quite similar apart from additional compounds present in the river water samples and small variations in resin acid abundances. The total concentrations of resin acids in river water at sites downstream of the paper mill during the 1992 study are shown in Table I. At site B, which is directly in front of the effluent discharge, the concentration of total resin acids was 0.19 mg l^{-1}. In the 1990 survey, the corresponding value was very similar (0.21 mg l^{-1}) which is approximately 14 fold less than typical concentration of 2.7 mg l^{-1} in the effluent. The distribution of resin acids was quite similar to that in the effluent sample collected on the same day (Table I), and the differences were within the range of variations found in effluent composition (unpublished data).

The concentration of resin acids in the water column at site C, 500 m downriver, was much less at only 0.018 mg l^{-1} suggesting that this sample was collected on the edge of the plume which was not well defined on the day of sampling. In 1990, the corresponding concentration at this site was 0.15 mg l^{-1} but, as we show later, such extreme variability can be caused by the state of the tide changing the position of the effluent plume in the river. Surface water concentrations further downstream at site I, 11.6 km from the mill, were even lower at 0.008 mg l^{-1}.

Fox [23] carried out a study of resin acids in waters of Lake Superior which received effluent from a kraft pulp mill. The concentration of dehydroabietic acid was 1.93 mg l^{-1} at 300 m from the effluent (*c.f.* 1.5 mg l^{-1} in the effluent in our study) and decreased to 0.018 mg l^{-1} at 1.7 km from the mill which is comparable to most of the downriver sites analysed here from the Derwent estuary. In the Lake Superior study, organic constituents in the effluent were reduced to background levels within the first 2 km due to efficient mixing processes, but in the Derwent they can still be detected in waters 10 km downriver (albeit in low concentration). Based on the volume of effluent discharge compared with the river flow, if there were complete mixing this would produce resin acid concentrations of 0.05–0.06 mg l^{-1} which is considerably in excess of most of the concentrations measured (0.008–0.04 mg l^{-1} at sites 500 or more meters from the effluent; Table I). Although the plume is well defined near the mill, one would expect that the waters would be reasonably mixed at sites more than 5 km from the mill such as G, H and I. The lower than expected con-

TABLE I

PERCENTAGE COMPOSITION AND TOTAL CONCENTRATION OF RESIN ACIDS IN PAPER MILL EFFLUENT AND SURFACE WATER SAMPLES FROM THE UPPER DERWENT ESTUARY

All samples were collected on April 1, 1992. Site A is upstream of the mill, and site B is immediately adjacent to the mill outfall. All other sites are downriver.

Resin acids	Effluent	Sites (distance from mill in km)							
		A (−2.1)	B (0)	C_4 (0.5)	E (3.6)	F (4.7)	G (5.8)	H (8.6)	I (11.6)
Pimaric	11.6	23.8	15.2	16.0	17.1	13.9	15.2	17.5	12.4
Sandaracopimaric	2.2	2.4	2.3	3.3	2.9	2.5	0.8	0.6	2.4
Isopimaric	10.6	9.0	10.3	10.0	10.3	11.1	12.7	10.7	10.8
Levopimaric + Palustric	18.9	0.0	16.4	0.3	0.4	11.4	8.3	4.9	9.2
Dehydroabietic	33.8	63.3	33.8	67.0	67.0	42.7	50.1	63.9	45.2
Abietic	14.9	1.5	17.1	2.1	1.8	17.4	12.3	2.4	17.4
Neoabietic	8.1	0.0	5.0	1.3	0.4	1.8	0.7	tr[a]	2.6
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Conc. mg l^{-1}	4.5	0.0023	0.192	0.0186	0.0312	0.0395	0.0172	0.0192	0.0084

[a] tr = trace; <0.1%.

centrations in the surface fresh water imply that a major part (perhaps about 50%) of the resin acid load is transferred to the underlying salt wedge and bottom sediments.

The changes in proportions of individual resin acids at the different sites cannot be explained in terms of a simple model of effluent mixing with the river water. At sites C and E, the proportions of individual resin acids were markedly different from those in the effluent with abietic, levopimaric, palustric and neoabietic acids present in much reduced amounts (Table I). This distribution more closely resembles that found at the upstream control site A, although the total concentrations were 15–20 times lower there. Compositional changes were also apparent at sites further downriver, but a regular trend was not apparent.

Although most resin acids are rapidly degraded by microorganisms in aquatic environments [24], dehydroabietic acid appears to be quite persistent [23] with an apparent half-life in water of about 0.12 years and in sediments of 21 years [17]. It seems unlikely that the rapid transport of resin acids downriver within the fresh water phase would allow enough time for microbial processes to change the resin acid proportions significantly. It are only those resin acids which contain conjugated double bonds (mainly of the abietane class) which show major changes in proportions. These are the same acids which are degraded and interconverted during paper pulping and processing suggesting that chemical processes are responsible. Since much of the resin acids in the effluent are associated with colloids, the sedimentation of this material to the sediment–water interface could account for the reduced concentrations found in the fresh water phase. Also, since these colloids and bottom sediments are readily resuspended this process could reinject the resin acids back into the water column, but over a longer time frame which is sufficient to allow for chemical modification of the resin acid distributions to take place.

Effects of tidal mixing on water column concentrations of resin acids

The concentrations of resin acids in surface waters of the estuary are strongly influenced by the mixing of fresh and salt waters, and by the effects of the tide. To assess this, water samples were collected at 4 times during the day from 4 stations across the river at site C, 500 m downstream of the effluent. An additional sample was collected from site F* near to Green Island 5 km downstream of the mill. Data were obtained on October 29, 1990; February 29, 1991; June 3, 1991; September 30, 1991; and on January 13, 1992. Data from the latter sampling,

which was closest in time to the river survey, are shown in Table II. It should be noted, however, that the concentration data from the other sampling dates showed considerable variation.

Samples collected at 08.35 in the morning showed very low concentrations of resin acids (most <0.01 mg l^{-1}) at all sites across the river, but two h later at 10.35 the concentrations had increased to 0.18 mg l^{-1} except near the inner bank where the concentration was still <0.01 mg l^{-1}. Concentrations were even higher after mid-day and reached maximum values of 0.78 mg l^{-1} in the early afternoon due to the incoming tide pushing the effluent stream to the far bank. The variations at site F* near Green Island are less affected by the tide, but even here the concentrations ranged from <0.01 to 0.05 mg l^{-1} which might indicate incomplete mixing of the plume (at least on some occasions). It is clear from these data that any attempt to model the transport of resin acids in the estuary must include the effects of water mass mixing due to currents and tides. Discrete water samples can only provide a snapshot of contaminant loads in the river and estuary and integrated samples which distinguish between the surface fresh waters and deeper waters of the salt water wedge are essential to obtain a better budget of contaminant loads and transport in the estuary.

TABLE II

CONCENTRATIONS (mg l^{-1}) OF TOTAL RESIN ACIDS, AMOUNT OF NON-FILTERABLE RESIDUE (NFR), pH AND CONDUCTIVITY IN SURFACE WATERS FROM TWO SITES IN THE UPPER DERWENT ESTUARY DOWNSTREAM OF THE MILL COLLECTED AT 2-h INTERVALS ON JANUARY 13, 1992

Site[a] number	Time	Total resin acids	NFR[b]	pH	Conductivity
C_1	08.35	<0.01	1.2	7.3	10.4
C_2	08.35	0.02	3.6	7.6	9.6
C_3	08.35	<0.01	2.8	7.7	9.6
C_4	08.35	<0.01	2.8	7.6	9.9
F*	08.35	<0.01	1.8	7.5	14.3
C_1	10.35	0.17	3.4	7.5	10.3
C_2	10.35	0.07	2.0	7.5	11.3
C_3	10.35	0.18	3.8	7.6	11.0
C_4	10.35	<0.01	1.4	7.6	11.3
F*	10.35	0.02	2.0	7.5	11.1
C_1	12.40	0.29	6.0	7.8	12.8
C_2	12.40	0.21	3.6	7.7	12.6
C_3	12.40	0.15	2.6	7.5	12.6
C_4	12.40	0.07	2.0	7.5	13.1
F*	12.40	0.02	0.6	7.5	10.8
C_1	14.35	0.78	13.8	8.2	13.2
C_2	14.35	0.42	6.4	7.8	13.1
C_3	14.35	0.08	2.4	7.5	13.1
C_4	14.35	0.07	2.8	7.5	13.0
F*	14.35	0.05	1.8	7.3	11.6

[a] Site C is 500 m.
[b] NFR is non-filterable residue which contains a large contribution of wood fibre from the paper mill effluent. downstream: samples 1–4 were collected across the river with sample 1 on the opposite bank to the mill. Site F* is on the north end of Green Island 5 km from the mill, slightly downriver from site F in the 1992 study.

Resin acids in sediments

Resin acids distributions in sediments collected on April 1st, 1992 are shown in Table III. A representative chromatogram of the resin acids in sediment from site G is shown in Fig. 3. Note that although this extract had been purified by passing it through a Bond Elut cartridge, the chromatogram still shows many peaks due to compounds other than resin acids. Straight-chain, branched-chain and unsaturated fatty acids account for many of the peaks present, but many unidentified compounds are also abundant.

Peaks due to fatty acids are of similar abundance to those for the resin acids, but this underestimates their true concentration since the extraction method is not designed for quantitative recovery of fatty acids. In another study [13] we showed using extraction with chloroform–methanol that the concentration of fatty acids in fibre-rich sludges from the Derwent estuary were as high as 2300 mg kg^{-1} compared with a background figure of about 50 mg kg^{-1} in deeper sediments deposited before the paper mill was operational. Note that the bacterially derived C_{15} iso- and anteiso-branched fatty acids are as abundant as the C_{16} and C_{18} fatty acids from wood fibre and higher plants testifying to the high bacterial biomass and intense anaerobic metabolism and breakdown of organic matter occurring in the sediments.

At site A 2.1 km upstream of the paper mill, some resin acids were detected in the sediment, but the amounts were extremely low (0.34 mg kg^{-1}). The salt wedge at the time of sampling extended well past site A almost to the New Norfolk bridge and

TABLE III

PERCENTAGE COMPOSITION AND TOTAL CONCENTRATION OF RESIN ACIDS IN SEDIMENTS FROM THE UPPER DERWENT ESTUARY

All samples were collected on April 1, 1992. Site A is upstream of the mill, and site B is immediately adjacent the mill outfall. All other sites are downriver.

Resin acids	Sites (distance from mill in km)								
	A (−2.1)	B (0)	C_4 (0.5)	D (1.5)	E (3.6)	F (4.7)	G (5.8)	H (8.6)	I (11.6)
Pimaric	9.0	7.5	14.9	13.7	30.3	9.3	9.5	9.1	8.5
Sandaracopimaric	0.7	0.7	5.3	1.6	3.1	5.5	3.3	3.0	3.8
Isopimaric	8.6	13.4	19.2	20.3	13.2	8.8	15.3	16.2	20.7
Levopimaric + Palustric	0.0	0.5	0.4	2.7	5.6	5.1	2.3	2.7	3.7
Dehydroabietic	80.1	62.0	38.3	43.4	29.2	51.2	49.4	51.7	51.4
Abietic	1.7	15.1	19.0	15.2	16.0	11.0	11.1	11.7	9.6
Neoabietic	0.0	0.8	2.9	3.1	2.6	9.1	9.1	5.5	2.3
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Concentration mg kg^{-1} (dry wt.)	0.34	87	1.2	34	0.7	7.0	5.3	4.0	1.5

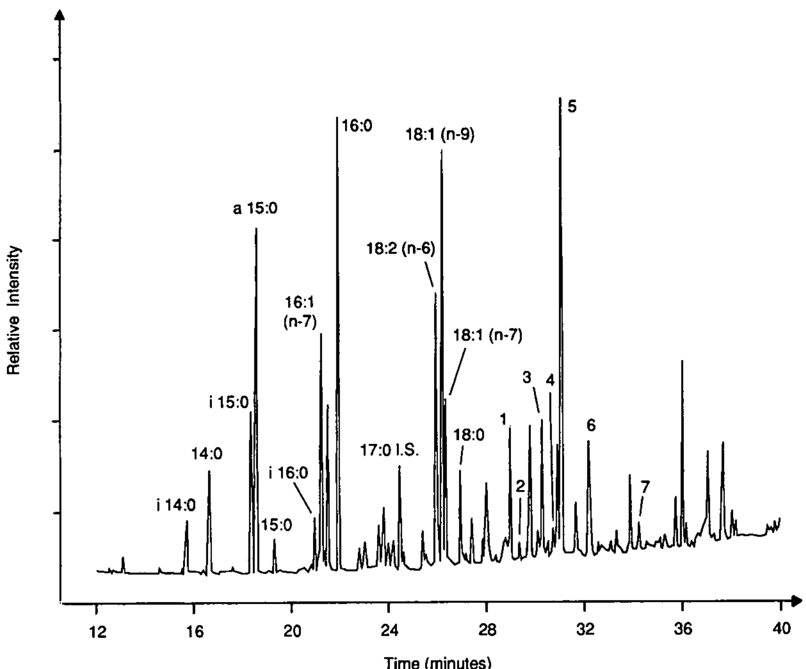

Fig. 3. Partial capillary GC–FID chromatogram of resin acids in sediment from site G. Peaks are methyl esters of (1) pimaric, (2) sandaracopimaric, (3) isopimaric, (4) levopimaric plus palustric, (5) dehydroabietic, (6) abietic and (7) neoabietic resin acids. Heptadecanoic acid methyl ester (17:0) was added as an internal standard (I.S.). Fatty acids are designated number of carbon atoms: number of double bonds. Branched fatty acids are indicated by iso (i) and anteiso (a). The group of compounds eluting after the resin acids was not identified.

this would have carried upstream a small amount of organic material from the effluent. Indeed, analysis of a saline bottom water sample from just below the outfall showed concentrations of resin acids approximately half those in the overlying fresh water (unpublished data). Dehydroabietic and pimaric acids dominated the distribution at site A (Table III), indicating that these resin acids are the most stable of those studied. The absence of the resin acids with conjugated double bonds such as levopimaric and palustric acids confirms that these resin acids are readily degraded.

At site B close to the discharge point, the total resin acid concentration in the sediment was 87 mg kg^{-1} (dry wt.). At site C_4 500 m downriver the concentrations of resin acids was only 1.2 mg kg^{-1} and yet at site D the concentrations were considerably higher at 34 mg kg^{-1}. This extreme variation is largely due to the changing composition of the bottom sediments. At site C_4 the sediment was very sandy and there was little evidence of wood fibre present. In contrast, at site D where the concentration of resin acids was higher, there has been extensive deposition of wood fibre around the inner bend. These sediments contained considerable silt and organic matter and they were strongly anoxic reflecting substantial microbial reworking of the organic matter present.

The presence of resin acids in water at sites H and I, which are over 10 km from the mill, is difficult to explain simply due to the water transport of dissolved constituents. The river is only 3 m deep at these sites in this part of the estuary (apart from a narrow central channel) and the river flow at the edges is much slower leading to considerable sediment build-up at sites H and I. These sediments still contain resin acids and other organic compounds from the paper mill, and these sediments can be resuspended especially during periods of high freshwater flow leading to higher than expected water column concentrations.

CONCLUSIONS

GC–FID, GC–MS and HPLC methods have been developed to study resin acids in effluents, river waters and sediments. The HPLC method provides concentration data for dehydroabietic acid and total non-aromatic resin acids. It is less time consuming than the GC–MS determinations and thus it is ideal for routine monitoring of total resin acid concentrations. The GC–MS method is better suited to environmental applications such as water and sediment analyses where concentration data are required for individual resin acids. Surface waters and river sediments from the upper Derwent estuary contain resin acids derived from paper mill effluent. The distribution of resin acids in the rivers shows considerably more variation than is found in the effluent providing evidence that the resin acids are degraded and remobilised in the estuary. The resin acid concentrations are much less than those which have been found to cause mortality in fish, but they are only just below the levels found to cause sublethal effects. A more extensive monitoring program would be needed to determine the three-dimensional transport of effluent constituents in the estuary and the relative importance of sediment resuspension as a contributing source of contaminants in the water column.

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We thank the Department of Environment (Tasmania) for the use of their vessel *Aqua* for water and sediment sampling. *Aqua* was ably skippered by Dave Bartlett who provided very useful local knowledge of the river and sediments. Teresa O'Leary, Rhys Leeming and Graeme Dunstan are thanked for their help with sample collection and laboratory work. Dr. Peter Nichols and Dr. Andy Revill provided useful comments on draft versions of the manuscript. The assistance of Dr. Tony Flowers and other staff from Australian Newsprint Mills is gratefully acknowledged.

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CHROMSYMP. 2761

Determination of the potent mutagen 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX) in water by gas chromatography with electron-capture detection

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ABSTRACT

A highly sensitive method for the determination of 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX) in water by gas chromatography with electron-capture detection is described. MX was derivatized with 2,2,3,3,3-pentafluoropropanol and the product was easily decomposed by UV irradiation. The disappearance of the peak on the chromatogram after the irradiation was used for the identification of MX; the amount of MX was calculated from the decrease in peak height. The method was applied to the analysis of five samples of chlorinated domestic sewage. MX (141.6–2.2 ng/l) was detected in all samples with a detection limit of 0.8 ng/l. The recovery of MX was more than 97.5% at the level of 20.0 ng/l added.

INTRODUCTION

3-Chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX), an involatile, direct-acting and highly potent Ames mutagen, has been identified as a major mutagen in pulp chlorination liquors [1,2]. MX is formed by chlorination of naturally occurring humic substances [3–5]. Recently, MX was detected in tap water disinfected with chlorine in Finland [3,5], the USA [4], the UK [6], Netherlands [7] and Japan [8], and according to these reports the mutagenicity contribution of MX in the sample water was about 7–60%. Because of the high mutagenic potency of MX (20 000 revertants/μg MX), it is expected that requirements for checking the amount of MX in drinking water disinfected by chlorination will increase.

The determination of MX in water has conventionally been carried out by gas chromatography–mass spectrometry with selected ion monitoring (GC–MS–SIM) after the extraction of MX from water followed by high-performance liquid chromatographic (HPLC) purification and methylation with methanol and sulphuric acid [9]. Because of the extremely low concentration of MX in tap water, the GC–MS–SIM method required large volumes of sample water, high resolution and high sensitivity for analysis. Gas chromatography with electron-capture detection (GC–ECD) is highly sensitive to methylated MX (Me-MX). In this work, pentafluoropropylation instead of the conventional methylation was employed for the derivatization of MX in GC–ECD. It was expected that ECD would be more sensitive to pentafluoropropylated MX (PFP-MX) than Me-MX. PFP-MX was easily decomposed by UV irradiation, hence the disappearance of the PFP-MX peak on a gas chromatogram after UV

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irradiation could be used to identify MX. The method was applied to the determination of MX in a sample of chlorinated domestic sewage that had been treated with an activated sludge system. The results were compared with those obtained by GC–MS–SIM and were in good agreement.

EXPERIMENTAL

Materials

MX was synthesized and purified according to the procedure of Padmapriya *et al.* [10]. The synthesized MX was identified by GC–MS and its purity was confirmed by GC and HPLC to be more than 98%. MX was stored in ethyl acetate at 4°C, where MX is completely stable [11].

A standard solution of MX was prepared by dilution of the ethyl acetate stock solution with ethyl acetate and was stored in the same manner as the stock solution. 2,2,3,3,3-Pentafluoropropanol (PFP) was obtained from Nacalai Tesque (Kyoto, Japan) and was used as received.

Isolation of MX from chlorinated sewage

The isolation of mutagenic substances including MX from chlorinated sewage was performed as described previously [12]. Samples of 1 l of chlorinated sewage were dechlorinated with sodium thiosulphate, adjusted to pH 2.0 with hydrochloric acid and applied to two sequential columns, one packed with Amberlite XAD-2 and the other with Amberlite XAD-8 resin (Rohm and Hass, Philadelphia, PA, USA). The columns were washed with distilled water acidified with hydrochloric acid (pH 2), then the adsorbates on the resins were eluted with ethyl acetate. The eluates were dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure at 40°C. The residue containing non-volatile mutagens was resolved with a definite volume of ethyl acetate and this XAD extract was stored at −15°C in a freezer.

Fractionation of XAD extracts by HPLC

The XAD extracts were evaporated to dryness under reduced pressure at 40°C immediately before use. The residue was dissolved in 1.0 ml of methanol and the solution was subjected to preparative HPLC. The HPLC conditions were as follows: apparatus, LC-4A (Shimadzu, Kyoto, Japan); column, Capcell Pak C_{18} (250 mm × 20 mm I.D.) (Shiseido, Tokyo, Japan); elution, methanol–0.05 *M* phosphate buffer (pH 3.0) (40:60) for 50 min followed by a linear gradient to 100% methanol during 30 min, then held at 100% methanol for 40 min; flow-rate, 5 ml/min; column temperature, ambient; detection, UV at 230 nm. Fractions of 5 ml up to 50 min and of 25 ml after 50 min were collected. Each fraction was evaporated to dryness under reduced pressure at 40°C, the residue was dissolved in ethyl acetate and the solution was stored in the same manner as the stock solution.

Pentafluoropropylation of MX

The solvent was removed from the standard solution of MX and the fractions obtained by preparative HPLC under a stream of dry nitrogen immediately before use. To the residue, 100 μl of PFP and 10 μl of concentrated sulphuric acid were added and the mixture was heated for 30 min at 70°C. After cooling, 1 ml of 8% sodium hydrogen carbonate solution was added, then PFP-MX was extracted twice with 1-ml volumes of *n*-hexane. The extract was concentrated to 0.5 ml.

Detection and determination of PFP-MX by GC–ECD

Part of the *n*-hexane solution of PFP-MX was injected into a GC–ECD system. The GC–ECD conditions were as follows: apparatus, Shimadzu GC-4BM equipped with an electron-capture detector; column, SPB-5 fused-silica capillary (15 m × 0.53 mm I.D.; film thickness 1.5 μm) (Supelco, Bellefonte, PA, USA); carrier gas, nitrogen at 6 ml/min; injection port and detector temperatures, 200°C; column temperature, 110°C. The remaining 0.25 ml of *n*-hexane solution was transferred into a small quartz vessel and irradiated with a UV lamp (Toshiba GL-15 bactericidal lamp, 18 W, 15 cm long) placed parallel to the surface of the solution and about 30 cm from it for 90 min. After irradiation, the solution was diluted to the original volume with *n*-hexane and analysed by GC–ECD. PFP-MX was decomposed by the UV irradiation and the peak corresponding to it disappeared. This peak showed a retention time identical with that of authentic PFP-MX. The amount of MX was calculated from the height of this peak.

TABLE I

RELATIONSHIP BETWEEN VOLUME OF PFP AND PEAK HEIGHT OF PFP DERIVATIVE OF MX

The reaction mixture containing 1 μg of MX, 10 μl of sulphuric acid and PFP was allowed to react for 30 min at 70°C and extracted three times with 1-ml volumes of *n*-hexane. The PEP derivative of MX was detected by GC–ECD.

PFP volume (μl)	Peak height (mm)
0	0.0
10	142.2
50	267.8
100	293.6
200	150.0
300	108.5

TABLE II

RELATIONSHIPS AMONG REACTION TIME, REACTION TEMPERATURE AND PEAK HEIGHT OF PFP DERIVATIVE OF MX

A reaction mixture containing 100 μl of PFP was used; the other conditions were as in Table I.

Reaction temperature (°C)	Reaction time (min)	Peak height (mm)
60	30	102.8
70	0	0.0
	15	249.2
	30	330.0
	60	335.7
	90	327.0
80	30	136.1
90	30	59.6

Determination of PFP-MX by GC–MS–SIM

Authentic PFP-MX and pentafluoropropylated samples obtained from chlorinated sewage were analysed by GC–MS–SIM. The GC and MS conditions were as follows: apparatus, Shimadzu GC-9A gas chromatograph and MS QP-1000 mass spectrometer; column, 3% silicone OV-101/Shimalite W (AW-DMCS) (1 m × 2.6 mm I.D.) (Shimadzu); column temperature, increased from 80 to 200°C at 6°C/min; carrier gas, helium at 30 ml/min; injection temperature, 250°C; electron energy, 70 eV; ion source temperature, 250°C. Fragment ions of *m/z* 265, 267, 199 and 201 were chosen for SIM.

RESULTS AND DISCUSSION

MX could be pentafluoropropylated in the same manner as the conventional methylation of MX. PFP-MX gave one peak in GC–ECD. A mass spectrum of the peak is shown in Fig. 1. A number of specific ion peaks, *m/z* 348 (M^+, weak), 265 and 267 ($M^+ - CHCl_2$, prominent), 199, 201 and 203 ($M^+ - OCH_2CF_2CF_3$), were observed. The results showed the hydroxyl group at the 5-position in MX was pentafluoropropylated. The set of specific ions of *m/z* 265, 267, 199 and 201 was used for GC–MS–SIM.

MX was allowed to react with various amount of PFP in the presence of concentrated sulphuric acid and the reaction products were extracted with *n*-hexane. As shown in Table I, the maximum peak height was obtained in the presence of 100 μl of PFP. In the presence of more than 200 μl of PFP, the

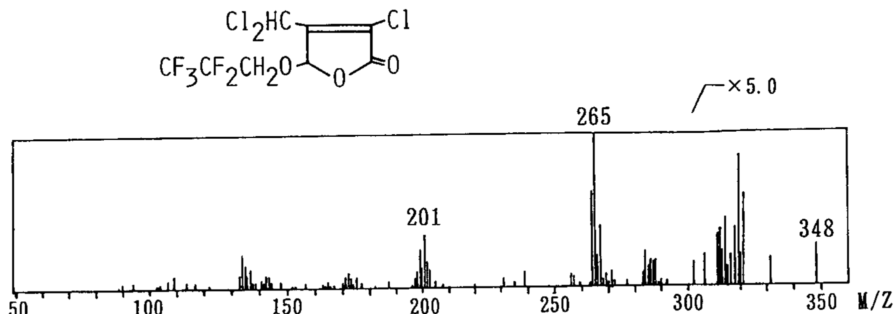


Fig. 1. Mass spectrum of PFP derivative of MX.

mixture was separated into three phases (water, PFP and n-hexane phases) in the extraction process and the peak height of PFP-MX decreased. It was thought that PFP-MX was distributed between the n-hexane and PFP phases, and the recovery of PFP-MX in the n-hexane phase was lowered. Table II shows the relationship among the peak height, the reaction temperature and the reaction time. The maximum peak height was obtained by heating at 70°C for 30 min. From these results, it was decided that pentafluoropropylation of MX was carried out in the presence of 100 μl of PFP and 10 μl of concentrated sulphuric acid with heating at 70°C for 30 min. The electron-capture detector was about 3.5 times more sensitive to PFP-MX than Me-MX.

When XAD extracts obtained from an actual sample were pentafluoropropylated and analysed by GC–ECD without further purification, there were large peaks of impurities in the chromatogram and the peak of PFP-MX was completely obscured (Fig. 2). Hence purification of the XAD extracts by preparative HPLC was necessary in the GC–ECD method. The gas chromatograms of authentic PFP-MX and purified XAD extracts are shown in Fig. 3A-1 and B-1, respectively. As shown in Fig. 3B-1, a peak showing a retention time (t_R) identical with that of PFP-MX was found in the actual sample. A method other than t_R for the identification of PFP-MX in the chromatogram was further investigated. As shown in Figs. 3A-2 and 4, the peak of authentic PFP-MX completely disappeared after UV irradiation for 90 min. The peak height identified as PFP-MX in the actual sample decreased after irradiation for 90 min and was constant after 90 min (Fig. 4). When authentic PFP-MX was added to the actual sample and irradiated for 90 min, the peak height also decreased to the same constant level as that in the actual sample (Fig. 4). These results suggested that PFP-MX was not separated from some component(s) of an actual sample and overlapped them. However, they also suggested that PFP-MX contained in the peak was completely decomposed by UV irradiation for 90 min. Thus, in addition to identification via t_R, the disappearance of the peak or the decrease in the peak height after UV irradiation for 90 min was used for the routine identification of MX. The decreased peak height was used for the calculation of the amount of MX.

The recovery of MX through the all analytical

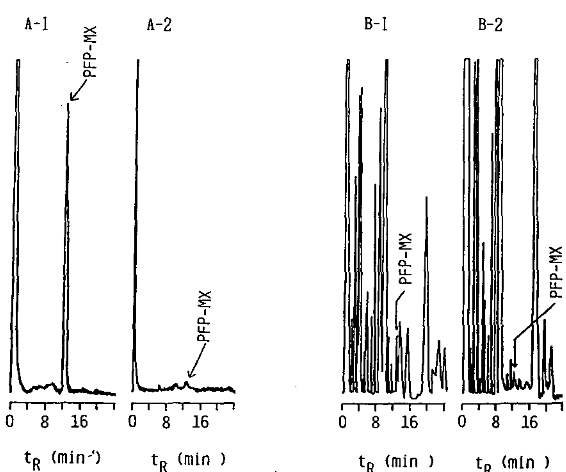


Fig. 3. Typical gas chromatograms with ECD of PFP derivatives of authentic MX and chlorinated sewage purified by preparative HPLC before and after UV irradiation. n-Hexane solutions (0.25 ml) containing PFP derivatives of authentic MX (0.2 μg, A) or chlorinated sewage purified by preparative HPLC (B) were analysed by GC–ECD before (A-1 and B-1) and after (A-2 and B-2) UV irradiation for 90 min.

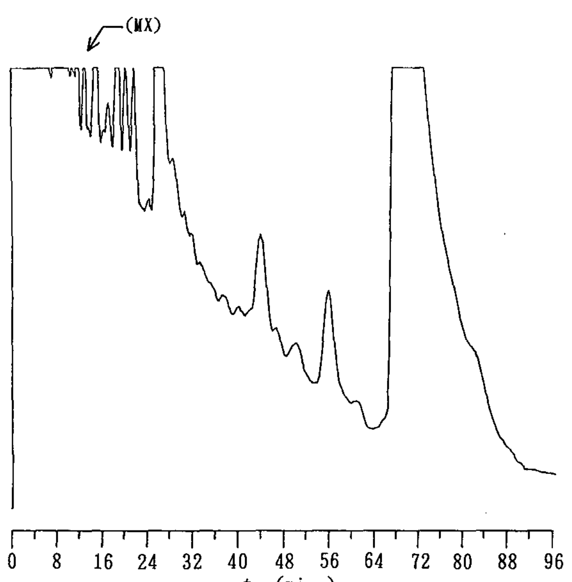


Fig. 2. Typical gas chromatogram with ECD of chlorinated sewage derivatized with PFP before preparative HPLC purification. XAD extracts corresponding to 1 l of original chlorinated sewage were derivatized with PFP without further purification and analysed by GC–ECD.

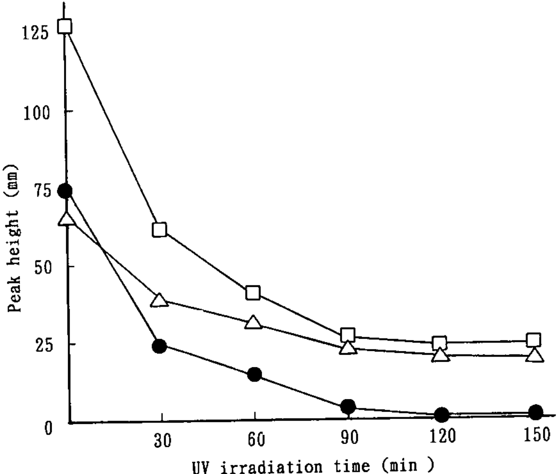


Fig. 4. Decrease in peak height of PFP-MX in gas chromatograms with ECD of authentic sample, chlorinated sewage and chlorinated sewage spiked with authentic MX on UV irradiation. *n*-Hexane solution (0.25 ml) containing PFP derivative of authentic MX (0.2 μg) (●), chlorinated sewage (△) or chlorinated sewage spiked with 0.2 μg of authentic MX (□) was subjected to UV irradiation for various times and was analysed by GC–ECD.

procedures was examined by use of the GC–ECD method with UV irradiation. Authentic MX was added to an actual sample immediately after the dechlorination process. As shown in Table III, the recoveries of 20 and 40 ng/l of MX added to an actual sample were more than 97.5%. Table IV shows the results of the determination of MX in five chlorinated domestic sewage samples by GC–ECD.

TABLE III

RECOVERY OF MX FROM CHLORINATED DOMESTIC SEWAGE SPIKED WITH MX

The amount of MX was calculated from decrease in peak height on UV irradiation.

Spike level (ng/l)	MX	
	Found (ng/l)[a]	Recovery (%)
0.0	30.8 ± 2.02	–
20.0	50.3 ± 0.59	97.5 ± 2.94
40.0	70.9 ± 5.23	95.3 ± 6.01

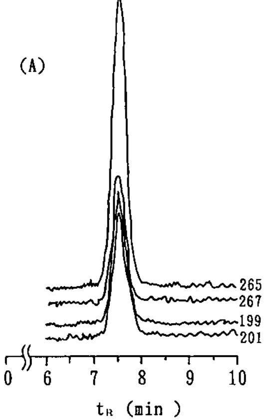
[a] Each value is the mean ± S.D. of three determinations.

TABLE IV

CONTENT OF MX IN CHLORINATED DOMESTIC SEWAGE

Sample No.	TOC[a] (mg/l)	Content of MX (ng/l)[b]	
		GC–ECD method[c]	GC–MS–SIM method
1	40.5	141.6 ± 8.2	134.0 ± 9.1
2	45.5	37.2 ± 3.5	34.6 ± 2.8
3	17.5	30.8 ± 2.0	28.5 ± 3.3
4	42.2	10.9 ± 0.7	N.D.[e]
5	11.0	2.2 ± 0.4	N.D.[e]
6[d]	353.2	N.D.[e]	N.D.[f]

[a] Content of total organic carbon.
[b] Each value is the mean ± S.D. of three determinations.
[c] The amount of MX was calculated from the decrease in peak height on UV irradiation.
[d] Domestic sewage not treated with activated sludge system.
[e] Not detected (below 0.8 ng/l).
[f] Not detected (below 23 ng/l).



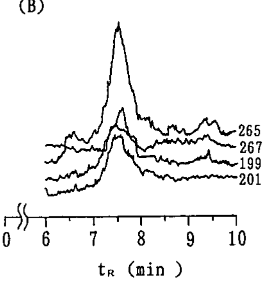


Fig. 5. Typical GC–MS–SIM traces of PFP derivatives of (A) authentic MX and (B) chlorinated sewage.

MX was found in all samples in the range 141.6–2.2 ng/l. The detection limit was lower than 0.8 ng/l of MX in water (signal-to-noise ratio = 2). Detection and determination by the GC–MS–SIM method (Fig. 5) was carried out simultaneously. In three out of five samples the results obtained by both the two were in good agreement. It was confirmed from these results that the decrease in the height of the peak that showed a t_R identical with that of authentic PFP-MX in the gas chromatogram obtained with ECD is attributable only to the decomposition of PFP-MX by UV irradiation, and not to the other substances coeluted with MX. Hence measurement of the peak height of PFP-MX after UV irradiation was reasonable for the determination of MX in the GC–ECD method. In the two samples, MX could be determined by the GC–ECD method, but could not be detected by the GC–MS–SIM method because the amount of MX was below the detection limit (23 ng/l of MX in water under the conditions used). MX was not detected by either method in a sample of chlorinated domestic sewage that had not been treated with an activated sludge system (sample No. 6). The mutagenicity contribution of MX detected has been reported elsewhere [13].

CONCLUSION

An advantage of the proposed GC–ECD method is that pentafluoropropylation of MX is used instead of conventional methylation. The electron-capture detector was about 3.5 times more sensitive to PFP-MX than Me-MX and the detection limit was lowered. When a GC separation was carried out, insufficient separation among PFP-MX and impurities derived from an actual sample was observed. This problem was solved by the purification of MX by preparative HPLC and by identification based on the disappearance of the peak of PFP-MX on UV irradiation. The method was successfully applied to chlorinated domestic sewage for the determination of MX. The method proposed here is useful and convenient for the routine determination of MX in water.

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CHROMSYMP. 2729

Determination of chlorophenoxy and other acidic herbicide residues in ground water by capillary gas chromatography of their alkyl esters formed by rapid derivatization using various chloroformates

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ABSTRACT

A simple method for the determination of several chlorophenoxy acid and other acidic herbicides as methyl, ethyl and butyl esters by means of capillary gas chromatography is described. Derivatization with chloroformates (methyl, ethyl or butyl chloroformate) to give the corresponding methyl, ethyl or butyl esters of the pesticides tested can easily be achieved. Fifteen chlorophenoxy acid and seven other acidic herbicides can easily be determined by gas chromatography with electron-capture or mass spectrometric detection at the relevant residue levels of 100 ng/l in water samples. Recoveries were determined with spiked tap and ground water samples at 50, 100 and 200 ng/l using solid-phase extraction with RP-18 material. Nineteen out of the 22 herbicides could be found with a recovery of more than 75% and the remaining three herbicides could be extracted with a recovery of more than 50%. Tentative confirmation can be achieved by simply esterifying two aliquots of the sample extracts with two different chloroformates.

INTRODUCTION

Phenoxy acid herbicides are in widespread use for weed control, which results in their presence as residues in surface and ground waters. Various methods for their determination have been published [1–4] and a comprehensive critical treatment of the methods available up to 1980 was given by Sirous *et al.* [5].

In recent years, solid-phase extraction (SPE) has grown in popularity and gained a reputation as a reliable method for the extraction of pesticide residues from water samples [6,7]. Phenoxy acid herbicides were also found to be extracted with good yields after acidification of the water samples to pH 2. Solid-phase extraction and elution are usually followed either by high-performance liquid chromatography (HPLC) with UV or photoconductivity detection [8] or by gas chromatography (GC) with electron-capture detection (ECD) [6,7,9–11]. Using GC–ECD, derivatization is necessary in order to reach the level of 100 ng/l per substance which has been fixed by the European Community.

The most common derivatives are the methyl esters, using, for example, H_2SO_4–methanol, diazomethane, BF_3–methanol or dimethyl sulphate for methylation [10,11]. Derivatization with pentafluorobenzyl bromide produces derivatives that exhibit very good ECD responses [10–13]. The drawback of this derivatization method, however, is the lack of selectivity of pentafluorobenzyl bromide, leading to the derivatization of a large number of matrix compounds. The results are very complex chromatograms, which make identification and quantification very difficult.

Chloroformates have been known as a possible source of mixed anhydrides since the beginning of this century [14]. In 1990, Husek *et al.* [15] published a rapid derivatization procedure for fatty acids using

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chloroformates as reagents. The method was subsequently extended to the derivatization of hydroxycarboxylic acids [16] and amino acids [17]. These chloroformates seem to be promising for the derivatization of acidic herbicides because of the simplicity of sample preparation. In this paper, a procedure for the derivatization of acidic herbicides using methyl, ethyl and butyl chloroformate to produce methyl, ethyl and butyl esters, respectively, is described.

EXPERIMENTAL

Materials

All pesticide standards were of analytical purity, purchased from Promochem (Wesel, Germany), or of Pestanal quality, from Riedel de Haën (Seelze, Germany). Sample vials, screw-caps and septa were purchased from Zinsser (Frankfurt, Germany) and 200-μl inserts for the sample vials were obtained from CS-Chromatographie Service (Langerwehe, Germany).

Stock solutions of all compounds were prepared in toluene or methanol. Standards and samples were finally dissolved in toluene. All solvents were Pestanal products from Riedel de Haën. Ethyl chloroformate, methyl chloroformate, butyl chloroformate and HCl were purchased from Merck (Darmstadt, Germany). Solid-phase extraction cartridges (6 ml) (polypropylene) and RP-18 material were obtained from Baker (Frankfurt, Germany). Adjustable Transferpettors (1–10 and 10–100 μl) were supplied by Brand (Wertheim, Germany).

The following reaction mixtures were used: (A) acetonitrile–ethanol–water–pyridine (5:2:2:1, v/v); (B) acetonitrile–methanol–water–pyridine (2:2:7:1, v/v); and (C) acetonitrile–butanol–water–pyridine (2:2:6:1, v/v).

Instrumentation

Gas chromatography–mass spectrometry. An HP 5890 gas chromatograph with an HP 5970 mass-selective detector and an HP 59970 MS ChemStation, equipped with an HP 7673 autosampler and a split–splitless injector for capillary columns, was employed.

For GC, a fused-silica capillary column (12 m × 0.20 mm I.D.), coated with SE-54 with a film thickness of 0.32 μm, was used with helium as the carrier gas. The injection port temperature was 210°C and the transfer line temperature 190°C. The column temperature programme was 2.9 min at 90°C, increased at 6°C/min to 240°C and held at 240°C for 20 min. A 1-μl volume of sample was injected by the autosampler using hot splitless injection with the split closed for 1 min.

For MS, the transfer line temperature was 190°C and the ion source temperature 200°C. The scanned mass range was 50–500 u, scan rate 1.22 scans/s and threshold 500. The solvent delay was 10 min. The voltages of the repeller, draw out, ion focus, entrance lens and X-ray and the parameters for the quadrupole mass filter were set according to the values proposed by the programme AUTOTUNE, which automatically optimizes these parameters using perfluorotributylamine (PFTBA) as a calibration standard.

Gas chromatography with electron-capture detection. An HP 5890 gas chromatograph with an electron-capture detector, an HP 7673 autosampler and a split–splitless injector for capillary columns was employed. Nelson analytical Software 2600 was used for data acquisition.

A fused-silica column (25 m × 0.20 mm I.D.), coated with SE-54 material with a film thickness of 0.32 μm, was used with helium as the carrier gas. The injection port temperature was 220°C and the detector temperature 300°C. The column temperature programme was 1 min at 100°C, increased at 30°C/min to 150°C, held for 2 min, then increased at 3°C/min to 205°C and at 10°C/min to 260°C, held at 260°C for 25 min. A 1-μl volume of sample was injected with the autosampler using hot splitless injection with the split closed for 1 min.

Sample preparation

A 1-l water sample was spiked with a mixture of pesticides to achieve a concentration of 100 ng/l per substance. The internal standard, 2,4-dichlorobenzoic acid, was added at the same concentration level. The sample was then acidified to pH 1.5 with HCl. Each SPE cartridge was filled with 1 g of RP-18 adsorbent. Conditioning was performed successively with 5 ml of acetone, 5 ml of methanol, 10 ml of distilled, deionized water and finally 5 ml of water acidified to pH 1.5. The solvents were drawn through the cartridges by means of a gentle vacuum and the cartridge was not permitted to run dry after

addition of the acidified water. The water sample spiked with the herbicides was then percolated through the cartridge at a flow-rate of *ca.* 8 ml/min. After drying the cartridge for 2–3 h under a gentle stream of nitrogen, the herbicides were eluted with 2 ml of methanol. The eluate was dried under a gentle stream of nitrogen.

Hydrolysis of acidic esters

As the pesticides diclofop, fenoxaprop and quizalofop are not available as free acids, they were prepared by alkaline hydrolysis. The ester was dissolved in a solution of ethanol (10 ml) and aqueous potassium hydroxide (40%, 30 ml) and the mixture was heated under reflux for 5 h. After cooling, the pH was adjusted to 2 with hydrochloric acid. The pesticide was then extracted with three portions of ethyl acetate (40 ml) in a separating funnel. The combined extracts were dried over anhydrous sodium sulphate and decanted into a 250-ml round-bottomed flask. The solvent was removed under vacuum with a rotary evaporator and the residue treated as described below.

Derivatization

The dry sample extract was placed in a reaction tube and dissolved in 100 μl of reaction mixture A followed by 7 μl of ethyl chloroformate to obtain the corresponding ethyl esters. To obtain the methyl esters, the dry sample eluate was dissolved in 100 μl of reaction mixture B followed by 7 μl of methyl chloroformate. To obtain the butyl esters, the dry sample eluate was dissolved in 100 μl of reaction mixture C followed by 7 μl of butyl chloroformate. The reaction tube was shaken gently for about 5 s, preferably against a pad, to initiate the gas evolution that can usually be observed. This is caused by decomposition of the reagent to alcohol and carbon dioxide. The derivatives were dried with a gentle stream of nitrogen and finally dissolved in 100 μl of toluene, from which an aliquot of 1 μl was injected into the GC–ECD or GC–MS system.

RESULTS AND DISCUSSION

The known simplicity of derivatizing fatty acids, hydroxy acids and amino acids in the field of clinical chemistry, as demonstrated by the work of Husek and co-workers [15–17], encouraged us to investigate whether chloroformates would also be suitable for the esterification of the carboxyl function of chlorophenoxy acid herbicides and similar acidic herbicides. Our attention was attracted by the possibility of producing parallel series of alkyl esters with the same sample extract. This would enable the analyst to make a first check for the presence of one of these pesticides by comparing the parallel chromatograms with the corresponding retention time tables. A second obvious advantage of parallel derivatization is that if the usually employed methyl ester of one of these pesticides is overlapped by a matrix compound it is not likely that the same situation would occur with its ethyl or butyl ester. The pesticides under investigation are shown in Fig. 1.

The reaction scheme is described by the following equation:

$$\text{R–COOH} + \text{Cl–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–O–R}' \xrightarrow[-\text{HCl}]{} \text{R–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–O–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–O–R}' \xrightarrow[-\text{CO}_2]{} \text{R–}\overset{\|}{\text{C}}\text{–O–R}'$$

$R' = CH_3,\ CH_2CH_3,\ (CH_2)_3CH_3$

To find the optimum conditions for the derivatization procedures, pyridine, triethylamine and sodium carbonate were tested as catalysts. Only with pyridine was an instantaneous evolution of carbon dioxide gas observed and a single product (methyl, ethyl or butyl ester) was found to be formed. These findings confirm the observations of Husek *et al.* [15], who studied extensively the reaction conditions to form methyl, ethyl and 2-chloroethyl esters with fatty acids. Using triethylamine or sodium carbonate, the equivalent ester was also formed but together with some by-products. The compositions of reaction mixtures A, B and C had also to be varied in order to find optimum derivatization conditions.

This detailed investigation resulted in the procedure described above. The surprising finding was that with a constant alcohol-to-pyridine ratio of 2:1 the optimum proportions of acetonitrile and water varied with the different chloroformates as reported. Our optimum for the formation of methyl esters did not exactly confirm the reaction procedure proposed

Fluroxypyr
Fenoprop
2,4-DB
MCPB
MCPA
2,4,5-T
Flurenol
Picloram
2,4-D
Triclopyr
Mecoprop
Dichlorprop
Acifluorfen
Chlorflurenol
Clopyralid
Fluazifop
Quizalofop
Haloxyfop
Diclofop
Benazolin
Flamprop
Fenoxaprop

Fig. 1. Structures of pesticides investigated.

by Husek *et al.* [15]. It must be noted, however, that our acids are of a different nature to the aliphatic fatty acids that they investigated. All the pesticides shown in Fig. 1 were derivatized to obtain methyl, ethyl and butyl esters. With all the pesticides the esters were produced in good yields.

Chromatograms of a mixture of pesticides obtained with the GC–ECD system are shown in Fig. 2. All the pesticides of the mixture can be readily recognized. Each group of esters exhibits the same sequence pattern. Although the sequence of the alkyl esters is the same with all alkyl moieties, different response ratios were observed. This is particular evident with the later eluting peaks 9–13. The variation of response factors depends on the structures of the individual pesticides (Fig. 1), but an influence by the alkyl ester group should not be expected. The varying peak sizes rather reflect the reproducibility of the derivatization of the compounds at the 100-ng level. With methyl esters, the first-eluting peak, the internal standard 2,4-dichlorobenzoic acid, is always found to be very small, indicating losses due to volatility. This is to be expected, because 2,4-dichlorobenzoic acid is only used to check the extraction and the derivatization procedure and is not used for any final quantification purposes.

It should be noted that the group of active substances contains herbicides with hydroxyl groups adjacent to the carboxyl group. These two particular herbicides were also found to be completely esterified. Under the conditions described under Experimental, the carboxyl group was found to form the corresponding alkyl ester as for all other acids. The hydroxyl group, however, was esterified to form the alkyl carboxy ester, as indicated by the mass spectral investigation.

Fig. 3 shows the GC–MS traces for flurenol methyl, ethyl and butyl ester. The reactions resulted in two peaks with all three esters. The mass spectra for peaks A and B in Fig. 3 indicate which products were formed during esterification. Peak A represents the esterified carboxylic function, which leads to a molecular ion at m/z 240 for the flurenol methyl ester, at m/z 254 for the ethyl ester and at m/z 282 for the butyl ester. Peak B in Fig. 3 represents flurenol esters in which the hydroxyl function is also esterified. The increase in the molecular ions at m/z 298 for the methyl, m/z 326 for the ethyl and m/z 382 for the butyl ester clearly indicates the formation of the corresponding alkyl carboxy esters. All the spectra show the molecular ion well displayed and the interpretation of the fragmentation pattern is convincing.

To demonstrate the applicability of the method in pesticide residue analysis, tap and ground water samples were spiked with acidic herbicides at a level of 100 ng/l and analysed as described. Screening analyses were carried out by capillary GC–ECD. The chromatograms of the extract of such a spiked ground water sample are shown in Fig. 4. All the pesticides, including the internal standard 2,4-dichlorobenzoic acid, can be identified.

In Table I, the retention times and recoveries of the three alkyl esters of all the pesticides tested are given. These analyses were carried out with the GC systems permanently used in our laboratory for pesticide multi-residue determinations in water and food samples with aldrin as internal standard. The carrier gas flow-rate in the GC–ECD system is always adjusted to give aldrin at 25.00 min. Therefore, the retention times reported in Table I fit in this overall frame. The retention times of all 22 pesticides were found to be very stable for the methyl, ethyl and butyl esters, matching our experience with most of the pesticides determined with the multi-residue method.

The recoveries were better than 75% for most of the pesticides investigated. However, that of clopyralid acid was only 51%, MCPA 63% and picloram 69%, which we do not consider to be satisfactory. Therefore, the recovery study was repeated with increased amounts of 1.5 and 2.0 g of adsorbent, but no better recoveries were obtained. This is the reason for recommending 1 g of RP-18 adsorbent for SPE. The relative standard deviation, however, was found to be acceptable for all pesticides at the low residue levels in ground water samples. It should be emphasized that all 22 herbicides could be easily determined with the method described at the low residue concentration levels.

The retention time differences of *ca.* 1.5 min observed between the methyl and ethyl esters, *ca.* 4.5 min between the methyl and butyl esters and *ca.* 3 min between the ethyl and butyl esters can be used to confirm the presence of a supposed pesticide. The sample extract is simply split and the aliquots derivatized to produce two or even all three of the alkyl esters.

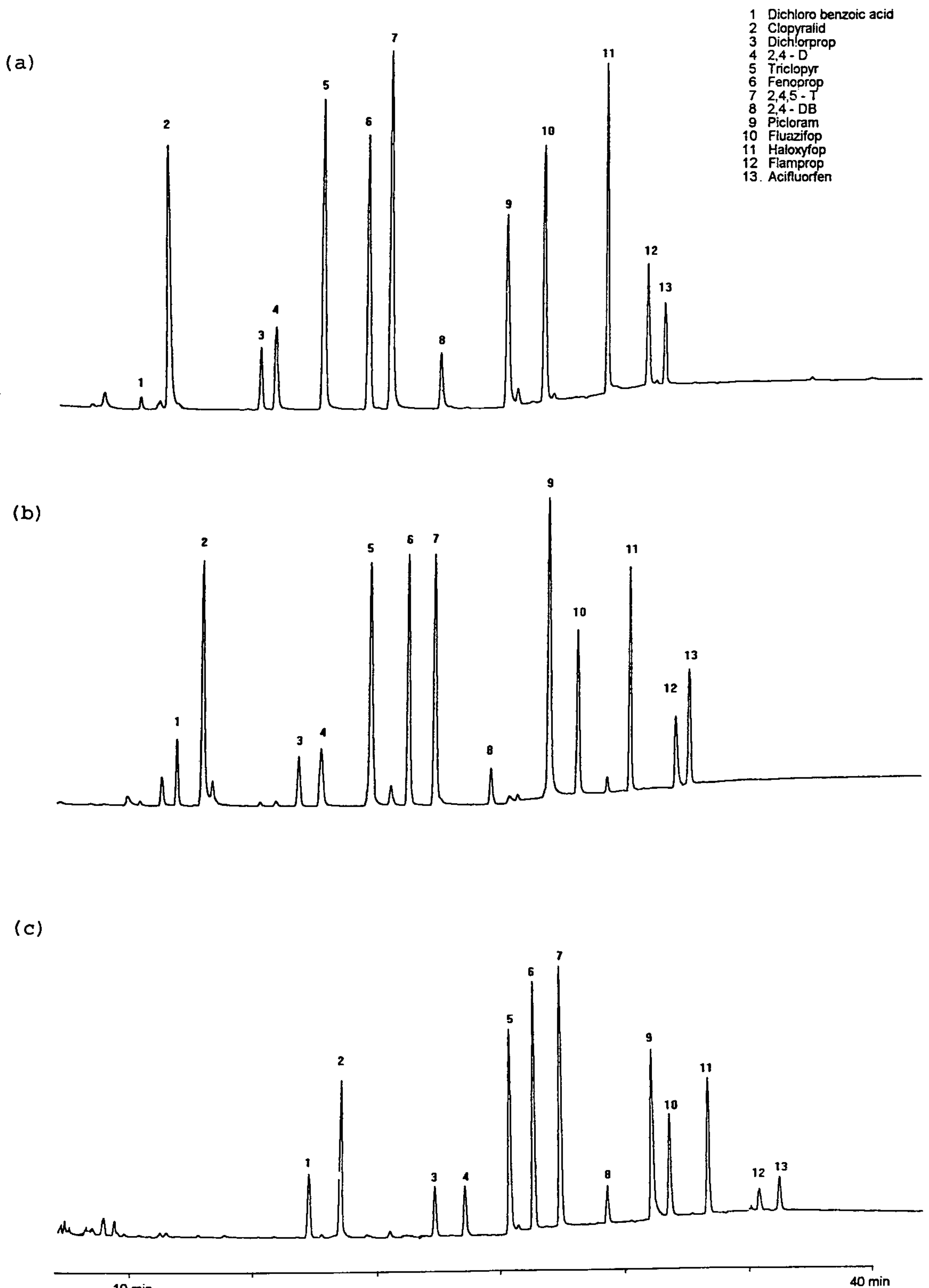

Fig. 2. Gas chromatograms of a standard mixture of thirteen acidic herbicides after derivatization to the (a) methyl, (b) ethyl and (c) butyl esters. Amount injected into the GC–ECD system = 1 ng. Peaks: 1 = dichlorobenzoic acid; 2 = clopyralid; 3 = dichlorprop; 4 = 2,4-D; 5 = triclopyr; 6 = fenoprop; 7 = 2,4,5-T; 8 = 2,4-DB; 9 = picloram; 10 = fluazifop; 11 = haloxyfop; 12 = flamprop; 13 = acifluorfen.

To demonstrate the practical use of this method, ground water samples were spiked each time at 100 ng/l with only one pesticide unknown to the analyst. An example chromatogram of a methylated extract is shown in Fig. 5a and that of the corresponding ethylated extract in Fig. 5b. According to the retention time table, the marked peak at 17.13 min was found to be triclopyr methyl ester. Ethylation of the second half of the sample extract showed that the peak of interest was shifted to the expected retention time of 19.02 min. The pesticide in the sample was identified as triclopyr, which could easily be confirmed by GC–MS. Fig. 5a and b indicate that most of the matrix compounds seen in

(a) Flurenol after methylation

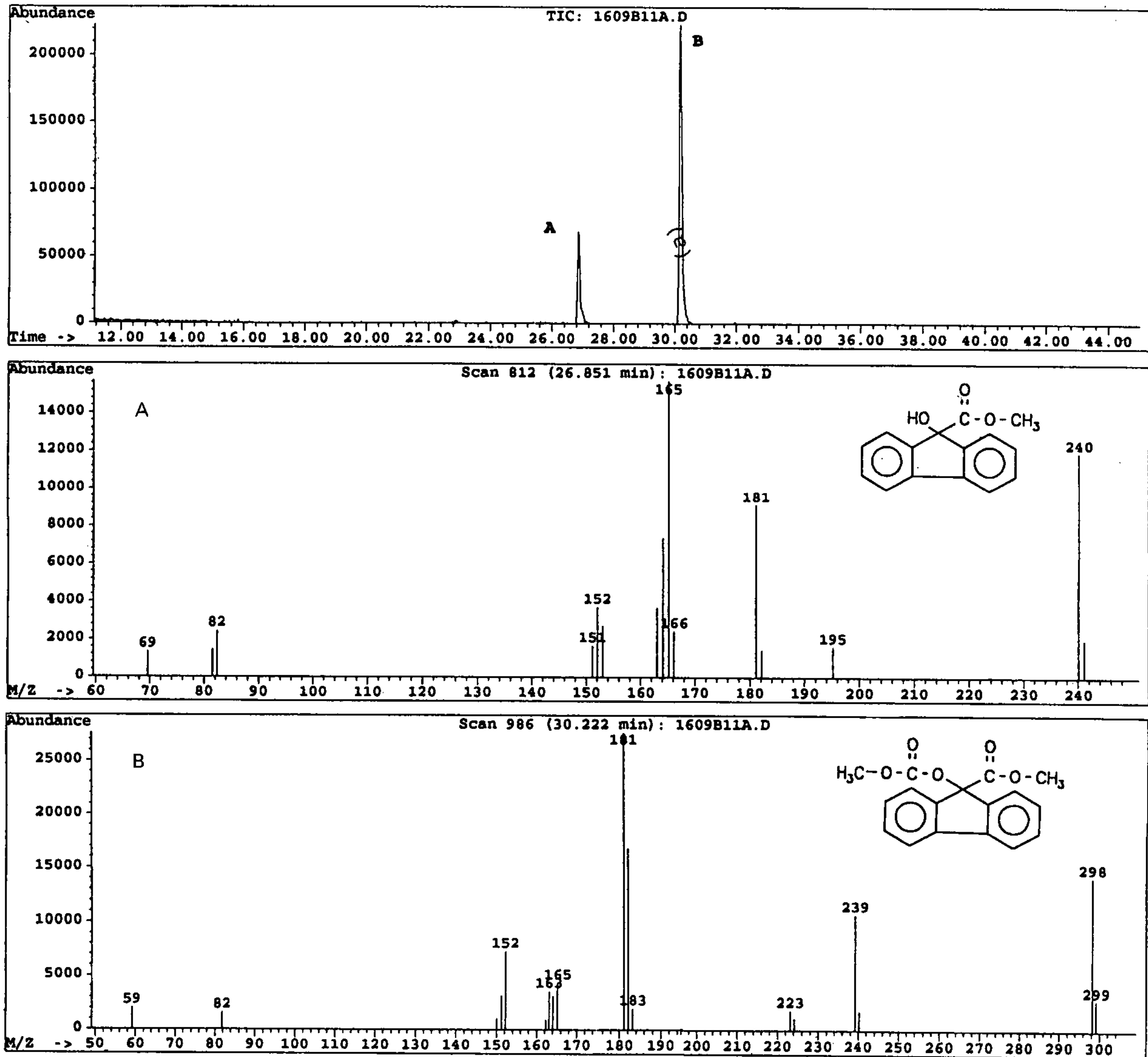

Fig. 3.

(Continued on p. 234)

the chromatograms obtained with ECD were not shifted when two different esterifications were applied, an indication that those peaks do not represent esters formed during derivatization. This is the reason why, in the samples treated as described, the acidic herbicides could always be easily seen.

To summarize, it can be stated that the determination of trace levels of 22 acidic herbicides can be achieved using these simple derivatization procedures. The obvious advantage of the use of chloroformates is the ease wiht which a tentative confirmation can be carried out by simply esterifying two aliquots of the extract with two different chloroformates. The described confirmation, however,

(b) Flurenol after ethylation

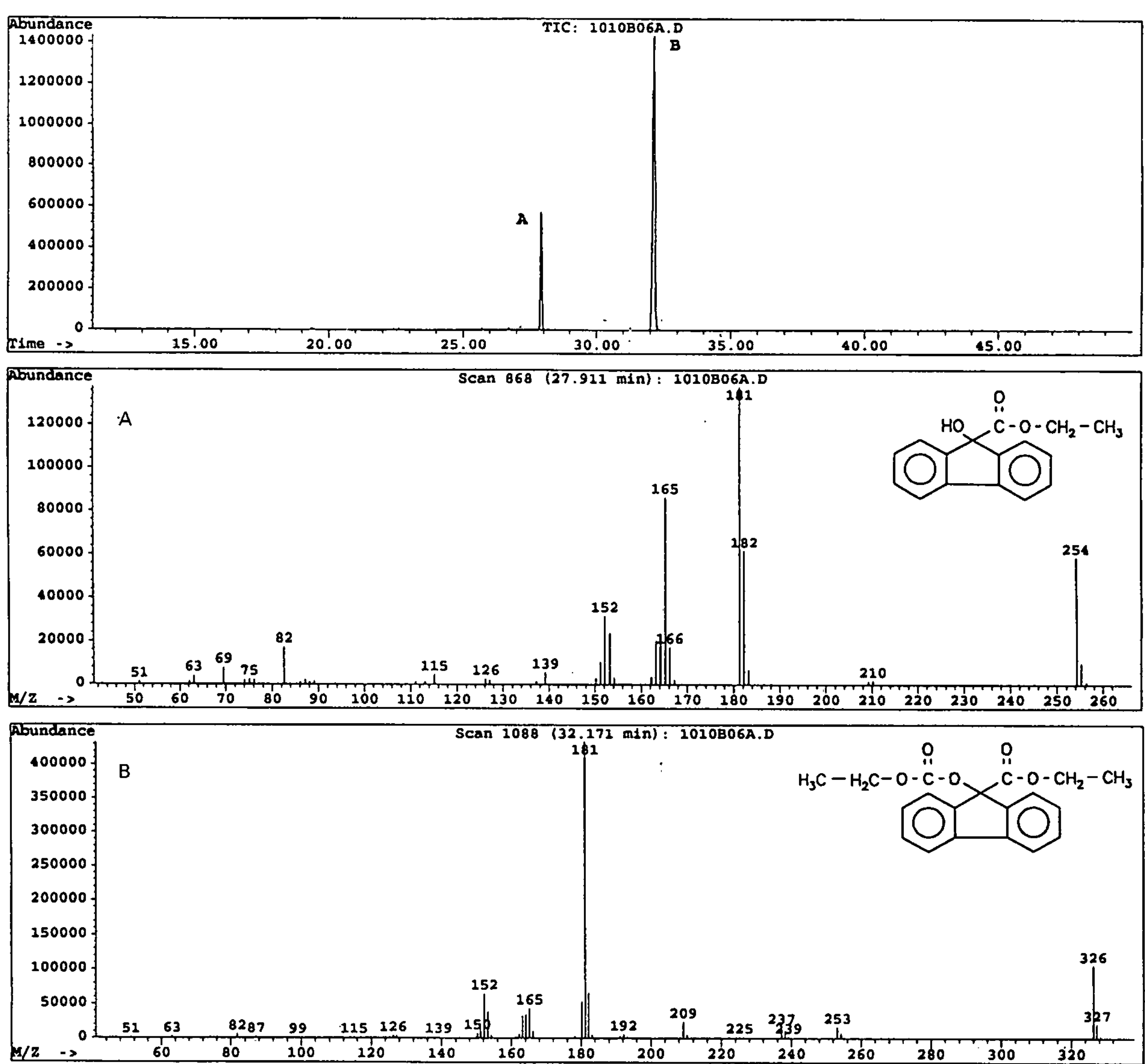

Fig. 3.

proves only that a peak shifting in the way described is produced by a substance that can be derivatized with chloroformates, in the first aliquot to the methylated and in the second to the ethylated or butylated derivative, for example. An unequivocal confirmation, however, needs the use of GC–MS.

CONCLUSIONS

With the simple derivatization procedure described, 22 acidic herbicides can be converted into the corresponding methyl, ethyl or butyl esters, which are then amenable to capillary GC. The

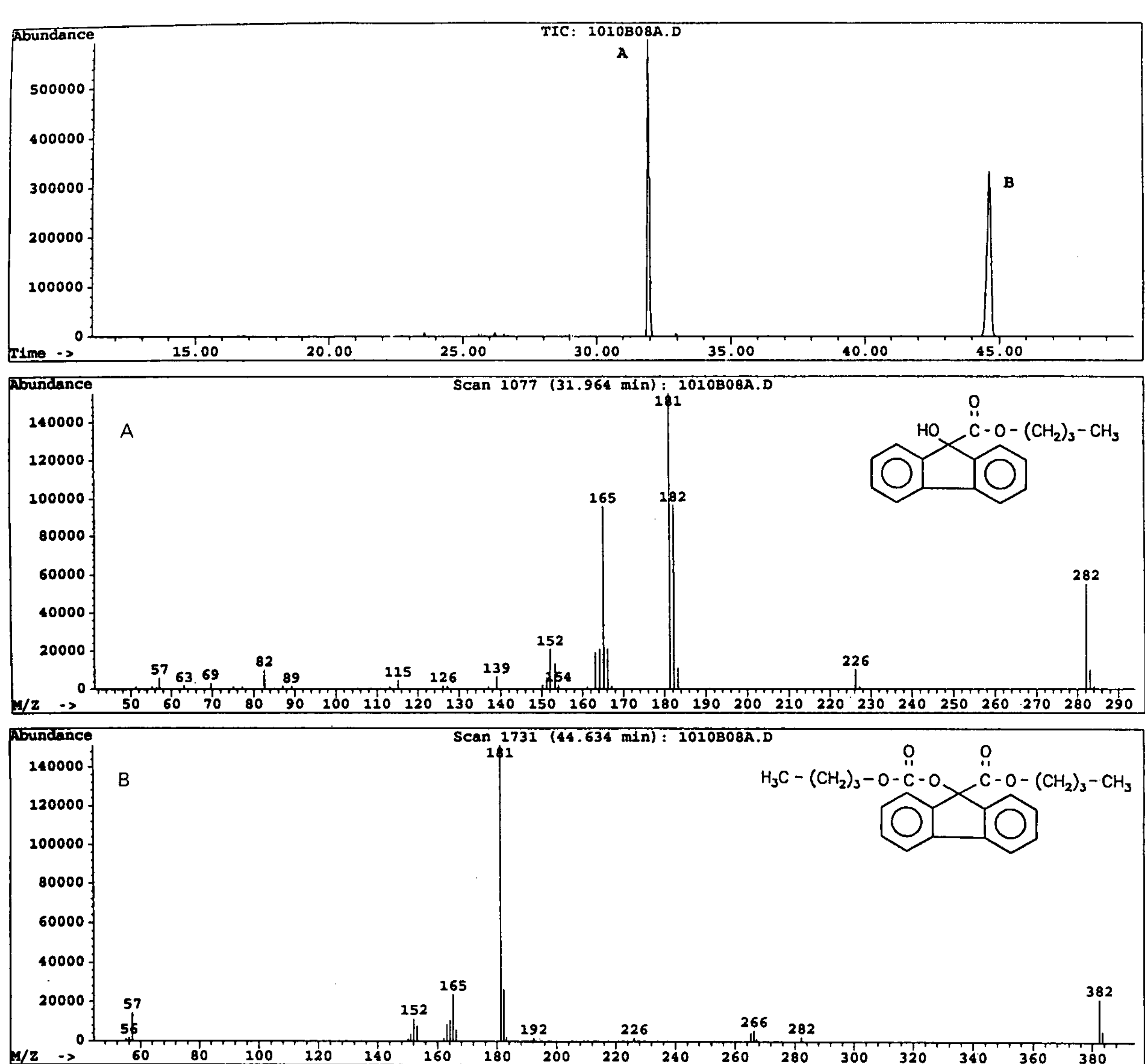

Fig. 3. TIC and mass spectra of flurenol after (a) methylation, (b) ethylation and (c) butylation.

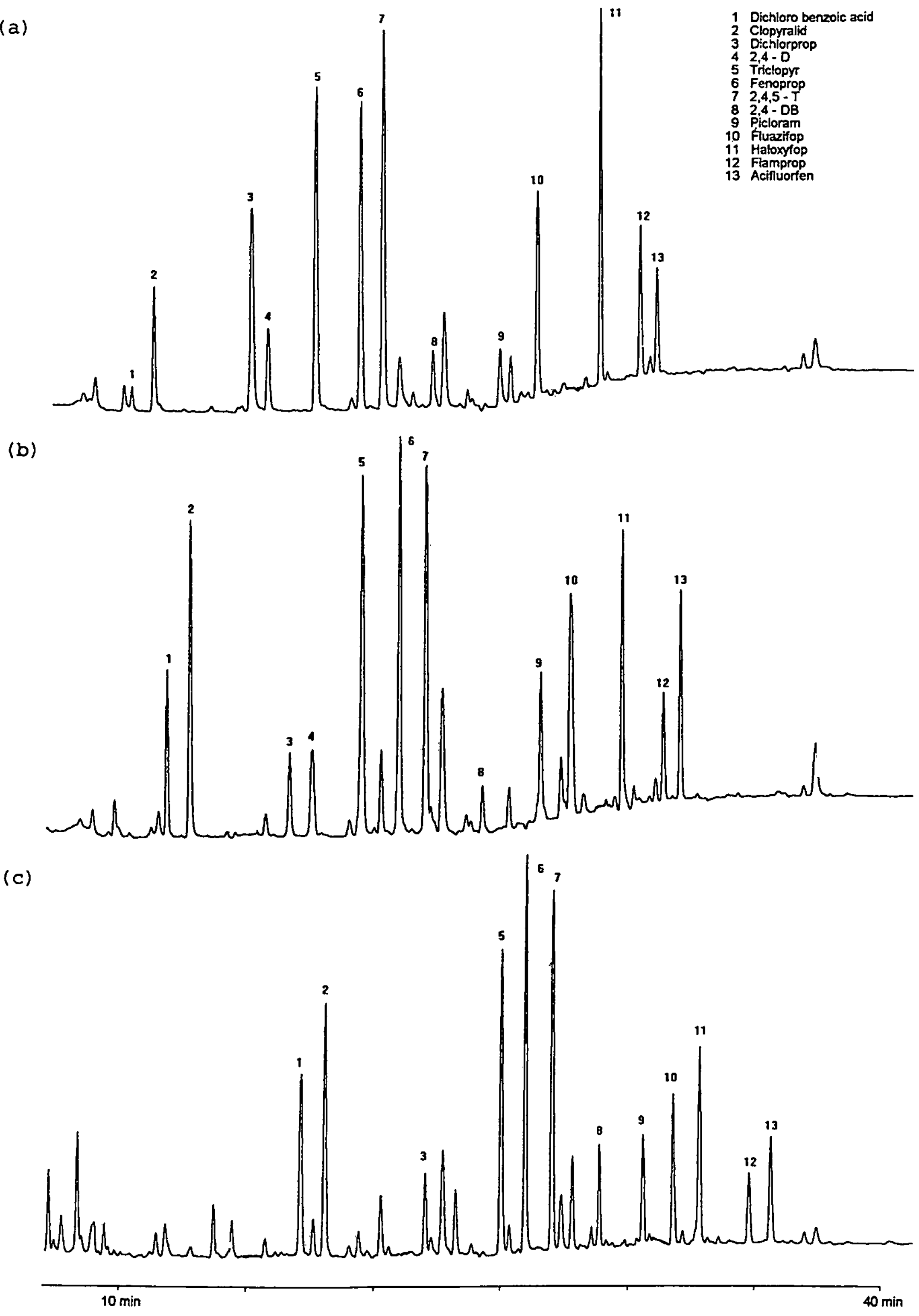

Fig. 4. Gas chromatogram of a ground water sample spiked with 100 ng/l of a mixture of thirteen acidic herbicides derivatized to their (a) methyl, (b) ethyl and (c) butyl esters. Detection: ECD. Peaks as in Fig. 2.

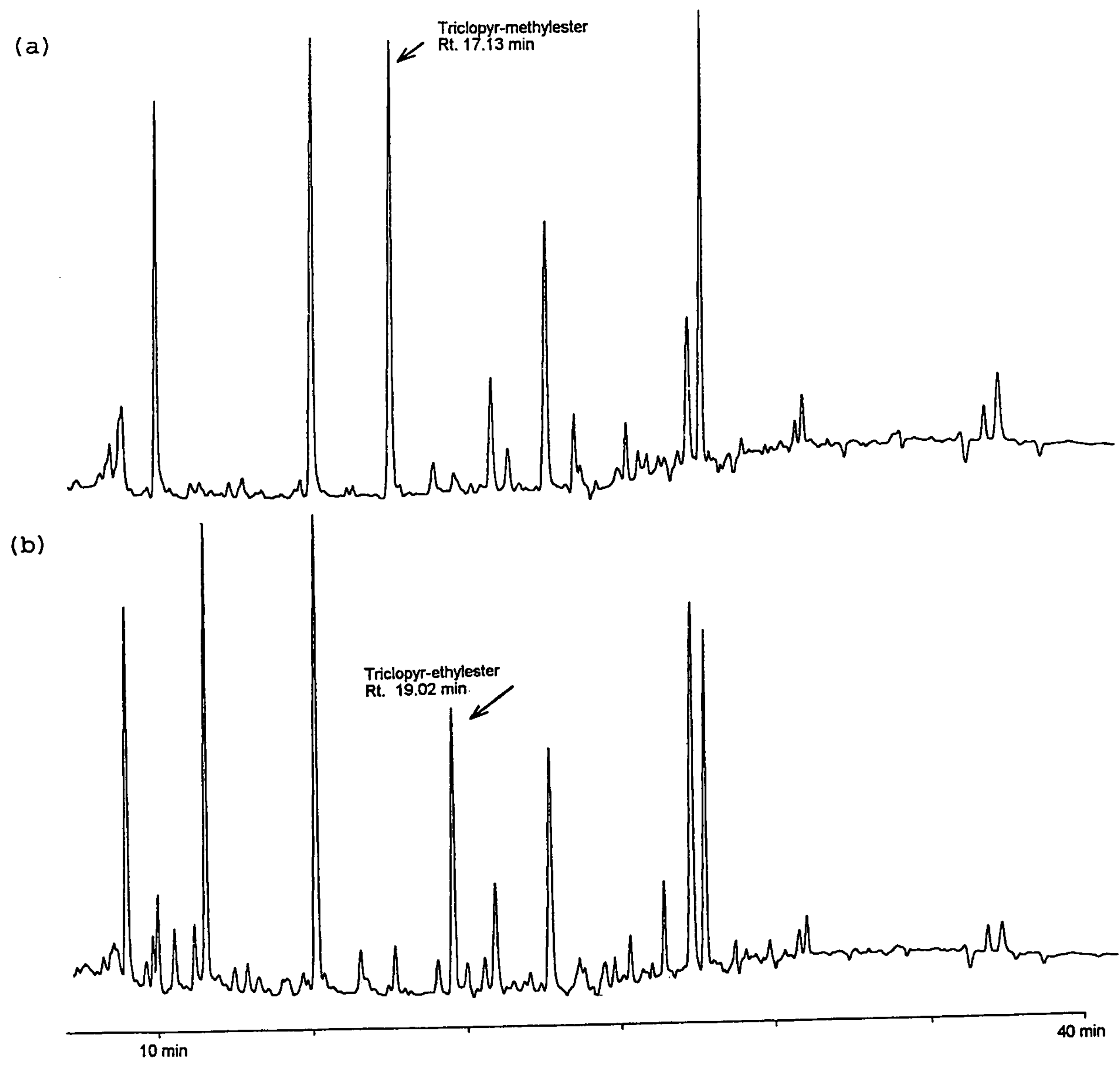

Fig. 5. Gas chromatogram of a ground water sample after derivatization to the (a) methyl and (b) ethyl esters. Detection: ECD.

derivatives are highly specific for their parent compounds. The preparation of a set of ten samples for GC takes about 10 min and requires only small amounts of inexpensive reagents. Almost without additional laboratory work a second set of derivatives can be prepared, allowing a tentative confirmation of suspected pesticide residues. Therefore, we consider that this derivatization method is unrivalled in ease, speed and flexibility for the preparation of alkyl esters.

TABLE I

RETENTION TIMES AND RECOVERIES OF THE ALKYL ESTERS OF 22 ACIDIC HERBICIDES

GC–ECD, 50 m × 0.20 mm I.D. SE-54 column, conditions as described under Experimental. All retention times were measured with the GC system adjusted to give aldrin with a retention time of 25.00 min (see text).

Pesticide	Retention time (min)			Recovery (%)	R.S.D. (%) ($n = 10$)
	Methyl ester	Ethyl ester	Butyl ester		
2,4-Dichlorobenzoic acid (ISTD)	10.15	11.25	16.67	80	4.5
Clopyralid acid	10.98	12.13	17.58	51	6.3
Mecoprop	12.93	14.08	18.50	82	5.9
MCPA	13.35	14.75	19.27	63	7.5
Dichlorprop	14.75	15.85	20.35	98	5.1
2,4-D	15.33	16.73	21.40	83	6.8
Triclopyr	17.13	19.02	22.55	93	7.2
Fenoprop	18.85	19.98	24.28	98	3.9
MCPB	19.53	20.03	23.98	82	5.6
2,4,5-T	19.72	21.15	25.18	88	6.2
Fluroxypyr	21.55	23.18	27.35	77	8.1
2,4-DB	21.63	23.45	27.42	87	6.4
Picloram acid	24.20	25.93	27.98	69	7.1
Benazolin	25.56	26.80	30.35	82	5.7
Fluazifop	25.62	26.92	29.68	83	6.1
Haloxyfop	27.98	28.88	31.82	79	5.4
Flurenol	28.33	29.21	33.30	82	6.3
Chlorflurenol	28.43	29.35	33.39	80	7.5
Flamprop	29.53	30.52	33.73	91	6.5
Acifluorfen	30.53	31.45	34.55	78	5.9
Diclofop	32.77	33.80	36.90	81	6.3
Fenoxaprop	37.53	38.68	41.76	78	5.9
Quizalofop	43.53	44.63	47.68	89	6.1

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Gas chromatographic determination of halogenated organic compounds in water and sediment in the Skagerrak

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ABSTRACT

Biogenic and anthropogenic volatile halocarbons and chlorinated phenolic compounds were monitored in the Skagerrak. Measurements were made both in water and in sediment by means of gas chromatography with electron-capture detection. The results show that the load of biogenic halocarbons exceeds that of the anthropogenic halocarbons and that the iodinated compounds show a more marked seasonal trend than the brominated compounds. Additionally, it was established that waters of different origins have exclusive sets of biogenic halocarbons. Pentachlorophenol is widely distributed throughout the Skagerrak. It is transported in its dissociated form. The concentration level of other chlorinated phenolics is less than 1 ng/l. However, it could be demonstrated that there is an accumulation of chlorinated phenolics in sediments in the deeper parts of the Skagerrak.

INTRODUCTION

Investigations of the distribution of halogenated organic compounds in a relatively large area in the marine environment are still rare, mainly owing to the difficulty of analysing large number of samples with low concentrations of analytes under complex hydrological conditions. In distribution studies the analytical procedure should ideally be simple, require small amounts of samples and be able to determine as many components as possible. Preconcentration is always necessary, as the concentrations are low and derivatization prior to analysis is needed for some compounds. Gas chromatography with electron-capture detection, with its high separation efficiency, speed of analysis and low detection limits, is by far the most commonly used technique for halogenated substances. In the coastal waters of Sweden and in the North Sea, only a few investigations have been made of individual compounds in the water phase [1–5]. However, for sediments several investigations have been made [2,6,7,8].

Marine organisms have the capability to form halogenated organic compounds. Hence, the halogenated organic compounds in sea water have both a biogenic and an anthropogenic origin. Of the naturally produced compounds, the volatile compounds are the most commonly investigated [9,10]. However, many substances are both biogenic and anthropogenic. For example, substances usually associated with the chlorination of water such as chloroform, bromoform and dibromomethane are also formed naturally [11]. In this investigation we focused on two groups of environmentally interesting compounds, volatile halocarbons and chlorinated phenolic compounds. The analytical techniques available permit the determination of these compounds in a vast number of samples. Consequently, we can study the dispersion of these compounds in sea water.

The volatile halocarbons are known to influence the atmospheric ozone. Several investigations have

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dealt with the distribution of halocarbons in sea water and the atmosphere, mainly the chlorofluorohydrocarbons (CFCs) and tetrachloromethane [12–14]. The naturally produced volatile halocarbons consist of chlorinated, brominated and iodinated compounds. The formation mechanism is still elusive, but there are indications that both macroalgae and planktonic organisms have the ability to produce such compounds. Earlier investigations have mainly dealt with brominated compounds [15,16,]. It has been shown that the flux of naturally produced brominated compounds from sea water was the main source of organobromine in the atmosphere [17]. Of the iodinated compounds, methyl iodide was regarded as the main compound governing the global iodine budget [18,19]. Recently, it has been shown that the diversity of iodinated halocarbons is larger than expected and that several iodinated compounds should be considered [20].

Chlorophenols have been used as flea repellents, fungicides, wood preservatives, mould inhibitors, etc. In 1980, about 200 000 tons of chlorophenols were manufactured annually worldwide [21]. For instance, Finnish saw mills, wood processing and textile industries used approximately 1300 tons of chlorophenols, mainly penta-, tetra- and trichlorophenols, against rot and blue stain [22]. Phenolics are also formed in the bleaching process of pulp. Pulp mills discharge a considerable volume of effluents (*ca.* 200 m^3 per metric ton of pulp) and the concentrations of chlorophenolics can vary between 0.1 and 300 μg/l of effluent water [2,23,24]. The atmospheric input, *e.g.*, from waste incineration, has not been estimated. Studies of the distribution of chlorinated phenolics in sea water have been performed in coastal waters by Xie *et al.* [2], Paasivirta *et al.* [21], Folke and Birklund [25] and Abrahamsson and Klick [4].

The aim of this investigation was to determine the amounts of chlorinated phenolics and biogenic halocarbons released in the coastal waters of Sweden and in the Skagerrak. In accordance with this aim, water and sediment samples were collected from August 1989 to April 1991.

EXPERIMENTAL

Chemicals

The chlorinated phenolics used were 2,4-dichlorophenol (DCP) (AB Reagents, Göteborg, Sweden), 2,6-dichlorophenol (2,6-DCP) (Nova Kemi, Göteborg, Sweden), 2,4,6-trichlorophenol (2,4,6-TCP) (Merck, Hohenbrunn, Germany), 3,4,5-trichlorophenol (3,4,5-TCP) (EGA-Chemie, Steinheim, Germany), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) (Aldrich, Gillingham, UK), 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP) (EGA-Chemie), pentachlorophenol (PCP) (Fluka, Buchs, Switzerland) and 2,6-dibromophenol (Kodak, Rochester, NY, USA). 3,4,5-Trichlorocatechol (TCC), tetrachlorocatechol (TeCC), 4,5,6-trichloroguaiacol (TCG) and tetrachloroguaiacol (TeCG) were kindly provided by Professor Wachtmeister, University of Stockholm, Sweden. Stock standard solutions were prepared in acetone (Merck, Darmstadt, Germany) and stored in a refrigerator.

The volatile halogenated hydrocarbons investigated were chloroform (Merck), 1,1,1-trichloroethane (Fluka), 1-iodopropane (Fluka), 2-iodopropane (Fluka), trichloroethene (Mallinckrodt, St. Louis, MO, USA), dibromomethane (Merck), bromodichloromethane (Fluka), chloroiodomethane (Fluka), 2-iodobutane (Fluka), dibromochloromethane (Fluka), tetrachloroethene (Merck), 1-iodobutane (Fluka), bromoform (Merck), diiodomethane (Fluka) and bromotrichloromethane (Fluka). Stock standard solutions were prepared in acetone (Merck) and stored at −18°C.

Sampling and description of sampling area

The Skagerrak is a fairly deep part of the North Sea with an average depth of 200 m. The water in the Skagerrak has its origin mainly in the north Atlantic, and enters through the northern North Sea. Along the Danish west coast the water entering the Skagerrak has its origin in the southern North Sea. The out-flowing surface water from the Kattegat influences only a minor part of the Skagerrak along the Swedish and Norwegian coast. The salinity in the Skagerrak is predominantly oceanic, except for the surface water along the Swedish west coast and the Norwegian south coast. The average current is a strong cyclonal circulation [26].

Water samples were collected along three transects on the Danish west coast (Table I) on three occasions: September 1990, November 1990 and April 1991. On each occasion each transect was sampled twice, except for September, where one of

TABLE I

DESCRIPTION OF TRANSECTS

Transect	Position
Hirtshals	N57°30′, E9°56′ to N58°01′, E9°36′
Hanstholm	N57°10′, E8°34′ to N57°41′, E8°12′
Tyborøn	N56°43′, E8°06′ to N56°43′, E6°38′

the transects was sampled just once. Along each transect seven stations were sampled and water was collected from different depths, giving a total of 30 water samples per transect. Salinity, temperature, oxygen, nutrients and chlorophyll were monitored throughout the investigation. Sediment samples were taken on two occasions along the Swedish west coast and in the deeper part of Skagerrak. The samples that were taken with a box corer were provided by Dr. B. Dennergård, Department of Marine Geology, University of Göteborg. A sediment core was also collected with a multiple corer [27] at depth 300 m, position N58°15′,E10°30′, and was provided by Dr. K. Nordberg, Department of Geology, University of Göteborg.

The sea water was collected from Niskin water sample bottles and stored in brown glass bottles (100 ml). The determination of the chlorophenols, chlorocatechols and chloroguaiacols were made shortly after the samples arrived at the department. Determination of the volatile hydrocarbons was made on board the ship less than 24 h after the samples were collected.

Analytical procedures

The chlorinated phenolics were determined as acetylated derivatives according to Abrahamsson and Xie [28] and Xie [29]. The determination of the volatile halocarbons were performed according to Abrahamsson and Klick [30].

The derivatization and liquid–liquid extraction procedures were performed directly in the sampling bottles. The phenolic compounds were extracted by adding 1 ml of hexane (Merck) containing 2,6-dibromophenol as internal standard (I.S.). The bottles were shaken for 3 min and the hexane phase (1 μl) was then used for gas chromatographic analysis. The volatile compounds were extracted by adding 1 ml of distilled pentane, containing bromotrichloromethane as internal standard, and shaking for 5 min. The extract was then injected on to the gas chromatographic column.

The chlorophenols, catechols and guaiacols were determined with a Carlo Erba HRGC 5300 gas chromatograph equipped with a nickel-63 electron-capture detector and on-column injector. The compounds were separated on a 30 m × 0.32 mm I.D. DB-1 fused-silica column (J & W Scientific). The GC conditions were as follows: hydrogen carrier gas flow-rate, 2 ml/min; nitrogen make-up gas flow-rate, 30 ml/min; injector and detector temperatures, 275°C, temperature programme, 100–230°C at 15°C/min, 230–260°C at 30°C/min.

The volatile halocarbons were determined with a Carlo Erba 4160 gas chromatograph equipped with a nickel-63 electron detector (275°C). The injector was an automatically driven Valco liquid chromatograpic valve with a 15-μl loop. The compounds were separated on two fused-silica columns connected with a capillary glass connector. The precolumn was a 30 m × 0.32 mm I.D. DB-1701 (J & W Scientific) column with a film thickness of 0.1 μm. The separation column was a 30 m × 0.32 mm I.D. DB-5 (J & W Scientific) column with a film thickness of 1 μm. The hydrogen carrier gas flow-rate was 2 ml/min and the nitrogen make-up gas flow-rate was 30 ml/min. The oven temperature was held at 35°C for 2 min and then increased to 120°C at 10°C/min.

The chromatographic peaks were integrated using a Jones Model JCL 6000 chromatographic system.

RESULTS AND DISCUSSION

Volatile halocarbons

Even though biogenic halocarbons have been a focus of attention for several years, there are still a number of questions that have to be answered. The development of the liquid–liquid extraction method [30] enabled us to determine fifteen anthropogenic and biogenic volatile halocarbons. The main improvement was the combination of two separation columns of different polarity, whereby we could separate dibromomethane, bromodichloromethane and trichloroethene, which had previously been impossible. A chromatogram of a water sample is shown in Fig. 1. The low detection limits (below the

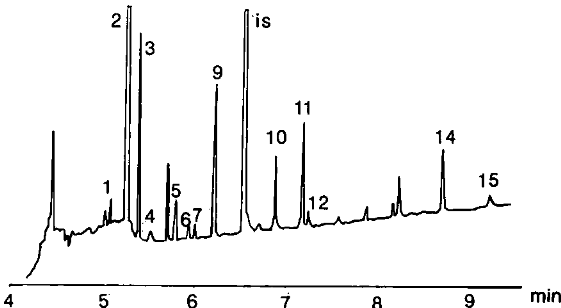

Fig. 1. Chromatogram for the volatile halocarbons. Transect Hirtshals, station 4 at 2 m depth, April 18th, 1991. 1 = 1.9; 2 = 41; 3 = 0.67; 4 = 1.2; 5 = 1.2; 6 = 0.46; 7 = 0.09; 9 = 1.9; 10 = 3.4; 11 = 0.14; 12 = 0.64; 14 = 3.6; 15 = 2.4 ng/l.

nanograms per litre level) and the precision of the method (Table II) make it suitable for distribution studies.

We have recently shown that biogenic volatile halocarbons are very diverse [20], and that both macroalgae and microorganisms are responsible for their formation. In order to establish the levels of anthropogenic and biogenic compounds in the Skagerrak, sampling was performed at several sampling sites over several years.

The biogenic compounds constitute the largest fraction of volatile halocarbons in the Skagerrak. Table III shows the mean values for both biogenic and anthropogenic halocarbons in surface waters (<6 m). The biogenic compounds are 1-iodopropane, 2-iodopropane, chloroiodomethane, 1-iodobutane, 2-iodobutane, diiodomethane, dibromomethane and bromoform. The anthropogenic compounds are tetrachloromethane, trichloroethene and tetrachloroethene. As can be seen from the chromatogram in Fig. 1, there were contamination problems from the ship, which interfered with the determination of chloroform and 1,1,1-trichloroethane. They are therefore excluded from Table III. Interestingly, the mean values for the biogenic halocarbons exceed the values for the anthropogenic compounds. The concentrations of chloroform and 1,1,1-trichloroethane are usually low (1–4 ng/l) in the Skagerrak.

TABLE II

PRECISION AND DETECTION LIMITS FOR HALOCARBONS ACCORDING TO ABRAHAMSSON AND KLICK [29]

No.	Compound	Precision (%)	Detection limit (ng/l)
1	$CHCl_3$	9	2
2	CH_3CCl_3	8	0.2
3	CCl_4	10	0.05
4	CH_3CHICH_3	9	0.7
5	$CHClCCl_2$	8	0.4
6	CH_2Br_2	10	0.3
7	$CHBrCl_2$	10	0.2
8	$CH_3CH_2CH_2I$	7	0.2
9	CH_2ClI	3	0.03
10	$CH_3CHICH_2CH_3$	11	0.3
11	$CHBr_2Cl$	7	0.2
12	CCl_2CCl_2	9	0.07
13	$CH_3CH_2CH_2CH_2I$	12	0.4
14	$CHBr_3$	5	0.2
15	CH_2I_2	7	0.4

TABLE III

MEAN SURFACE WATER CONCENTRATIONS OF BIOGENIC AND ANTHROPOGENIC HALOCARBONS ALONG THREE TRANSECTS IN THE SKAGERRAK

The values are given in ng/l. Biogenic compounds: 1-iodopropane, 2-iodopropane, chloroiodomethane, 1-iodobutane, 2-iodobutane, diiodomethane, dibromomethane and bromoform. Anthropogenic compounds: tetrachloromethane, trichloroethene and tetrachloroethene.

Transect[a]	September		November		April	
	Anthropogenic	Biogenic	Anthropogenic	Biogenic	Anthropogenic	Biogenic
1	2.6 ± 1.5	5.1 ± 2.1	4.8 ± 1.6	24 ± 14	3.8 ± 2.8	26 ± 14
2	1.7 ± 0.74	7.3 ± 6.6	3.4 ± 1.5	24 ± 42	1.8 ± 0.48	23 ± 5.8
3	2.1 ± 1.0	6.0 ± 2.8	2.9 ± 0.50	14 ± 15	5.8 ± 1.9	13 ± 3.4

[a] Transect 1: September, n = 10; November, n = 12; April, n = 10. Transect 2: September, n = 10; November, n = 10; April, n = 9. Transect 3: September, n = 8; November, n = 13; April, n = 14.

TABLE IV

COMPARISON OF MEAN CONCENTRATIONS (ng/l) OF INDIVIDUAL BIOGENIC COMPOUNDS FOR DIFFERENT SURFACE WATERS

Compound	Skagerrak, August 1989[a]	Skagerrak coastline, April 1990[b]	Skagerrak coastline, May 1990[b]	Skagerrak, September 1990	Skagerrak, November 1990	Skagerrak, April 1991	Antarctic Ocean[c], November 1989	Arctic Ocean[d], August 1991
Dibromomethane	0.43 ± 0.41	0.57 ± 0.23	0.87 ± 0.54	0.21 ± 0.25	0.31 ± 0.17	0.45 ± 0.22	0.30 ± 0.62	1.0 ± 0.6
Bromoform	5.1 ± 1.5	23 ± 16	22 ± 18	4.5 ± 4.2	4.9 ± 5.0	4.3 ± 6.8	2.7 ± 1.2	3.4 ± 1.6
Chloroiodomethane	1.3 ± 0.88	7.1 ± 2.6	7.6 ± 4.0	0.40 ± 0.26	0.90 ± 0.63	3.0 ± 3.0	0.07 ± 0.21	0.12 ± 0.07
2-Iodobutane	n.d.[e]	2.4 ± 0.48	1.6 ± 0.31	0.35 ± 0.21	1.3 ± 0.30	3.8 ± 0.22	0.32 ± 0.61	n.d.
Diiodomethane	5.2 ± 6.3	63 ± 26	.45 ± 46	n.d.	15 ± 25	7.7 ± 8.3	n.d.	n.d.
No. of samples (n)	26	22	20	27	35	35	210	22

[a] Abrahamsson and Klick, unpublished data, centre of the Skagerrak.
[b] Abrahamsson and Klick, unpublished data, N58°,E11°5′.
[c] Klick and Abrahamsson [20].
[d] Abrahamsson, unpublished data, N86°4′,E55°3
[e] Not detected.

There are both seasonal and spatial variations in the amounts of biogenic halocarbons present in the water. Surprisingly, the levels of biogenic compounds did not differ between November and April. It would be easy to predict that the bloom of phytoplankton in April should lead to increased levels of brominated and iodinated substances. However, the chlorophyll content was measured throughout the investigation and no correlation could be found. This finding is not contradictory to the idea that phytoplankton could be involved in the formation of biogenic halocarbons, but it does imply that the amount of biogenic halocarbons is not related to the number of living cells present. One explanation might be differences in degradation rate.

Several individual compounds show seasonal variations. Table IV gives the mean values from different sampling occasions for surface waters in the Skagerrak. In addition, values from the Antarctic and the Arctic are given as references. In principle, the highest values were measured at stations close to the coastline (April and May 1990), which is due to the large macro algae belts. In the open ocean waters of the Skagerrak, the composition of biogenic substances varies with the seasons. This is pronounced for the iodinated compounds, but the same effect cannot be seen for the brominated compounds, for which the concentrations seem to be fairly constant during the year. The values for bromoform correspond well with other reported values in this area [5].

Waters of different origins contained different compositions of biogenic halocarbons. In the Skagerrak, where the mixing is complicated and the residence times are short, this could prove to be a valuable tool for the identification of different water masses. For example, bromoform and chloroiodomethane correlated well. For water with salinity (S) >35 there is a positive correlation (slope $= 0.55$; $r = 0.95$). However, a negative correlation was observed for waters with $33.8 < S < 34.0$ (slope $= -0.01$; $r = 0.90$). Interestingly, the correlation between bromoform and chloroiodomethane did not change with season. This allows us to infer that water, with its origin in the North Atlantic, transports these compounds conservatively and that their half-life in sea water is long. Additionally, bromoform and chloroiodomethane should have the same pelagic source.

We have shown that the load of biogenic volatile halocarbons exceeds that of the anthropogenic compounds. There are seasonal variations of biogenic iodinated halocarbons in the Skagerrak. The variation is less pronounced for brominated species. The amount of biogenic halocarbons cannot be correlated with the chlorophyll content in the water. In other words, biogenic halocarbons could be formed for a specific reason, or microorganisms other than phytoplankton could be responsible for their formation. Additionally, waters of different origins contain different proportions of biogenic halocarbons, and this relationship seems to prevail for an extended period of time.

Chlorinated phenolics

For trace determinations of chlorinated phenolic compounds in sea water and sediment, gas chromatography with electron-capture detection is the commonest chromatographic technique. A simple and rapid pretreatment procedure to increase the volatility and stability of the phenolics is derivatization with acetic anhydride to convert them to their non-polar derivatives. The sea water samples are buffered with $NaHCO_3$ prior to derivatization in order to ensure pH 9 and thus favour the dissociated form of the phenol. The acetylation step only takes a few min. Sample manipulation is minimized as both the derivatization step and the extraction of the derivatives into the organic phase can be performed directly in the sample bottles. With a 100-ml sea water sample we can detect chlorophenols at levels as low as a few ng/l or less. The sediment samples were extracted with sodium carbonate solution. The phenolate anions formed were acetylated with acetic anhydride and then extracted into the organic phase. With this pretreatment method and chromatographic technique we were able to determine chlorophenols in sediment at a level of 0.1 ng/g dry sediment. The method requires only 1 g of sediment. These factors make it possible to analyse large numbers of samples in a relatively short time.

Fig. 2 shows a typical chromatogram of chlorinated phenols in a sediment sample. Earlier investigations in our laboratory using GC–MS confirmed the identity of the derivatives [31].

The distribution of most chlorinated organics in the environment is determined by their lipophilicity. It has been shown that a convenient way to describe

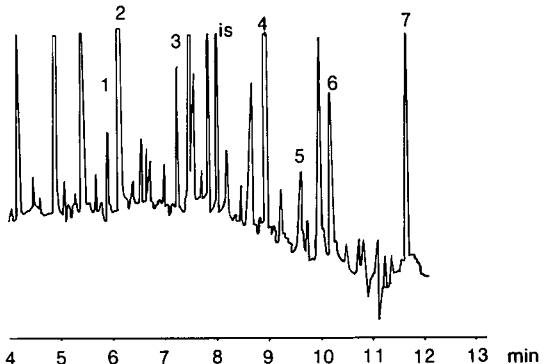

Fig. 2. Chromatogram of chlorinated phenols in the sediment core at level 22–24 cm. The sediment core was collected at position N58°15′, E10°30′. The concentrations of the individual chlorophenols are given in Table IV.

the lipophilicity of a compound is the partition coefficient between octanol and water (K_{ow}). It has been shown that this parameter is linearly correlated with partition coefficients between water and soil, sediment or biota [32]. The distribution of chlorophenolics is governed by both their hydrophilic and their lipophilic properties. It was shown by Xie *et al.* [2] that the ionizable phenolic compounds were distributed according to the distribution ratio (D) rather than K_{ow}. The distribution ratio depends on both the dissociation constant (K_a) and K_{ow}, and thus reflects their fate in the marine environment. The value of D will accordingly indicate the ability of the compounds to associate with organic matter and/or to bioaccumulate (Table V).

As can be seen from Table V, the effect is dramatic for the strongest acids. Consequently, at the pH encountered in sea water pentachlorophenol and possibly 2,3,4,6-tetrachlorophenol will be transported mainly in their dissociated form. In sea water additional features have to be considered such as the formation of ion pairs. It has been shown by Westall *et al.* [34] and Abrahamsson *et al.* [35] that the phenolate ions can form ion pairs with preferably sodium and potassium ions, and this will consequently influence the distribution ratio. This will effect D at pH > 8.

2,3,4,6-Tetrachlorophenol and PCP were the only phenolics that could be determined throughout the transects. The range was <1–100 ng/l. The precision, given as the relative standard deviation, was 6% for PCP at concentrations close to the detection limit of 1 ng/l (ten times the standard deviation of the blank). The average concentration of PCP did not vary significantly between the transects or the sampling occasions: September 53 ± 33 ng/l, November 36 ± 62 ng/l, April 34 ± 37 ng/l. In an investigation in the North Eastern part of the Skagerrak, close to a paper and pulp mill, the average concentration of PCP was of the same magnitude [4].

The residence time in the Skagerrak is short and

TABLE V

pK_a, LOG K_{ow}, LOG D AND DETECTION LIMITS FOR PHENOLIC COMPOUNDS

Compound[a]	pK_a[b]	Log K_{ow}[b]	log D (pH 7)	Detection limit (ng/l)
2,4-DCP	8.09	3.21	3.18	2
2,6-DCP	6.79	2.84	2.42	2
2,4,6-TCP	6.21	3.75	2.89	1
3,4,5-TCP	7.81	4.36	4.3	1
2,3,4,5-TeCP	6.61	4.82	4.28	1
2,3,4,6-TeCP	5.62	4.42	3.02	1
PCP	4.9	5.04	2.94	1
4,5,6-TCG	7.2	3.74	3.52	1
TeCG	6.26	4.45	3.64	1
3,,4,5-TCC		3.71		

[a] DCP = Dichlorophenol; TCP = trichlorophenol; TeCP = tetrachlorophenol; PCP = pentachlorophenol; TCG = trichloroguaicol; TeCG = tetrachloroguaiacol; TCC = trichlorocatechol.

[b] pK_a and log K_{ow} from Xie and Dyrssen [33].

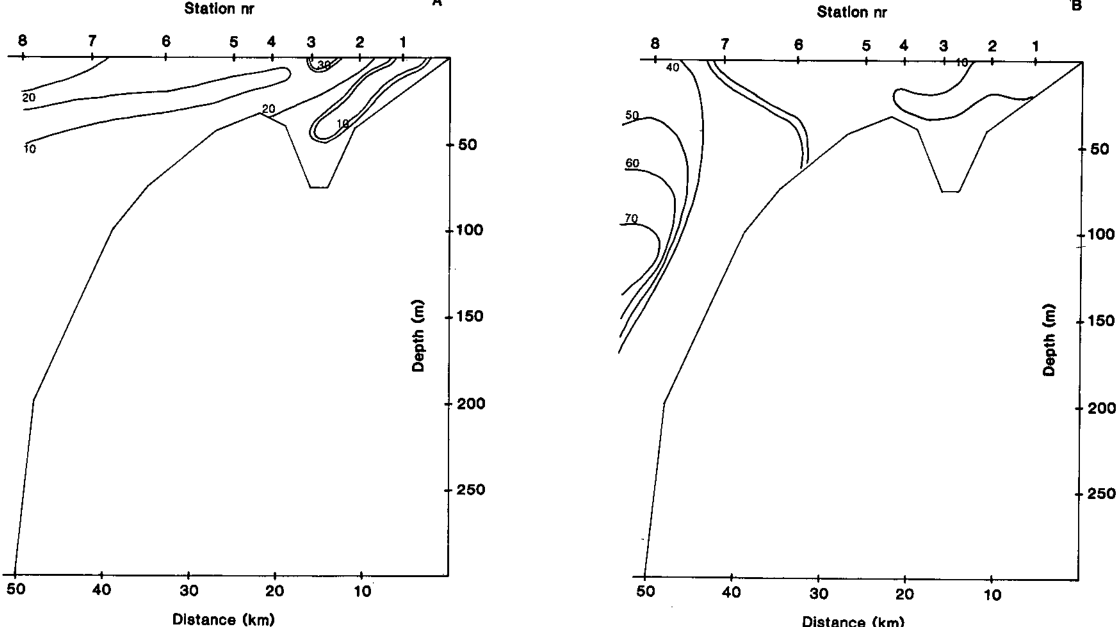

Fig. 3. Distribution of pentatchlorophenol at transect 1 in November 1990. The concentrations are given in ng/l. (A) November 12th, 1990; (B) November 14th, 1990.

it exhibits complex mixing. This implies that the distribution of different constituents in the water changes rapidly. In Fig. 3 the isopleths for PCP at transect 1 are shown for two different sampling dates in November. Within this period of only 2 days, the distribution pattern changed drastically.

In order to study the fraction of PCP associated with the organic layer of particles, water samples

TABLE VI

CONCENTRATIONS OF CHLOROPHENOLS IN A SEDIMENT CORE COLLECTED AT POSITION N58°15′, E10°30′

The concentrations are given in ng/g wet sediment.

Depth (cm)	2,6-CP	2,4-DCP	2,,4,6-TCP	3,4,5-TCP	2,3,4,6-TeCP	2,3,4,5-TeCP	PCP
0–2	57	440	210	240	16	32	230
2–4	180	250	18	–[a]	19	–	4.4
4–6	120	150	7.7	41	13	–	4.7
6–8	–	30	–	–	–	–	22
8–10	195	359	7.3	54	7.7	30	24
22–24	140	950	42	130	7.6	47	39
28–30	–	–	–	–	–	–	–
30–32	–	–	–	–	–	–	–
32–34	–	22	9.8	–	–	–	–
34–36	150	650	26	120	37	29	–
36–38	–	–	–	–	–	–	–
38–40	120	–	–	–	24	–	–
40–42	250	580	39	220	58	94	11

[a] Dashes indicate not detected.

were filtered with 0.45-μm filters and were compared with unfiltered water samples. No significant difference could be observed.

The deeper parts of the Skagerrak are known as accumulation areas for suspended matter from the North Sea [6]. Accordingly, sediment samples were analysed for their content of chlorinated phenolics at several positions in the Skagerrak. For comparison, sediment was collected from accumulation areas in the Kattegat.

The sediment from the deepest parts (water depths of 300 and 650 m) had a high load of chlorinated phenolics. Unfortunately, at the time of the analysis at the station at 650 m, we did not have sufficient standard compounds for quantification. However, it could be seen that the sediments contained chlorinated phenolics, guaiacols and cathecols. A sediment core was collected with a multiple corer at a depth of 300 m. The core was sliced into 2-cm depth intervals; the total length was 42 cm. The age of the core was determined to be at least 50 years and not older than 150 years [36]). Table VI shows the concentrations of the individual chlorophenols in the different 2-cm layers of the sediment core. In this sediment, no chlorinated guaicols or catechols could be determined. This could be due to degradation and/or transformation reactions [37,38].

A number of paper and pulp mills are situated in the coastal areas of Sweden and Norway and have been operational since the beginning of this century. At a core depth of more than 22 cm, the sediment was deposited before 1950. We still found fairly high concentrations of chlorinated phenols. The levels could probably be related to these industrial activities. The higher relative content of dichlorophenols indicates degradation activity, probably microbiologically mediated.

To conclude, the load of chlorinated phenolics in the water phase is low, except for pentachlorophenol. However, they are accumulated in the deeper parts of the Skagerrak.

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Review

Determination of benomyl and its degradation products by chromatographic methods in water, wettable powder formulations, and crops

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ABSTRACT

Chromatographic methods, used for the determination of methyl [1-(butylcarbamoyl)-1H-benzimidazol-2-yl]carbamate (benomyl) and methyl 1H-benzimidazol-2-ylcarbamate (carbendazim or MBC) in water, wettable powder (WP) formulations, and crops have been discussed. Because of the instability of benomyl in water and common organic solvents, most methods reported for the analytical determination of benomyl use an indirect approach. Since the kinetics of degradation of benomyl in water and common organic solvents is important in the development of analytical methods of benomyl, kinetic rates of various degradation reactions of benomyl are also discussed. The methods, based on the conversion of benomyl into MBC, and stabilization of benomyl in the presence of excess butyl isocyanate (BIC), will over-estimate benomyl with wide range of errors. Since MBC is a natural degradation product of benomyl and is present in different media at varying concentrations with benomyl, it should be determined individually with the intact concentrations of benomyl.

CONTENTS

1. Introduction ... 250
2. Kinetics of benomyl degradation ... 250
   2.1. Degradation in partially aqueous and aqueous solutions ... 250
   2.2. Degradation in common organic solvents ... 253
3. Review of chromatographic methods ... 253
   3.1. TLC methods ... 254
   3.2. GC methods ... 255
   3.3. HPLC methods ... 255
      3.3.1. Determination of benomyl as MBC, after its quantitative conversion to MBC ... 255
      3.3.2. Determination of benomyl as benomyl after its stabilization in organic solvents by BIC ... 257
      3.3.3. Simultaneous determination of benomyl and MBC after selective conversion of MBC to MBC–PIC (*n*-propyl isocyanate) and stabilization of benomyl by the addition of excess BIC ... 257

* Corresponding author.

3.3.4. Simultaneous determination of benomyl and MBC after quantitative conversion of benomyl to STB (STB method) 257
3.3.5. Determination of benomyl and MBC after their respective conversions to BBU and 2-AB 257
3.3.6. Direct determination of benomyl 258
4. Recent developments 258
5. Critial comments and concluding remarks 258
References 260

1. INTRODUCTION

Methyl [1-(butylcarbamoyl)-1H-benzimidazol-2-yl]carbamate (benomyl) is one of the most widely used systemic fungicides. For many years, benomyl has been successfully used for the control of many plant diseases [1–6]. Recently, the National Research Council (U.S.A.) has estimated the use of benomyl at two million lbs (1 lb ≈ 0.45 kg) of active ingredient per year [7]. Benomyl is a non-volatile white crystalline solid which has small solubility in water [8].

Because of its extensive use and suspected carcinogenic activity, determination of benomyl (and its degradation products) in environmental water, soil extracts, and crops is required. Accurate determination of benomyl (and MBC) in wettable powder formulations and pathological solutions (used in the studies to determine the efficacy and mode of action of benomyl) is also required. Instability of benomyl in common organic solvents and water has been used as an excuse by many researchers to determine benomyl as its degradation product MBC (after quantitative conversion of benomyl to MBC) in crops and other matrices. Early methods of benomyl determination are reviewed by Slade [9] and Gorbach [10]. In this review we critically discuss different chromatographic methods that are reported in the literature for the determination of benomyl and its degradation products. In most methods reported for benomyl determination, benomyl is converted into a stable compound, normally into a degradation compound of benomyl. Therefore, kinetics of degradation of benomyl in water (at different pH values) and common organic solvents is important in the development of analytical methods for the determination of benomyl. Keeping this in mind, in this paper, we have also reported kinetics of the degradation of benomyl.

2. KINETICS OF BENOMYL DEGRADATION

2.1. Degradation in partially aqueous and aqueous solutions

In aqueous solutions benomyl is not stable and converts to methyl 1H-benzimidazol-2-ylcarbamate (carbendazim, more commonly MBC), 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB) and, 1-(2-benzimidazolyl)-3-*n*-butylurea (BBU), depending upon the pH of the solutions. In highly alkaline solutions (pH > 13) MBC slowly converts to 2-aminobenzimidazole (2-AB). The degradation of benomyl in aqueous solutions can be represented as depicted in Fig. 1.

The distribution profiles of various degradation products of benomyl, in benomyl saturated aqueous solutions of different pH values, have been depicted in Figs. 2 and 3.

The kinetics of decomposition of benomyl to its different degradation compounds was first studied by Calmon and Sayag [11,12], in methanol–water (50:50, v/v) using a spectrophotometric method. On the basis of the absorbance *vs.* time plots, these authors were able to determine the pseudo-first-order rate constants of benomyl decomposition to MBC, STB, and BBU, graphically. However, due to the low solubility of benomyl in water, Calmon and Sayag had to use a mixture of aqueous buffers and methanol to dissolve large amounts of benomyl in the working solution, which was required to study its decomposition kinetics by the spectrophotometric method. On the basis of a detailed investigation of the UV spectra of solutions at the end of reaction, they concluded that in the pH range 2.5–7.0, the final product was MBC. Their plots of the logarithms of observed pseudo-first-order rate constants (k_{obsd}) against pH indicated that in strongly acidic media (pH < 2.5) the decomposition reaction was inhibited by hydronium ions whereas over the pH range 2.5–7.0 the reaction rate was pH independent.

Very Fast in water, pH 13
Na salt of STB
Slow pH 13
BBU
Very Fast pH 7
STB
Benomyl
Fast
in org. solvent
Carbendazim
+ O=C=N-C_4H_9
Butyl isocyanate
Relatively Slow in water, pH 7
Carbendazim
+ HO-C(=O)-NH-C_4H_9
Butyl carbamic acid
Fast
CO_2 + $H_2NC_4H_9$
Carbon dioxide
Butylamine
in water pH 13
Carbendazim
Stable in water, pH 13
No Change
Slow in water, pH > 13
2-AB

Fig. 1. Degradation of benomyl in aqueous and organic solvents at room temperature.

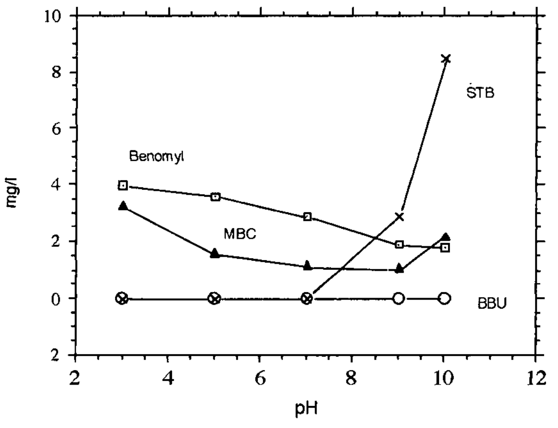

Fig. 2. Composition of benomyl (wettable powder) saturated aqueous buffers of pH 3–10, with respect to benomyl, MBC, STB and BBU.

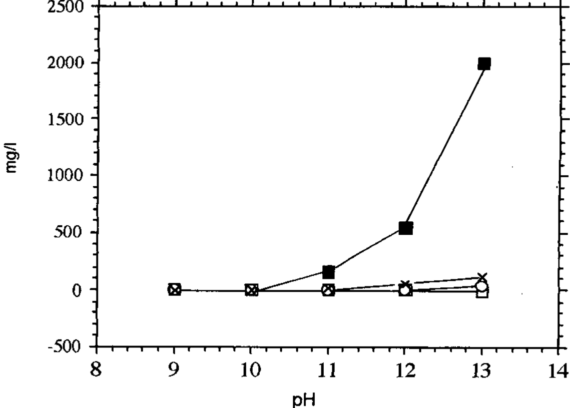

Fig. 3. Composition of benomyl (wettable powder) saturated aqueous buffers of pH 9–13, with respect to benomyl (□), MBC (×), STB (■) and BBU (○).

The conversion of benomyl to STB and BBU in alkaline medium was first studied by White *et al.* [13]. These authors reported that in alkaline medium benomyl was converted into STB, which after its formation, converted slowly to BBU in standing solutions. The amount of BBU in solution increases with time, temperature, and alkalinity. The work of White *et al.* [13] may have prompted Calmon and Sayag [12] to study the kinetics and mechanism of benomyl degradation to STB and BBU in alkaline media. Their studies were carried out in aqueous alkaline buffers–methanol (50:50, v/v), at constant ionic strength maintained at 1.0 mol/l. On the basis of their investigation Calmon and Sayag [12] concluded that the kinetics of hydrolysis of benomyl in alkaline media proceeded through several reaction mechanisms. In mildly alkaline media (pH < 12), conversion of benomyl to STB proceeds via $E_{1C}B$ elimination mechanism, followed by a fast cyclization. In strongly alkaline media (pH > 12), benomyl is converted into STB via a dianion. The conversion of STB to BBU occurred in very strongly alkaline media (pH > 13.5) and followed a first order rate kinetics with respect to hydroxide ion.

A knowledge of stability of benomyl in water at different pH values is important not only to agricultural and analytical chemists but also to plant pathologists. Although the work of Calmon and Sayag [11,12] on the kinetics of degradation of benomyl at various pH values in aqueous buffers–methanol (50:50, v/v) media have shown a trend of benomyl degradation, their results cannot be taken quantitatively for the development of analytical methods of benomyl determination.

Keeping this in mind Singh *et al.* [14] studied the kinetics of decomposition of benomyl in pure aqueous solutions of different pH values, using reversed-phase high-performance liquid chromatography (RP-HPLC). In the RP-HPLC method, benomyl in aqueous solutions was directly analyzed using a suitable mobile phase. The decrease in benomyl peak height with time was used to calculate the kinetic rate constants (k) according to the equation

$$k = 2.303/\Delta t \log C_1/C_2$$

where C_1 is the benomyl peak height at time t_1, C_2 is the benomyl peak height at time t_2, and $\Delta t = t_2 - t_1$.

The rate constant (k) of benomyl conversion to

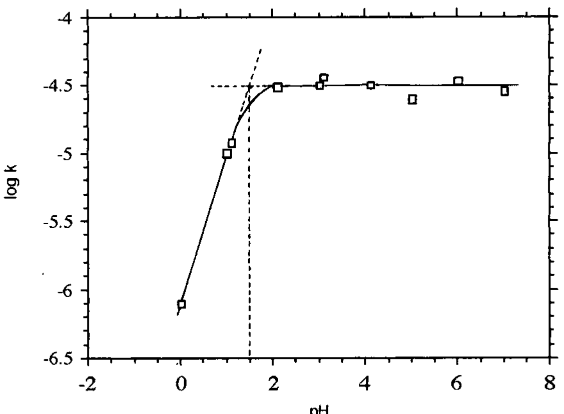

Fig. 4. Plot of log k_{obs} (rate constant of benomyl decomposition to MBC) *versus* pH of aqueous solutions.

MBC in the aqueous solutions remained nearly constant between pH 2 and 7 with mean k at $(3.16 \pm 0.38) \cdot 10^{-5}$ s^{-1}. However, in acidic solutions, below pH 2, protonation of benomyl (at the nitrogen of benzimidazole group) starts taking place. The plot of logarithm of k *versus* pH (pH 0–7) is shown in Fig. 4. In pure aqueous solutions the trend in the log k *vs.* pH plot was similar to that observed by Calmon and Sayag [11]. However, the mean k at $(3.16 \pm 0.38) \cdot 10^{-5}$ s^{-1}, in pure aqueous media in th pH range 2–7, was 60% lower than $5.0 \cdot 10^{-5}$ s^{-1}, the mean value of k reported by Calmon and Sayag [11], in aqueous buffers–methanol (50:50, v/v) in the same pH range.

A pK_a value of 1.5 was graphically determined for ionization of benomyl-H^+, in pure aqueous solutions on the basis of the plot in Fig. 4.

Kinetic study of degradation of benomyl in pure auqeous alkali solutions (pH ≥ 13) was also carried out by us [15]. In a solution of sodium hydroxide (0.125 mol/l) of pH 13, a very fast conversion of benomyl to STB was observed. The first order rate constant (k_{obs}) for this reaction was estimated at approximately 0.01 s^{-1}. This value of k_{obs} is about 9 times higher than that reported by Calmon and Sayag [12] in methanol–sodium hydroxide (50:50, v/v) solution of pH 13. The value of k_{obs} for the degradation of STB to BBU depends upon the alkalinity and temperature. Again, the degradation of STB to BBU was 4 times higher in pure aqueous

solution than in methanol–sodium hydroxide (50:50, v/v) solution of the same pH.

Degradation of MBC to 2-AB was observed only in strong alkali solutions. In 1.0 mol/l sodium hydroxide, the value of k_{obs} was $1.32 \cdot 10^{-4}$ s^{-1} at 22°C. However, at high temperature, this reaction was faster. A value of approximately 0.034 s^{-1} was obtained in 0.125 mol/l sodium hydroxide at 80°C.

2.2. Degradation in common organic solvents

Rapid degradation of benomyl to MBC in common organic solvents was first reported by Chiba and Doornbos [16]. Calmon and Sayag [17] confirmed their finding and determined the rate constants of the decomposition of benomyl to MBC. They further reported that the conversion of benomyl to MBC proceeds by spontaneous intramolecular catalysis. The values of observed rate constants do not show any correlation with the existing empirical solvent parameters. However, the observed rate constant data can be explained in terms of solvent–solute interactions. The spontaneous intramolecular catalysis is markedly slowed down by the presence of water.

Chiba and Cherniak [18] have studied the kinetics of the decomposition of benomyl in common organic solvents such as chloroform, dichloromethane, ethyl acetate, benzene, ethanol, methanol, and dioxane using a spectrophotometric method at 25°C. These authors discovered that the reaction of the decomposition of benomyl was reversible (Fig. 1), *i.e.*,

$$\text{Benomyl}\,(k_{21}) \xleftrightarrow{\text{organic solvents}} \text{MBC} + \text{BIC}\,(k_{12}).$$

where k_{12} and k_{21}, are the specific rates of benomyl degradation and reformation, respectively [18], and BIC is butyl isocyanate.

The values of the specific rate constants k_{21} and k_{12} were determined for benomyl decomposition reaction in different (above-mentioned) organic solvents. The values of k_{12} showed no correlation with existing solvent parameters. However, k_{21} was found to be larger for less polar solvents. The reversible nature of the decomposition reaction was different in different solvents. The largest percentage of intact benomyl at equilibrium was found for benzene while the smallest intact concentration of intact benomyl was found in methanol.

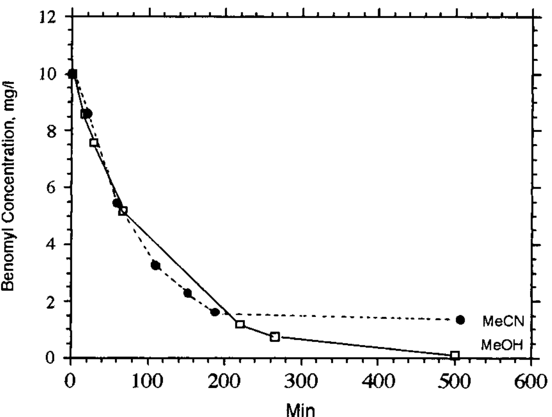

Fig. 5. Decomposition kinetics of benomyl to MBC in acetonitrile (MeCN) and methanol (MeOH).

The decomposition of benomyl in organic solvents, acetonitrile and methanol, was also studied by Singh *et al.* [14] using a RP-HPLC method. The RP-HPLC method also confirmed the reversibility of benomyl decomposition to MBC and BIC in acetonitrile. After the decomposition reaction attained the equilibrium, a constant peak height of benomyl was obtained by RP-HPLC analysis of the benomyl solution in acetonitrile for several days. In methanol, however, the decomposition of benomyl to MBC was almost quantitative. These results are shown in Fig. 5. The RP-HPLC results also confirmed the observations reported by Calmon and Sayag [17], *i.e.*, the decomposition of benomyl in organic solvents was slowed down by the addition of water in the reaction medium. The observed rate constant (k) for the forward decomposition reaction of benomyl to MBC in pure methanol at $2.5 \cdot 10^{-4}$ s^{-1} decreased to $6.2 \cdot 10^{-5}$ s^{-1} in methanol–pH 7 phosphate buffer (50:50, v/v).

3. REVIEW OF CHROMATOGRAPHIC METHODS

It is obvious from the above-mentioned kinetic results that benomyl, once dissolved in solutions, is not stable, either in an aqueous medium or in common organic solvents. As a result, for the determination of benomyl in different media, it is first converted to a stable compound which was then determined using chromatographic methods, most notably, thin-layer chromatography (TLC), gas chro-

matography (GC) and HPLC. A brief description of chromatographic methods reported for the determination of benomyl in water, wettable powder (WP) formulations and crops by TLC, GC and HPLC methods is presented in the following paragraphs.

3.1. TLC methods

Benomyl and its degradation products MBC and 2-AB were separated on TLC sheets coated with silica gel (100 μm). The coated material contained a fluorescent indicator [19]. Two-dimensional solvent systems containing benzene–methanol (9:1) and ethyl acetate–chloroform (6:4) were used to obtain the best separation of benomyl, MBC, 2-AB, thiophanate methyl, and benzimidazole. The detection of the compounds on TLC plates was carried out under 254 nm UV light as dark spot. A chromogenic spray reagent (0.5% solution of N-2,6-trichloro-*p*-benzoquinoneimine) was used for selective determination of MBC. The two dimensional TLC offered excellent accuracy and better separation than those developed in one dimension only.

White and Kilgore [20] used TLC for the determination of benomyl (as MBC) and MBC in food crops such as apple, apricot, cherry, grape, nectarine, peach, and plum treated with benomyl. In this method freshly prepared aliquots of benomyl solution were added to fruit-control macerates prior to extraction. The compound was first extracted with benzene and partitioned into 0.1 mol/l hydrochloric acid. The acidic layer was washed several times with chloroform and then neutralized to pH 7.8–8.2 with concentrated sodium hydroxide. During the extraction procedure all benomyl converted to single residual product, MBC, which was then partitioned into ethyl acetate. The compound was concentrated by evaporation of ethyl acetate and subsequently developed on a commercially prepared TLC sheets (polyamide precoated plastic sheets containing a fluorescent indicator). The TLC sheets were first developed in a mixed solvent system containing chloroform–ethyl acetate–acetic acid (190:10:4) to eliminate the streaking of MBC. On dry sheets samples were spotted (in 0.5 cm diameter) approximately 3 cm apart along an imaginary line, 2 cm from the bottom of the TLC sheet. A standard solution of MBC was spotted on each TLC plate to serve as a reference. Separation was achieved in a mixed solvent, chloroform–ethyl acetate–acetic acid (190:10:4) and the spots were identified under UV light (2537 Å). The spots corresponding to MBC R_F values were extracted from the TLC plate with a vacuum-assisted spot collector and the compounds were eluted from the TLC support medium with absolute methanol. Quantitative determination of extracted MBC was carried out spectrophotometry at 287 nm utilizing semimicro quartz cells having 10-cm light paths for residues with MBC concentrations lower than 0.2 mg/l. For residues containing large concentrations of MBC, a 1-cm quartz cell was used.

The lower limit of detection for this method was reported at 0.05 mg/l. Overall average recovery of benomyl residues (determined as MBC), obtained from fortified control samples was, 87%.

In another TLC method, Baker *et al.* [21] used aluminium oxide F_{254} neutral (type E) and silica gel 60 F_{254} coated TLC plates. The separation of benomyl and MBC from six other fungicides was achieved in the following four solvent systems: diethyl ether–glacial acetic acid–methanol (100:5:2), acetone (100%), light petroleum (b.p. 60–80°C)–acetone (3:1), and diethyl ether–methanol (40:1). The fungicides were visualized under UV light or by spraying potassium iodobismuthate solution followed by exposure to bromine vapour. The detection limit for benomyl determination was estimated at 0.8 μg.

It is obvious from the above that TLC can be used as a simple method for the separation of benomyl (MBC) from coextractives obtained from water, WP formulations, and crops. As reported by White and Kilgore [20], TLC can also be used for the quantitative determination of benomyl (MBC). However, the limitation of the TLC method, where benomyl is determined as MBC, is the overestimation of benomyl quantity. Being a natural degradation product of benomyl, MBC is always present in the samples with benomyl, and determined as benomyl (in the methods where benomyl is determined as MBC). This results in a positive error in the determination of benomyl. Another problem with this method is that it cannot be used for the determination of the fate of benomyl after its spray on crops. Baude *et al.* [22] have reported the only TLC method where determination of intact concentrations of

benomyl and MBC in crops was made. In this method crop tissues containing [^{14}C]benomyl were first macerated in 1 mol/l NaOH. The mixture was immediately refluxed. As a result all the [^{14}C]benomyl was converted into [^{14}C]BBU. Any concentration of [^{14}C]BBU which may be present in the crop tissues was transformed into [^{14}C]2-AB. After the reflux step, it was possible to analyze aliquots of sodium hydroxide solution directly by TLC, using ^{14}C-readout techniques. Quantitative determinations were made by scraping TLC spots corresponding to [^{14}C]BBU and [^{14}C]2-AB, and their subsequent liquid scintillation counting. However, when green plant tissue samples were involved, extraction of BBU and 2-AB from refluxed solution was necessary before TLC analysis could be performed. The extraction of BBU and 2-AB in the refluxed solutions was carried out with ethyl acetate. Hexane washes of the basic solutions, prior to ethyl acetate extraction, were sometimes used as an additional clean-up step.

3.2. GC methods

Rouchaud and Decallone [23] developed a GC method for the analysis of MBC in plants and soil. Benomyl and MBC were extracted from melon plants and soil using an extraction method similar to that reported by Kirkland [24] (described under HPLC methods). Residual benomyl and MBC were extracted with benzene and partitioned into 0.1 mol/l HCl. The acidic layer was washed several times with chloroform and then neutralized. The single residual product MBC (present initially in the plant and soil, and formed during the acidic clean-up by the quantitative hydrolysis of benomyl) is partitioned into ethyl acetate. MBC was then derivatized into MBC–trifluoroacetylate (MBC–TFA) for its determination by GC, using ^{3}H electron-capture detection (ECD). A glass column, 1.5 m × 2.2 mm I.D. packed with 5% SE-30 on 80–100 mesh Chromosorb, was used for the separations of desired analytes. For MBC-TFA determination, column temperature was maintained at 140°C. A volume of 1.5 μl of residue was injected into the injector, set at 250°C. The temperature of detector was set at 225°C. Nitrogen, at 40 ml/min, was used as carrier gas. After 10 injections (one day's work) the column temperature was raised to 210°C during the night in order to clean the column from high boiling natural products, unrelated to MBC. The overall recovery of benomyl residues obtained from fortified control samples ranged from 80 to 100%. The minimum detectable concentration of the method was estimated at 0.02 mg/l.

Pyysalo [25] described a modification of the Rouchaud and Decallonne [23] method. In this modified method MBC is derivatized into mainly 2-AB acetate which was determined by capillary GC with nitrogen–phosphorus selective and electron-capture detectors. A 50-m long glass capillary column (0.25 mm I.D.) coated with OV-101 liquid phase was used for achieving the desired separations. Hydrogen was used as carrier gas with the splitless injection technique. Sensitivity of this method at 0.00001 mg/l (for benomyl as MBC) was a significant improvement over the Rouchaud and Decallonne [23] method. The method was tested for various vegetables and fruits.

Cline *et al.* [26] also used a GC method (with ECD) for the determination of very small concentrations of MBC in black walnut fruits by derivatizing MBC into a pentafluorobenzyl bromide derivative. Other chromatographic conditions of this method were similar to that used in the Rouchaud and Decallonne [23] method.

3.3. HPLC methods

The HPLC methods, developed for the determination of benomyl, can be divided into six categories. A brief description of these methods is presented in the following sections.

3.3.1. Determination of benomyl as MBC, after its quantitative conversion to MBC

The first HPLC method based on this approach was reported by Kirkland [24]. In this method benomyl was first quantitatively converted to MBC, by hydrolyzing the sample in aqueous acid. The compounds of interest were then extracted from the acidic solution by organic solvents. The extract was cleaned up by a solvent–solvent partitioning process, and the compounds were determined by high-speed strong cation-exchange liquid chromatography, using a 1000 mm × 2.1 mm I.D. stainless-steel column containing Zipax SCX strong cation-exchange packing. The eluent, a mixture of 0.15

mol/l each of sodium acetate and acitic acid mixed in a 7:3 ratio, was used at a flow-rate of 0.5 ml/min. The column temperature was maintained at 60°C. A highly sensitive ultraviolet (UV) photometer operating at 254 nm was used as a detector. The method was used to determine benomyl in cow milk, urine and tissues. The recoveries of benomyl and its metabolites (other than MBC) averaged about 80% in cow milk and urine. Lower benomyl recoveries (50–80%) were obtained from tissue samples and feces. The minimum detectable concentrations for benomyl/MBC were 0.02 mg/l in milk, and 0.01 mg/l in urine, feces and cow tissues.

The same method with slight modification in the extraction step was used by Kirkland *et al.* [27] for the determination of benomyl in soils and plant tissues. In the modified method any 2-AB, present in the samples, was also extracted and simutaneously determined with MBC. Recoveries of benomyl (as MBC), MBC and 2-AB from various types of soils averaged 92, 88, and 71%, respectively. The minimum detectable concentration of benomyl, MBC and 2-AB was estimated at 0.05 mg/l.

Spittler *et al.* [28], using similar extraction and chromatographic procedures as described by Kirkland [24], determined benomyl (as MBC) and 2-AB in water, brussels sprouts, snap beans, grapes, endive, bok choy, cauliflower, and beet tops. Detection limits for benomyl in the commodities ranged from 0.002 to 0.02 mg/l. Recoveries for MBC ranged between 75 and 114%. Recoveries for 2-AB at 57 to 67% in crops were significantly lower, as compared to MBC.

Zweig and Gao [29] determined benomyl, after its quantitative conversion to MBC in acetonitrile. These authors mentioned that a waiting period of 3 h, after the extraction of benomyl in acetonitrile, ensured the quantitative conversion of benomyl to MBC, at room temperature. RP-HPLC with a RP-18 Spheri 5, Brownlee Labs. bonded reversed-phase column (25 cm × 2 mm I.D.) was used for the determination of MBC. The eluent, a mixture of aceonitrile–water (50:50, v/v) was used at a flow-rate of 1.5 ml/min. Peak detection was made by a UV detector. The recoveries of added MBC and benomyl from surgical gauze patches ranged from 87.0 to 100.4%.

RP-HPLC was also used by Farrow *et al.* [30] [Spherisorb ODS packed into a 150 mm × 4.6 mm I.D. stainless-steel column; eluent methanol–water–ammonia (60:40:0.6, v/v/v); flow-rate, 1 ml/min; UV detector] and Sanchez-Brunete *et al.* [31] [RP-18, 5 μm phase into a 250 × 4.6 mm I.D. stainless-steel column; eluent, methanol–0.3% ammonia (60:40, v/v) flow-rate, 1 ml/min; UV detection] for the determination of benomyl as MBC. The determination was made after quantitative conversion of benomyl to MBC during acid hydrolysis and extraction in organic solvents from crops. Kiigemagi *et al.* [32] employed HPLC with UV detection for the determination of benomyl (as MBC) residues in postharvest-treated pears in cold storage. A CN column (10 cm × 5 mm I.D., 10 μm μBondapak) was used for the separation and determination of MBC in pears. A tetrahydrofuran–water mixture (60:40, v/v) was used as mobile phase at 1.5 ml/min flow-rate. Their clean-up procedure included the extraction of benomyl (MBC) in acetone from acidified pear puree followed by partitioning with ethyl acetate.

A Diol column (LiChrosorb Diol, 5 μm, 15 cm × 4.6 mm I.D.), a mixture of hexane and isopropanol as mobile phase, and UV detection at 285 nm was used by Bicchi *et al.* [33] for the separation and determination of MBC. Their method is based on a clean-up procedure carried out on an Extrelut 20 cartridge. The average recovery for MBC in apples, pears and their pulps was reported at 83.8%.

Liu *et al.* [7] reported a HPLC–mass spectrometry–selected ion monitoring method for the determination of benomyl after its quantitative conversion to MBC. These authors used a 25 cm × 4.6 mm I.D. Whatman Partisil 5 ODS-3 column with a particle size of 5 μm, and a mixture of acetonitrile–0.1 mol/l ammonium acetate (85:15, v/v) as mobile phase running at 1 ml/min flow-rate for the separation of MBC in fruits and vegetables. The mass spectrometer was operated in the positive ion filament mode and selected ion monitoring of m/z 192 was performed. The method was tested for the determination of benomyl in tomatoes, peaches and apples. The minimum detectable level of benomyl in apples, peaches, and tomatoes was estimated at 0.025 mg/l. Recoveries of fortified benomyl at 0.1 mg/l were in the range 85 to 110% in all three commodities.

3.3.2. Determination of benomyl as benomyl after its stabilization in organic solvents by BIC

Determination of benomyl as benomyl in wettable powder (WP) formulations by RP-HPLC has been reported by Stringham and Teubert [34]. In this method WP formulations were extracted with acetonitrile that contained 3% *n*-butyl isocyanate (BIC), and chromatographed on a reversed-phase C_{18} column. This method is based on the finding by Chiba and Cherniak [18] who reported that the degradation of benomyl to MBC in most organic solvents is reversible *i.e.*, benomyl (k_{21}) $\leftrightarrow$ MBC + BIC (k_{12}), as described earlier in this review. The method showed good reproducibility with relative standard deviation (R.S.D.) of 1.95%. Mean recovery of standard from the sample pool was estimated at 95% with a standard deviation of 3.50%. A collaborative study of this method was also carried out [35]. The collaborators stated the method as simple, rapid, and reproducible. Statistical analysis of analytical data showed the method to be precise and free from expected interferences.

3.3.3. Simultaneous determination of benomyl and MBC after selective conversion of MBC to MBC–PIC (n-propyl isocyanate) and stabilization of benomyl by the addition of excess BIC

Chiba and Veres [36] developed a method for the determination of residual benomyl and MBC on apple foliage. Samples leaves, in a Mason jar, were freeze-dried and tumbled for extraction in $CHCl_3$ containing 5000 μg *n*-propyl isocyanate/ml at 1°C. During this treatment free MBC present in the sample was quantitatively converted to methyl 1-(*n*-propylcarbamoyl)-2-benzimidazole carbamate (MBC–PIC). *n*-Butyl isocyanate was added to the extract at 5000 μg/ml to prevent the degradation of benomyl during HPLC analysis. A volume of 20 μl of this mixture was injected onto the column, a Brownlee LiChrosorb silica gel column with a guard column in series. The mobile phase contained a mixture of chloroform–hexane (4:1, v/v) that was saturated with water. MBC–PIC, formed from MBC, was separated from benomyl and simultaneously determined. The detection limit for both the compounds in apple leaves was estimated at 0.2 mg/l.

3.3.4. Simultaneous determination of benomyl and MBC after quantitative conversion of benomyl to STB (STB method)

A HPLC method was developed by Chiba and Singh [37] for the simultaneous determination of benomyl and MBC in water and WP formulations. In the method benomyl was converted to 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB) in 0.125 mol/l sodium hydroxide. After the conversion (at room temperature in 20 min), STB was determined simultaneously with MBC (which remained intact with the sodium hydroxide treatment) at 286 nm after HPLC separation on a C_{18} column (15 cm × 4.6 mm I.D., Regis HiChrom reversible, 5-μm Spherisorb ODS or ODS-II). A mixture of acetonitrile–water–phosphate buffer (pH 7) (40:45:15, v/v) was used as the mobile phase at flow-rate of 0.8 ml/min. The resolution of STB and MBC was influenced by the composition and pH of the mobile phase, and also by the composition of sample solutions prepared for HPLC injections. To obtain a good resolution between STB and MBC, the factors to be considered were the percentages of organic solvents (acetonitrile), pH and buffer concentration [37] in the mobile phase. The method worked well for the analysis of samples containing varying concentrations of benomyl and MBC. The method was also applied for the determination of benomyl and MBC in WP formulations [38], organic solvents [14], and pathological samples [3]. The method was also used for the determination of benomyl, MBC, STB and BBU in aqueous solutions of different pH values, saturated with benomyl [8].

The conversion of benomyl to STB (the conversion on which this method is based) was found to be quite selective. The method was found accurate and showed good reproducibility with R.S.D. of 0.7% for benomyl and 2.2% for MBC determinations, respectively in WP formulations.

3.3.5. Determination of benomyl and MBC after their respective conversions to BBU and 2-AB

Chiba [39] reported a method for the determination of benomyl and MBC in apples. In this method benomyl and MBC were quantitatively converted to BBU and 2-AB, respectively. Macerated apples were mixed with sodium hydroxide solution (pH 13.2) and the mixture was refluxed for 2 h at 100°C.

During this high-temperature alkali treatment, benomyl quantitatively converted to BBU, and MBC converted to 2-AB. The converted compounds were extracted in diethyl ether–2-butanol (50:50, v/v). The residue, obtained after evaporation of the extractant, was dissolved in a solvent mixture containing methanol–water (30:70). Determinations were made by HPLC with UV detection at 280 nm. BBU and 2-AB were separated from coextractives on a short size-exclusion column connected to a CN analytical column via a switching valve. Separation of BBU and 2-AB was achieved on the CN column.

Maeda and Tsuji [40] determined benomyl in plant tissue by HPLC (Hitachi Gel No. 3010-CH_2OH, 20–25 μm particle size, 500 × 2.1 mm I.D. column; column temperature 45°C; methanol containing 0.1% acetic acid as the mobile phase; flow-rate 1.2 ml/min) after quantitative conversion of benomyl to 2-AB (via MBC). Recoveries of benomyl were reported at 90.5–102.9% with no interference from plant tissue components. The method can detect up to 0.02 mg/l benomyl.

3.3.6. Direct determination of benomyl

Benomyl can be separated in its intact form from other pesticides and its degradation products by HPLC, as reported by Austin *et al.* [41] [Zorbax-silica column and propan-2-ol–hexane (5:95, v/v) at flow-rate of 0.25 ml/min; UV detection], Cabras *et al.* [42] (C_{18} column at temperature 20–50°C; mixtures of water, acetonitrile and phosphate buffer as mobile phase; UV detection at 221.0 nm), Singh and Chiba [8] [Regis Hi-Chrom reversible, 5-μm Spherisorb, 15 cm × 4.6 mm I.D.; mixtures of acetonitrile–water–phosphate buffer (pH 7) as mobile phase; UV detection at 286 nm], Chiba and Northover [43] and Northover and Chiba [44] [Regis Hi-Chrom reversible ODS-1, 5-μm Spherisorb, 15 cm × 4.6 mm I.D. column; mixture of methanol–0.034 mol/l phosphate buffer, pH 7.0 (40:60, v/v) at flow-rate 0.5 ml/min; UV detection at 286 and 294 nm], Singh *et al.* [14] (Regis Hi-Chrom reversible, 5-μm Sperisorb 15 cm × 4.6 mm I.D., and a Phenomex ODS-2 (C_{18}) 15 cm × 4.6 mm I.D.; mixtures of acetonitrile–water–phosphate buffer (pH 7) as mobile phase; flow-rates 0.8–1.5 ml/min; UV detection at 220 and 286 nm), and Marvin *et al.* [45,46] (3 cm × 4.6 mm I.D. C_8 precolumn; acetonitrile–water gradient mobile phase at flow-rates 1–1.5 ml/min; UV detection at 220 nm). However, due to the unavailability of benomyl stock solutions of desired strength in aqueous media at room temperature, it was not possible to exploit the HPLC separation of benomyl for its direct analytical determination until recently. Nevertheless, direct determination of benomyl by HPLC made it possible to study the kinetics of benomyl degradation in solutions [14]. The method was also used for the study of biological activity of benomyl [43,44].

4. RECENT DEVELOPMENTS

Recently, Singh *et al.* [47] reported that preparation of stable stock solutions of desired strength of benomyl in aqueous media is possible. Therefore, direct determination of intact concentrations of benomyl (and MBC) can accurately be made now. Actually, the decomposition of benomyl in water and in the mixed water and organic solvents is not very fast as reported (and believed by many researchers) earlier. According to the decomposition kinetics results [14], approximately 2% benomyl will decompose to carbendazim in 5 min in a solution containing acetonitrile–water (60:40). This suggested that during chromatographic determination of benomyl, the decomposition of benomyl to MBC will not be significant enough to introduce large error in its analytical determination. Preliminary results of direct quantitative analysis of benomyl by HPLC and fast-atom bombardment mass spectrometry (FAB-MS) have recently been reported by Singh *et al.* [48].

5. CRITICAL COMMENTS AND CONCLUDING REMARKS

It is clear from the above that majority of the methods used for the analytical determination of benomyl are based on quantitative conversion of benomyl to its degradation compound, MBC which is determined by HPLC, GC or TLC techniques. The principal drawback of these methods is the over-estimation of benomyl with a wide range of errors. The over-estimation of benomyl is due to the fact that MBC, which is a natural degradation product of benomyl, is present in different samples with benomyl, and is also determined as benomyl. MBC is also a known degradation compound of thiophanate-methyl, a fungicide not as widely used

as benomyl but registered for use on some fruits and vegetables. Therefore, determination of benomyl as MBC would be subjected to positive errors. Moreover, since MBC is also fungitoxic and its level of activity is different from that of benomyl these compounds should be determined individually, particularly for pathological studies. For these reasons methods based on the determination of benomyl as MBC have only limited application.

We would also like te mention that in most of these methods, acid hydrolysis of benomyl is believed to be causing the conversion of benomyl to MBC. However, in our recent work we found that the presence of acid in solution actually stabilized benomyl. Protonation of benomyl at the nitrogen of benzimidazole ring is believed to be responsible for the stabilization of benomyl. This contrasts with the findings of many researchers who reported to achieve a quantitative conversion of benomyl to MBC in acidic solutions with the presence of some organic solvents. We suspect that in these methods temperature [49], duration of time, and presence of organic solvents have caused the conversion of benomyl to MBC and not the presence of acid.

It is also to be pointed out that in common organic solvents (except in methanol and ethanol) quantitative conversion of benomyl to MBC is not possible, at room temperature [18]. This is due to the reversible nature of the decomposition reaction of benomyl to MBC and BIC in organic solvents, as mentioned earlier in this review. This contrasted with the report made by Zweig and Gao [29], who claimed to have achieved quantitative conversion of benomyl to MBC in acetonitrile, at room temperature in three hours.

The method reported by Stringham and Teubert [34] and Teubert and Stringham [35], where benomyl and MBC are determined as benomyl, is also subjected to over-estimation of benomyl. In this method wettable powder formularions were extracted in acetonitrile that contained 3% BIC. Excess amount of BIC was added in the solution to stabilize benomyl. However, excess amount of BIC in solution will also produce benomyl from MBC, which being a natural degradation compound of benomyl, is always present at variable quantities in any kind of benomyl containing samples.

The over-estimation of benomyl by the method of Stringham and Teubert may be explained on the basis of Fig. 6a and c, which show the chromatograms of a WP formulation, dissolved in cold (1°C) acetonitrile in the absence and presence of excess BIC. The presence of chromatographic peaks due to MBC and benomyl in Fig. 6a reveals that both of these compounds were present in the WP formulation (as benomyl does not decompose to MBC in cold acetonitrile). However, in excess of BIC, only one peak due to benomyl was observed (Fig. 6c), due to the conversion of MBC to benomyl. The benomyl peak in Fig. 6c was approximately 20% higher than benomyl peak in Fig. 6a. Concentrations of MBC and intact benomyl in WP formulation can be accurately determined using a method similar to that reported by Chiba and Veres [36] (Fig. 6b) or by the STB method [37]. Although chromatographic methods, especially HPLC methods, are more popular for the determination of benomyl, the spectrophotometric method reported by Chiba [50,51] can also be used for the determination of intact concentrations of benomyl and MBC in WP formulations. The determination of MBC and

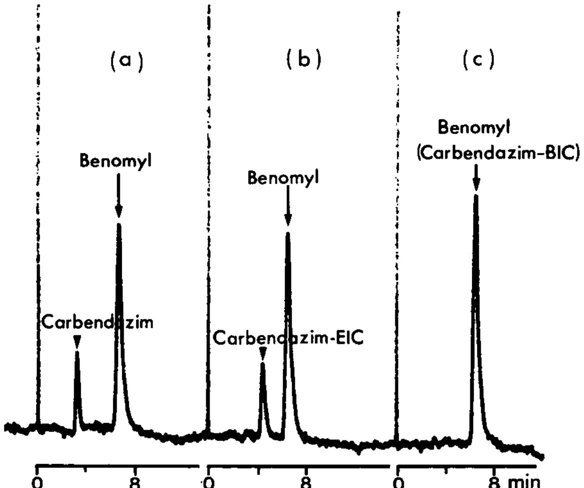

Fig. 6. Chromatograms of MBC, benomyl and MBC-EIC (ethyl isocyanate). Column: 15 cm × 4.6 mm I.D. Regis ODS, mobile phase: acetonitrile–water–buffer (60:30:10). (a) Chromatogram of 50% WP sample dissolved in cold acetonitrile, showing MBC and benomyl peaks; (b) chromatogram of 50% WP sample dissolved in cold acetonitrile in which 2% (v/v) EIC was added, showing the peaks of MBC–EIC and benomyl; and (c) chromatogram of benomyl after 50% WP formulation was dissolved in acetonitrile containing 1000 mg/l of BIC showing benomyl peak only.

intact concentrations of benomyl in crops can be carried out by the methods of Baude *et al.* [22] and Chiba [39].

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Review

Use of insoluble polyvinylpyrrolidone and isoelectric focusing in the study of humic substances in soils and organic wastes

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ABSTRACT

The use of insoluble polyvinylpyrrolidone and isoelectric focusing in the study of humic substances is reviewed. Chromatography on insoluble polyvinylpyrrolidone is widely used to characterize humic materials extracted from soils, organic fertilizers, soil amendments and organic wastes in order to evaluate their degree of humification. The isoelectric focusing technique is employed mainly to evaluate the stability of the organic matter in organic wastes before their use in agriculture.

CONTENTS

1. Introduction 261
2. Insoluble polyvinylpyrrolidone (PVP) 262
   2.1. PVP in studies of soil humic substances 262
   2.2. PVP in studies of organic amendments 264
   2.3. PVP in studies of organic fertilizers and wastes 264
3. Isoelectric focusing (IEF) 266
   3.1. IEF of soil humic substances 266
   3.2. IEF of organic wastes 267
4. Acknowledgements 269
References 269

1. INTRODUCTION

The total amount of soil organic matter worldwide has been estimated to be *ca.* $2 \cdot 10^{12}$–$3 \cdot 10^{12}$ kg [1,2], while the amount of soil humic substances can be estimated as 50% of the total soil organic matter.

In general, organic matter (especially humic substances) plays an important role in soil and water environments. Hayes *et al.* [3] have summarized the role of organic matter in soils as follows: (1) formation and maintenance of a good soil structure; (2) improvement of water capacity; (3) retention in available form of plant nutrients by cation-exchange processes; (4) slow release of nitrogen, sulphur, phosphorus and some trace elements; (5) transport of metals into plant roots; (6) stimulatory effects on plant growth; (7) immobilization of some anthro-

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pogenic chemicals (*e.g.*, pesticides) added to the soil to influence crop growth; (8) enhancement of the buffering capacities of soils; and (9) raising the soil temperature due to increased adsorption of solar radiation. Humic substances are also present in all waters and are most abundant in watersheds which drain acid or sodic soils [3,4]. The agronomic functions of the organic matter through its effect on the physical, chemical and biological properties of the soil directly involve the environment because the soil *sensu strictu* is an important compartment of the environment itself [5]. On the Earth the life of plants and consequently via food chains of the animals, including man, depends mainly on the quality of the soils. In fact, a soil polluted for instance due to the addition of organic wastes containing heavy metals or pesticides may cause pollution of surface waters, ground waters and plants and via food chains the pollutants may reach animals. In this way the global environment can become polluted [6].

For these reasons it is important to be able to assess the presence of humic substances in organic materials that reach the soil and to determine their degree of humification [7]. In fact, when applied to the soil, non-humified organic carbon is converted into humic substances plus metabolic energy, which has a considerable effect on the activities of microflora and microfaunal organisms [5]. The evolution towards humified compounds of the organic matter in materials that undergo comparatively rapid transformations (*e.g.*, sewage sludges, pig slurries, dung, composts) is of both agronomic and environmental importance [8]. The occurrence of such transformations corresponds to stabilization of the organic matter and avoids adverse reactions in the soil environment, such as the production of phytotoxic substances or anoxic environments.

Many methods have been used for the extraction, separation and characterization of soil humic substances (*e.g.*, [9–18]). Specific analytical methods also have been proposed for organic wastes used in agriculture, such as organic fertilizers or amendments, which, however, produce environmental risks if a proper period of stabilization of the organic matter has not been observed (*e.g.*, refs. 7 and 19–25).

Evaluation methods based on the use of chromatography with insoluble polyvinylpyrrolidone (PVP) resin and the isoelectric focusing (IEF) technique in studies of soil humic substances and humic materials in organic wastes are reviewed in this paper.

2. INSOLUBLE POLYVINYLPYRROLIDONE (PVP)

The basic structure of PVP and the postulated hydrogen bonding with phenol groups [26] are shown in Fig. 1. PVP resin forms strong hydrogen bonds mainly with phenolic, hydroxyl and carboxyl groups [26].

PVP in the insoluble form has been used in many fields of research, including studies of humic substances in soils beginning in 1968 [27]. In the food industry, for example, insoluble PVP has been employed to remove polyphenol substances in the clarification of wines and beer [28,29]. In thin-layer chromatographic separations insoluble PVP has been used as the stationary phase in separations of anthocyanins [30–32], anthocyanidinglucosides [33], chlorogenic acids [34] and flavonoids from plant materials in aqueous media [35,36]. Also, aromatic acids, aldehydes and phenols present in aqueous media have been retained on PVP columns [37], *e.g.*, during the purification of plant hormones from tissue extracts [38–40].

2.1. PVP in studies of soil humic substances

The procedure for the extraction of the organic matter from soils, before the application of the PVP method [18], is the same as reported in the literature [5]. After extraction the organic extract is fractionated in humic acids (HA) and fulvic acids (FA) according to the methodology proposed by Schnitzer [5]. The fulvic fraction, however, also contains non-humic substances (*i.e.*, carbohydrates, peptides and amino acids) that must be separated from FA.

Fig. 1. Basic structure of polyvinylpyrrolidone (PVP) and the postulated hydrogen bonding to phenol groups. From ref. 26.

With this aim the fulvic fraction is purified from non-humic (NH) substances by using small columns (10 cm) packed with insoluble PVP (3–5 cm^3) after acidification of the alkaline extract [17,18,41,42]. The retained fraction on PVP (generally brown) is then re-eluted by adding 5–10 ml of 0.5 *M* NaOH and collected (FA), while the non-adsorbed fraction (NH, non-humic fraction) is discharged.

Humic substances have been defined as amorphous, polymeric, brown compounds [43,44] and other classes of organic compounds, such as polysaccharides, polypeptides or altered lignins, are not considered to be humic compounds. It has recently been demonstrated [45] that carbohydrates, peptides, amino sugars and amino acids contained in the fulvic fraction separated from three different types of soil are concentrated mainly in the fractions which are not adsorbed on PVP. Indeed, the FA fraction adsorbed on the PVP resin contained a large number of aromatic rings and carboxyl groups, while non-humified (NH) compounds remained in the fraction not adsorbed on the PVP [45].

The International Humic Substances Society (IHSS) has recommended the use of Amberlite XAD-8 resin as the adsorbent to obtain only humic substances in the FA fraction. However, Kuwatsuka *et al.* [46] have recently demontrated that fractionation using insoluble PVP gives a higher recovery and more distinct fractions of FA than the resin proposed by the IHSS. In fact, after extraction and fractionation of FA from forest soils and from ando soils, the amounts of FA recovered using the Nagoya method [46] were 5 and 40 times higher, respectively, than those found after application of the IHSS method.

Recently, in studies of soil organic matter, some workers (*e.g.*, refs. 17, 18, 47 and 48) have used insoluble PVP to calculate various humification parameters, namely (i) a humification index [17], $HI = NH/(HA + FA)$, *i.e.*, the ratio between non-humified (NH) and humified compounds (HA + FA), (ii) the degree of humification [49], $DH\ (\%) = [(HA + FA)/TEC] \cdot 100$, *i.e.*, the percentage of humified compounds with respect to total extracted organic carbon (TEC) and (iii) the humification rate [49], $HR\ (\%) = [(HA + FA)/TOC] \cdot 100$, *i.e.*, the percentage of humified compounds with respect to total organic carbon (TOC) in the sample.

Some of the results obtained after application of PVP in the separation of the fulvic fraction of organic extracts arising from the A horizons of typical Italian soils are reported in Table 1. The calculated humification rate (*HR*) and degree of humification (*DH*) are values typical of these two parameters for soil samples [18]. The role of PVP in the separation of humified (FA) from non-humified (NH) compounds in the fulvic fraction is well indicated by the *DH* values. In fact, the difference

TABLE 1

TOTAL ORGANIC CARBON AND NITROGEN, HUMIFICATION RATE (*HR*) AND DEGREE OF HUMIFICATION (*DH*) OF REPRESENTATIVE SAMPLES OF SOILS

All samples were taken from the A horizon of the soil profile and the data are expressed on the basis of the air-dry mass.

Soil samples	Total organic carbon (%)	Total organic nitrogen (%)	Humification rate, *HR* (%)	Degree of humification, *DH* (%)
Typic Xeropsamment	1.72	0.17	21.2	65.7
Typic Xerorthent	2.71	0.13	13.6	63.4
Mollic Xerorthent	6.30	0.34	18.4	67.4
Fluventic Xerochrept	1.91	0.21	17.9	68.2
Typic Haplumbrept	5.13	0.37	20.9	68.7
Typic Pelloxerert 1	3.78	0.25	21.2	80.3
Typic Pelloxerert 2	0.99	0.10	26.3	88.9
Typic Chromoxerert	2.21	0.16	36.5	97.7
Mollic Andept 1	13.1	0.65	27.5	79.1
Mollic Andept 2	12.0	0.90	32.9	78.4

between the *DH* value found and 100 represents the amount (as a percentage) of the NH fraction present in the fulvic fraction of the soil organic extract. Without the use of the PVP resin, these fractions, mainly composed of polysaccharides, amino acids and amino sugars [44], are often erroneously included in the humic fraction. The mean values of the overestimate are around 30%; the overestimate is less only in the case of samples of Vertisols (*e.g.*, Pelloxerert and Chromoxerert). It should be emphasized that the role of PVP in the separation of FA from NH compounds in the fulvic fraction is higher in the soil samples where the organic matter is less humified (*e.g.*, Entisols, Inceptisols, Mollisols), where the amount of NH compounds is generally high (Table 1).

2.2. PVP in studies of organic amendments

The procedure for the extraction and separation of the soil organic matter reported in section 2.1. has also been used to characterize organic amendments, such as peats, lignins and leonardites [18]. Owing to the high organic carbon content of these samples (normally 30–55%) [7], as reported above, the ratio between the volume of the extractant solution and the mass of the sample used is greater than that for soil humic substances (50:1 compared with 10:1, v/w, respectively). This procedure has recently been introduced by the Italian Ministry of Agriculture and Forestries (MAF) [50] as the official method for determining humic substances in peats, leonardites, suspensions of humic acids and farmyard samples.

Some selected results after fractionation of the organic carbon extracted from samples of peats, lignites and leonardites are reported in Table 2. Organic amendments are generally characterized by a high content of organic carbon and small amounts of organic nitrogen. The percentage of humic substances (HA + FA) with respect to total organic carbon in the sample (TOC) is low for the lignite samples, around 60% for the peat samples (except peat sample 2 with $HR = 26.7\%$) and over 80% for the leonardite samples. As expected, the presence of NH substances in organic amendments is generally low, owing to the progressive decrease in easily oxidized compounds (*i.e.*, polysaccharides and amino sugars). Indeed, the *DH* of the organic extracts is very high for all samples and close to 100% for the leonardite samples and highly humified peats [7].

2.3. PVP in studies of organic fertilizers and wastes

In the case of organic fertilizers and wastes the use of insoluble PVP in the separation of humified

TABLE 2

TOTAL ORGANIC CARBON AND NITROGEN, HUMIFICATION RATE (*HR*) AND DEGREE OF HUMIFICATION (*DH*) OF SOME SAMPLES OF ORGANIC AMENDMENTS

The data are expressed on a dry mass basis.

Samples	Total organic carbon (%)	Total organic nitrogen (%)	Humification rate, *HR* (%)	Degree of humification, *DH* (%)
Peat 1 (Czechoslovakia)	50.7	2.38	60.2	89.2
Peat 2 (Russian Federation)	58.4	1.04	26.7	72.2
Peat 3 (Ireland)	57.8	1.27	60.5	90.4
Peat 4 (Ireland)	55.7	1.69	59.2	94.8
Peat 5 (Italy)	33.7	2.11	64.0	96.5
Peat 6 (Italy)	46.2	2.53	53.4	91.3
Peat 7 (Norway)	52.6	3.23	59.4	89.0
Peat 8 (Scotland)	52.0	1.40	52.3	87.5
Lignite 1 (Italy)	51.2	0.79	6.8	77.4
Lignite 2 (Italy)	52.3	0.74	11.6	88.9
Leonardite 1 (USA)	40.5	0.52	84.4	99.7
Leonardite 2 (USA)	41.3	0.55	82.7	94.9

compounds from the organic extract presents many problems [49,51]. According to the definition of humic substances given by Hayes and Swift [43] and Aiken *et al.* [44], without a proper period of maturation organic fertilizers and organic wastes do not contain humic materials. However, during the application of the procedure for the extraction and separation of the organic matter many interferences have been found [18]. In fact, after acidification some of the organic compounds extracted precipitate, or others in the fulvic fraction are adsorbed on PVP (humic-like substances). Ciavatta *et al.* [49] experimented with the use of an acid or an alkaline hydrolysis of the organic extract in order to reduce these interferences, but the results were unsatisfactory. More recently, the same group [51] studied the possibility of using a series of aspecific enzymes added sequentially to the extract to reduce the interferences arising from the humic-like substances. The results obtained were, in most instances, satisfactory and the interferences were reduced or completely eliminated [51,52]. In the determination of the humification parameters the chromatography on PVP of the hydrolysed organic extract only permits the separation of phenolic substances and the reduction of non-humic organic carbon [51,52].

For wastes containing large amounts of organic carbon (*e.g.*, slurries, compost produced from municipal solid wastes and sewage sludges) it is very important to assess the stability of the organic carbon before its addition to the soil. Indeed, the addition of easily oxidized organic compounds to soil, such as those generally present in large amounts in organic wastes, can produce an anoxic environment, phytotoxic substances, leaching to deep waters and damage to crops. For these reasons various humification parameters have been used to characterize, or better to evaluate, the degree of stabilization of the organic matter from a series of materials, including animal manures after digestion by earthworms [21] and raw composts [20,22,24,25,53].

Reported in Fig. 2, for example, is the trend of the *DH* observed during stabilization of the organic matter extracted from samples of compost from municipal solid wastes. Of the two humification parameters, *HR* and *DH*, the latter appears to be the most useful to follow the stabilization processes in organic materials. The values of the *DH* during the period of stabilization are characterized by a sharp increase, which is then followed by an asymptotic trend. In other words, the evolution of organic matter during maturation of an organic waste is characterized by a continuous increase in humified or humic-like substances in the alkali-soluble fraction, so that the *DH* effectively represents the development of the process. Sometimes, but not always, the *HR* also has a similar trend to that of the *DH* [8]. Especially with liquid or semi-liquid wastes, the entire mass of organic matter is simultaneously involved in the stabilization process and only the *HR* can accurately describe the process [8,22]. When the processes are effective for a small proportion of the material and involve progressively only further limited parts, while the bulk remains unaltered, the *DH* can describe the process better than the *HR* [7].

In contrast, as shown in Fig. 2, in all instances the total extracted organic carbon (TEC) is not a suitable parameter for following the stabilization processes, because its trend is irregular and characterized by a decrease during the formation of humic and humic-like substances.

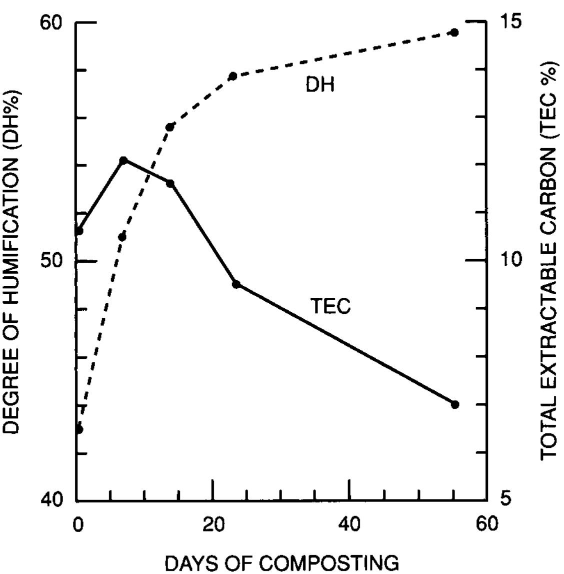

Fig. 2. Trend of both the degree of humification (*DH*) and total extracted organic carbon (TEC) during the stabilization process of the organic matter from a pile of compost with municipal solid wastes. From ref. 25.

3. ISOELECTRIC FOCUSING (IEF)

In general, the electrophoretic separation of a charged compound is based on its mobility in an electric field [54–56]. One of the electrophoretic techniques, isoelectric focusing (IEF), is an electrophoresis carried out on a medium with a preformed pH gradient. The principle of this technique has been described well by Righetti and Drysdale [56]: "a stable pH gradient increasing progressively from anode to cathode is established by electrolysis of carrier ampholytes (CAs) in a suitable anticonvective medium". In this system a charged molecule migrates and reaches a zone where its net electric charge is zero. The final result is that all the molecules of an unknown mixture are fractionated in the pH gradient on the basis of their different isoelectric points (p*I*).

The IEF technique was first reported in 1912 [57] when a mixture of amino acids from hydrolysed vegetable proteins was fractionated in a three-chambered electrolysis cell. One of the most important problems in this and other pioneering studies [58,59] was the absence of an uniform and stable pH gradient, mainly owing to the lack of a suitable CA.

Later, as reported by Righetti and Drysdale [56], the theoretical basis for IEF was developed and can be summarized as follows: the importance of using electrolytes with a high buffering capacity and of stabilizing the pH gradient against convective mixing [60,61] was pointed out; and the law of the monotony of the pH was introduced and the idea of developing a natural pH gradient by electrolysis of amphoteric molecules [62–65] was advanced. Ideally, the ampholytes should have good conductivity, good buffering capacity and good solubility at their isoelectric point and also be easily distinguishable and separable from proteins. In 1969 [66], practical means of synthesizing CAs with many of the properties described by Svensson [62–64] and Rilbe [65] were achieved.

3.1. IEF of soil humic substances

The first electrophoretic separations of humic substances were carried out nearly 70 years ago [67] in free solutions. Then several media, such as a filter-paper matrix [68,69] or cellulose powder [70], were used. The first electrophoresis of humic substances carried out on polyacrylamide (PAA) gel was reported by Stepanov and Pakhonov in 1969 [71]. At present, PAA gel is the most widely used medium in electrically driven separations of humic substances.

The first application of the IEF technique in studies of humic substances appeared in 1972 [72], when the fractionation of humic substances from freshwater was described. Later, IEF was used to characterize humic substances from soils, fertilizers, soil amendments and organic wastes.

One of the most important objectives of researchers who use IEF to characterize soil humic substances is to fractionate the compounds of the humic extract and then to conduct further studies on the single fractions. This second objective is not so easy to achieve and, consequently, the use of IEF has often been limited to the characterization of humic substances only by the evaluation of the pattern.

The first applications of IEF to soil humic substances were published in the 1970s. In early work [73], this technique was applied to soil humic substances without preforming the pH gradient, but by just mixing the CAs with the sample to be characterized. Later it was found [74], that the use of IEF produced a greater number of electrophoretic and isotachophoretic bands.

It has been reported [75] that the CAs interfered with the humic substances during the IEF fractionation. It was pointed out that urea is apparently unable to break up these interactions and that its presence causes an increase in the intensity in the bands focused in the most acidic region of the pH gradient and a shift in the p*I* of the bands focused at the higher p*I* values. These data, however, in the opinion of De Nobili [76], were not sufficient to demonstrate the presence of interactions because similar results had been obtained previously during the fractionation of humic substances with electrophoresis when CAs were not present.

Another fact that is a cause for discussion about the reliability of the IEF technique for the characterization of humic substances is that, with the exception of the bands focused in the acidic region of the pH gradient, the IEF profile of a single band after refocusing has been found to be characterized by a more or less complex pattern [75]. However, more recently, it was found that the refocusing of a single band obtained in the presence or absence of urea was resolved in its original pattern [77].

In another study [76], humic substances were fractionated with electrophoresis at pH 6.5 into two groups which were subjected to IEF. The group of substances that had migrated towards the anode focused in the pH gradient region below pH 6.5, while the other group focused in the remaining pH gradient region.

As suggested by Duxbury [78] on the basis of experimental data, the formation and stability of complexes between humic compounds and CAs should be pH dependent and it could be possible that the alkaline re-dissolution of a humic–ampholyte complex would create a variety of new complexes that, after refocusing, could resolve in a new IEF profile. Duxbury [78] also reported that the resolution of the bands of the humic substances also depends on the prefocusing time and it was concluded that the technique used was not IEF. In a previous paper published in the 1988 [76], De Nobili used the term electrofocusing (EF) instead of IEF and also more recently the EF of soil humic substances has been reported [48].

Another problem is that commercial CAs are chemically different because their methods of synthesis are protected by patents.

It is not difficult to imagine that the characterization of humic substances with IEF could be influenced by the CA used. Recently, it has been shown [79] that the IEF separation of soil humic compounds using different CAs leads to the formation of different IEF profiles.

Ceccanti *et al.* [80] characterized a soil organic extract and its two fractions previously obtained by ultrafiltration. They found that the higher molecular mass fractions focused in the higher pH gradient region, whereas the fractions with lower molecular mass focused in the lower pH gradient region. Successively, these results were substantially confirmed by combining gel electrophoresis with IEF [81].

More recently [77], using IEF to characterize the different molecular sizes of humic substances in the presence or absence of 8 *M* urea, the relationship found between molecular mass and the position of resolution of a band in the pH gradient was confirmed but, in addition, it was noticed that the shift in pH due to the presence of urea had a greater influence in the fractions with $M_r > 10^5$. These and other results have demonstrated that most humic substances are fractionated on the basis of their p*I* or, at least, by charge neutralization. The lower molecular mass fraction appears to be constituted mainly of polyphenolic compounds with less polymerization than in the other fractions [82].

In agreement with other results, humic compounds with a lower electrophoretic mobility focus at higher pH values whereas the humic compounds with a higher electrophoretic mobility focus at lower pH values [83]. A further evaluation by use of infrared spectrometry and pyrolysis–gas chromatography–mass spectrometry has shown a lower content of carboxyl groups and an higher content of ketonic and quinonic carboxyl groups in the fractions with lower electrophoretic mobility. The fraction with lower electrophoretic mobility was more complex and contained a higher content of polysaccharides and also proteins or peptide residues. It was concluded that humic substances with different origins, but which focused in the same pH interval, show evident structural similarities [83].

The application of IEF in the characterization of humic substances extracted from different types of soils showed that different IEF profiles were obtained. This evidence suggested that the systematic application of IEF in the study of soil humic substances could lead to great improvements in the soil taxonomy [84].

The application of IEF in the study of organic matter extracted from soils that had been fertilized differently for 22 years with different types and rates of organic materials showed that the IEF profile obtained depended on the type of organic material, whereas mineral fertilization did not affect the IEF profiles of the native soil humic substances [48].

The estherase activity of a humic extract in the single focused bands has been measured and it was found that the activity was present in each band of the fractionated soil humic extracts [85]. However, in a more recent study [86], it was found that protease activity was present only in some bands and that this activity was higher in the band focused at pH 4.44.

3.2. IEF of organic wastes

The main objective of workers who apply IEF in studies of organic wastes is to define an analytical method that can be useful in order to characterize

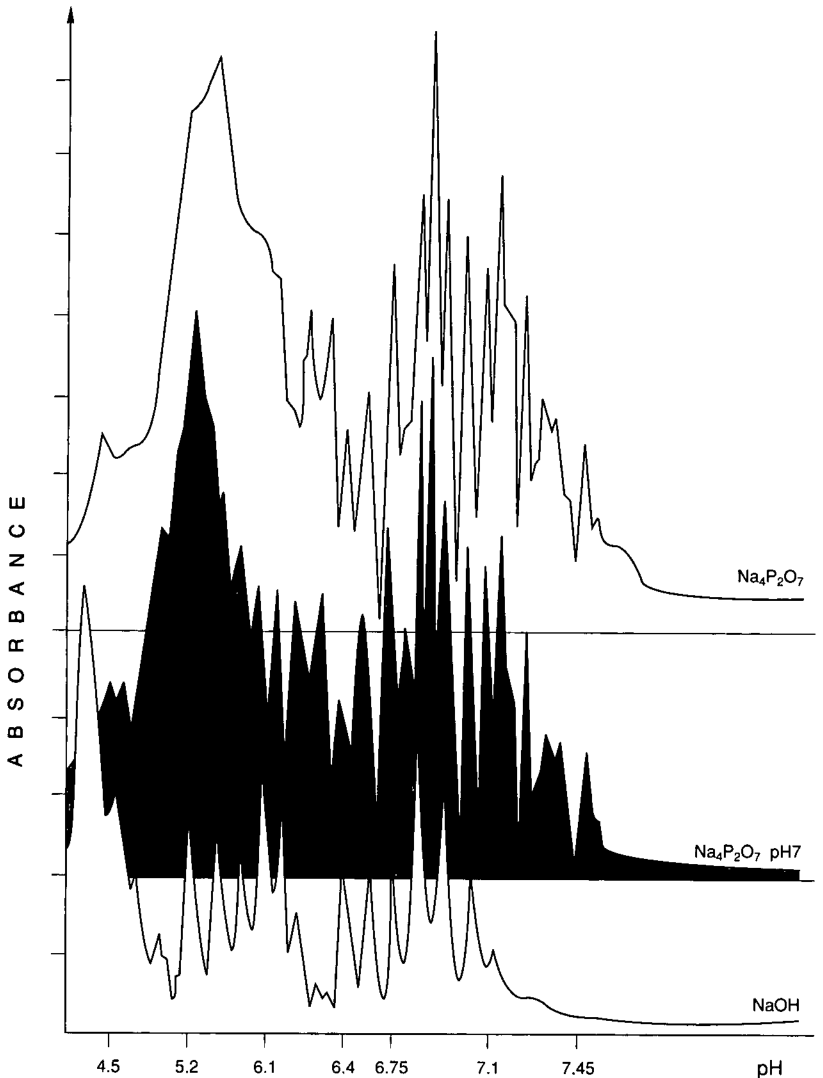

Fig. 3. IEF profiles of a digested sewage sludge extracted with 0.5 *M* NaOH, 0.1 *M* $Na_4P_2O_7$ adjusted to pH 7 with H_3PO_4 and 0.1 *M* $Na_4P_2O_7$. The carrier ampholyte used was Biolyte 3-10 (Bio-Rad). Each scale mark on the ordinate corresponds to 0.1 unit of absorbance.

the organic matter of a waste and accurately evaluate its stability before its use in agriculture. Obviously, this objective is of considerable interest for both agricultural and environmental purposes. Evaluation of the IEF profiles in this case is very different from the case of the soil humic substances because here the main factor is the stabilization of a raw organic material and not the characterization of humic substances. It is not difficult to understand why for this application the problems of the interactions of the organic compounds with the CAs appear to be of secondary importance. The most important question is whether or not an IEF profile of an organic waste can give enough information about its stabilization so that its proper use in agriculture can be assured.

The IEF profiles of raw and mature poultry manure, farmyard manure, worm compost and compost from municipal waste treatment plants have been evaluated [87]. The IEF profiles of the

first three materials were characterized by considerable heterogeneity of the bands in the pH gradient region from 4.5 to 6.5, whereas the composts from municipal waste treatment plants showed an IEF profile with a simpler pattern resolved below pH 5.5. The oldest sample, however, displayed fewer bands in the acidic region.

Comparison of the IEF profiles of differently aged sewage sludges showed the presence of resolved bands in the pH gradient region below pH 5 for the raw samples, whereas the *ca.* 6-month-old samples also focused in a less acidic region (up to pH 6.5) [53]. Similar results were also obtained in another study in which the organic matter was extracted using several extractant solutions [88]. Fig. 3 shows an example of the IEF profiles of a digested sewage sludge extracted using three different extractant solutions.

The evolution of the organic matter of pig slurries has been followed both in summer and in winter [89]. The IEF profiles of the raw samples were poor in bands in the pH gradient region above 5 whereas the IEF profile of the slurry, matured in the summer season, was rich in bands also in the pH gradient region from 5 to 7. The IEF profile of the slurry matured in the winter season was much less complex than that of the summer-matured slurry. These results have also been confirmed in a study of the evolution of organic matter during the stabilization of composts from municipal solid wastes [25].

The most important aims of the use of IEF are (i) to find the relationships between the IEF profiles of organic wastes and their degree of stabilization and (ii) to find the relationships between IEF profiles and the presence of phytotoxic compounds.

Studies to optimize the IEF technique should also provide for comparisons of the IEF profiles obtained using different CAs in order to choose the best CA for this type of work.

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Review

Thin-layer chromatographic methods for use in pesticide residue analysis

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ABSTRACT

A selective review is presented, focusing mainly on stationary phases, mobile phases, detectors and TLC techniques used for the detection, separation, determination and identification of pesticide residues in various environmental samples. The results from numerous papers are presented in tabular form.

CONTENTS

1. Introduction 271
2. Results 271
References 285

1. INTRODUCTION

TLC has grown rapidly in recent years and is now widely accepted as a rapid and efficient detection technique. TLC has replaced paper chromatography in pesticide residue analysis because of its higher resolution and shorter development time. It is lacking in the precise specificity of gas–liquid chromatography but it is more precise and sensitive than PC. Although most advances in pesticide analysis during the past few years have taken place in the field of GC and HPLC, TLC has retained its status as a valid and simple method for the qualitative and quantitative analysis of pesticide residues and their metabolites.

2. RESULTS

A number of books, reviews [1–88] and research papers have been published in this area. Several new coating materials have been discovered and tested for pesticides analysis. The older coating materials have been re-investigated using new solvents systems, especially mixed solvents. Many of the papers published on this subject originate from researchers in the less developed countries, probably because of the lack of more sophisticated instrumentation.

The results from numerous publications are presented in Tables 1 and 2.

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TABLE 1

ABBREVIATIONS

Stationary phases	
P1	Admixtures of barium sulphate and calcium sulphate
P2	Admixtures of silica gel and calcium sulphate
P3	Alumina G
P4	Aluminium oxide, Silica gel KCK and Silufol
P5	Aluminium oxide 60 F_{254} (Type E)
P6	Barium sulphate
P7	Calcium sulphate containing ammonium molybdate, aluminium oxide, calcium carbonate, copper sulphate, iron(III) chloride, magnesium sulphate, phthalic anhydride and zinc oxide
P8	Cellulose
P9	Chromarods-A
P10	C_8 and C_{18}
P11	C_{18} chemically bonded RP layer
P12	C_{18} SPE and Silica gel 60
P13	KC_{18} reversed-phase impregnated with Bratton–Marshall reagent
P14	Kieselgel 60, silica gel 60, Kieselguhr F_{254}, Aluminium oxide G, Polygram $SILUV_{254}$, Polygram cell 300 and Silufol
P15	Polyamide
P16	Polygram SILG UV_{254}
P17	Preadsorbed silica gel layers impregnated with silver nitrate
P18	Precoated plate CNF_{254S}
P19	RP-18-W layers
P20	Silica gel
P21	Silica gel G
P22	Silica gel–plaster of paris
P23	Silica gel LS/40m
P24	Silica gel impregnated with cresol (0.5%)
P25	Silica gel impregnated with metal ion or phenol
P26	Silica gel containing zinc acetate (1% w/w)
P27	Silica gel–Kieselguhr (2:3)
P28	Silica gel GF_{254}
P29	Silica gel impregnated with paraffin in hexane (5%)
P30	Silica gel impregnated with diethylene glycol (20%)
P31	Silica gel precoated with fluorescence indicator
P32	Silica gel, Merck No. 5721
P33	Silica gel impregnated with 2-diphenylacetyl-1,3-indandion-1-imir
P34	Silica gel SPF
P35	Silica gel 60
P36	Silica gel Sep-Pak or C_{18} preadsorbent TLC plate
P37	Silica gel F_{1500}
P38	Silica gel HF_{254}
P39	Silica acid-impregnated glass-fibre sheets
P40	Silufol
P41	Silufol UV_{254}
P42	Silver nitrate-impregnated alumina G
P43	Sorbfil HPTLC plates, silica gel with silicic acid
P44	Soil
P45	Whatman $LKC_{18}D$ chemically bonded reversed-phase plates
Mobile phases	
S1	Dichloromethane–heptane (2:9)
S2	Hexane–benzene (1:1) and hexane–diethyl ether (1:1)
S3	Acetonitrile–water (75:25)
S4	Hexane–chloroform (60:40) and hexane–benzene (45:55)
S5	Acetonitrile–water–ammonia (40:9:1) followed by hexane–diethyl ether (90:10)

TABLE 1 *(continued)*

Mobile phases	
S6	Acetonitrile–water–ammonia (40:9:1) followed by hexane–methanol–acetic acid (5:5:1)
S7	Chloroform–diethyl ether–hexane–toluene (293:257:250:200)
S8	Hexane–acetone (70:30) or benzene–chloroform (70:30)
S9	Chloroform–benzene (9:1) and chloroform–methanol (1:1)
S10	Hexane–acetone (4:1) and hexane–trichloromethane (1:1)
S11	Light petroleum–benzene–ethyl acetate (65:30:5)
S12	Ethyl acetate–chloroform (1:9)
S13	Hexane–acetone (1:1) followed by chloroform–acetone–methanol (1:1:1)
S14	Acetic acid
S15	Toluene–ethyl acetate (85:15)
S16	Hexane, acetone and ethyl acetate
S17	Hexane–acetone (2:1) or benzene–ethyl acetate (2:1)
S18	Hexane–diethyl ether (3:1)
S19	Benzene–chloroform–methanol (9:3:2) and chloroform–methanol (3:1)
S20	Water–acetonitrile (1:9) and water–methanol (25:75)
S21	Benzene, chloroform, carbon tetrachloride, distilled water, 1,4-dioxane and ethyl acetate
S22	Hexane–acetone (1:1) and diethyl ether–hexane–ethanol (77:20:3)
S23	Cyclohexane–benzene–acetic acid–liquid paraffin and cyclohexane–benzene–acetone
S24	Benzene–hexane–acetic acid (5:13:2) and hexane–acetic acid (7:3)
S25	Diethyl ether–toluene (1:3 or 2:1) and chloroform–nitromethane (2:1)
S26	Dichloromethane–acetone, toluene–acetone, chloroform–ethyl acetate and benzene–acetic acid (9:1)
S27	Toluene–acetone, ethyl acetate–chloroform and chloroform–acetone
S28	Hexane–butyl acetate
S29	Toluene–acetone (85:15)
S30	Chloroform–ethyl acetate or hexane–ethyl acetate
S31	Chloroform–acetone (95:5)
S32	Hexane–butyl acetate (60:60)
S33	Acetic acid–chloroform–isooctane (5:20:75) or acetic acid–chloroform (3:7)
S34	Butanol–formic acid–water (7:2:1)
S35	Hexane–diethyl ether (3:1) followed by hexane–acetone–acetic acid (35:25:0.05)
S36	Chloroform–methanol (49:1)
S37	Cyclohexane–acetone (10:1) and light petroleum–benzene–ethanol (65:30:5)
S38	Chloroform–acetone and hexane–acetic acid–diethyl ether
S39	Hexane–benzene (45:55)
S40	Carbon tetrachloride–light petroleum (60:40)
S41	Acetone–hexane (1:9)
S42	Hexane–methanol–diethyl ether (3:1:1)
S43	Hexane, hexane–benzene (1, 2, 3:1, 2, 3), benzene, hexane–benzene–acetonitrile (10:10:1, 10:10:2), chloroform–benzene (2:1, 9:1), chloroform, chloroform–acetone (9:1), chloroform–ethyl acetate–acetonitrile (9:1:1), dichloromethane–ethyl acetate (7:3), ethanol and acetone–water (1:1)
S44	Hexane–acetone (4:1)
S45	Hexane–chloroform (2:1, 4:1)
S46	Hexane–acetone (2:1)
S47	Hexane–chloroform (2:1) and hexane–acetone (3:1)
S48	Hexane–xylene–ethyl acetate–water (50:15:5:18)
S49	Hexane–acetone (3:1)
S50	Chloroform–light petroleum (1:2)
S51	Chloroform–acetone (68:32)
S52	Acetonitrile–water (80:20)
S53	Benzene–hexane (2:1), acetone–benzene (100:0.4), benzene and methanol
S54	Chloroform–acetone
S55	Hexane, heptane, chloroform, benzene, ethanol, ethyl acetate, carbon tetrachloride, diethyl ether, light petroleum, acetone and their mixtures

(Continued on p. 274)

TABLE 1 *(continued)*

Mobile phases	
S56	Chloroform
S57	Methanol, methanol–water (1:1, 9:1), water–formic acid–methanol (4:1:5), water–acetone (69:4), methanol–25% ammonia solution (30:1), acetone, methanol–diisopropyl ether (1:1, 1:4), hexane–acetone (9:3, 7:3), chloroform–acetone (9:1) and diethyl ether
S58	Acetone, acetonitrile, diethyl ether, ethyl acetate, acetone–hexane, diethyl ether–hexane, ethyl methyl ketone–light petroleum, acetone–acetonitrile–cyclohexane and acetone–cyclohexane–ethanol
S59	Hexane–ethyl acetate (13:7)
S60	Hexane–ethyl acetate–acetone (9:1:1)
S61	Benzene–ethyl acetate (9:1)
S62	Benzene, chloroform, carbon tetrachloride and distilled water
S63	Chloroform–methanol (1:1) and glacial acetic acid–methanol–benzene (1:1:3)
S64	Benzene–ethyl acetate (50:10)
S65	Benzene–ethylmethyl ketone (9:1)
S66	2-Propanol–ammonia–water (10:1:1)
S67	Cyclohexane–acetone (4:5)
S68	Dichloromethane–acetone–acetic acid (8:1:1), acetone–toluene–acetic acid (2:2:1), dichloromethane–toluene–acetic acid (2:2:1), toluene–benzene–acetic acid (2:2:1), dichloromethane–heptane–acetic acid (2:2:1), ethyl acetate–dichloromethane–acetic acid (8:1:1), ethyl acetate–acetic acid (49:1), acetone–acetic acid (97:3), dichloromethane–acetone–acetic acid (5:4:1), acetone–chloroform–heptane–acetic acid (3:3:3:1) and toluene–benzene–dichloromethane–acetic acid (3:3:3:1)
S69	Organic solvents, mixtures of water and acids
S70	Dichloromethane–methanol (99:1) and acetonitrile–water (85:15)–3% sodium chloride
S71	Chloroform–acetone (6:1)
S72	Dichloromethane
S73	Hexane impregnated with paraffin oil in light petroleum (8%), in acetonitrile–acetone–methanol–water (40:18:40:2), acetone, hexane–diethyl ether–acetone (7.5:2:0.5) or hexane–diethyl ether (8:2)
S74	Chloroform–diethyl ether (5:2) and hexane–acetone (20:1)
S75	Benzene–ethanol (9:1), chloroform–ethyl acetate (9:1), chloroform–ethyl acetate–acetone (17:2:1), light petroleum–acetone (7:3), chloroform–cyclohexane–ethyl acetate (15:2:3) and chloroform–ethyl acetate–acetone (13:5:3)
S76	Pentane–diethyl ether
S77	Methanol (25%)–potassium iodide–butanol (80:15:5)
S78	Carbon tetrachloride and hexane–acetone (95:5)
S79	Hexane–ethyl acetate (3:1)
S80	Dichloromethane–acetone–acetic acid (8:1:1), acetone–toluene–acetic acid (2:2:1), benzene–ethanol–acetic acid (6:3:1), acetone–light petroleum–acetic acid (2:2:1), acetone–methanol–acetic acid (6:3:1), acetone–diethyl ether–acetic acid (2:2:1), dichloromethane–toluene–acetic acid (2:2:1), acetone–chloroform–acetic acid (2:2:1), toluene–benzene–acetic acid (2:2:1), dichloromethane–heptane–acetic acid (2:2:1), dichloromethane, dichloromethane–acetone–benzene (2:1:1), acetone–benzene–acetic acid (5:4:1), dichloromethane–heptane–ethanol (2:2:1), chloroform–hexane–dichloromethane (1:1:1), chloroform, diethyl ether–toluene (1:1:1), hexane–heptane–ethanol (2:2:1), dichloromethane–light petroleum (2:3) and toluene–heptane–diethyl ether (1:1:1)
S81	Chloroform–methanol (1:1)
S82	Hexane–acetone (3:1), benzene–acetone (6.6:3.4), hexane–acetone (9:1) and hexane–acetone (4:1)
S83	Benzene–ethyl acetate–water (5:4:1), benzene–ethyl acetate–acetic acid (15:4:1) and toluene–ethyl acetate–acetic acid (25:15:2)
S84	Hexane–acetone (4:1) or hexane–diethyl ether (2:1), hexane–benzene (1:1) or hexane–toluene (1:1), benzene–methanol–acetone (7:1:2) or benzene–methanol–diethyl ether (7:1:2.5)
S85	Hexane–acetone (90:10), hexane–acetone (150:45), chloroform–nitromethane (100:100), chloroform–acetic acid (190:10) and benzene–hexane–acetic acid (50:100:20)
S86	Distilled water
S87	Hexane–dioxane–acetic acid (79:20:1), hexane–dioxane (80:20), hexane–dichloromethane (30:70) and chloroform–diethyl ether (80:20)

TABLE 1 *(continued)*

Detection	
D1	$SnCl_2$ in 50% HCl–aqueous fuchsin dye solution
D2	3-Methylbenzidine, N,N′-dimethylbenzidine, N,N′-tetramethylbenzidine and 3,5,3′,5′-tetramethylbenzidine in presence of sunlight
D3	Ammoniacal silver nitrate solution and densitometric scanning
D4	*o*-Tolidine reagent and densitometric scanning ranging from 300 to 900 ng
D5	Preadsorbed $AgNO_3$ and UV light
D6	1,4-Dihydroxybenzene
D7	Pyrolysis technique with IR laser and electron capture
D8	Iodine
D9	Cholinesterase
D10	$KI_3 + KI$ solution
D11	Mass spectrometry
D12	Ammonium molybdate (15%) in HNO_3 (2:1)
D13	$PdCl_2$ or *p*-dimethylaminobenzaldehyde
D14	Copper(II) acetate in dilute HCl followed by KI
D15	*In situ* densitometry
D16	Acetylcholinesterase
D17	*p*-Dimethylaminobenzaldehyde–acetic acid (10:1)
D18	4-Amino-N,N′-dimethylaniline · 2HCl
D19	Swine liver hommogenate–alcoholic *β*-naphthol acetate
D20	Palladium(II) chloride–iodine
D21	KOH–*p*-nitrobenzenediazonium fluoroborate
D22	Dimethylaminobenzaldehyde, *p*-nitrophenyldiazomine or 2,6-dibromo-N-chloroquinoimine
D23	Iron(III) chloride (1%) in butanol and 2,4-dinitrophenylhydrazine (3%) in chloroform–methanol (3:1)
D24	$CuCl_2$ (1%, w/v) followed by metavanadate or potassium hexacyano–ferrate(III) (0.5%, w/v) in sodium hydroxide (0.5%, w/v)
D25	Diphenylamine
D26	Diazotized *p*-nitroaniline or diazotized *p*-aminoacetophenone
D27	Fluorescamine
D28	Ninhydrin
D29	Hill reaction inhibition detection technique
D30	*o*-Phthalaldehyde in 7 *M* sulphuric acid and ethanol (10%) and UV (350 nm)
D31	Bromophenol blue
D32	Silver nitrate and UV light
D33	UV light (365 nm) or densitometry
D34	Reflection–absorption photometry at 240 nm
D35	UV light and *o*-tolidine–Mitchells' reagent
D36	4,4′-Tetramethyldiaminodiphenylmethane
D37	Fluorimetry
D38	Photolysis, morin derivatization and fluorescence
D39	Dragendorff reagent
D40	Dithionite
D41	Microbioassay using *C. cucumerinum* spores
D42	Chloroplast homogenate and 2,6-dichloroindophenol, exposure to white neon light
D43	Aqueous K_2CO_3 (10%) and diazotized *o*-dianisidine solution or orthanilic acid or *o*-dianisidine or diazotized orthoanilic acid
D44	Aqueous sodium hydroxide (20%) followed by nickel aminine reagent [aqueous nickel chloride solution (5%, w/v)–ammonia (30%) (1:1)]
D45	3,5,3′,5′-Tetramethylbenzidine (0.2%)
D46	Differential-pulse polarography
D47	Gas chromatography with electron-capture detection

(Continued on p. 276)

TABLE 1 *(continued)*

Detection	
D48	$Hg(NO_3)_2$ + diphenylcarbazone, iodine, $HgNO_3$ and heating, acidic potassium permaganate
D49	Ammonia solution–water (1:4) followed by beef liver homogenate, indoxyl acetate, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$
D50	Magnesium chloride (5%) followed by N,2,6-trichlorobenzoquinonimine (0.3%)
D51	N,2,6-Trichlorobenzoquinonimine
D52	Triethanolamine exposed to mercury lamp, 254 nm
D53	Diazotization with sodium nitrite solution after thermal degradation with 4-aminoantipyrine in presence of ammonium persulphate (betanol), or *p*-dimethylaminobenzaldehyde (asulam, betanol), bromophenol blue reagent or *o*-toluidine after N-chlorination (eptane, tillam, yalan), Dragendorff reagent or sulphuric acid (roneet)
D54	UV quenching, NaOH, $AgNO_3$, *p*-nitrobenzenediazonium tetrafluoborate, fisetin and enzyme inhibition
D55	Rhodamine B–UV, *p*-dimethylaminobenzaldehyde, potassium permanganate, silver nitrate + bromophenol blue, pinacriptol yellow–UV, sodium fluoresceinate–UV, sodium fluoresceinate–UV (modified method), iodine vapour and iodine spray
D56	2,6-Dibromo-N-chlorobenzoquinonimine (0.5%) or N,2,6-trichlorobenzoquinonimine (Gibbs reagent) in acetic acid
D57	Diazotized *p*-nitroaniline
D58	*p*-Nitrophenyldiazonium chloride
D59	Methanolic potassium hydroxide (1%) followed by *p*-nitrobenzenediazonium tetrafluoroborate (0.1%) in acetone
D60	Sodium nitrite in 1 *M* hydrochloric acid (1%) followed by N-(1-naphthyl)ethylenediaminedihydrochloride (1%) in 2 *M* hydrochloric acid
D61	Ethanolic Fast Blue B (1%) followed by sodium hydroxide (20%), UV light (254 nm), 2,6-dibromoquinonechlorimide (0.5%) in dimethylformamide
D62	Autoradiography using Kodak X-ray film and analyte was eluted with aqueous ethanol (50%), 10 ml toluene–Triton X-100 (2:1) + 2,5-diphenyloxazole (4 g/l) and 1,4-bis(5-phenyloxazdyl-2) benzene (0.1 g/l), radioactivity measured using Philips PW 4540 liquid scintillation spectrometer
D63	3,5-Dichloro-*p*-benzoquinonechlorimine
D64	Fluorescence quenching or UV
D65	Scanning or autoradiography
D66	Silver nitrate–2-phenoxyethanol, 4-(4-nitrobenzyl)pyridine (NBP), 2,6-dibromobenzoquinone-N-chloroimine (DBBC) and its analogue
D67	Reflectance densitometry
D68	Flame ionization detection
D69	Spectrodensitometry
D70	UV light
D71	Phosphomolybdic acid
D72	GC
D73	Enzyme (mouse liver), then substrate containing β-naphthyl ethyl ester
D74	Palladium chloride or dibromo-4-chlorimide, 4-(4-nitrobenzyl)pyridine reagent
D75	Ammonium molybdate reagent
D76	Spectrophotometry
D77	Indoxyl acetate and human serum, exposed to 366-nm UV irradiation, fluorimetric scanning
D78	Zeiss ERI 65m spectrometer and integrator
D79	Enzyme (pig liver acetone powder), sprayed with 1-naphthylacetone solution followed by *p*-nitrobenzenediazoniumfluoroborate in acetone
D80	UV at 254 nm or 2,6-dichloroquinonechloroimide or indoplatinate reagent
D81	Inhibition of esterase, 1-thionaphthyl acetate and 2,2′-azo(1-naphthol-8-chloro-3,6-disulphonic acid) 4,4′-diphenyl disulphide, as post-chromatographic reagent
D82	Ammoniacal silver nitrate solution (0.5%) in aqueous acetone (1:3)
D83	*o*-Tolidine plug potassium iodide
D84	2-Methylthioacridone solution

TABLE 1 *(continued)*

Techniques, pesticides, etc.	
AMDTLC	Automated multiple development thin-layer chromatography
BPMC	2-*sec*.-Butylphenyl N-methylcarbamate (osbac)
C	Colorimetry
C_8	C_8 alkyl-bonded silica gel
C_{18}	C_{18} alkyl-bonded silica gel
2-D	Two-dimensional
2,4-D	2,4-Dichlorophenoxyacetic acid
DDD (mixed)	2,2-Bis(chlorophenyl)-1,1-dichloroethane and related compounds
o,p'-DDD (2,4'-DDD)	1-(*o*-Chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane
p,p'-DDD (4,4'-DDD)	2,2-Bis(*p*-chlorophenyl)-1,1-dichloroethane
o,p'-DDE (2,4'-DDE)	1-(*o*-Chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethylene
p,p'-DDE	2,2-Bis(*p*-chlorophenyl)-1,1-dichloroethylene
DDT (mixed)	Dichlorodiphenyltrichloroethane (mixture of metabolites of *ca.* 80% *p,p'*- and 20% *o,p'*-)
o,p'-DDT (2,4-DDT)	1-(*o*-Chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane
p,p'-DDT (4,4'-DDT)	1,1-Bis(*p*-chlorophenyl)-2,2,2-trichloroethane
DNOC	2,6-Dinitro-*o*-cresol
DDP	Differential-pulse polarography
EI	Enzyme inhibition
EPTC	S-Ethyldipropylthiocarbamate
FID	Flame ionization detection
GC	Gas chromatography (gas–liquid chromatography)
α-HCH (α-BHC)	Hexachlorocyclohexane (α-isomer)
β-HCH	Hexachlorocyclohexane (β-isomer)
γ-HCH (lindane)	Hexachlorocyclohexane (γ-isomer)
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
IAA	Indole-3-acetic acid
FC (IPC)	Isopropyl carbanilate
IPRPTLC	Ion-pair reversed-phase thin-layer chromatography
Llt	Lower limit of detection
MCPA	4-Chloro-2-methylphenoxyacetic acid (isooctyl ester)
MDTLC	Multiple-development thin-layer chromatography
MIPC	2-Isopropylphenyl-N-methylcarbamate (Isocarb)
MS	Mass spectrometry
β-NPA	β-Naphthaleneacetic acid
β-NPXA	β-Naphthoxyacetic acid
OC	Organochlorine
OP	Organophosphorus
OPTLC	Over-pressurized thin-layer chromatography
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PFB	Pentafluorobenzyl derivatives
ppb	Parts per billion (w/w)
ppm	Parts per million (w/w)
R_F	Migration distance relative to solvent front
RP	Reversed-phase
RTLC	Rod thin-layer chromatography
STLC	Sequential thin-layer chromatography
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
TCA	Trichloroacetic acid
TLC	Thin-layer chromatography
TGTLC	Temperature gradient (30–40°C) thin-layer chromatography
UV	Ultraviolet

TABLE 2

APPLICATION OF TLC TO PESTICIDE ANALYSIS

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Abate	Environmental water samples; RPTLC; determination	P45	S52	D50	89
Abate, actellic, anthionon, aphox, bromophos, chlorofos, cyanox, etaphos, heptrophos, malathion, parathion-methyl, phozalon, phthalophos, Rogor, ricid and trichlormethaphos	HPTLC–EI, determination (Llt = 0.001–0.01 mg/)	P43	S82	D81	90
Abscisic acid	TLC; separation	P36	–	–	91
Abscisic acid	TLC; determination (Llt = 5 ng)	P28	S83	D5	92
Aearicides fungicides and insecticides	Fresh and processed apples; 2-DTLC; determination	P21	S37	D70/D31	93
Actellic and basudin(II)	Green, dry tobacco leaves; TLC–GC; determination (Llt = 0.5 μg, 0.1 mg/kg)	–	S44	D72	94
Acifluorfen, bifenox, chloroxuron, diphenoxuron, fluorodifeny, fomesafen, nitrofen and oxyfluorfen	Mixturex; STLC; separation	P32	S35	D69	95
Aldicarb, Baygon, bendiocarb, BPMC, carbaryl, carbofuran, MIPC, zineb and ziram	TLC; separation	P26	S64	–	96
Aldicarb, Baygon, carbaryl, carbofuran, lannate, mancozeb, thiram, zineb and ziram	*Post mortem* material; TLC; detection, separation (Llt = 0.5 μg)	P20	S65	D61	97
Aldicarb, carbaryl, diuron and propoxur	Fruits vegetables; HPTLC; detection	P20	S56	D54	98
Aldrin, α-BHC, dieldrin, heptachlor, heptachlor epoxide and methoxychlor	RPTLC; detection, determination	P11	S3	D4	99
Aminocarb and its major metabolites	TLC–EI, separation	P20	S22	D28	100
Amino acids, bile acids, hormones and pesticides	2-DTLC; detection	P18	–	–	101
Anilide, carbamate and urea herbicides	TLC; determination	P13	–	D15	102
Aphox	Air, water and soil; TLC–GC; determination (Llt = 0.5 μg, 0.5 mg/kg, 0.05 mg/m^3)	P40	S46	D32	103
Aromatic acid herbicides residues	Plant material; TLC; determination	P20	S24	D32	104
Arylcarbamates (aniline, asulam, bental, Carbyne, chlor-IFC, *m*-chloroaniline and IFC), phenylurea derivatives (3,4-dichloroaniline, diuron and dosanex), thiocarbamates (diptal, Eptam, roneet and yolan)	TLC–GLC; determination (Llt = 10 μg, 0.025 μg)	P4	S55	D53	105
Asulam and its degradation products	Soil; TLC; determination	P20	–	–	106
Asulam and its degradation products	HPTLC; determination (Llt = 2–200 ppb)	P20	–	D60	107
Asulam, sulphanilamide and sulphanilic acid	TLC; detection (Llt = 10 ng)	P28	S63	D27	108
Atrazine	Drinking water, ground water; TLC; determination (Llt = 20 ng)	P20	S29	D36	109

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Atrazine, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, α-HCH, γ-HCH, metaphos, phosalone, phosphamide, prometryn and simazine	Environmental samples; 2-DTLC; GC; detection, determination	P40	S74	D72	110
Atrazine and simazine	Water and sewage; TLC; determination (Llt = 0.1 $\mu g/dm^3$)	P20	S31	–	111
Azinophos-ethyl, azinophos-methyl, coumaphos, diazinon, dimethoate, disulfoton, ethion, fenchlorphos, Malathion, oxydemeton-methyl, parathion, parathion-methyl and phorate	TLC; detection, separation, determination	P28	S43	D48	112
Azinophos-ethyl, diazinon, parathion-methyl and malathion	TLC; separation	P20	S14	D51	113
Bendiocarb	Workplace air; TLC; determination	P20	S17	D22	114
Benthiocarb, drepamon and yolan	Environmental samples; TLC; determination	–	–	–	115
Benzoate derivative of pentachlorophenol	TLC; detection	P37	–	D45	116
Benzoic acid, cinnamic acid, 2,4-D, IAA, β-NPA, β-NPXA, TCA and 2,4,5-T	STLC; separation	P7	S21	D31	117
Biphenyl residues, anilide carbamate and urea	Citrus; TLC; determination	P20	–	D15	118
Bolstar residues	Plants, soil and water; TLC–GC; detection, determination (Llt = 0.01 mg/kg)	P41	S47	–	119
4-Bromo-2,5-dichlorophenol, debromoleptophosoxon, leptophos, *o*-methylphenylphosphonothioate and phenylphosphoric acid	STLC; separation	P39	S5	D8	120
Bromophos residues	Peanut crops; TLC; detection	P20	S53	D52	121
Buturon, chlortoluron, diuron, fenuron, isoproturon, methabenzthiazuron, metonuron, monuron and neburon	TLC; separation	P16	S25	D15	122
Butylate, chlorfenvinphos, cycloate, *p,p'*-DDE, *p,p'*-DDT, dimethoate, EPTC, fenthion, lindane, mevinphos, molinate, parathion-methyl and trichlorphon	TLC; detection, determination	P14	S73	D66	123
Captafol, captan, Difolatan and folpet	Water, lettuce, apples; TLC; determination	–	D15	124	
Carbamates and OP residues	Fruits vegetables; TLC; detection, separation	–	–	–	125
Carbamates, phenylureas and triazines	Drinking water; HPTLC–AMD; determination	P12	–	D15	126
Carbaryl	TLC; detection (Llt = 1 μg)	P20	–	D24	127
Carbaryl residues	Apples; TLC; determination	P20	S61	–	128
Carbaryl	TLC, detection (Llt = 5 μg, 1 μg, respectively)	P21	–	D26	129
Carbaryl	Water; TLC; detection, determination (Llt = 20 μg, 40–200 μg, respectively)	P21	S62	D59	130

(Continued on p. 280)

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Carbaryl and related compounds	TLC; detection, determination	P21	S21	D59	131
Carbaryl, dichlorvos and malathion	TLC–EI; determination	P21	–	D9	132
Carbendazin	TLC; separation, semi-quantitation	P21	–	–	133
Carbofuran	Fresh water, plant tissues, soil; TLC; separation	P20	–	D21	134
Carbofuran and quinalphos	Air; TLC; determination	P20	–	–	135
Carboxylic acid herbicides	TLC, 2-D TLC; separation	P7	S21	D31	136–138
Carboxylic acid herbicides	TLC; determination	P1	S69	D31	139, 140
Carboxylic acid herbicides	TLC; separation	P2	S21	D31	141
Carboxylic acid herbicides	TLC; separation	P40/P27	S23	–	142
Carboxylic acid plant growth regulators	TLC; separation	P6	S21	D31	143
Chlorbromuron, chloroxuron, linuron, methoxuron, metribuzine, prometryne, simazine, terbuthylazine and terbutryne	TLC; detection, determination	P40	S75	D42	144
Chlorocaragard, metazine and methoxy-caragard herbicides	Air; TLC; determination	P40	–	D72	145
Chlorocholine chloride (plant growth regulator)	Grain, grain products; TLC; determination (Llt = 0.1 mg/kg)	P8	S34	D39	146
Chlorophenols	Water;TLC; detection	P20/P40	–	–	147
Chlorophenoxy acid herbicides (2,4-D, 2,4,5-T) and triazines	TLC; determination	P17	S38	–	148
Chloropyrifos and its metabolites	TLC; determination	P20	–	D5	149
Chlorpropham	Onions; TLC; determination	–	–	–	150
Curacron or selecron residues	Environmental samples; TLC; determination (Llt = 1–2 μg)	P40	S46	–	151
Cyanox	Plant samples; TLC; determination	–	–	–	152
Diazinone, dimethoate, ethion, malathion, parathion and parathion-methyl	TLC; detection, determination (Llt = 0.03 μg)	P35	S8	D12	153
Diazinone, eptam, γ-HCH, lenacil, phen-medipham and phosphamide	TLC, 2-D TLC; identification, separation (Llt = 0.5–1 μg)	P41	S84	–	154
Dicarboximide fungicides, iprodion, procymi-done and vinclozolin	Drinking water; HPTLC; determination	P12/P35	–	D34	155
2,4-D, MCPA, 2,4,5-T and their PFB bromide derivatives	TLC; separation	P35/P5	S68	D63	156
DDT	TLC; detection	P20	–	D6	157
p,p'-DDT and dieldrin	Soil; TLC; determination	P3	–	–	158
DDD, DDE, DDT, β-HCH, lindane and methoxychlor	TLC; detection (Llt = 0.20, 0.20, 0.20, 0.20, 0.25 and 1.00 μg, respectively)	P35	S1	–	159
p,p'-DDE, *p,p'*-DDT, methoxychlor and parathion	Spiked samples; TLC; separation	P20	S78	–	160
Dichlorvos, dimethoate, malathion and phosphamidon	TLC; mobility	P44	S86	D82	161
Dicrotophos, ethion, fensulfothion, oxydeme-ton-methyl, phorate, phosmet, phospholan and trichlorfon	TLC; detection, separation	P20	–	D3	162
Dieldrin derivatives	TLC–GC; identification	P20	–	D72	163
Diflubenzuron urea herbicide	TLC; determination (Llt = 0.1 μg per spot, 2 ng/g)	P42	–	D3	164

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Dimethoate, dimethoate oxygen analogue, dioxathion, disulfoton, fonogos, fonogos oxygen analogue and oxydemetonmethyl	STLC; separation	P20	–	–	165
DNOC	Urine; TLC; identification	P20	–	–	166
DNOC and dinoseb	Water; TLC–DPP; determination	P20	–	D46	167
Disulphoton, disulphoton oxygen analogue, fenthion, fenthion oxygen analogue, phorate and phorate oxygen analogue	TLC; detection, determination	P28	S49	D48	168
Disuphoton, monocrotophos and quinalphos	TLC; identification, determination (Llt = 10 μg)	–	–	D14	169
O-Ethyl-O-4-ntrophenyl phenylphosphonothioate and related compounds	STLC; separation	P39	S6	D8	170
Endrin	TLC; detection	P20	–	D1	171
Endosulphan	Biological materials in forensic toxicology; TLC; detection (Llt = 1 μg per spot)	P21	S44	D44	172
Fenitrothion	Water; TLC–EI; detection, determination	P21	S41	D79	173
Fenitrothion	TLC–EI; determination	–	–	D9	174
Fenitrothion and parathion	TLC; detection, determination	–	–	–	175
Fluchloralin, formothion, malathion and thiometon	TLC; mobility	P44	S86	D82	176
Flurecol	Formulations; TLC; detection, separation	P20	–	D15	177
Flucythriate insecticide residues	TLC; separation	P42	S39	D70	178
Flungicides	TLC; detection	P20	–	D41	179
Furadan and its metabolites	Biological materials (blood, urine); TLC; determination (Llt = 0.5 μg)	P40	–	D57	180
Furadan and its metabolites	Air; TLC; determination	P20	S59	D58	181
Gammacarbatox (carbaryl–lindane mixture)	Potatoes; TLC–EI; detection (Llt = 0.1 ppm, 1, pbb, respectively)	P20	S60	D25	182
Gardona (tetrachlorvinphos) residues	Apples; TLC; determination	P20	S50	D77	183
Gardona residues	Water, fish tissues; TLC; determination (Llt = 0.01 mg/l, 0.1 mg/kg)	P41	S54	–	184
Gibberelin A_3 and $A_4 + A_7$	Fermentation broths; HPTLC; determination	P35	S67	D15	185
Glyphosate and its metabolite (aminoethylphosphonic acid)	TLC; determination	–	–	–	186
Glyphosate and its metabolite (aminoethylphosphonic acid)	Water; TLC; determination	P8	–	–	187
Halogenated synthetic pyrethroid insecticide, *cis* and *trans* isomers of permethrin and cypermethrin from fenvalerate and decamethrin	Fruits, plants, soil and tomato; TLC; detection (Llt = 0.10 mg/l)	P40	S4	D5	188
Hazardous phenols	TLC; separation	P21	S36	D43	189
Herbicides	TLC; detection	P20	–	D29	190
Herbicides	TLC; separation	P31	–	D67	191

(Continued on p. 282)

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Herbidice residues	TLC; identification	P20	–	D15	192
Herbicide residues	Agricultural crops, food, soil and water; TLC; determination (Llt = 1–10 μg/kg)	P20	–	D29	193
Herbicides	Sugar beet and sugar; TLC; determination	P20	–	–	194
Herbicides and related compounds	RTLC; determination	P9	S76	D68	195
Hexazinone metabolites	Rat-liver microcosmes, peanut seedlings, sugarcane; TLC–MS; determination	–	–	D11	196
Imidan and its degradation products, N-hydroxymethylphthalimide imidoxon, phthalic acid and phalmic acid	TLC; separation	P22	S9	D8	197
Indole-3-acetic acid	TLC; detection	P20	S66	D62	198
Indol-3-acetic acid	TLC; detection (Llt = 5–100 ng per spot)	P20	–	D30	199
Ioxynil residues	Animal tissues; TLC; determination (Llt = 0.1 ppm)	P29	–	–	200
Isouron and its metabolite	TLC; determination (Llt = 0.25–1.5 μg per spot)	P21	S71	D34	201
MCPA and its soil metabolites	TLC; detection, separation	P31	S80	D63	202
MCPA and its two metabolites	Soil, water; TLC; determination	P35	S72	D25	203
MCPA and terbacil	Apples; TLC–GC; determination	P20	–	D15/D47	204
Mecarbam and its degradation products	Crops; TLC–GC; determination	–	–	–	205
Mephosfolan, phosfolan and related compounds	TLC; separation	–	–	–	206
Methamidophos	Potato tubers and foliage; STLC; detection, separation	P40	S13	D19	207
Methamidophos	TLC–EI; detection; identification (Llt = 15 ng)	P20	S51	D49	208
Methanearsonic acid	Rice, soil; TLC; detection, separation	–	–	–	209
Methidathion and methoxychlor	Clinical samples; TLC; RPTLC; identification	P20	–	–	210
Methomyl	Serum and urine; RTLC; determination	P20	–	D68	211
Methomyl, parathion, parathion-methyl and sumithon	TLC; separation, determination	–	S12	D76	212
Methoxuron and its breakdown product (3-chloro-4-methoxyaniline)	Potato, soil and water; TLC; HPLC; determination (Llt = 0.02, 0.2, 0.001 mg/kg, respectively)	P45/P28	S70	D33	213
Methylnitrophos and its metabolites (fenitrooxon and *p*-nitrocresol)	Grain; TLC; detection, determination (Llt = 0.1, 0.1, 0.005 mg/kg, respectively)	P26	–	D17	214
Monocrotophos	TLC; detection	P21	S44	D10	215
OC	HPTLC; separation	P20	–	–	216
OC	TLC; detection (Llt = 0.20 μg)	P20	–	D2	217

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
OC	TLC–GC; identification, determination	P20	–	–	218
OC	TLC; separation	P20	S2	D3	219
OC	TLC; detection	P20	–	D7	220
OC, petroleum distillates, PCBs, phenols and explosives	TLC; determination	P20	–	–	221
OC and OP	Animal tissues; TLC–EI; determination	–	–	D16	222, 223
OP and its metabolites	TLC–MS; detection, determination	P15	–	D11	224
OP residues	Dried fruits; TLC; determination, separation	P20	–	–	225
OP	HPTLC; determination	P35	S7	D74	226
OP	Vegetables and human blood; TLC; determination	P24	S48	–	227
OP (warfare agents)	2-D OPTLC; separation	P20	–	D15	228
OP	TLC; separation	P20/P3	S11	–	229
OP	Water; HPTLC; separation, identification	P20	–	D84	230
OP residues	TLC; detection (Llt = 100 ng)	–	–	D75	231
OP (sulphur-containing OP)	TLC; detection (Llt = 1 μg)	–	–	D10	232
OP residues	Vegetables and toxicological investigation; TLC; detection, separation	–	–	–	233, 234
OP	TLC; separation	P25	–	–	235
OP	Sewage sludge and drinking water; TLC–GC; R_F values	–	S16	–	236
Organotin compounds	HPTLC; determination	P20	–	–	237
Paraquat	Marijuana; TLC; determination	P20	–	D40	238
Paraquat	Plasma, urine; RTLC; determination	P10/P34	–	D68	239
Paraquat	Biological samples (blood, urine and tissue samples); TLC; determination	P38	S77	D39	240
Paraquat and related compounds	MDTLC; separation	–	–	–	241
Parathion	Crops; TLC–C; detection, determination	–	–	D18	242
Parathion and its metabolite (paraoxon)	Crops (rice); TLC–EI; detection, determination	P28	S42	D9	243, 244
Parathion residues and its metabolite (paraoxon)	Crops; TLC–EI; determination (Llt = 10^{-10} g)	P28	S79	D73	245
Pesticides (crop protection agents)	Drinking water, ground water; AMDTLC; STLC; detection, determination	–	–	–	246
Pesticides	Drinking water, ground water, surface water at trace level; AMDTLC; detection, determination	P35	–	–	247
Pesticides (acidic, basic, neutral)	TLC; 2-D TLC; detection, separation (Llt = 0.5–2 μg)	P21	S85	–	248

(Continued on p. 284)

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Pesticides (predominantly fungicides and insecticides)	Standard solutions; HPTLC; detection, determination	–	–	–	249
Pesticides	Multi-residue analysis to confirm GLC results; HPTLC; detection, determination	P35/P19	–	–	250
Pesticides	TLC; detection	P33	–	D70	251
Phenoxy acid herbicides	IPRPTLC; detection, separation (Llt = 20–60 μg)	P7	S21	D31	252
Phenoxycarboxylic acid herbicides and organic acids	Drinking water; TLC; determination	P20	S69	D15	253, 254
Phenylcarbamate residues	Carrots, potatoes, wine; TLC; determination (Llt = 0.1 ppm)	–	–	–	255, 256
Phenyltin fungicides	TLC; detection, separation (Llt = 0.01 ppm)	P20	S33	D38	257
Phenylureas	TLC; determination	–	–	–	258
Phosmethylan and its major metabolite	Crops, meat, milk, soil, water; TLC; detection (Llt = 500 ng)	P20	S87	D13/D83	259
Propamocarb	Peppers; TLC; determination	P21	S57	D55	260
Pyramine herbicide residues	Soil; TLC; detection, separation (Llt = 0.02 ppm)	–	–	–	261
Pyrazophos	Plant products; TLC; separation, determination	–	–	D76	262
Radiolabelled pesticides	Soil surface; TLC; mobility	P20/P44	–	D65	263
Rodenticides and vitamins	TLC; separation (Llt = 1–8 μg)	P20	–	D71	264
Semicarbazone herbicides	TLC; RPTLC; separation	P20	S19/S20	D23	265
Tebuthiuron and related compounds	TLC; mobility	–	–	–	266
Terbufos and its four oxidative metabolites	TLC; separation	P35	S15	D20	267
Thiabendazole	Fruit, peel; TLC; determination	P20	–	D37	268
N,N′-Bis(1,3,4-thiadiazol-2-yl)methanediamine residues	Rice; TLC; determination	P20	–	-	269
Thiocarbamate herbicides	Foods; TLC; determination	P20	–	–	270
Thiocarbamate herbicides and their sulphoxide and silufon metabolites	TLC; detection	P40	S58	D56	271
Thiocarbamic acid	TLC; separation, detection (Llt = 0.01–0.02 mg/l)	P40	S18	D14	272
2-Thiouracil, 4(6)-methyl-2-thiouracil and 6(4)-propyl-2-thiouracil	Feed additives, biological materials; TLC; separation, identification	P28	S81	D80	273
Toxaphene	Bee honey; TLC; determination (Llt = 0–100 μg per spot)	P20	–	D78	274
Toxaphene	Surface water, standard solutions, water; TLC; determination (Llt = 3 μg)	P20	S40	D3	275
Triazine residues	Water; TLC; detection, determination (Llt = 10 ppb)	P20	–	D32	276
Triazines	TLC; separation	P20	S26	D35	277
Triazines	TLC; determination	P28	S32	D64	278
Triazine herbicides	TLC; separation	P40/P28	S27/S28	D70	279

TABLE 2 *(continued)*

Pesticide(s) studied	Sample; comments	Stationary phase	Mobile phase	Detection	Ref.
Triazine herbicides (degradation product)	TLC; separation	–	–	–	280
Triazine herbicides	TLC; R_F values	P30	–	–	281
Triazine herbicides	Milk; OPTLC; determination (Llt = 5 μg/kg, 10 μg/kg)	P20	S30	–	282
Triazine derivatives	RPTLC; detection, separation	P29	–	–	283
Triazine derivatives	TLC; determination	P10	–	–	284
Triazine and urea herbicides	Water; TLC; determination	P20	–	D29	285
Trichlorometaphos-3 residues	Grain; TLC; determination (Llt = 1–20 μg)	P20	S45	D3	286
Urea and carbamate herbicides	Drugs; TLC; separation	–	–	–	287
Urea herbicides	Drinking water; determination	P20/P11/P3	–	D64	288
Valexon residues	Grains, vegetables; 2-D TLC; determination	P23	S10	D14	289
Zineb	Foliage; TLC; determination	P20	–	–	290

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Review

Chromatographic methods in the determination of herbicide residues in crops, food and environmental samples

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ABSTRACT

The state of the art of chromatographic methods used in the determination of herbicide residues in crops, food and environmental samples is reviewed. The main structural groups of herbicides, *i.e.*, triazines, phenyl- and sulphonylureas, carbamates, uracils and phenoxyalkanoic and arylphenoxypropanoic acids, and important degradation products (dealkylated triazines, substituted anilines, chlorophenols) are considered. Advantages and drawbacks of gas (GC), liquid (LC) and thin-layer chromatography in this type of analysis are discussed. The characteristics of a modern chromatographic method for the determination of herbicide residues are summarized and trends in the development and combination of current GC and LC methods discussed.

CONTENTS

1. Introduction . . . 291
2. Analytical methods for herbicide residues . . . 292
  2.1. Triazines . . . 292
  2.2. Phenylureas . . . 296
  2.3. Carbamates, uracils, pyridazines . . . 297
  2.4. Phenoxyalkanoic acids . . . 297
  2.5. Aryloxyphenoxypropanoic acids . . . 298
  2.6. Sulphonylureas . . . 298
  2.7. Diquat and paraquat . . . 299
3. Present trends in the analysis of herbicide residues . . . 299
4. Conclusions . . . 301
References . . . 301

1. INTRODUCTION

Modern agricultural production depends considerably on the use of pesticides, especially in the major agricultural countries of North America and Europe. In most of them, herbicides represent more than 50% of all pesticides used; in the USA and Germany the proportion of herbicides is *ca.* 60%. In the USA alone over 10^8 ha are currently being treated with herbicides, which is more than half of the total cropland [1]. It is therefore not surprising that herbicides contribute significantly to the con-

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tamination of the environment, particularly of soil and surface and ground waters.

In drinking and environmental waters, atrazine belongs to the most frequent contaminants [2–4]. Owing to the phytotoxic nature of herbicides and their low mammalian toxicity, their residues in crops generally do not present serious risks, but contamination of some food commodities by carry-over through contaminated water and feed has been observed. In feeding experiments, transfer of herbicide residues to milk has been reported for compounds of the uracil group [5,6], triazines [7] and, to some extent, phenoxyalkanoic acids [8]. Low levels of triazines, primarily atrazine, have been found in dairy milk [9,10] and butter [10] and even in sugar [11]. There are indications that atrazine may occur in the human organism [12]. Even though in general the risk of humans ingesting toxic doses of herbicide residues in food seems low, it is important to monitor their levels in the environment and in food commodities because of their extensive use and documented occurrence both in the environment and in foods.

2. ANALYTICAL METHODS FOR HERBICIDE RESIDUES

The general characteristics of analytical methods for residues of herbicides and their degradation products are the same as those for other pesticide residues. The analysis involves sampling and sample handling, for which the recommended approaches are described in refs. 13 and 14, extraction and clean-up procedures [15–17], the determination and evaluation and interpretation of the results. The individual steps of the analytical procedure are designed according to the chemical structure of the analyte compounds and according to the character of the matrix.

The detection and determination limits required for routine analytical methods for herbicide residues should not be higher than 10–50% of the corresponding maximum residue limit (MRL) as recommended by Frehse [18]. This puts the highest requirements on methods for the determination of residue in drinking water where the maximum permissible levels are sometimes as low as 0.1 $\mu g\ l^{-1}$.

The present trends in the development of residue analysis are towards multi-residue methods with adequate recovery characteristics (over 80% as a rule, but not less than 70%), good reproducibility and low determination limits. These are methods that permit the simultaneous determination of herbicides of different structural types, *e.g.*, triazines and ureas, or the simultaneous determination of parent herbicidal compounds and their degradation products, such as triazines and dealkylated and/or hydroxytriazines, phenylureas and substituted anilines, phenoxyalkanoic acids and chlorophenols.

Chromatographic methods, in particular capillary gas chromatography (cGC) and high-performance liquid chromatography (HPLC), are the methods of choice for this purpose. Thin-layer chromatography (TLC), which was popular in late 1960s and the 1970s has been almost completely superceded by the more precise, faster and more convenient instrumental chromatographic techniques, but in special cases it can be of valuable help.

In this paper we review the development of analytical methods for herbicide residues over the last 5–7 years. The herbicidal compounds considered are listed in Table 1 and their classification according to the chemical structure is given in Table 2. The analytical methods for the individual structural groups are reviewed in the sections 2.1–2.7 and are summarized in Table 3.

2.1. Triazines

Triazines belong to the oldest and most commonly used herbicides. Data from the world pesticide market show that the greatest volume (*ca.* 30%) of all herbicides applied in agriculture can be attributed to *s*-triazines [116]. Consequently, and also because of their relative stability in the environment, they also belong to the herbicides most frequently found in environmental samples. This is reflected in the vast number of published methods for the determination of triazine residues. More recently, the use of triazines, especially atrazine, is being limited and they are gradually being replaced with less environmentally hazardous herbicides.

Symmetrical 1,3,5-triazines are well chromatographed by GC and give good responses with nitrogen–phosphorus detection (NPD), owing to the nitrogen atoms in their molecules. Therefore, GC–NPD, usually on DB-1, OV-1 or polyethylene glycol-based stationary phases, is the method mostly

TABLE 1

ALPHABETIC LIST OF THE HERBICIDAL COMPOUNDS REVIEWED AND REFERENCES RELATING TO THEIR RESIDUE ANALYSIS

Common name	Class (see Table 2)	Refs.
Ametryn	Ia	9, 26, 27, 39
Atrazine	Ia	7, 9, 10, 19, 26, 27, 30, 31, 39, 41, 74, 96
Bensulphuron-methyl	VI	83
Bentazone	X	63, 73, 74, 96, 112
Bromacil	VIII	5, 57, 60, 61, 74, 96
Buturon	II	34, 38
Chlorbromuron	II	31, 32, 34, 41, 42, 47, 49, 74
Chloridazone	IX	19, 31, 56, 59, 61, 74, 96
Chlorimuron-ethyl	VI	84
Chloroxuron	II	30, 31, 34, 37, 38, 47, 49, 74, 96
Chorpropham	III	30, 32, 61, 74, 96
Chlorsulphuron	VI	49, 82, 86, 88, 89, 90
Chlortoluron	II	30–32, 34, 36, 38, 39, 47, 49, 74
Cyanazine	Ia	7, 9, 10, 27, 30, 39, 41, 74
Desmedipham	III	31, 55
Desmetryn	Ia	7, 9, 26
Dichlorprop	IV	62–64, 66, 73, 74
Diphenoxuron	II	34, 74
Diquat	VII	91–95
Diuron	II	30, 32, 34, 36, 38, 47, 48, 74
Fenoprop (2,4,5-TP)	IV	62, 64–66, 69, 74, 96
Fenuron	II	30, 34, 36, 38, 39, 47
Fluazifop-butyl	V	61, 76–80
Fluometuron	II	34, 36, 47, 74
Haloxyfop-ethoxyethyl	V	63, 76, 77
Isoproturon	II	30, 31, 34, 36, 37, 38, 41, 47, 74
Lenacil	VIII	19, 31, 58, 61
Linuron	II	30–32, 34, 36, 38, 39, 41, 42, 47, 49, 74, 96
MCPA	IV	62–66, 68, 69, 72–74
MCPB	IV	62, 63, 65, 73, 74, 96
Mecoprop (MCPP)	IV	62–64, 66, 68, 69, 72–74, 96
Metabenzthiazuron	II	30, 31, 38, 39, 54
Metamitron	Ib	29, 30, 56, 61
Metobromuron	II	30, 34, 36, 38, 42, 47
Metoprotryn	Ia	9, 26, 39
Metoxuron	II	30, 31, 34, 36, 37, 38, 39, 41, 46, 47, 74, 96
Metribuzine	Ib	26, 28, 30, 31, 41, 61, 74, 96
Metsulphuron-methyl	VI	90
Monolinuron	II	30–32, 38, 39, 41, 42, 47, 49, 74, 96
Monuron	II	34, 36, 38, 47, 74, 96
Neburon	II	34, 38, 39, 47, 48, 74
Paraquat	VII	91–95
Phenmedipham	III	31, 32, 53–56
Prometryn	Ia	7, 9, 10, 26, 27, 30, 31, 39, 41
Propazine	Ia	9, 26, 27, 30, 39, 74
Propham	III	96
Quazalofop-ethyl	V	76, 77
Siduron	II	47
Simazine	Ia	7, 9, 10, 26, 27, 30, 31, 39, 41, 74
Sulphometuron-methyl	VI	85

(Continued on p. 294)

TABLE 1 *(continued)*

Common name	Class (see Table 2)	Refs.
Terbacil	VIII	6
Terbutryn	Ia	7, 9, 10, 26, 27, 30, 31, 41
Terbuthylazine	Ia	7, 9, 30, 31, 74
Thiazafluron	II	31, 54
2,4-D	IV	62–66, 68, 69, 72–74, 96
2,4,5-T	IV	62–66, 68, 69, 74, 96
2,4-DB	IV	62, 64–66, 73, 74, 96
2,4,5-TB	IV	74
2,4-DP	IV	65, 66, 68, 69, 72

used for determining their residues. Recently published methods involve almost exclusively capillary GC columns. Víden *et al.* [9] determined residues of triazines in forage and milk, the identity of the residues being confirmed by GC–mass spectrometry (MS). Tekel' and co-workers used GC–NPD on OV-1 for the determination of triazine residues in butter [10] and sugar [19]. There are several methods permitting the simultaneous determination of the parent compounds and their degradation products, *e.g.*, those described by Bardalaye and co-workers for the determination of prometryn in parsley [20] and ametryn in tropical root crops [21], or the method [22] for the determination of terbutryn and its metabolites in sorghum grain. In the last study, the identity of the residues was confirmed by MS.

TABLE 2

STRUCTURAL GROUPS OF HERBICIDAL COMPOUNDS

Class	Structural group
Ia	1,3,5-Triazines
Ib	1,2,4-Triazines
II	Phenylureas
III	Carbamates
IV	Phenoxyalkanoic acids
V	Aryloxyphenoxypropanoic acids
VI	Sulphonylureas
VII	Bipyridylium cations
VIII	Uracils
IX	Pyridazines
X	Others

LC offers another possibility for the determination of residues of triazines. Ultraviolet (UV) detection is very suitable as *s*-triazines exhibit strong absorbance at 220–240 nm. The chlorotriazine herbicides atrazine, cyanazine and simazine and their dealkylated degradation products have been determined in soil by LC with diode-array detection (DAD) and by GC–NPD [23]. GC–MS and thermospray LC–MS were employed as confirmatory characterization techniques. With LC–DAD, the detection limit was much higher than with GC–NPD (0.3–0.5 mg kg^{-1} *vs.* 5–10 μg kg^{-1}), but the other advantages of LC–DAD, such as the possibility of choosing different wavelengths to avoid matrix interferences, and partial degradation of some chlorotriazines under GC conditions, were pointed out. A lower detection limit in the LC–UV determination of eight triazines in soil, *i.e.*, 1 μg kg^{-1}, was reported by Battista *et al.* [24]. They used a special extraction–isolation procedure on two minicolumns connected in series. A one order of magnitude lower sensitivity for LC–UV determination compared with GC–NPD was also reported by Hajšlová *et al.* [7] in a comparative study of chromatographic methods for the determination of *s*-triazines in milk. Moreover, an additional clean-up step had to be included prior to the LC–UV determination. The detection limit with GC–MS was comparable to that achieved with GC–NPD.

In the determination of residues of triazines in water, the detection limit of the method seems to depend more on the isolation and enrichment procedure chosen than on the method adopted for the

TABLE 3

MULTI-RESIDUE METHODS FOR THE DETERMINATION OF DIFFERENT HERBICIDE GROUPS IN ENVIRONMENTAL AND FOOD MATERIALS

Herbicide group	Method	Commodity	Ref.	Notes (Derivatization)
1,3,5-Triazines	GC–NPD (SE-30)	Milk	9	
1,3,5-Triazines	GC–NPD (OV-1)	Butter	10	
1,3,5- and 1,2,4-triazines Dealkylated atrazine Phenylureas Carbamates	LC–DAD ($RP\ C_{18}$)	Water (ground, drinking, surface)	30	
1,3,5-Triazines Phenylureas Uracils Pyridazone Carbamates Phenoxyalkanoic acids	Environmental ($RP\ C_{18}$)	LC–DAD water	74	
Phenylureas Substituted anilines	GC–ECD. GC–NPD (CP Sil 5) LC–ECD ($RP\ C_{18}$)	Environmental samples	33, 34	Hydrolysis on silica gel HFBA
1,3,5- and 1,2,4-triazines Dealkylated products of atrazine	GC–NPD (DB-17)	Water	26	
Phenylureas Substituted anilines	GC–NPD (SE-54)	Water	42	
Phenylureas	GC–NPD (DB-5)	Water	36	Methyl iodide
Phenylureas	LC–photodegradation ($RP\ C_{18}$)	Crops	47	After UV photodegradation, OPA (postcolumn)
1,3,5- and 1,2,4-triazines Phenylureas Carbamates Uracils Pyridazone Bentazone	TLC on silica gel	Crops Foods Water Soil	31	Only for inhibitors of Hill reaction
Phenoxyalkanoic acids Chlorophenols	GC–ECD (SE-54)	Cereal grain	68	PFBB
Aryloxyphenoxypropanoic acids (esters, free acids)	GC–ECD (HP-5)	Crops	77	PFBB
Phenoxyalkanoic acids	GC–NPD (DE-1 or DB-5)	Water Soil	72	CEMDSDEA
1,2,4-Triazines Carbamates Uracils Pyridazone Aryloxyphenoxypropanoic acids (esters)	GC–MS	Crops	61	

final determination. When classical liquid–liquid extraction with methylene chloride was used followed by clean-up on a Floriril column and GC–NPD determination, a detection limit of 25 ng l^{-1} was obtained for eleven triazines [25]. Grandet *et al.* [26] reported a detection limit of <100 ng l^{-1} for the GC–NPD determination of triazines and their metabolites in drinking water after liquid–liquid extraction. On the other hand, a detection limit of <10 ng l^{-1} was achieved in the determination of seven triazine herbicides in drinking water and ground water when solid-phase extraction (SPE) was employed [27].

The non-symmetrical 1,3,4-triazines can be also determined by GC–NPD. Jarczyk determined metribuzine in water, soil, cereals and vegetables [28] and metamitron in soil, water, sugar and fodder beet, strawberries and peas [29]. Metribuzine and metamitron, along with several *s*-triazines, were determined in water by LC–DAD [30].

2.2. Phenylureas

The use of this herbicide group is growing, partially because they are gradually replacing the more persistent triazine herbicides. The lower stability of phenylurea herbicides contributes to their faster degradation in crops and the environment but it also makes their analysis more complicated.

For the determination of both phenylurea herbicides and their degradation products, substituted anilines, GC or HPLC methods are almost exclusively used. TLC with selective biochemical detection [31] has a limited applicability to the parent compounds only.

The GC determination of phenylurea herbicides has to cope with the problem of thermal instability of these compounds. This is usually overcome either by derivatizing them to more stable products or by hydrolysing them to their corresponding anilines, which are subsequently measured. The latter approach was used by Dornseiffen and Verwaal [32], who determined the anilines obtained by alkaline hydrolysis of the parent herbicides. The anilines were determined after bromination to 2,4,6-tribromo derivatives by GC with electron-capture detection (ECD). The method is not suitable for the determination of metoxuron and difenoxuron but it covers some carbamate herbicides. De Kok and co-workers [33,34] developed a technique of catalytic hydrolysis on silica gel. The anilines obtained were again determined by cGC–ECD following derivatization with heptafluorobutyric anhydride (HFBA). The anilines originally present in the sample were determined in parallel. The advantage of this approach is the possibility of determining degradation products (anilines) in addition to parent herbicides. However, most methods involve derivatization of the phenylureas and the use of GC–NPD. Ogierman [35] used derivatization with trimethylanilinium hydroxide (TMAH), Oehmichen *et al.* [36] alkylation with methyl iodide and Pérez *et al.* [37] alkylation with ethyl iodide. Stan and Klaffenbach [38] determined phenylurea herbicides by GC–ECD after derivatization with HFBA. To avoid derivatization of both the phenylureas and the substituted anilines prior to the final GC determination, attempts have been made to find conditions for direct GC analysis. This was first done by Deleu and Copin [39] for the parent compounds only and later by Böer *et al.* [40] for the substituted anilines in water. Tekel' and co-workers [41,42] established conditions for the simultaneous determination of seven phenylureas and four anilines in water by GC–NPD without derivatization.

All urea herbicides can be determined by HPLC. Without derivatization and after thorough clean-up of the extracts, determination limits in the range 0.015–0.02 mg kg^{-1} could be achieved for plant materials using UV detection [43–45]. Three linuron metabolites, including 3,4-dichloroaniline, could be determined simultaneously with the parent compounds [45]. The sensitivity of the analysis can be improved by derivatizing the analytes and using a selective detector. Fluorescence detection was used by Lantos *et al.* [46] for the determination of metoxuron in potatoes, soil and water. The compound was first hydrolysed and the product converted into a fluorescent derivative with dansyl chloride. Luchtefeld [47] inserted a module for photodegradation of the separated phenylureas between the LC column and the fluorescence detector. The photodegradation products were then derivatized with *o*-phthalaldehyde (OPA). Limits of detection for the six phenylureas investigated ranged between 0.001 and 0.006 mg kg^{-1} for eight different crops and the limits of determination between 0.003 and 0.022 mg kg^{-1}. Zahnow [48] used photocon-

ductivity detection (PCD) in the LC determination of linuron, diuron and three diuron metabolites in crops with a detection limit of 0.01 mg kg^{-1}. An improved sensitivity of LC–UV measurement was reported for the micro-HPLC technique used in the determination of linuron and monolinuron in milk [49]. Liu *et al.* [50] determined the residues of six phenylureas in fruits and vegetables by LC with thermospray MS single-ion monitoring.

2.3. Carbamates, uracils, pyridazines

In earlier reviews [51,52], information was summarized on analytical methods for carbamate pesticides in general, which, apart from herbicides, include insecticides, acaricides and fungicides. Only a small proportion of analytical work on carbamate residues concerns the carbamate herbicides. The most important carbamate herbicides are phenmedipham, desmedipham, propham and chlorpropham. Bromacil, lenacil and terbacil are uracil-type herbicides and chloridazone belongs to the pyridazine group. Their residues are determined mostly by GC.

Dornseiffen and Verwaal [32] included propham, chlorpropham and phenmedipham in a multi-residue method for herbicides that generate anilines on alkaline hydrolysis. The corresponding anilines are determined by GC–ECD after bromination. The method has been tested for the determination of herbicide residues in various crops, with a detection limit of *ca.* 0.01 mg kg^{-1}. Alkaline hydrolysis to *m*-toluidine has been also used in a method [53] for the determination of phenmedipham in spinach, but in this instance the *m*-toluidine was determined directly without derivatization by GC with flame ionization detection (FID). A determination limit of 0.03 mg kg^{-1} was reported. Stan and Klaffenbach [54] used GC–MS for the determination of thermolabile carbamates (phenmedipham) and ureas (metabenzthiazuron, thiazafluron) after derivatization with acetic anhydride. Residues of desmedipham and phenmedipham in drinking water were determined by LC–UV after enrichment by SPE [55]. LC–DAD was applied to residues of phenmedipham and chloridazone in soil [56].

GC–NPD was described for the determination of bromacil residues in strawberries [57], lenacil in sugar beet roots and tops [58] and in sugar [19] and chloridazone residues in sugar beet [59] and sugar [19]. Residues of bromacil [5] and terbacil [6] in milk were determined by GC–ECD. Goewie and Hogendoorn [60] determined residues of bromacil and other herbicides in well water by LC–UV. Tuinstra *et al.* [61] worked out a multi-residue–multi-matrix method for the determination of nitrogen-containing herbicides. The method, which is based on GC–MS determination, has been evaluated for bromacil, lenacil, chlorpropham, chloridazone, fluazifop-ethyl, metamitron and metribuzine.

2.4. Phenoxyalkanoic acids

Phenoxyalkanoic acids are the oldest group of synthetic herbicides, introduced in agriculture as early as the 1940s. They still retain an important position, especially in the control of weeds in cereal crops.

Because of their highly polar nature and low volatility, phenoxyalkanoic acids cannot be directly determined by GC at residue levels and they have to be derivatized to esters, usually methyl or pentafluorobenzyl (PFB) esters. Chlorophenols, which are important degradation products of phenoxyalkanoic acids, are derivatized to the corresponding methyl and PFB ethers. Methylation is conveniently done with methanol and sulphuric acid [62,63]. Diazomethane is an efficient methylating agent [63,64] but less convenient for toxicity reasons. The residues in the form of methyl esters are determined by GC–ECD [63] or GC–MS [62], which is less demanding with respect to the clean-up and has a lower determination limit. Derivatization with pentafluorobenzyl bromide (PFBB) has been reported [65–68]. This method results in a higher sensitivity of GC–ECD analysis, but a comparative evaluation showed that the results obtained with the methylation method were in general more reliable. The PFB method may be advantageous if lower detection limits are required and if a narrower GC–ECD quantification range can be tolerated [66]. Other derivatization agents have been used for phenoxyalkanoic acids, such as trifluoroethanol [69], acetyl chloride [70] and iodoethane [63]. Derivatization with 2-cyanoethyldimethyldiethylaminosilane (CEDMSDEA) has been reported for use with GC–NPD [71] and was applied to the determination of acidic herbicides in water and soil [72]. The advantage of this method is an almost instantaneous

formation of the CEDMSDEA derivative and its detectability by NPD, which is much more selective than ECD.

Phenoxyalkanoic acids in water were also determined by HPLC with simultaneous UV, fluorescence and electrochemical detection [73]. The herbicides could be detected at levels between 20 and 90 ng l^{-1} without the necessity for derivatization. Di Corcia and Marchetti [74] determined phenoxyalkanoic acids and other herbicides (triazines, ureas, carbamates, uracils) in environmental waters by LC–UV. Novel clean-up techniques for a polymeric precolumn for the subsequent determination of eight phenoxy acid herbicides and bentazone in surface water by HPLC–UV were described [75]. Detection limits of 50–100 ng l^{-1} were reported and, owing to automation, the total analysis time was *ca.* 30 min.

2.5. *Aryloxyphenoxypropanoic acids*

Esters of aryloxyphenoxypropanoic acids are a new series of highly selective post-emergence herbicides often termed "phenoxyphenoxys". In the treated plants they decompose fairly rapidly, yielding the corresponding free acids as the main metabolites. Fluazifop-butyl, haloxyfop-methyl and -ethoxyethyl, quazalofop-ethyl and others belong to this group.

The number of studies dealing with trace analysis for aryloxyphenoxypropanoates in plant materials is limited. The residues are hydrolysed to their corresponding acids directly in the matrix and then extracted together with the free acids present as degradation products. The free acids are converted into methyl esters by methylation with diazomethane and determined by GC–MS or GC–ECD [76,77]. For ECD, fluazifop esters had to be brominated prior to GC analysis. NPD detection was also used [77,78], but the determination limit was higher (0.05 mg kg^{-1} for NPD, 0.01 mg kg^{-1} for ECD and MS).

Worobey and Shields [79] determined fluazifop-butyl and fluazifop acid using LC with oxidative amperometric detection (LC–AD). Fluazifop-butyl was hydrolysed to fluazifop acid prior to the extraction, similarly to the procedures used with GC analysis, but no methylation of the free acid was needed for the LC separation. Extracts of soybeans and soybean oil could also be analysed using LC–UV detection and no adverse effects of co-extracted compounds were observed; however, the sensitivity was approximately one order of magnitude less than with LC–AD where the limit of detection was $\leqslant 0.01$ mg kg^{-1}. To improve the sensitivity of detection, fluazifop-butyl was derivatized with 4-bromoethyl-7-methoxycoumarin to give a fluorescent derivative that was determined by HPLC [80]. A detection limit of 0.5 ng for the derivative was reported, but no real samples were analysed with this method.

2.6. *Sulphonylureas*

Herbicides of the sulphonylurea group were developed by DuPont in the 1970s for weed control in cereal crops. They are characterized by high effectiveness, resulting in low application doses, usually of the order of 10–150 g of active ingredient per hectare. Their herbicidal properties, mode of action, degradation and persistence in soil were thoroughly reviewed by Blair and Martin [81].

Owing to the low application doses, low residue levels in soil, water and crops can be expected. Hence methods for residue analysis should exhibit an adequate sensitivity.

For the determination of the residues of chlorsulphuron in cereal crops, Slates [82] developed an LC method with photoconductivity detection. The detection limits were 0.01 mg kg^{-1} for grain and 0.05 mg kg^{-1} for straw and green plants. No residues were detected in grain and straw even at treatment up to 2240 g of active ingredient per hectare. In green plants, residues were detected shortly after the post-emergence treatment. The same author later determined the residues of bensulphuron-methyl in rice grain and straw by LC–photoconductivity detection (PCD) [83], with similar detection limits. Chlorimuron-ethyl was determined by LC–PCD in soybeans and some soybean rotational crops [84] and sulphometuron-methyl in fish and in green plants (alfalfa, corn, rice, wheat) [85].

For the analysis of sulphonyl urea herbicides in runoff water, a detection limit of $\leqslant 50$ ng l^{-1} is required. Ahmed [86] found this impossible to reach with LC–UV for chlorsulphuron and used GC–ECD. However, due to the polar nature of the compound, GC of chlorsulphuron was poor and

methylation with diazomethane was needed. Methylation conditions could be optimized to obtain mainly monomethyl chlorsulphuron and a detection limit of 25 ng l^{-1} was reached. The same principle was applied in the analysis of chlorsulphuron in soil [87]. In this case, the detection limit was 0.001 mg kg^{-1}.

A different approach was adopted by Long *et al.* [88] who determined chlorsulphuron residues in milk by GC–NPD. Chlorsulphuron was found to undergo a thermally induced decomposition to give 2-amino-4-methoxy-1,3,5-triazine which was detected and quantitated. The products of thermal decomposition of chlorsulphuron were characterized by GC–MS [89]. Cotterill [90] determined the residues of chlorsulphuron and metsulphuron-methyl by GC–ECD following derivatization with PFEB. The PFB derivative was characterized by GC–MS as N,N-bis(pentafluorobenzyl)-2-chlorobenzene sulphonamide. The method was more sensitive than those described above and was found to be suitable for the determination of these residues in soil and water. However, it was less successful in plant materials for which the clean-up method used was inadequate.

2.7. Diquat and paraquat

The bipyridinium derivative diquat and paraquat are widely used general non-selective weed killers. Both are quite toxic for man and warm-blooded animals. Owing to their cationic nature, bipyridinium herbicides are prone to sorption interactions and their displacement from the bonding sites of an organic matrix requires special conditions, mostly achieved by refluxing with strong sulphuric or hydrochloric acid. This results in large amounts of co-extractives which may interfere with the determination. The older methods were often based on spectrophotometric determination and lacked specificity and sensitivity. At present, LC methods are most commonly used. GC determination is only possible after conversion into volatile products.

Worobey [91] analyzed the residues of diquat and paraquat simultaneously in potatoes by HPLC–UV on a reversed-phase column. The method works with 5-g samples and a detection limit of approximately 0.05 ppm was achieved. Nagayama *et al.* [92] reported a detection limit of approximately 0.02 ppm for their method which was also based on reversed-phase LC–UV. The method which includes clean-up on an Amberlite CG-50 column is relatively simple and rapid and it was tested for a variety of crops (cereal grains, potatoes, peaches, cabbage). Chichila and Walters [93] developed a method with a detection limit of 0.01 mg kg^{-1} which was achieved by using pH-controlled silica SPE, clean-up of the hydrochloric acid (6 *M*) digest and ion-pairing LC–DAD for the final determination. The method is suitable for the analysis of high-moisture crops. For the analysis of diquat and paraquat in well water, Simon and Taylor [94] used HPLC–DAD after SPE on bare silica columns. Followig the direct detection with DAD, postcolumn reaction with sodium hydroxide and sodium hydrosulphite was performed and the derivatives were detected with a variable-wavelength UV detector. The detection limit of 0.1 $\mu g\ l^{-1}$ was achieved with 100-ml samples, 1 $\mu g\ l^{-1}$ can be detected in 20-ml samples.

For the GC analysis, diquat and paraquat have to be volatilized, usually by hydrogenation. Hajšlová *et al.* [95] analyzed diquat and paraquat in potatoes and rapeseed by GC–NPD and GC–MS following hydrogenation with sodium borohydride–nickel(II) chloride. Comparable detection limits (0.005 ppm) were achieved with NPD and mass fragmentography; for the analyses of rapeseed the latter method was preferred owing to higher selectivity.

3. PRESENT TRENDS IN THE ANALYSIS OF HERBICIDE RESIDUES

Multiresidue methods are a response to the demand for decreasing the cost of analyses and increasing the productivity of laboratories. Most such procedures have been developed for the particular structural groups of herbicides in different commodities. Multiresidue methods require universality of the isolation and clean-up procedure and, as far as possible, unification of the conditions of the chromatographic separation.

In isolation of residues, efforts have been devoted to optimize the extraction and clean-up procedures [15–17]. Apart from the classical solvent extractions, other processes are being introduced. In the determination of herbicides in water, SPE became generally accepted for all major herbicide groups [27,30,36,40, 55,74]. In addition to the regularly used C_{18}-bonded

silica cartridges, graphitized carbon black cartridges seem to be advantageous for specific applications [96].

This technique makes it possible to concentrate the residues so that levels below 0.1 $\mu g\ l^{-1}$ can be determined, 0.1 $\mu g\ l^{-1}$ being the maximum residue limit of many herbicides in drinking water [117]. In the determination of herbicide residues in solid matrices, supercritical fluid extraction (SFE) has recently been introduced [97–99]. This technique contributes to decreasing the use of hazardous organic solvents and to giving shorter extraction times. SFE can be coupled with cGC. With this on-line modification, lower detection limits may be reached.

For clean-up, gel permeation chromatography (GPC) is increasingly being used whereas traditional column chromatography on alumina, silica and Florisil, which had been almost ubiquitous in the earlier clean-up procedures, is gradually losing its exclusive position. Detailed information on the utilization of GPC on Bio-Beads SX-3 has been published [100–102].

Unification can be observed in the types of columns used for GC and also for HPLC. Wall-coated open-tubular (WCOT) columns are used for GC where capillary columns and operation with optimized temperature programming are currently a standard requirement. For the determination of herbicide residues, capillary columns with immobilized or cross-linked stationary phases are employed. WCOT columns of length 15–30 m and I.D. *ca.* 0.3 mm and with a stationary phase film thickness of 0.2–0.4 μm are most frequently encountered. Capillary columns with non-polar or low-polarity stationary phases (SE-30, SE-54, OV-1, DB-1, DB-5 or equivalent) dominate.

Of the different GC detector types, those used for pesticide residue analysis have been reviewed [103]. For herbicide residue analysis, the two detection methods most frequently used are nitrogen–phosphorus-selective detection (NPD) and electron-capture detection (ECD). Both can be used either for direct detection (the procedures not requiring derivatization), or after conversion of the analytes into suitable derivatives. The derivatization in turn is employed for two reasons: (1) to improve the chromatographic behaviour of the analyte (*e.g.*, for thermolabile or highly polar compounds), or (2) to increase the sensitivity and/or selectivity of the detection (*e.g.*, by introducing more halogen atoms into the molecule).

It is essential for a derivatization technique that well defined reaction product(s) are formed with the derivatizing agent in a reasonable time and in sufficiently high and reproducible yields.

NPD is routinely used for most herbicidal compounds and their important degradation products in crops, foods of plant and animal origin, soils and water. It predominates in the determination of residues of triazines and is often used for phenylureas and uracils. In the determination of residues of phenoxyalkanoic acids and aryloxyphenoxypropanoic acids, ECD is the method of choice. For some herbicides that have both nitrogen and halogen atoms in their structures, both NPD and ECD can be used.

LC is a good method for the determination of a wide variety of different herbicides, especially in water samples. DAD is effective for the identification of the compounds. LC–DAD after SPE can serve as a means of determining polar, non-polar or thermolabile compounds in a simple run. The universal UV detector is usually insufficiently selective for this purpose. Fluorescence detection is highly sensitive but pre- or postcolumn derivatization of the analyte to fluorescent products is necessary in most instance. In the LC determination of sulphonylurea herbicides, PCD proved useful [82–85]. This method is highly sensitive and selective for sulphur, halogens, nitrogen and phosphorus. In the determination of phenylurea herbicides and substituted anilines, LC–ECD has also been applied [33,34], but it requires derivatization and technical adjustment of the LC equipment. Recently, the combination of reversed-phase LC or GC with NPD has been described for the determination of herbicide residues [104]. The sensitivity and selectivity of the LC determination can be increased by column switching. Comprehensive information on the application of this technique in the HPLC of pesticide residues was presented by Hogendoorn *et al.* [105].

Most of the LC work on herbicide residues is done on a C_{18} reversed-phase. Amino- and cyano-bonded stationary phases are less common. Both isocratic and gradient elution are employed.

As indicated earlier, TLC, even though of only marginal importance in modern residue analysis,

may still be of value especially as an inexpensive routine screening method not requiring sophisticated instrumentation. Technical developments (automated sample application and other high-performance TLC techniques, densitometric evaluation) have contributed to the value of TLC results. The use of HPTLC for the identification and determination of pesticide residues was evaluated by Gardyan and Thier [106], but only a few of th *ca.* 150 compounds discussed are herbicides. They also used HPTLC for confirmation of the identities of the residues [107]. The separation of phenylurea and triazine herbicides has been optimized using overpressured layer chromatography [108]. TLC methods in pesticide residue analysis have been thoroughly reviewed by Sherma [109–111].

For the TLC of herbicide residues, biochemical detection based on their ability to inhibit the enzyme systems of isolated chloroplasts, known as the Hill reaction inhibition, proved to be very sensitive and selective. This biochemical detection even permits quantification by evaluating the dependence between the lifetime of the spots and the amount herbicide present in them. The utilization of this chronometric technique for the determination of herbicide residues in soil, water, food commodities and plant materials has been summarized [31]. Residues of herbicides inhibiting the Hill reaction can be determined by this method, *i.e.*, triazines, phenylureas, carbamates, uracils and pyridazone. The method has been used for the determination of bentazone in soil, water and crops [112] and of thiazafluron in drinking water [113].

4. CONCLUSIONS

Chromatographic methods are indispensable in the determination of herbicide residues. A variety of selective detectors permit the analysis of compound mixtures or mixtures of parent compounds and degradation products. cGC–NPD and –ECD are the dominant methods for routine control analyses. The use of LC is growing, especially for the analysis of less complex matrices, *e.g.*, water. In spite of technical improvements, TLC is losing importance and is used, if at all, as a screening method.

In research work, the mass-selective detector is indispensable for identity confirmation studies, especially using GC–MS, whereas LC–MS has so far been applied less frequently. For the characterization of the chromatographic and spectral properties of the compounds investigated, GC–Fourier transform infrared spectrophotometry (FT-IR) has been applied [114]. Two-dimensional chromatography broadens the potential of the GC method [115].

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Residue levels of polynuclear aromatic compounds in urban surface soil from Japan

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ABSTRACT

Samples of surface soil from urban areas of Japan were analysed for polynuclear aromatic hydrocarbons (PAHs). Concentrations of benzo[*a*]pyrene were in the range 12–225 μg/kg. Profiles of polynuclear aromatic materials were obtained by glass capillary gas chromatography. The overall distribution of PAHs is similar to the distribution of PAHs in air particulate matter and combustion sources. Benzanthrone is found at concentrations ranging from 10 to 160 μg/kg. The presence of benzanthrone and other polynuclear aromatic ketones also indicates that polynuclear aromatic materials in surface soil have originated from combustion sources. A comparison with air particulate matter showed that the concentrations of PAHs in air particulate matter are about 100-fold higher than in surface soil. Perylene seems to be more resistant to degradation in the soil environment than in air particulate matter. Subsurface layers of soil contained PAHs at concentrations below 2 μg/kg, which was the detection limit.

INTRODUCTION

Organic substances in soil can be translocated into plants. Therefore, knowledge of the presence of toxic or carcinogenic substances in soil is important [1]. Polynuclear aromatic hydrocarbons (PAHs) have been found in soil and soil-related samples like sediments, and the occurrence of PAHs in soil–plant systems has been investigated extensively [2–4].

Previous work has shown that PAHs released into the air with particulate matter from combustion sources occur together with polynuclear aromatic ketones (PAKs) and other derivatives of PAHs [5–8]. Since air particulate matter settles out and will be incorporated into the soil environment, polynuclear aromatic substances are transferred into surface soil by this process [9].

PAKs are more polar than PAHs and may be more rapidly dissipated by leaching and degradation. PAHs, however, are lipophilic and almost insoluble in water. They should be only slightly translocated from surface layers of soil by leaching. In order to obtain data on the fate of PAHs and PAH-related compounds, samples of surface soil obtained from urban areas in Japan are investigated in this work. Polynuclear aromatic compounds were isolated by clean-up on XAD-2 [10] in order to obtain unique information about PAKs occurring together with PAHs.

EXPERIMENTAL

Chemicals

n-Pentane was purchased from Nakarai (Kyoto, Japan). Ethanol and toluene were purchased from Wako (Osaka, Japan). All solvents were of chromatographic grade and used as supplied. Polycyclic aromatic hydrocarbons, 1-nitropyrene and benzan-

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throne (7-*H*-benz[*de*]anthracene-7-one) were also purchased from Wako and used as supplied.

Samples

Sampling sites were chosen in park areas in downtown Nagoya, Tomakomai and Sapporo, and on a field near Nagoya Airport. One sample was taken from each site at a depth of 0–3 cm. Further samples were taken from surface soil in a forest inside Nagoya University campus. The soil was then separated from residues of vegetation (grass). Samples were dried in a desiccator over silica gel at room temperature for 24 h, and transferred into glass fibre extraction thimbles. They were extracted by the Soxhlet method with a mixture of ethanol and toluene. Total carbon contents of the samples were determined after drying at 110°C using a Yanaco MT-500 C-N-corder (Yanagimoto, Kyoto, Japan).

Isolation of polynuclear aromatic compounds

Extracts were concentrated to 0.5 ml and subjected to clean-up on XAD-2. Sample extracts were transferred to the top of a column of XAD-2 (50–100 μm particle size, Serva Feinbiochemica, Heidelberg, Germany). The column had an internal diameter of 1.4 cm, and the height of the resin packing was 9 cm. The packing of the column was done with a slurry of XAD-2 in ethanol. Stepwise elution was carried out with ethanol, *n*-pentane and toluene. Polycyclic compounds were eluted with the toluene fraction, which was concentrated to 0.5 ml under vacuum (water aspirator) using a rotary evaporator. The concentrated extracts were directly subjected to capillary GC. Details of the analytical procedure are published elsewhere [11,12].

Gas chromatography

A Hitachi 263-50 gas chromatograph equipped with a flame ionization detector and adapted for capillary columns was used. Nitrogen was used as a carrier gas. The injection port was maintained at a temperature of 260°C, and the detector temperature was 300°C. A temperature programme from 110 to 260°C was chosen throughout the work, and the heating rate was 5°C/min. A fused-silica capillary column coated with SE-54 (25 m × 0.3 mm I.D., Gaskuro Kogyo, Tokyo, Japan) was used for all determinations by GC. The injection port was glass-lined and allowed split or splitless injections. All injections were done in the splitless mode.

RESULTS AND DISCUSSION

Polynuclear aromatic compounds were isolated by clean-up on XAD-2. This method allows a selective isolation of PAHs, PAKs and nitro- and other derivatives of PAHs [13]. Further determination of polynuclear aromatic compounds was carried out by capillary GC, and profiles of polynuclear aromatic compounds were obtained. One of these profiles from the surface soils is displayed in Fig. 1.

PAHs and PAKs were not detected in the subsurface layers of soil in urban areas. These data, however, do not prove that polar metabolites of PAHs cannot be translocated to deeper soil layers, which remains to be investigated in the future. PAHs and PAKs were also not detectable at concentrations above 2 μg/kg in surface soil from a forest in the Nagoya University campus grounds.

The qualitative compositions of polynuclear aromatic materials in the four soils analysed are similar. Several PAHs and benzanthrone are present in higher concentrations and are visible as major peaks in the gas chromatograms. There also are a large number of minor peaks present.

The patterns of polynuclear aromatic compounds found in the samples resemble the pattern of polynuclear aromatic compounds in air particulate matter. In the soils from downtown Nagoya and Sapporo, benzo[*a*]pyrene is present in a smaller amount than benzo[*e*]pyrene. This fact indicates a more rapid decomposition of benzo[*a*]pyrene than benzo[*e*]pyrene as both isomers are released in a ratio of 1:1 from most combustion sources. A ratio of benzo[*a*]pyrene and benzo[*e*]pyrene close to 1:1 is encountered in the soil sample from Nagoya Airport, and this ratio is also found in aircraft turbine particulate emissions [14].

Another remarkable result is the presence of benzanthrone among polynuclear aromatic compounds in all four surface soil samples. This indicates an origin of polynuclear aromatic compounds from combustion processes, where benzanthrone always accompanies PAHs.

Table I lists the absolute concentrations of some PAHs found in the four samples of surface soil investigated. Concentrations of PAHs in the sample

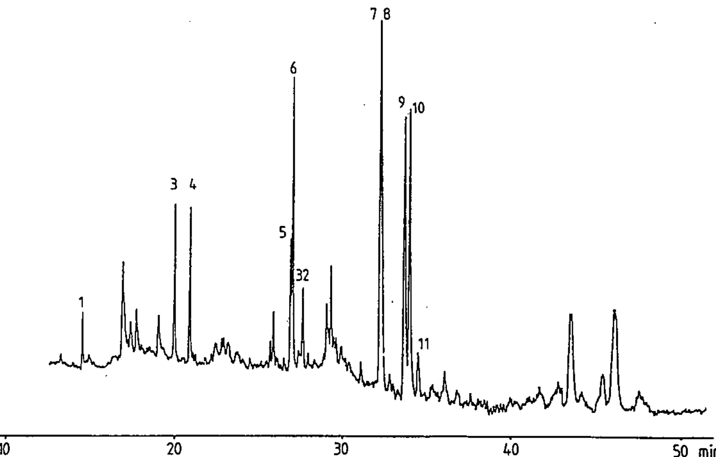

Fig. 1. Gas chromatogram of polynuclear aromatic substances found in surface soil from Nagoya Airport. GC conditions: stationary phase, SE-54; carrier gas, nitrogen; temperature programme, 110–260°C, 5°C/min; column, fused silica, 25 m × 0.3 mm I.D.; stationary film thickness, 0.2 μm. Peaks: 1 = phenanthrene; 3 = fluoranthene; 4 = pyrene; 5 = benz[*a*]anthracene; 6 = chrysene; 7, 8 = benzofluoranthenes; 9 = benzo[*e*]pyrene; 10 = benzo[*a*]pyrene; 11 = perylene; 32 = benzanthrone.

TABLE I

CONTENT OF SOME POLYCYCLIC AROMATIC COMPOUNDS IN SURFACE SOIL

Numbers in italics are relative amounts based on benzo[*e*]pyrene = 1.

Substance	Content (μg/kg)				
	Air particulate matter	Nagoya centre	Nagoya Airport	Sapporo centre	Tomakomai industry
Fluoranthene	7800	38	128	160	375
	4.9	*2.1*	*0.58*	*1*	*2.3*
Pyrene	54000	31	118	160	570
	3.4	*1.7*	*0.5*	*1*	*3.5*
Benz[*a*]anthracene	1500		99	160	203
	0.93	118	*0.45*	*1*	*1.2*
Chrysene	7400	*6.5*	225	320	345
	4.6		*1*	*2*	*2.1*
Benzofluoranthenes	27000	70	278	440	329
	1.7	*0.25*	*1.3*	*2.8*	*2*
Benzo[*e*]pyrene	16000	18	220	160	165
	1	*1*	*1*	*1*	*1*
Benzo[*a*]pyrene[a]	7000	12	225	100	120
	0.43	*0.7*	*1*	*0.63*	*0.73*
Perylene	500	<2	34	20	38
	0.03		*0.15*	*0.13*	*0.23*
Benzanthrone	2000	10	60	160	45
	0.13	*0.55*	*0.27*	*1*	*0.27*

[a] Benzo[*a*]pyrene is co-eluted with 6-*H*-benzo[*cd*]pyrene-6-one on SE-54, and quantitative values are the sum of both compounds.

from downtown Nagoya are as low as 10% of the concentrations found in the other three samples. The range of concentrations encountered was 10–500 μg/kg. This is 100 times lower than concentrations of PAHs in air particulate matter. This reduction in the concentrations of PAHs in surface soil compared with air particulate matter indicates that PAHs are not accumulated, but degraded in surface soil.

The concentrations of PAHs in air particulate matter were obtained by high-volume sampling and extraction of precipitated matter with toluene and clean-up on XAD-2 [15]. The quoted concentrations are average values encountered in urban air particulate matter, and individual values can be 10 times higher or lower [7,16,17].

Degradation of PAHs is also indicated if an excess of benzo[*e*]pyrene over benzo[*a*]pyrene is found in PAH profiles. While these isomeric benzopyrenes are released from most combustion sources in ratios close to 1:1, benzo[*a*]pyrene is less stable and disappears more rapidly in an environment where degradation of PAHs can take place. Perylene occurs in rather low concentrations in air particulate matter compared with benzo[*e*]pyrene, whereas in soil the ratio of perylene to benzo[*e*]pyrene is higher. This may indicate that perylene is more resistant to degradation in the soil environment than in air particulate matter. There was also a large number of minor polynuclear aromatic compounds present, many of which where not resolved as single components by capillary GC. Such components are probably isomeric or otherwise structurally related derivatives formed during primary degradation of PAHs in soil. Primary derivatization of PAHs can be the well known epoxide/diol formation [18]. Another interesting finding is the presence of PAKs among PAHs in the samples investigated. Their half-lives in soil are therefore similar to those of PAHs.

The concentrations of PAHs found in samples investigated here are of the same order of magnitude as concentrations of PAHs found in other samples of surface soil from Japan [19] or Norway [20]. Much higher concentrations of PAHs are found in surface soil from waste disposal sites [21] or railway ditches [22]. The concentrations of PAHs directly near a road with heavy automobile traffic in Great Britain were 10 times higher than the concentrations of PAHs reported here [23,24]. It should also be pointed out that present-day concentrations of PAHs are the result of a higher intake of PAHs by precipitation of air particulate matter, and overall concentrations of PAHs in surface soil are higher nowadays than they were 100 years ago [25–27].

The concentrations of PAHs are affected by the sampling depth, since concentrations in deeper layers of soil will be much lower. However, some accumulation of PAHs in deeper soil layers under exceptional circumstances has been reported [28]. The ratio of benzo[*a*]pyrene to benzo[*e*]pyrene in air particulate matter is often less than 1, since during atmospheric exposure benzo[*a*]pyrene is degraded more rapidly than benzo[*e*]pyrene. The concentrations of benzo[*a*]pyrene were also lower than concentrations of benzo[*e*]pyrene in all samples of surface soil investigated here.

Table II lists relative retention times of some compounds based on benzo[*e*]pyrene, which had a retention time of 33.5 min.

TABLE II

RELATIVE RETENTION (α) OF IDENTIFIED COMPOUNDS BASED ON BENZO[*e*]PYRENE

Substance	α
Penanthrene	0.432
Fluoranthene	0.569
Pyrene	0.623
Benz[*a*]anthracene	0.800
Chrysene	0.804
Benzofluoranthenes	0.957
Benzo[*e*]pyrene	1.000
Benzo[*a*]pyrene	1.011
Perylene	1.025
Benzanthrone	0.821

CONCLUSION

The profiles of PAHs in surface soil from urban areas in Japan show a distribution of polynuclear aromatic compounds similar to air particulate matter. PAKs are also present, which indicates that half-lives of such compounds in surface soil are similar to those of PAHs. Perylene seems to be more resistant to a degradation in the soil environment than in air particulate matter.

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CHROMSYMP. 2701

High-performance liquid chromatographic method for the determination of oxolinic acid residues in crops[☆]

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ABSTRACT

A procedure for the high-performance liquid chromatographic (HPLC) determination of oxolinic acid in crops was examined. Oxolinic acid was extracted with acidic methanol and re-extracted into dichloromethane. After successive clean-up by liquid–liquid partition and Sep-Pak silica column chromatography, oxolinic acid was determined by HPLC with fluorimetric detection (excitation at 270 nm, emission at 370 nm). The HPLC column was Inertsil ODS and the eluent was 1 m*M* tri-*n*-octylamine in 0.45% citric acid–tetrahydrofuran–acetonitrile (7:1:2, v/v/v) (pH 2.9). The limit of detection was 0.01–0.02 ppm and the recoveries from crops (spiked with 0.5–1.0 ppm, w/w) were 78–95%. The method was shown to be applicable to residue analysis of oxolinic acid in crops sprayed with Starner in fields.

INTRODUCTION

Oxolinic acd (5-ethyl-5,8-dihydro-8-oxo[1,3]-dioxolo[4,5-*g*]quinoline-7-carboxylic acid) is a synthetic antibacterial agent used in human and piscine medicine. Recently, oxolinic acid was found to be effective in agricultural disease control, *e.g.*, *Pseudomonas* and *Erwinia* species in rice crops; it was introduced as a fungicide by Sumitomo Chemical under the trade name Starner (Fig. 1).

Several methods for the determination of oxolinic acid, such as bioassay [1,2], fluorimetry [3] and high-performance liquid chromatography (HPLC) [4–8] have been described. However, these methods have been developed for the assay of pharmaceutical products and/or biological specimens, *e.g.*, plasma, urine and fish; none of the methods has been reported for residue analysis of oxolinic acid in crops.

HOOC O O CH$_2$ O N C_2H_5

Fig. 1. Structure of oxolinic acid.

This paper reports a simple, sensitive and specific HPLC method for the residue analysis of oxolinic acid in crops.

EXPERIMENTAL

Reagents and materials

Tri-*n*-octylamine, an ion-pairing reagent, was purchased from Tokyo Chemical Industry. A Sep-Pak silica cartridge (Part No. 20520, amount of silica gel 680 mg per cartridge) was obtained from Waters Assoc. Pure standard oxolinic acid was supplied by Sumitomo Chemical. The HPLC column, Inertsil ODS-2, was obtained from GL Sciences and the guard column, Brownlee RP-8, from Applied

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Biosystems. The organic solvents were of pesticide residue grade (or their equivalent) and all the other reagents were of analytical-reagent grade.

Apparatus

A Tri-Rotar SR-1 chromatograph (Japan Spectroscopic) with a fluorescence detector (Hitachi 650-10S) was used for HPLC.

Sample preparation

Brown rice and rice straw. Brown rice grain and chopped rice straw were ground to a powder with a pulverizer (Retsch). A subsample (20 g of brown rice and 10 g of rice straw) was placed in a 300-ml flask and 20 ml of water and 100 ml of methanol were added. After the contents had been blended with a Polytron homogenizer for 1–2 min, 10 ml of 12 *M* hydrochloric acid were added, and the flask was shaken mechanically for 30 min.

Other crops. Crops were homogenized with a mixer. A 20-g subsample was placed in a 300-ml flask, 10 ml of 12 *M* hydrochloric acid and 100 ml of methanol were added and the flask was shaken mechanically for 30 min.

Extraction

The contents were filtered through a filter-paper under suction. The filter cake and the flask were washed with 30 ml of methanol–12 *M* hydrochloric acid (10:1, v/v) and the washings were filtered. The filtrates were pooled and transferred into a 500-ml separating funnel. To the funnel, 200 ml of 5% sodium chloride solution were added, and oxolinic acid was extracted twice with 50 ml of dichloromethane by shaking for 5 min. The dichloromethane extract was filtered through a Whatman 1PS filter-paper and the filtrate was evaporated to dryness under reduced pressure in a water-bath at <40°C. The dried residue was dissolved in 5 ml of 4 *M* potassium hydroxide solution–methanol (1:3, v/v) and transferred into a 300-ml separating funnel. To the funnel, 50 ml of 10% sodium chloride solution and 50 ml of dichloromethane were added and the funnel was shaken for 5 min. After the dichloromethane layer had been discarded and the residual aqueous solution acidified wih 30 ml of 1.2 *M* hydrochloric acid, oxolinic acid in the aqueous solution was extracted twice with 50 ml of dichloromethane by shaking for 5 min. The dichloromethane extract was filtered through a Whatman 1PS filter-paper and the filtrate was evaporated to dryness under reduced pressure in a water-bath at <40°C.

Clean-up

The dried residue was dissolved in two 5-ml portions of dichloromethane and passed through the Sep-Pak silica cartridge. The cartridge was washed with 8 ml of acetone. After the cartridge had been acidified with 0.8 ml of 8.5% phosphoric acid, oxolinic acid in the cartridge was eluted with 15 ml of dichloromethane.

The eluate was evaporated to dryness under reduced pressure in a water-bath at <40°C. The dried residue was dissolved in 4 ml of 0.25 *M* potassium hydroxide solution–methanol (1:9, v/v) and a 10-μl aliquot (equivalent to 25 or 50 mg of the sample) was subjected to HPLC.

Samples used for the stability study of oxolinic acid in frozen samples were prepared as follows: crop homogenate or ground sample (10 or 20 g) was placed in a 300-ml flask and spiked with 10 μg of oxolinic acid. The flask was stoppered, shaken thoroughly and then stored in a freezer at −20°C. After storage, the samples were thawed in a water-bath at 22°C for 10 min, then analysed immediately.

HPLC determination

The HPLC conditions were as follows: column, Inertsil ODS-2 (150 mm × 4.6 mm I.D.); guard column, Brownlee RP-8 (30 mm × 4.6 mm I.D.); eluent, 1 m*M* tri-*n*-octylamine in 0.45% citric acid–tetrahydrofuran–acetonitrile (7:1:2, v/v/v) (pH 2.9); flow-rate, 1.0 ml/min; column oven temperature, 40°C; excitation wavelength, 270 nm; emission wavelength, 370 nm; and sensitivity setting, range 1, fine 5.

Standard solutions of oxolinic acid were prepared as follows: 50 mg of pure standard material were accurately weighed into a 100-ml volumetric flask and the flask was filled up to the mark with 0.25 *M* potassium hydroxide solution. This solution (0.5 mg/ml) was diluted in 0.25 *M* potassium hydroxide solution–methanol (1:9, v/v) to provide standard solutions of concentrations in the range 0.5–3.0 μg/ml.

A 10-μl aliquot of the standard solutions was subjected to HPLC. A calibration graph was pre-

pared by plotting the peak heights against the amounts of oxolinic acid injected. The amount of oxolinic acid in the sample extract was determined by comparing the observed peak height with the calibration graph.

RESULTS AND DISCUSSION

HPLC conditions

Oxolinic acid in the HPLC eluent has strong fluorescence with maximum emission at 370 nm, when excited at 270 and 340 nm. Excitation at 270 nm gives a 2–3 times higher sensitivity than at 340 nm when determined by HPLC.

As oxolinic acid is an organic acid, adjustment of the HPLC eluent is required for its determination. Acidification of the eluent (ion suppression) was not satisfactory as this resulted in tailing peaks, giving non-reproducible elution volumes and poor sensitivity (Fig. 2A). Addition of tetrabutylammonium phosphate or tri-*n*-octylamine to the eluent as an ion-pairing reagent resulted in a sensitive and reproducible determination (Fig. 2B and C). Tri-*n*-octylamine was better than tetrabutylammonium phosphate; the latter, widely used as an ion-pairing reagent, gave broad peaks resulting in lower sensitivity. Further, tetrabutylammonium salts are likely to be deposited in the HPLC line because of lack of solubility, hence requiring conditioning before use and washing after use.

The effect of the alkyl chain length of the trialkylamine on determination was investigated (Table I). Among the trialkylamine compounds tested as ion-pairing reagents, longer alkyl chain compounds gave sharp and symmetrical peaks of oxolinic acid, resulting in higher sensitivity. It was thought that longer alkyl chain compounds formed ion pairs with oxolinic acid, which behaved like a lipophilic compound and prevented irreversible adsorption to residual silanol. Adding tetrahydrofuran to the eluent improved the solubility of tri-*n*-octylamine in the eluent.

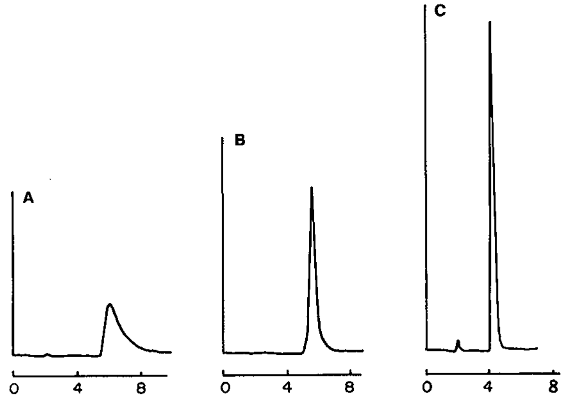

Fig. 2. HPLC determination of oxolinic acid. Detected peak: 20 ng of oxolinic acid standard. HPLC eluent: (A) 0.45% citric acid–tetrahydrofuran–acetonitrile (7:1:2 v/v/v) (pH 2.9); (B) 5 m*M* tetrabutylammonium phosphate/citric acid–tetrahydrofuran–acetonitrile (7:1:2 v/v/v) (pH 2.9); (C) 1 m*M* tri-*n*-oxtylamine/citric acid–tetrahydrofuran–acetonitrile (7:1:2 v/v/v) (pH 2.9). Retention times in min.

Clean-up

For clean-up of the crude extract, liquid–liquid partitioning and column chromatography were used.

TABLE I

EFFECT OF ALKYL CHAIN LENGTH OF ION-PAIR REAGENT ON HPLC DETERMINATION OF OXOLINIC ACID

Ion-paired reagent	Carbon number of alkyl chain	Content[a] (m*M*)	Relative peak height[b]	Retention time (min)[b]
Triethylamine	C_2	2	33	4.4
Tri-*n*-hexylamine	C_6	1	52	4.2
Tri-*n*-octylamine	C_8	0.5	76	4.2
		1	96	4.2
		2	100	4.2

[a] Content in HPLC eluent, 0.45% citric acid–tetrahydrofuran–acetonitrile (7:1:2, v/v/v) (pH 2.9).
[b] 20 ng of oxolinic acid standard were detected.

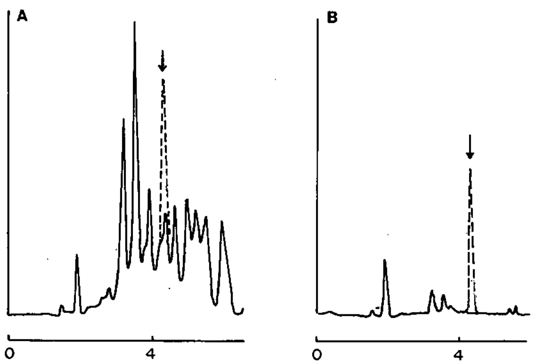

Fig. 3. Chromatograms of rice straw extract (A) before and (B) after clean-up with a Sep-Pak silica cartridge. A 25-mg rice straw sample was injected. Solid lines, control sample; dashed lines, control ground sample spiked at 1.0 ppm.

Oxolinic acid is an organic acid compound, so washing of the alkaline solution, which contained oxolinic acid, with dichloromethane, then extraction into dichloromethane after acidification of the aqueous layer was effective for the removal of co-extractives.

Further clean-up, however, was needed prior to HPLC determination. Silica gel column chromatography (Sep-Pak silica cartridge) was evaluated. Organic solvents were unsuccessful as eluents; oxolinic acid was adsorbed to tightly to the adsorbent that it could not be eluted with acetone or methanol. It was found that oxolinic acid could be eluted from silica gel with dichloromethane when the cartridge was acidified. The chromatographic procedure was as follows: the sample extract (dichloromethane solution) was loaded on the column and the column was washed with 8 ml of acetone. After the column had been fortified with 0.8 ml of 8.5% phosphoric acid, oxolinic acid on the silica gel was eluted with 15 ml of dichloromethane. This method was very effective for purification of the extract; acetone could be used for washing the column, so almost all co-extractives were eliminated (Fig. 3), while oxolinic acid was recovered quantitatively. For acidification of the Sep-Pak silica cartridge, 0.8 ml of acid solution was sufficient to fortify the adsorbent uniformly.

We have successfully applied this chromatographic method to the residue analysis of some acidic metabolites of pesticides.

Recovery and limit of detection

A known amount of oxolinic acid was added to the crop homogenate (or ground sample) and determined by the proposed procedure. The recoveries were between 78 and 95% at fortification levels of 0.5 or 1.0 ppm (Table II), and the limit of detection was 0.01–0.02 ppm; the minimum detectable amount was 0.5 ng and 25–50 mg of the sample was chromatographed. The method was also reproducible (Table II). Typical chromatograms are shown in Fig. 3B. Almost the same chromatograms were obtained for other samples; a few co-extractive peaks were observed on the chromatogram but the oxolinic acid peak was clearly separated from them.

TABLE II

RECOVERIES OF OXOLINIC ACID ADDED TO CROP HOMOGENATES OR GROUND SAMPLES

Crop	Fortification (ppm)	Recovery[a] (%)	Crop	Fortification (ppm)	Recovery[a] (%)
Lettuce	0.5	90 ± 6.4	Onion	0.5	95 ± 0.0
Chinese cabbage	0.5	87 ± 5.9	Japanese radish, leaves	0.5	88 ± 3.6
White potato	0.5	88 ± 5.3	Japanese radish, root	0.5	90 ± 3.6
Cucumber	0.5	91 ± 5.8	Konnyaku	0.5	87 ± 3.1
Melon	0.5	87 ± 3.6	Brown rice	0.5	81 ± 5.1
Broccoli	0.5	82 ± 2.7	Rice straw	1.0	81 ± 3.3
Cabbage	0.5	78 ± 0.9			

[a] Mean ± standard deviation (n = 4–6).

Stability of oxolinic acid in crop homogenates (or ground samples) frozen during storage

For residue analysis, samples are often stored in a freezer before analysis. Therefore, it is important to check the stability of pesticides in samples frozen during storage [9,10]. The stability of oxolinic acid was examined when crop homogenates (or ground samples) were stored at −20°C. It was found that oxolinic acid was fairly stable in crop homogenates (or ground samples) when stored at −20°C; recoveries above 84% were obtained for every sample (listed in Table II) after storage for 16–326 days.

Starner is used for a wide variety of crops, and the proposed simple, sensitive and selective method is applicable to residue analysis of oxolinic acid on crops sprayed with Starner in fields. The principle of this method has been applied to the official method notified by the Environmental Agency of Japan.

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CHROMSYMP. 2679

Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high-performance liquid chromatography with the use of fluorescence detection and a single *o*-phthalaldehyde post-column reaction

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ABSTRACT

A single-stage post-column fluorescence HPLC method for the analysis of N-methylcarbamates and N-methylcarbamoyloximes is presented as an alternative to the traditional two-stage method. The two-stage technique involves post-column hydrolysis with sodium hydroxide followed by derivatization with *o*-phthalaldehyde and β-mercaptoethanol in a borate buffer. In the proposed method only one reagent, consisting of sodium hydroxide, *o*-phthalaldehyde, and N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor), is used. The stability of the reagent is compared to single-stage alternatives involving β-mercaptoethanol or 3-mercaptopropionic acid, which have been previously reported. It is reported that Thiofluor provides the best stability and is satisfactory for overnight chromatographic runs. The sensitivity of the method is reported to be equal to that of the two-stage method. This innovation simplifies reagent preparation and equipment maintenance, while shortening start-up time without any loss in sensitivity.

INTRODUCTION

The extensive agricultural application of toxic N-methylcarbamates, *e.g.*, carbofuran and carbaryl, and of toxic N-methylcarbamoyloximes, *e.g.*, aldicarb and methomyl, has led the United States Environmental Protection Agency (U.S. EPA) to regulate levels of carbofuran [1], and to require monitoring the levels of oxamyl, methomyl, 3-hydroxycarbo-furan, and carbaryl [2] in community drinking water sources. The prevalent analytical technique for these compounds is the high-performance liquid chromatography (HPLC) fluorescence method first developed by Moye *et al.* [3] and extensively studied by Krause [4–7].

N-Methylcarbamates and N-Methylcarbamoyloximes in environmental samples are normally determined using two post-column reactions. In the first reaction the analytes are hydrolyzed using sodium hydroxide to methylamine and in the second the latter reacts with β-mercaptoethanol and *o*-phthalaldehyde (OPA) in a borate buffer to form a fluorescent isoindole.

Attempts at simplifying carbamate HPLC analysis have involved replacement of the hydrolysis reagent by a basic solid-phase catalyst contained in a heated cartridge in the post-column stream [8,9]. Ion-exchange resins in the basic form [8] and magnesium oxide [9] have been used in these schemes, which eliminate the need for one of the two post-column pumps normally used.

McGarvey [10] has eliminated one pump by placing the base together with the OPA and β-mercaptoethanol in the same bottle. The use of 3-mercaptopropionic acid is presented as an alternative to

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β-mercapto-ethanol. McGarvey's paper does not address the critical issue of reagent stability over a time period needed to process a group of samples. Our work indicates that the stability of the β-mercaptoethanol reagent is unsatisfactory, and that N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) gives a somewhat more stable reagent than does 3-mercaptopropionic acid. The odor associated with either β-mercapto-ethanol or 3-mercaptopropionic acid is not present with Thiofluor.

We have found that both reactions can be carried out using a single reagent consisting of o-phthalaldehyde and Thiofluor in 0.05 *M* sodium hydroxide. The single reagent is adequately stable for runs of as many as 30 injections, without the need to fabricate and maintain solid-phase cartridges. Use of a single-stage post-column reaction decreases equipment cost, simplifies reagent preparation, equipment setup, and system maintenance without any loss in sensitivity.

EXPERIMENTAL

Chemicals

All carbamate analytes used in the preparation of calibration standards were supplied by U.S. EPA (Research Triangle Park, NC, USA). The method was tested with a carbamate mixture of the ten analytes plus 1-naphthol purchased from Crescent Chemical (Hauppauge, NY, USA). The internal standard, 4-bromo-3,5-dimethylphenyl N-methylcarbamate (BDMC), was supplied by Aldrich (Milwaukee, WI, USA). Aldrich also supplied 3-mercaptopropionic acid and β-mercaptoethanol. Pickering Labs. (Mountain View, CA, USA) supplied Thiofluor. Fisher Scientific (Orlando, FL, USA) provided HPLC-grade water, acetonitrile, and all other chemicals, which were of reagent grade.

Instrumentation

Samples were injected using a Perkin Elmer ISS-100 autosampler (Perkin Elmer, Norwalk, CT, USA). The analytical pump was a Perkin Elmer Series 410 with gradient capability. The signal was monitored with a Perkin Elmer LS-1 fluorescence detector. Data handling was carried out using the Perkin Elmer OMEGA IV Workstation equipped with a GP-100 Graphics Printer. The post-column derivatization equipment including pump, flow conditioners, reaction coils, and oven were supplied by Pickering Labs.

Chromatographic conditions

An Uptight guard column (Upchurch Scientific, Oak Harbor, WA, USA), packed with C_{18} bonded-phase preceded a Perkin Elmer 150 × 4.6 mm I.D. HS-3C_{18}, 3 μm column. Well water samples were directly injected after being filtered. The injection volume was always 400 μl. A water–acetonitrile gradient began at 5% acetonitrile and reached 20% in 13 min and 65% during the next 15 min. The original composition of 5% was restored during the next 2 min and held for 8 min before the next injection. All changes were made in linear fashion. Pumping was at 1 ml/min throughout the gradient program. Mobile phase components were continuously sparged with helium during chromatography. Excitation wavelength of the pulsed xenon source was set at 340 nm, and emission was set at 460 nm. The detector gain was set at 2 and the response at 3.

Post-column reaction conditions. In two-stage studies both post-column pumps were run at approximately 0.1 ml/min. The hydrolysis reaction was carried out at 95°C in a 500-μl coil and the OPA reaction at ambient temperature in a 200-μl reaction coil. In single-stage studies the post-column pump was set at approximately 0.1 ml/min and the reaction was carried out at 95°C in a 500-μl reaction coil. A 34.4-bar pressure-release valve was installed ahead of the detector in all studies to enhance performance of post-column pumping.

Reagent for single-stage method. To approximately 100 ml of water in a 250-ml volumetric flask was added 1.25 ml of 10 *M* sodium hydroxide. A solution of 180 mg of Thiofluor in *ca.* 10 ml of water and a solution of 25 mg of OPA in 2.5 ml of methanol were added with water rinsings. The solution was diluted to 250 ml, filtered through a 0.45-μm nylon filter, and degassed with helium for approximately 10 min before starting the sample run. The reagent was prepared fresh daily.

Reagents for the two-stage post-column reaction method. The conditions are essentially those recommended in U.S. EPA method 531.1 [11]. The reagent used in the first (95°C) hydrolysis reaction is 0.05 *M* sodium hydroxide. The second reagent was prepared by dissolving 4.78 *g* sodium borate deca-

hydrate in 250 ml water and adding 25 mg of OPA in 2.5 ml of methanol. Both reagents were filtered and degassed as in the single-stage method. Just before use, 25 μl of 50% (v/v) β-mercaptoethanol in acetonitrile was added to the OPA reagent. The OPA reagent was prepared fresh daily.

Preservation of standards and samples. The preservative used was that recommended in U.S. EPA method 531.1 [11] and was prepared by mixing 156 ml 2.5 *M* monochloroacetic acid and 100 ml 2.5 *M* potassium acetate. Each 10 ml of standard or control solution contained 300 μl of preservative. Samples were stored at -23°C.

RESULTS AND DISCUSSION

Comparison of chromatograms developed using the present single-stage method and using the traditional two-stage post-column method is shown in Fig. 1. Examination of Fig. 1 reveals that the chromatograms are essentially equivalent. Retention times, recoveries, and estimated detection limits (EDLs) for the ten analytes are shown in Table I. Both single-stage and two-stage estimated detection limits for a signal-to-noise ratio of 5 were found to be in the range of 0.5 to 1.2 μg/l. This is in reasonably close agreement to the literature values of 0.2 to 0.6 μg/l [12].

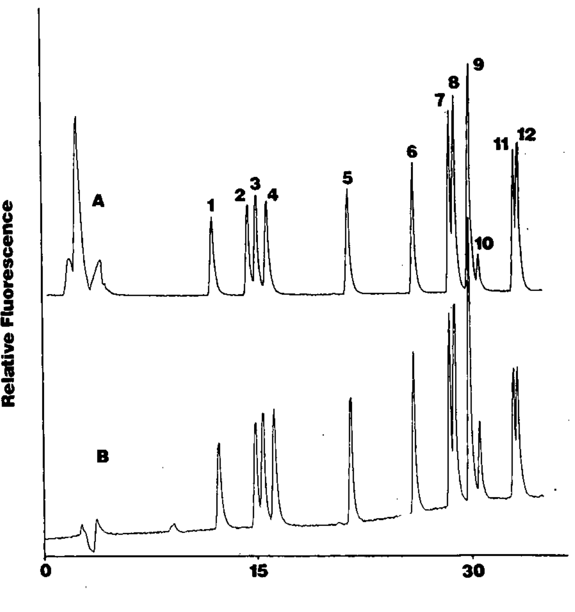

Fig. 1. Chromatogram using two-stage post-column reaction method (A) and using single-stage post-column reaction method (B). For both chromatograms: 1 = aldicarb sulfoxide, 2 = aldicarb sulfone, 3 = oxamyl, 4 = methomyl, 5 = 3-hydroxycarbofuran, 6 = aldicarb, 7 = propoxur, 8 = carbofuran, 9 = carbaryl, 10 = 1-naphthol, 11 = methiocarb, 12 = BDMC (internal standard). Both A and B resulted from the injection of 400 μl of water containing 20 μg/l of each analyte.

TABLE I

CHROMATOGRAPHIC RESULTS FOR TEN ANALYTES USING A SINGLE-STAGE POST-COLUMN REACTION

Retention times and recoveries were based on seven measurements at 10 μg/l. EDL is the estimated detection limit at a signal-to-noise ratio of 5.

Analyte	Retention time (min)	Recovery at 10 μg/l (%)	EDL (μg/l)
Aldicarb sulfoxide	12.08	100.4	1.2
Aldicarb sulfone	14.38	101.0	1.0
Oxamyl	15.07	99.4	0.9
Methomyl	15.69	99.6	0.9
3-Hydroxycarbofuran	21.23	102.1	1.1
Aldicarb	25.79	99.6	0.7
Propoxur	28.35	100.6	0.6
Carbofuran	28.71	100.4	0.5
Carbaryl	29.78	101.0	0.4
Methiocarb	32.92	99.4	0.7

Recoveries at 10 μg/l, averaged over seven determinations, were in the narrow range from 99.4 to 102%. Recoveries were based on the observed concentrations of analytes in a quality control mixture purchased from Crescent Chemical, after a single-point calibration using a standard prepared in our laboratory.

The precision at 10 μg/l was demonstrated by determining the relative standard deviation for seven measurements. The relative standard deviation varied from 1.8% for methomyl to 3.1% for 3-hydroxycarbofuran, with an average value of 2.1%.

Linearity of the single-stage post-column reaction method was tested using four concentrations, 3, 10, 20, and 50 μg/l. The *r* value was found to be 0.999 for each of the ten analytes.

In order to compare the stability of the single-stage post-column reagent used in the present method with that used by McGarvey [10], peak heights were followed over the course of twenty injections. In McGarvey's work the reagent was prepared using either β-mercaptoethanol or 3-mercaptopropionic acid. Using McGarvey's β-mercaptoethanol formulation peak heights for the ten analytes decreased to 35% of their original magnitude, on average, during 20 injections. Using McGarvey's preparation employing 3-mercaptopropionic acid peak heights were, on average, 91% of their starting magnitude after twenty injections. Use of the present method gave peak heights, on average, of 97% of their original magnitude after twenty injections. Sparging with helium throughout the run resulted in improvement for β-mercaptoethanol bringing peak heights to 71% of their initial values, but did not improve stability for Thiofluor or 3-mercaptopropionic acid. Though use of 3-mercaptopropionic acid is practical, we recommend the use of Thiofluor and suggest recalibration after every ten injections for best accuracy. Thiofluor, besides providing a somewhat more stable reagent, does not have an objectionable odor as does 3-mercaptopropionic acid.

In our laboratory we process as many as thirty vials containing samples and controls in a single overnight run. Provided that reasonably fresh water was used in making the gradient, we have encountered no interferences or unusual behavior with this method.

CONCLUSIONS

Carbamates and carbamoyloximes can be determined in the μg/l range using a single-stage reagent containing Thiofluor and OPA dissolved in 0.05 *M* sodium hydroxide. The single-stage method has no disadvantages compared to the traditional two-stage technique and leads to faster reagent preparation and considerable operational simplification.

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Thin-layer chromatographic behaviour of carbamate pesticides and related compounds

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ABSTRACT

The thin-layer chromatographic behaviour of carbaryl, carbendazim (Bavistin), carbofuran, propoxur, phenol, 4-chlorophenol, *o*-nitrophenol, α-naphthol and β-naphthol was examined on alumina, barium sulphate, calcium carbonate, calcium phosphate, calcium sulphate, cellulose and silica gel G in solvents such as acetone, benzene, carbon tetrachloride, chloroform, ethanol and distilled water. The important separations achieved were carbendazim from carbaryl, propoxur, 4-chlorophenol, *o*-nitrophenol, α-naphthol a··· β-naphthol; carbaryl, propoxur, α-naphthol and β-naphthol from carbofuran, phenol, 4-chlorophenol and *o*-nitrophenol; and *o*-nitrophenol from carbofuran, phenol and 4-chlorophenol.

INTRODUCTION

Thin-layer chromatography (TLC) is used for the qualitative and quantitative analysis of a wide variety of compounds [1]. Pandalikar *et al.* [2] have developed a plain thin-layer chromatographic (P-TLC) procedure for the detection of carbaryl at trace levels in biological fluids. Srivastava and Reena [3] developed a suitable P-TLC scheme for the separation of carbaryl, bendiocarb, carbofuran, baygon, ziram, zineb, aldicarb, 2-isopropyl-phenyl-*N*-methylcarbamate (MIPC) and 2-sec.-butyl-*N*-methylcarbamate (BPMC) on plates coated with silica gel containing 1% zinc acetate using benzene–ethyl acetate (50:10) as the solvent. Residues of carbofuran and its two carbamate metabolites have been extracted with HCl, partitioned into CH_2Cl_2, chromatographed on silica gel and detected with KOH–*p*-nitrobenzenediazonium fluoroborate [4].

Our previous work showed that papers impregnated with an ion-pair reagent or reversed-phase reagent can be used for the separation of herbicides and plant growth regulators [5]. P-TLC and sequential TLC (S-TLC) can be used for the detection and determination of carbaryl in water [6,7]. In continuation of our previous work, the chromatographic behaviour of carbamate pesticides and related compounds has been examined on thin layers of alumina, barium sulphate, calcium carbonate, calcium phosphate, calcium sulphate, cellulose and silica gel G.

EXPERIMENTAL

Apparatus

A Stahl apparatus with a universal applicator (thickness of the applied layers adjustable from 0.25 to 2.00 mm), glass plates (20 × 4 cm), glass jars (25 × 5 cm), a hot-air drier, a temperature-controlled electric oven (Tempo), a Spectronic-20 spectrophotometer (Bausch and Lomb), an electrical hot-plate with magnetic stirrer (Sunvic) and a micropipette (10–100 μl) (Gilson) were used.

Reagents and chemicals

Compounds were of laboratory-reagent (LR), general-reagent (GR), wettable powder (WP) or analytical-reagent (AR) grade as indicated. Aluminium hydroxide gel (Qualigens), barium sulphate (Ranbaxy), calcium carbonate and calcium phos-

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phate (LR) (GSC), calcium sulphate dihydrate (Merck), microcrystalline cellulose (LR) (CDH), silica gel G (Merck), 50% carbaryl (WP) (Paushak), 3% carbofuran (GR) (Pesticides), 1% (w/w) propoxur (Bayer), 50% Bavistin (WP) (BASF), phenol, *p*-chlorophenol (LR) (BDH), *o*-nitrophenol (LR) (CDH), α- and β-naphthol (AR) (CDH), 4-nitrobenzenediazonium tetrafluoroborate (Merck), potassium hydroxide (LR) (CDH), sulphanilic acid, sodium nitrite (AR) (CDH) and sodium hydroxide (LR) (Qualigens) were used. All other reagents were of analytical-reagent grade.

Preparation of solutions

Solutions (1%) of carbaryl, carbendazim, carbofuran, propoxur, phenol, 4-chlorophenol, *o*-nitrophenol, α-naphthol and β-naphthol were prepared in ethanol and 4-nitrobenzenediazonium tetrafluoroborate solution (0.1%) was prepared in acetone. Methanolic potassium hydroxide (1%) and aqueous solutions of sodium nitrite (0.3%) and sodium hydroxide (16%) were used. Sulphanilc acid (0.2%) solution was prepared in 10% HCl. When a 1% solution of a pesticide could not be prepared, the saturated solution was used.

Preparation of TLC plates

The following slurries were applied to the glass plates with the help of the applicator so that the thickness of the coating was 0.5 mm: (1) aluminium hydoxide gel (30 g) in distilled water (DW) (100 ml); (2) barium sulphate (30 g) in DW (60 ml); (3) calcium carbonate (25 g) in DW (60 ml); (4) calcium phosphate (40 g) in DW (60 ml); (5) calcium sulphate (30 g) in DW (70 ml); (6) microcrystalline cellulose (20 g) in DW (75 ml); and (7) silica gel G (25 g) in DW (65 ml).

The plates were first allowed to dry at room temperature (25 ± 2°C) and then in an oven at 110°C for 30 min for the cellulose coating and 1 h for the other coatings for activation.

Spotting of test solution

Test solutions were applied to the TLC plates with a fine capillary or micropipette. After drying at room temperature, the plates were developed to a distance of 10 cm with a suitable mobile phase. The solvents investigated, with dielectric constants [8] in parentheses, were hexane (1.89), 1,4-dioxane (2.21), carbon tetrachloride (2.24), benzene (2.28), chloroform (4.81), chlorobenzene (5.71), ethyl acetate (6.02), acetone (20.7), ethanol (24.3), methanol (32.63), nitrobenzene (34.82) and distilled water (78.54).

Detection of pesticides

The carbamates and phenols were detected on the TLC plates by spraying first with potassium hydroxide solution and then with *p*-nitrobenzenediazonium tetrafluoroborate solution. Coloured spots on a white background appeared for all the compounds.

Recording of R_F values

For tailing spots, the distance from the origin for the leading (R_L) and trailing (R_T) fronts were measured and R_m values were calculated with the equation

$$R_m = \frac{\text{average of } R_L \text{ and } R_T \text{ (cm)}}{\text{distance travelled by solvent front (cm)}}$$

For compact spots, R_F values were calculated in the conventional manner [5], *i.e.*, R_F = migration distance relative to solvent front.

Qualitative separations

To achieve the separation of pesticides, one of the compounds was spotted, the solvent was removed, the second compound was spotted, the solvent was removed again, then the plate was developed, dried and pesticides were located as above.

Quantitative separations

The pesticide solutions were spotted on fresh plates, solvents were removed and the plates were developed as above. The previously demarcated area of the plate was scratched off and carbaryl was extracted with methanol (5 ml) and determined by the following procedure [9].

Spectrophotometric determination of carbaryl

To the carbaryl extract (5 ml) in a 50-ml volumetric flask, 10 ml of distilled water and 2 ml each of sodium nitrite and sulphanilic acid solutions were added. After 10 min, 5 ml of 16% NaOH were added and the solution was made up to the mark with distilled water and mixed thoroughly. The absorbance was recorded against a blank after 10 min at 520 nm. The blank contained all the reagents except carbaryl.

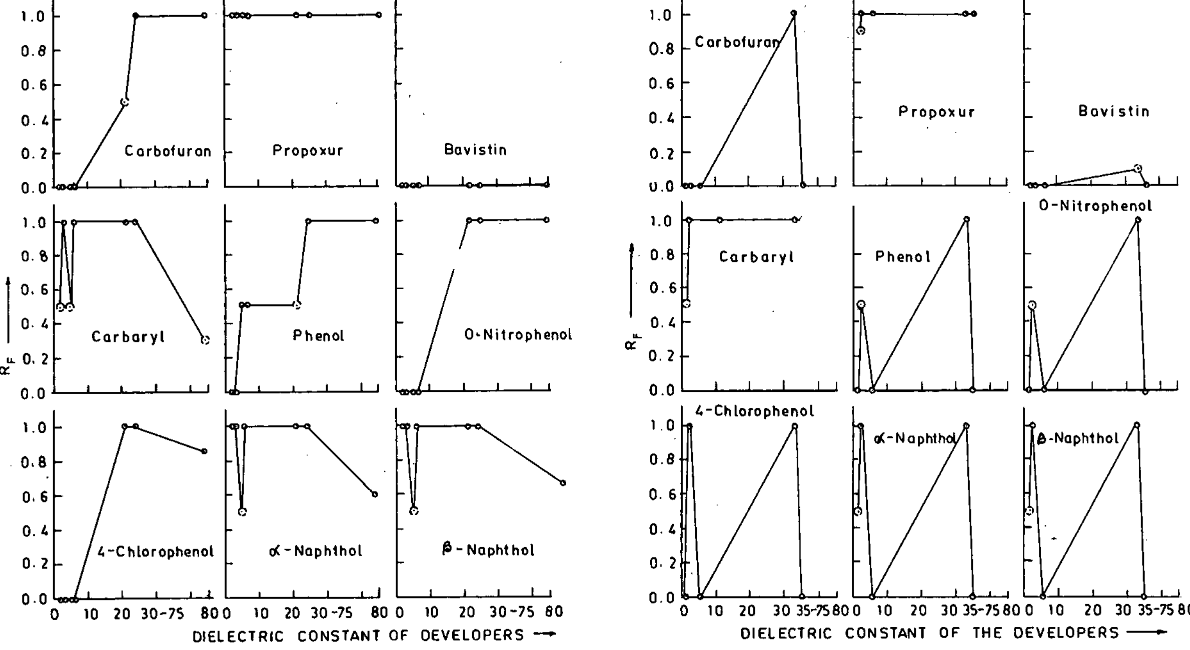

Fig. 1. TLC behaviour of some carbamates and related compounds on cellulose using different solvents. Solid circles, R_F; dashed circles, R_m.

RESULTS

Plots of R_F values *versus* the dielectric constants of the solvents are shown in Figs. 1–7. The separations achieved are recorded in Tables I–VII. Thin-layer chromatograms of some analytically important separations are shown in Fig. 8. Analytical results for the quantitative separations are summarized in

Fig. 2. TLC behaviour of some carbamates and related compounds on aluminium hydroxide gel. Symbols as in Fig. 1.

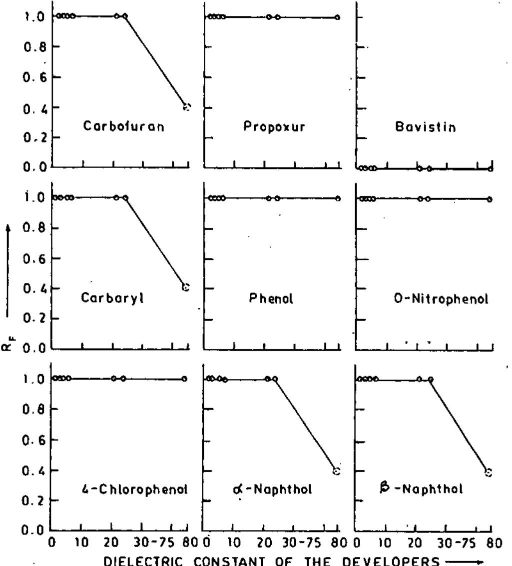

Fig. 3. TLC behaviour of some carbamates and related compounds on barium sulphate. Symbols as in Fig. 1.

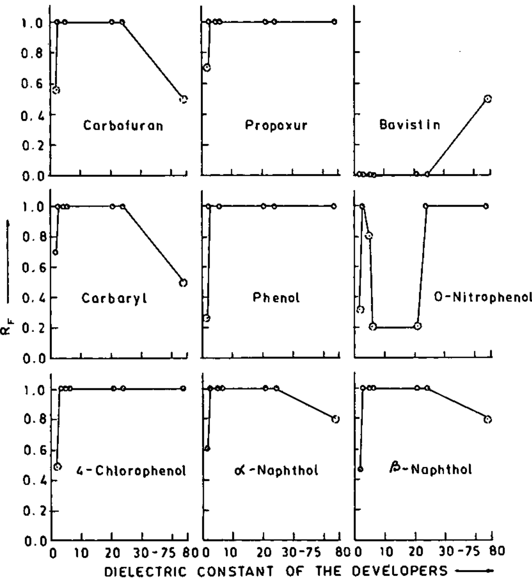

Fig. 4. TLC behaviour of some carbamates and related compounds on calcium carbonate. Symbols as in Fig. 1.

Table VIII. The times required for developing the chromatograms with different coatings are given in Table IX.

Statistical analysis of the spectrophotometric data was applied using the following expressions:

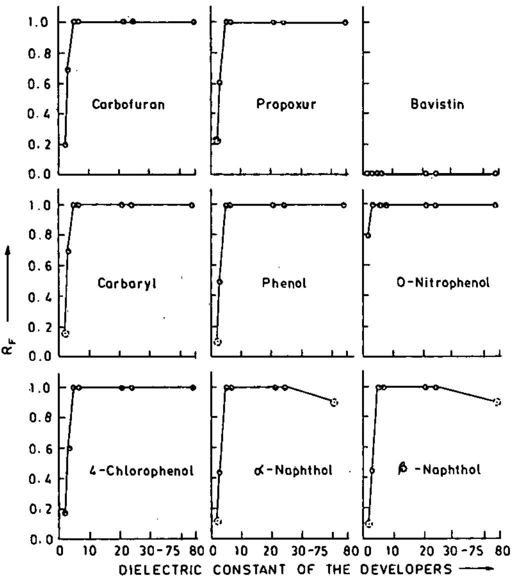

Fig. 5. TLC behaviour of some carbamates and related compounds on calcium phosphate. Symbols as in Fig. 1.

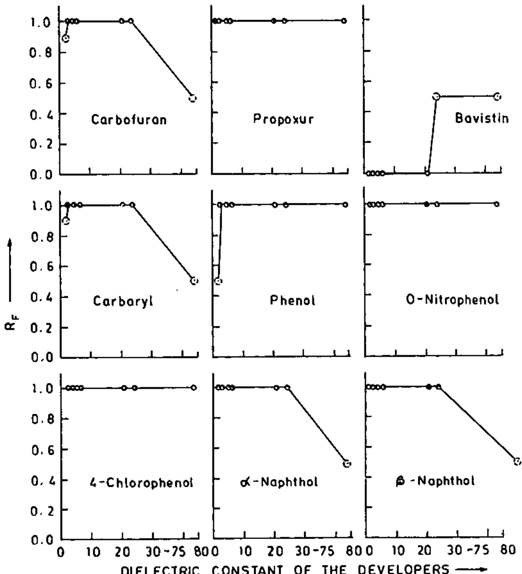

Fig. 6. TLC behaviour of some carbamates and related compounds on calcium sulphate. Symbols as in Fig. 1.

$$\sigma = \sqrt{\frac{(x_1 - \mu)^2 + (x_2 - \mu)^2 + (x_3 - \mu)^2 + \ldots}{N - 1}}$$

$$\text{R.S.D.} = \frac{\sigma \cdot 100}{\mu}$$

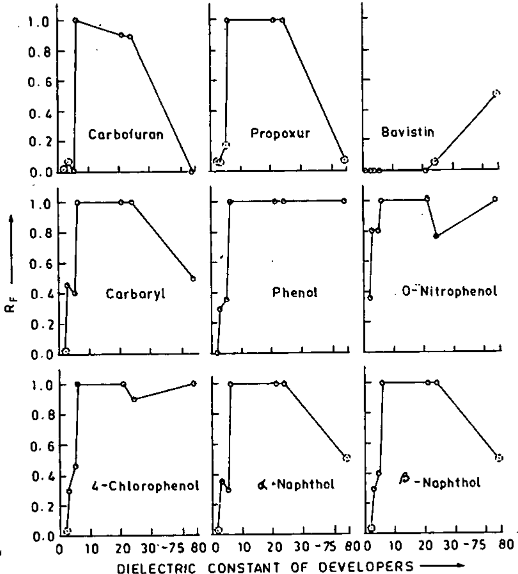

Fig. 7. TLC behaviour of some carbamates and related compounds on silica gel G. Symbols as in Fig. 1.

TABLE I

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS ON ALUMINIUM HYDROXIDE GEL

Compounds[a]	Separated from[a]	Solvent
Bavistin (0.0) and o-nitrophenol (0–1 cm)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), phenol (1.0) and propoxur (0.9)	Acetone
Carbaryl (7–9 cm), carbofuran (1.0) and propoxur (1.0)	Bavistin (0.0), 4-chlorophenol (0–3 cm), o-nitrophenol (0–1.5 cm) and phenol (0–2.5 cm)	Chloroform
4-Chlorophenol (1.0), o-nitrophenol (1.0) and phenol (1.0)	Bavistin (0.0), carbaryl (0.0), carbofuran (0–1 cm) and propoxur (0–2 cm)	Distilled water
Bavistin (0.0) and o-nitrophenol (1–4 cm)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0) β-naphthol (1.0), phenol (1.0) and propoxur (1.0)	Ethanol

[a] R_F values or migration distances (cm) are given in parentheses.

TABLE II

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS ON BARIUM SULPHATE

Compound[a]	Separated from[a]	Solvent
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Acetone
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Benzene
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Carbon tetrachloride
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Chloroform
Bavistin (0.0)	4-Chlorophenol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Distilled water
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethanol
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethyl acetate

[a] R_F values or migration distances (cm) are given in parentheses.

TABLE III

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS ON CALCIUM CARBONATE

Compounds[a]	Separated from[a]	Solvent
Bavistin (0.0) and o-nitrophenol (0–4 cm)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), phenol (1.0) and propoxur (1.0)	Acetone
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Benzene
Bavistin (0.0)	Carbaryl (1.0), carbofuran (4–7 cm), 4-chlorophenol (2–7 cm), α-naphthol (0.6), β-naphthol (0.45) and propoxur (0.7)	Carbon tetrachloride
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), phenol (1.0) and propoxur (1.0)	Chloroform
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), phenol (1.0) and propoxur (1.0)	Ethanol
Bavistin (0.0) and o-nitrophenol (0–4 cm)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), phenol (1.0) and propoxur (1.0)	Ethyl acetate

[a] R_F values or migration distances (cm) are given in parentheses.

TABLE IV

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS ON CALCIUM PHOSPHATE

Compound[a]	Separated from[a]	Solvent
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Acetone
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (0.7), β-naphthol (0.45), o-nitrophenol (1.0), phenol (0.5) and propoxur (0.6)	Benzene
o-Nitrophenol (0.8)	Bavistin (0.0), carbaryl (0–2 cm), carbofuran (0.2), 4-chlorophenol (o.15), α-naphthol (0–2.5 cm), β-naphthol (0–2 cm), phenol (0–2 cm) and propoxur (0.25)	Carbon tetrachloride
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Chloroform
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Distilled water
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethanol
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethyl acetate

[a] R_F values or migration distances (cm) are given in parentheses.

where x_1, x_2, x_3, etc. = measured values, μ = average value, N = number of sets, σ = standard deviation and R.S.D. = relative coefficient of variation standard deviation.

DISCUSSION

Fig. 1–7 show that amongst the seven adsorbents studied aluminium hydroxide gel possesses a critical separation potential, *i.e.*, the pattern of R_F values is entirely different to those on barium sulphate, calcium carbonate, calcium phosphate and calcium sulphate. The TLC behaviours of silica gel G, cellulose and aluminium hydroxide gel are very similar; the degree of polarity of the adsorbents may be responsible for this deviation.

Tables I–VII show that several separations can be achieved on these adsorbents. The definite size and

TABLE V

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS OH CALCIUM SULPHATE

Compound[a]	Separated from[a]	Solvent
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Acetone
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Benzene
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Carbon tetrachloride
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Chloroform
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), o-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethyl acetate

[a] R_F values or migration distances (cm) are given in parentheses.

TABLE VI

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS ON CELLULOSE

Compounds[a]	Separated from[a]	Solvent
Bavistin (0.0)	Carbaryl (1.0), chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), *o*-nitrophenol (1.0) and propoxur (1.0)	Acetone
Carbaryl (1.0), α-naphthol (1.0), β-naphthol (1.0) and propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0), *o*-nitrophenol (0.0) and phenol (0.0)	Benzene
α-Naphthol (1.0), β-naphthol (1.0) and propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0), *o*-nitrophenol (0.0) and phenol (0.0)	Carbon tetrachloride
Carbaryl (1.0) and propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0), α-naphthol (0.0), β-naphthol (0.0), *o*-nitrophenol (0.0) and phenol (0.0)	Chlorobenzene
Propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0) and *o*-nitrophenol (0.0)	Chloroform
Bavistin (0.0) and carbofuran (0.0)	Carbaryl (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0) and and propoxur (1.0)	Dioxan
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), *o*-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethanol
Bavistin (0–2 cm), carbaryl (0–6 cm), α-naphthol (0.6) and β-naphthol (0.55)	Carbofuran (1.0), *o*-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Distilled water
Bavistin (0–2 cm)	α-Naphthol (0.6) and β-naphthol (0.55)	Distilled water
Carbaryl (1.0), α-naphthol (1.0), β-naphthol (1.0) and propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0) and *o*-nitrophenol (0.0)	Ethyl acetate
Propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0), *o*-nitrophenol (0.0) and phenol (0.0)	Hexane
Bavistin (0-2 cm)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0), *o*-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Methanol
Carbaryl (1.0) and propoxur (1.0)	Bavistin (0.0), carbofuran (0.0), 4-chlorophenol (0.0), α-naphthol (0.0), β-naphthol (0.0), *o*-nitrophenol (0.0) and phenol (0.0)	Nitrobenzene
Bavistin (0–1 cm), carbaryl (0–6 cm), α-naphthol (0.6) and β-naphthol (0.5)	Carbofuran (1.0), 4-chlorophenol (1.0), *o*-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Tap water
Bavistin (0–1 cm)	α-Naphthol (0.6) and β-naphthol (0.5)	Tap water

[a] R_F values or migration distances (cm) are given in parentheses.

TABLE VII

SEPARATIONS ACHIEVED IN DIFFERENT SOLVENTS ON SILICA GEL G

Compounds[a]	Separated from[a]	Solvent
Bavistin (0.0)	Carbaryl (1.0), carbofuran (0.9), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0) *o*-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Acetone
o-Nitrophenol (0.80)	Bavistin (0.0), carbaryl (0.45), carbofuran (0.0), 4-chlorophenol (0.30), α-naphthol (0.35), β-naphthol (0.30), phenol (0.30) and propoxur (0–1 cm)	Benzene
o-Nitrophenol (0.40)	Bavistin (0.0), carbaryl (0–0.05 cm), carbofuran (0.05), 4-chlorophenol (0–0.05 cm), phenol (0.0) and propoxur (0–1 cm)	Carbon tetrachloride
o-Nitrophenol (0.80)	Bavistin (0.0), carbaryl (0.4), carbofuran (0.6), 4-chlorophenol (0.45), α-naphthol (0.45), β-naphthol (0.50), phenol (0.35) and propoxur (1–0.5 cm)	Chloroform
Carbofuran (0.0) and propoxur (0–1.5 cm)	4-Chlorophenol (1.0), *o*-nitrophenol (1.0) and phenol (1.0)	Distilled water
Bavistin (0–1 cm)	Carbaryl (1.0), carbofuran (0.9), 4-chlorophenol (0.9), α-naphthol (1.0), β-naphthol (1.0) *o*-nitrophenol (0.75), phenol (1.0) and propoxur (1.0)	Ethanol
Bavistin (0.0)	Carbaryl (1.0), carbofuran (1.0), 4-chlorophenol (1.0), α-naphthol (1.0), β-naphthol (1.0) *o*-nitrophenol (1.0), phenol (1.0) and propoxur (1.0)	Ethyl acetate

[a] R_F values or migration distances (cm) are given in parentheses.

TABLE VIII

ANALYTICAL PARAMETERS FOR QUANTITATIVE SEPARATIONS OF CARBARYL FROM OTHER PESTICIDES ON CELLULOSE

Amount of carbaryl applied (μg)	Separated from[a]	Carbaryl found (μg)	Developer	Error (%)[b]	$\mu \pm \sigma^b$	R.S.D. (%)[b]
25	Bavistin	22.666	Ethanol	9.36	0.136 ± 0.0058	4.207
50		44.642		10.72	0.250 ± 0.010	4.000
75		70.178		6.43	0.393 ± 0.0057	1.450
100		93.928		6.07	1.254 ± 0.0066	1.254
25	Carbofuran	22.166	Benzene	11.336	0.133 ± 0.011	8.164
50		46.428		7.144	0.26 ± 0.01	3.846
75		70.178		6.429	0.393 ± 0.0208	5.297
100		93.928		6.072	0.526 ± 0.0231	4.393
25	*o*-Nitrophenol	23.75	Benzene	5.000	0.133 ± 0.00578	4.345
50		45.714		8.572	0.256 ± 0.0058	2.265
75		68.928		8.096	0.386 ± 0.0058	1.502
100		93.392		6.608	0.523 ± 0.0057	1.089
25	Propoxur	22.166	Distilled water	11.336	0.133 ± 0.0057	4.285
50		47.499		5.002	0.266 ± 0.0058	2.180
75		70.178		6.429	0.393 ± 0.0115	2.798
100		93.928		6.072	0.526 ± 0.0152	2.908

[a] 5 μg in each instance.
[b] $N = 4$.

shape of the spots obtained on the TLC plates are shown in Fig. 8. The analytical data in Table VIII show that cellulose is a good TLC material for the clean-up and separation of carbaryl from other materials. TLC detection on cellulose was found to be much more sensitive than that on the other adsorbents. Table IX shows that the development is fast on cellulose (10 min) but very slow on barium sulphate (1020 min) in carbon tetrachloride. The development times with the different adsorbents

TABLE IX

TIME REQUIRED FOR ASCENDING CHROMATOGRAM (10 cm) IN DIFFERENT SOLVENTS

Coating	Time (min)						
	Acetone	Benzene	Carbon tetrachloride	Chloroform	Distilled water	Ethanol	Ethyl acetate
Aluminium hydroxide gel	25	30	135	95	30	90	30
Barium sulphate	360	510	1020	615	460	1045	375
Calcium carbonate	25	40	65	50	35	70	30
Calcium phosphate	25	35	60	40	50	100	35
Calcium sulphate	10	15	30	18	10	30	15
Cellulose	7	10	15	10	15	25	5
Silica gel G	37	50	125	70	45	145	55

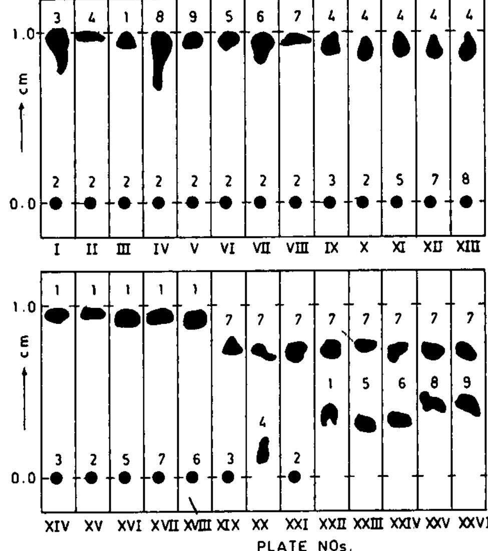

Fig. 8. Separations of some carbamate pesticides and related compounds on cellulose (I–XVIII) and silica gel G (XIX–XXVI) with the developers ethanol (I–VIII), chlorobenzene (IX–XVIII) and chloroform (XIX–XXVI). 1 = Carbaryl; 2 = carbendazim; 3 = carbofuran; 4 = propoxur; 5 = phenol; 6 = 4-chlorophenol; 7 = o-nitrophenol; 8 = α-naphthol; 9 = β-naphthol.

increase in the order cellulose < calcium sulphate < calcium phosphate < calcium carbonate < silica gel G < aluminium hydroxide gel < barium sulphate and the sequence of development with different solvents is acetone < ethyl acetate < distilled water < benzene < chloroform < carbon tetrachloride < ethanol.

Barium sulphate, calcium sulphate, calcium phosphate and cellulose have a good analytical potential as TLC materials for carbamate pesticides.

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Separation of some chlorophenoxyacetic acid congeners on a porous graphitized carbon column

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ABSTRACT

The retentions of twelve phenoxyacetic acid derivatives were measured on a porous graphitized carbon (PGC) column using dioxane–water mobile phases without additives and with added sodium acetate, acetic acid and lithium chloride. Good linear correlations were found between the capacity factors and the dioxane concentration in the mobile phase. The number of substituents on the benzene ring and their hydrophilicity most strongly affected the retention behaviour of phenoxyacetic acid derivatives with dioxane–water eluents. Sodium acetate had the greatest effect on both retention and selectivity, emphasizing the considerable role of the degree of dissociation of solutes on their retention on a PGC column.

INTRODUCTION

Chlorophenoxyacetic acid herbicides are applied extensively in chemical plant protection of rice [1], winter wheat [2,3] and soybean [4]. To increase their effect, phenoxyacetic acids are frequently used in combination with other herbicides [5,6]. Many HPLC methods have been developed for the determination of phenoxyacetic acid herbicides [7]. Separation of phenoxyacetic acids has been carried out on octadecylsilica using methanol–water as the eluent, acidified with trifluoroacetic acid [8] or acetic acid [9]. An octylsilica column has also been used for the separation of these herbicides with acetic acid and methanol–water as eluents [10]. The addition of the ion-pairing agent iron(II) 1,10-phenanthroline to the mobile phase considerably improved the separation of the herbicides [11]. The detection sensitivity was enhanced by the formation of 9-anthryldiazomethane derivatives and by using fluorescence detection [12] and particle beam mass spectrometry [13]. Porous polymer sorbents such as PLRP-S [14] and PRP-1 have also been used for the separation of chlorophenoxyacetic acid congeners [15,16].

Porous graphitized carbon (PGC) supports have gained increasing acceptance and application in HPLC. A carbon adsorbent with organic solvents as the eluent was suitable for the separation of isomers [17]. PGC showed lower equilibration times than a DIOL column and the water content of the organic mobile phase had no significant effect on the equilibration time [18]. Polar phenol derivatives have been successfully separated on a PGC column without buffering the eluent [19]; steric and electronic parameters of the solutes had the greatest effect on retention [20]. The application of PGC in biomedical research has recently been reviewed (21).

The retention mechanism on PGC columns has not been elucidated in detail. It has been established that the retention of anionic compounds is dominated by electronic interactions between the solute and the delocalized electron bonds on the graphitized carbon, whereas cationic compounds are retained mainly by reversed-phase interactions with the hydrophobic carbon surface [22].

The objectives of this investigation were to determine the retentions of twelve phenoxyacetic acids

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on a PGC column with various eluent systems, to assess the effect of several eluent additives on the separation power of the column and to evaluate the results with multivariate methods.

EXPERIMENTAL

A PGC column (Shandon Hypercarb, 100 × 4.7 mm I.D., particle diameter 7 μm) was purchased from Shandon Scientific (Runcorn, UK). The HPLC system consisted of a Liquopump Model 312 pump (Labor MIM, Budapest, Hungary), a Cecil (Cambridge, UK) CE-212 variable-wavelength UV detector, a Valco (Houston, TX, USA) injector with a 20-μl loop and a Waters Model 740 integrator (Waters–Millipore, Milford, MA, USA). The flow-rate was 0.6 ml/min and the detection wavelength was 230 nm. Dioxane–water mixtures were used as eluents with dioxane concentrations ranging from 30 to 85% (v/v) (in steps of 5%). To study the effect of eluent pH and salt concentration, the retention of the solutes was also determined in dioxane–water (7:3, v/v) containing 50, 25, 10 and 5 m*M* LiCl, 50 m*M* sodium acetate or 50 m*M* acetic acid (end concentration). As sodium acetate caused a large decrease in the retention, its effect was also determined in dioxane-water (4:6, v/v).

The structures of the phenoxyacetic acid congeners are shown in Table I. They were dissolved in the eluents at a concentration of 0.1 mg/ml. The retention time of each compound in each eluent was determined with three consecutive injections. As the correlation between log k' and the organic phase concentration is generally linear in HPLC, we applied linear equations to describe the relationship between he capacity factor and the dioxane concentration in the mobile phase:

$$\log k' = \log k'_0 + bC \qquad (1)$$

where k' = capacity factor, k'_0 = capacity factor extrapolated to zero concentration of organic component in the mobile phase (intercept, related to the retention strength of the solute), b = change in log k' caused by unit change (1%, v/v) in dioxane concentration (slope, related to the specific surface area of the solute contacting the stationary phase) and C = concentration of organic component (%, v/v).

To prove the validity of the hypothesis outlined in ref. 23 that for a homologous series of solutes the slope and intercept values are stongly intercorrelated, the linear correlation was calculated between the slope and intercept values of eqn. 1. A modified Free–Wilson analysis [24] was applied to select the substituents of the solutes having the greatest effect on the retention behaviour of these compounds. Free–Wilson analysis was developed for the calculation of the contributions of substituents to the biological activity of a homologous series of compounds, the activity of the unsubstituted molecule being equal to zero. In the traditional Free–Wilson analysis, the presence of independent variables (substituents) that exert no significant influence on the dependent variable (biological activity) lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, the modified Free–Wilson analysis automatically eliminates from the selected equation the insignificant independent variables (substituents), increasing in this manner the information power of the calculation. The same calculation can be used for the selection of substituents of solutes having the greatest effect on their retention under given chromatographic conditions. The slope and intercept values of eqn. 1 were the depen-

TABLE I

STRUCTURES OF PHENOXYACETIC ACID CONGENERS

[Structure: benzene ring with substituents R_2–R_6, bearing O–CH(R_1)–COOH]

R_{1-6} = H except where stated otherwise.

No.	R_1	R_2	R_3	R_4	R_5	R_6
1						
2				Cl		
3		Cl		Cl		
4		Cl	Cl			
5		Cl				Cl
6		Cl			Cl	
7			Cl	Cl		
8			Cl		Cl	
9		Cl		Cl	Cl	
10	CH_3	CH_3		Cl		
11	CH_3	Cl		Cl	Cl	
12	β-Naphthoxyacetic acid					

dent variables and the individual substituents and the sum of substituents on the benzene ring were the independent variables. The acceptance level for the independent variables was set to the 95% significance level. Owing to the small number of compounds, the number of accepted variables was limited to one.

To correlate the retention behaviour of phenoxyacetic acids with their physico-chemical parameters, stepwise regression analysis [25] was applied. The physico-chemical parameters included in the stepwise regression analysis as independent variables were as follows: π = Hans–Fujita substituent constant characterizing hydrophobicity; M-RE = molar refractivity; F and R = Swain–Lupton electronic parameters characterizing inductive and resonance effects, respectively; σ = Hammett's constant, characterizing the electron-withdrawing power of a substituent; E_s = Taft's constant, characterizing the steric effects of a substituent; and B_1, B_4 = Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to the attachment bond axis. The other conditions were the same as in the Free–Wilson analysis.

The effects of various eluent additives on the solvent strength and selectivity were calculated by the spectral mapping technique [26–28]. The capacity factors of the phenoxyacetic acids were the observations and the following eluents were the variables: dioxane–water (7:3, v/v) without additive, with 50 mM LiCl and acetic acid and dioxane–water (4:6, v/v) with 50 mM sodium acetate.

To study the effect of eluent additives on the performance of the PGC column, the theoretical plate number and the asymmetry factor were calculated for each solute in each eluent.

RESULTS AND DISCUSSION

Eluent additives do not change the retention order of chlorophenoxyacetic acids (Fig. 1). Sodium acetate considerably decreases the retention whereas the effects of acetic acid and LiCl are similar; however, the separation is better with LiCl as additive. This observation can be explained by the assumption that sodium acetate increases the dissociation of the polar solutes, and the dissociated form shows a lower retention on the carbon surface. Both acetic acid and LiCl probably suppress the dissociation, resulting in enhanced retention.

Each phenoxyacetic acid shows a regular retention behaviour (Fig. 2), the retention decreasing linearly with increasing concentration of dioxane in the eluent. The parameters of eqn. 1 are compiled in Table II. The relationship between log k' and the dioxane concentration in the eluent was significantly linear in each instance, that is, the retention of

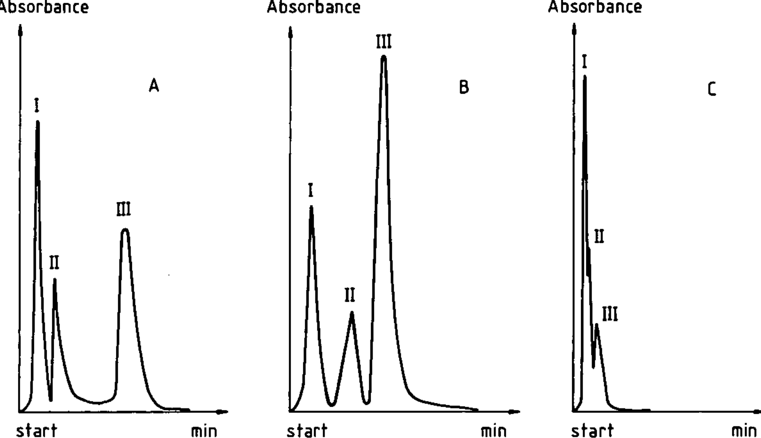

Fig. 1. Separation of chlorophenoxyacetic acids on the PGC column. I = 4-Chlorophenoxyacetic acid; II = 2,4-dichlorophenoxyacetic acid; III = 2, 4, 5-trichlorophenoxyacetic acid. A = dioxane–water (7:3, v/v), 50 mM acetic acid end concentration; B = dioxane–water (7:3, v/v), 50 mM LiCl end concentration; C = dioxane–water (7:3, v/v), 50 mM sodium acetate end concentration.

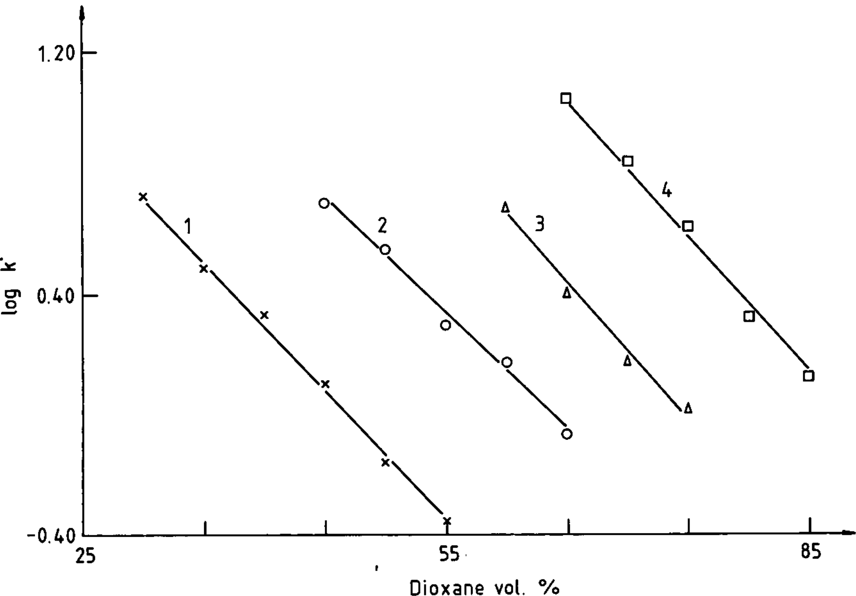

Fig. 2. Dependence of the capacity factor of phenoxyacetic acid derivatives on the dioxane concentration in the eluent. 1 = Phenoxyacetic acid; 2 = 4-chlorophenoxyacetic acid; 3 = 2,4-dichlorophenoxyacetic acid; 4 = 2,4,5-trichlorophenoxyacetic acid.

phenoxyacetic acid derivatives decreases linearly with increasing concentration of the organic component. The significance level in most instances was higher than 99.9%, confirming the applicability of eqn. 1. This indicates that the phenoxyacetic acid derivatives follow the general rule on PGC columns, and no anomalous retention behaviour was observed. The intercept values differ considerably from each other, which means that the these derivatives can be separated easily on the PGC column.

TABLE II

PARAMETERS OF THE LINEAR CORRELATION BETWEEN THE CAPACITY FACTOR (LOG k') OF PHENOXYACETIC ACID DERIVATIVES AND THE DIOXANE CONCENTRATION [C, (v/v)] IN THE MOBILE PHASE

Compounds numbers refer to phenoxyacetic acid derivatives in Table I.

$\log k' = \log k'_0 + bC$

Compound	Log k'_0	$-b \times 10^2$	Significance level (%)
1	1.96 ± 0.46	4.18 ± <0.01	99.9
2	2.47 ± 0.33	3.85 ± 0.02	99.9
3	3.43 ± 0.35	4.59 ± 0.3	95.0
4	3.18 ± 0.35	4.39 ± <0.01	99.9
5	2.35 ± 0.16	3.78 ± 0.03	99.9
6	3.28 ± 0.38	4.39 ± 0.02	99.0
7	3.48 ± 0.36	4.53 ± 0.01	95.0
8	3.45 ± 0.39	4.52 ± 0.02	99.0
9	3.94 ± 0.35	4.46 ± <0.01	99.9
10	3.52 ± 0.29	4.45 ± 0.02	99.9
11	3.88 ± 0.36	4.56 ± <0.01	99.9
12	3.08 ± 0.24	3.69 ± 0.01	99.9

The parameters in Table II make possible the calculation of retention time differences for each pair of derivatives at each eluent composition:

$$t_1 - t_2 = t_0(10^{a_1+b_1C} - 10^{a_2+b_2C}) \quad (2)$$

where a and b are the intercept and slope values for compounds 1 and 2 at a dioxane concentration C.

The relationship between the intercept (hydrophobicity) and the slope (specific hydrophobic surface area) values of eqn. 1 is significant, but the correlation is not strong enough to substitute the parameters with each other:

$$\log k'_0 = -(2.42 \pm 0.61) + (1.31 \pm 0.42) \cdot 10^2 b$$
$$r = 0.6966; n = 12 \quad (3)$$

According to ref. 23, a series of solutes can be considered homologous from the chromatographic point of view when there is a strong linear correlation between the corresponding slope and intercept

values. The relatively low correlation coefficient indicates that the phenoxyacetic acids cannot be considered as a homologous series of solutes according to their retention behaviour on the PGC column.

The results of modified Free–Wilson and stepwise regression analysis are compiled in Table III. The number of substituents on the benzene ring exerts the greatest effect on both the intercept (a values) and slope (b values) of eqn. 1 (see eqns. 4 and 5 in Table III). This result emphasizes the importance of steric parameters in the retention mechanism of PGC. The lipophilicity of phenoxyacetic acids significantly influences their retention (see eqn. 6 in Table III). As the substituents are apolar, and the differences between their lipophilicity is not very high, the conclusions drawn from eqns 4–6 are in good agreement: in this special case the higher the number of substituents, the higher is the lipophilicity of the compound.

The retention of the polar phenoxyacetic acids increases with increasing LiCl concentration (Fig. 3). This finding is in good agreement with the findings in refs. 29 and 30 that the dissociated ions markedly modify the retention (lipophilicity) of bioactive compounds containing one or more polar

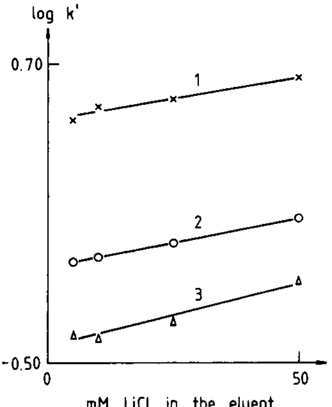

Fig. 3. Dependence of the capacity factors of phenoxyacetic acid derivatives on the LiCl concentration in the dioxan–water (7:3, v/v) eluent. 1 = 2,4,5-Trichlorophenoxyacetic acid; 2 = 3,5-dichlorophenoxyacetic acid; 3 = 2,3-dichlorophenoxyacetic acid.

substructures. The data emphasize again the role of lipophilicity in the retention. The salt suppresses the dissociation of polar substituents, increasing the apparent lipophilicity of the solute (salting-out effect).

The retention of solutes in the eluents dioxane–water (7:3, v/v) decreased in the sequence 50 mM sodium acetate > 50 mM acetic acid > water > 50 mM LiCl. This finding emphasizes again the importance of the degree of dissociation of the solute in the retention of polar molecules on a PGC column. The eluent additives form two separate clusters on the two-dimensional non-linear selectivity map (Fig. 4). The distribution of eluent additives indi-

TABLE III

RELATIONSHIP BETWEEN THE RETENTION PARAMETERS OF PHENOXYACETIC ACID DERIVATIVES AND THE PHYSICO-CHEMICAL PARAMETERS OF THE SUBSTITUENTS

Results of Free–Wilson and stepwise regression analysis (n = 12).

$$\log k'_0 = a + b_1x_1 \quad (4)$$
$$b = a + b_1x_1 \quad (5)$$
$$\log k'_0 = a + b_1x_2 \quad (6)$$

x_1 = number of substituents on the benzene ring; x_2 = lipophilicity of substituents; $r_{95\%}$ = 0.5760; $r_{99\%}$ = 0.7079; $r_{99.9\%}$ = 0.8233.

Parameter	Number of equation		
	4	5	6
a	2.35	3.89	1.98
s_a	0.61	0.33	0.61
b_1	0.47	0.22	0.82
s_{b_1}	0.14	0.08	0.16
r	0.7356	0.6561	0.8561

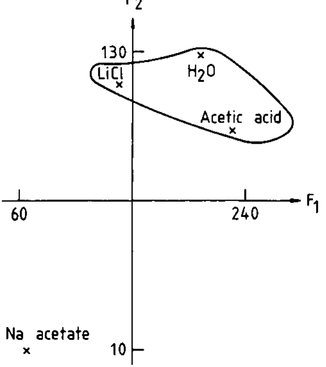

Fig. 4. Two-dimensional non-linear selectivity map of eluent additives. Number of iterations: 41. Maximum error: $4.02 \cdot 10^{-3}$.

TABLE IV

EFFECT OF ELUENT ADDITIVES ON THE THEORETICAL PLATE NUMBER (TPN) AND ASYMMETRY FACTOR (ASF) OF CHLOROPHENOXYACETIC ACID CONGENERS

Compound numbers refer to solutes in Table I.

No. of solute	Eluent additive							
	None		Sodium acetate		Acetic acid		LiCl	
	TPN	ASF	TPN	ASF	TPN	ASF	TPN	ASF
4	269	0.71	224	0.72	243	0.90	505	0.86
5	318	0.83	413	0.79	457	0.93	1022	0.93
10	209	0.66	201	0.95	299	0.83	918	0.95
11	255	0.45	192	0.91	316	0.84	920	0.91

cates that sodium acetate has the greatest effect on the selectivity whereas the effects of acetic acid and LiCl are of secondary importance.

The highest theoretical plate number and the best asymmetry factor were found with eluents containing LiCl (Table IV). The theoretical plate numbers were similar in eluents without an additive and with sodium acetate and acetic acid, whereas the peak asymmetry was the highest in the eluent without an additive. As far as we are aware, the influence of various eluent additives on the performance of PGC columns has not been studied in detail. Unfortunately, our data are not sufficient to be able to draw valid theoretical conclusions about the effect of eluent additives on theoretical plate number and peak asymmetry. These effects are not clearly understood, but our data lend support to the hypothesis that the addition of salts to the eluent may improve the separation efficiency of PGC columns for polar solutes.

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CHROMSYMP. 2583

Determination of diflubenzuron residues in water by solid-phase extraction and quantitative high-performance thin-layer chromatography

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ABSTRACT

A high-performance thin-layer chromatography (HPTLC) method using channeled, preadsorbent silica gel plates and Bratton–Marshall detection reagent was combined with C_{18} solid-phase extraction for quantification of diflubenzuron residues in water. The sensitivity of the technique for diflubenzuron was 0.1 μg, and residues in water at a concentration of 50 μg/l were determined with recoveries of 95–97% and relative standard deviations of 2–3%. Residues could be semi-quantitatively determined at concentrations down to 125 ng/l.

INTRODUCTION

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] (DFB) is a substituted benzoylurea insecticide that acts by interference with deposition of insect chitin. The only published thin-layer chromatography (TLC) method for DFB [1] qualitatively determines residues in water by methylene chloride extraction, separation on homemade silver-impregnated alumina layers, and detection by irradiation with UV light.

In an earlier paper [2], we reported the densitometric quantification of seven substituted urea herbicides, which have structures related to DFB, on C_{18} reversed-phase thin layers using Bratton–Marshall detection reagent after *in situ* hydrolysis to produce aromatic amines. This paper describes the determination of DFB residues in water by a similar densitometric TLC method after isolation on a C_{18} solid-phase extraction (SPE) column using procedures analogous to those we reported previously for the SPE of organochlorine insecticides [3], organophosphorus insecticides [4], and chlorinated herbicides [5].

EXPERIMENTAL

Pesticide solutions

Diflubenzuron standard was obtained from the EPA Pesticide Repository (Research Triangle Park, NC, USA). A stock standard solution was prepared in ethyl acetate at a concentration of 1.0 mg/ml, and this solution was quantitatively diluted with ethyl acetate to prepare a 0.10 mg/ml TLC standard solution and a 0.050 mg/ml spiking solution.

TLC procedure

TLC was carried out on 10 × 20 cm Whatman (Clifton, NJ, USA) LHP-KDF high-performance silica gel plates containing 19 channels and a preadsorbent spotting area. Plates were precleaned by development with methylene chloride–methanol (1:1). Standard and sample solutions were applied using a 25-μl Drummond (Broomall, PA, USA) digital microdispenser. Plates were developed for a distance of 6 cm beyond the preadsorbent-silica gel interface (*ca.* 12 min.) with ethyl acetate–toluene

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(1:3) in a paper-lined, solvent-equilibrated glass HPTLC chamber, and the plate was removed from the chamber and air-dried. Zones were detected as described earlier [2] by spraying in turn with 6 *M* ethanolic hydrochloric acid, 1% sodium nitrite in ethanolic HCl, and 1% ethanolic *N*-(1-naphtyl)ethylenediamine dihydrochloride. The layer was covered with a clean glass plate and heated at 180°C for 10 min after the first spray. The detection procedure is most successful when the spray solutions are prepared freshly within 4 h of use. DFB zones were scanned at 550 nm using a Shimadzu CS-930 densitometer in the single-beam, single-wavelength reflectance mode.

Water analysis

Recovery samples were prepared at a concentration of 50 μg/l by adding 1.0 ml of the spiking solution to exactly 1 l of water known form previous analysis to contain no DFB. The SPE method was adapted from an unpublished procedure supplied by Solvay Duphar B.V. (Weesp, Netherlands) [6]. A C_{18} disposable SPE column (J. T. Baker, Philipsburg, NJ, USA, No. 7020-3, 3 ml) was connected to a 75-ml reservoir, placed in a Baker-10 vacuum manifold operated at 15 inches of Hg, and washed in turn with 5-ml portions of acetonitrile, methanol, and deionized water. The 1 l water sample was passed through the column, followed by 35 ml of acetonitrile–water (3:7). The column was taken from the manifold and the reservoir removed, and the DFB was eluted with 2 ml of acetonitrile into a 2-ml graduated vial with a tapered bottom using gentle pressure from a rubber bulb or syringe. The vial was clamped in a 40°C water bath and the solution evaporated just to dryness under a stream of nitrogen gas. The residue was dissolved in exactly 1.0 ml of ethyl acetate to prepare the sample solution for TLC analysis.

Duplicate 5.0-μl portions from the 1 ml reconstituted sample solution were spotted on a TLC plate along with 1.0, 2.0, 4.0, 8.0 , and 12.0 μl (100–1200 ng) of the TLC standard. After development, detection, and scanning, the equation of the calibration curve (peak area of standards *vs*. weight spotted) was calculated, and the weight of DFB in the sample zones was interpolated from the standard curve. The percent recovery from spiked samples was calculated by dividing the average weight of DFB in the duplicate sample aliquots by the theoretical weight for 100% recovery (50 μg · 5 μl/1000 μl = 250 ng) and mulitplying by 100.

RESULTS AND DISCUSSION

The HP silica gel layer was found to be superior to the C_{18} layer used earlier [2] for the determination of substituted urea herbicides in terms of spot definition and detection sensitivity. On silica gel, DFB was detected as a compact purple-blue band on a white background with an R_F value of 0.40. The three detection solutions should not be sprayed too heavily or the spots will be blurred and the layer may pucker; spots appear as soon as the third solution, *N*-(1-naphthyl)ethylenediamine, is sprayed and reach maximum intensity within about 15 min.

The *in situ* spectrum of a sprayed 1.2-μg standard spot was obtained using the spectral mode of the densitometer, and the wavelength of maximum absorption was found to be 550 nm. In all subsequent analyses, DFB zones were scanned at this wavelength as soon as detection spray 3 dried, because the plate background becomes irreversibly purple after about 30 minutes.

The calibration equation calculated from the areas of the five standards typically had linearity coefficient (R) values of 0.97–0.99. Since slope and intercept values are somewhat variable, bracketing standards were applied and a separate calibration equation was calculated for each plate used to analyze samples.

Carbopack graphitized carbon cartridges were shown [7] to be more efficient than C_{18} for the extraction of phenylurea herbicides from water. However, the C_{18} SPE procedure proved to efficiently extract the less polar DFB from water and provided a quick and convenient alternative to the usual separatory funnel extraction prior to TLC. In the SPE method, the 30% aqueous acetonitrile eluent removes co-extracted impurities more polar than DFB, while the DFB is retained on the column. DFB is then completely eluted with 2 ml of acetonitrile, thereby achieving a 500-fold concentration increase from a 1-l water sample.

Recovery studies were carried out using 1-l water samples fortified with 50 μg of DFB (50 μg/l). A 5-μl aliquot from the 1000-μl reconstituted sample was spotted for TLC analysis, which represented

250 ng if recovery was 100%. Assuming that DFB quantities as low as 0.10 μg can be detected on the silica gel plate and a recovery of 90% through the SPE column, the ultimate sensitivity of the method for 1 l of water if the entire reconstituted extract residue was spotted would be approximately 111 ng/l. However, because of the experimental difficulties involved in dissolving and spotting the entire residue and working at the lowest sensitivity level of the detection method, results at this concentration would be semi-quantitative at best.

Three duplicate samples each of deionized water and local river water spiked at 50 μg/l were analyzed to test the accuracy and precision of the method. The average recovery (± S.D.) was 95 ± 2% for the deionized water and 97 ± 3% for the river water. The percentage difference between the areas of the duplicate sample aliquots spotted ranged from 2.8–8.1% with a mean of 5.9%. One sample of river water was spiked at 125 ng/l, and recovery was estimated to be 83% when the analysis was carried out after reconstituting the residue in 50 μl of ethyl acetate and spotting the entire residue onto the preadsorbent.

The earlier TLC method [1] claimed a detection limit of 0.1 μg of DFB on an absolute basis and a concentration detection limit of 2 μg/l. To achieve this limit, 100% recovery and a 50-μl sample would be required, but neither the sample size nor recovery were specified in the paper [1]. The method involved homemade silver-impregnated alumina layers, which are difficult to prepare reproducibly and turn black quickly on storage, and separatory funnel extraction. Since the layer did not contain a preadsorbent spotting area, precise application of a 50-μl sample in a narrow initial zone would be difficult and time consuming. The SPE–HPTLC method is much faster and convenient since it involves disposable C_{18} extraction columns and application of only 5 μl of sample solution to a commercial preadsorbent plate, and recovery studies show it is a reasonably accurate and precise quantitative procedure.

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CHROMSYMP. 2714

Efficient screening method for determining base/neutral and acidic semi-volatile organic priority pollutants in sediments

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ABSTRACT

An efficient screening method capable of recovering base/neutral and acidic organic priority pollutants from sediment samples has been developed and applied to field samples. The procedure involves solvent extraction with sonication, solid-phase extract clean-up, and quantitative analysis by gas chromatography–ion-trap mass spectrometry. The method was applied to over 300 samples of both freshwater and marine sediments. Quality control data indicate that the accuracy and precision of the method are comparable to those of other techniques reported in the literature, including US Environmental Protection Agency methods for waste samples. The spatial variation of organic pollutants in adjacently collected sediments was found to be greater than anticipated and does not appear to be a result of analytical imprecision.

INTRODUCTION

Sediments are an important component of aquatic ecosystems, serving both as sources and sinks for nutrients, carbon and toxicants [1]. There is an increasing awareness of the need to assess the quality of sediments with respect to the potentially toxic pollutants which they may contain. One class of these pollutants, hydrophobic organic compounds, is frequently among the most reported contaminants in sediments [2]. The concentrations of hydrophobic organic pollutants in sediments have important implications for benthic organisms and other biota in the aquatic systems [3–7].

Despite the increasing interest in sediment contamination, techniques used to assess contaminant concentrations in sediments are neither standardized nor simple to perform and they remain the subject of much ongoing research [8–28]. The complex nature of the sample matrix is one of many factors contributing to the difficulty of these efforts. Co-extracted natural products are another difficulty that often require tedious extract clean-up for their removal. An additional complicating factor is the large number of target analytes with widely ranging chemical properties that are likely to be found in the aquatic environment [29,30].

Reported recovery values and other method performance criteria typically originate from studies conducted under research conditions optimized for specific compound classes [23,28]. Such studies generally produce small data sets over relatively short time spans [8,9,18–21]. These idealized recovery efficiencies may be difficult to obtain under normal laboratory operating conditions with methods that are designed to recover a wide range of chemicals.

The organic priority pollutants (OPPs) are a group of analytes which contain a variety of chemical classes and are often the target analytes in contaminant investigations. The priority pollutants are the result of a 1976 consent decree between the US

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EPA and several environmental groups and include metals, volatile and semi-volatile organic compounds [31]. The semi-volatile OPPs are the subject of many investigations of contamination in sediments and soil.

This study presents an efficient and expedient screening method capable of reliably recovering different classes of OPPs from sediments. Sediments are solvent extracted with sonication, the extracts are concentrated, and natural product interferences are removed using solid-phase extraction columns. Quantification is accomplished with capillary gas chromatograph–ion-trap mass spectrometry (GC–ITMS). The method has been successfully used to screen freshwater and marine sediment samples for a wide variety of organic pollutants. The performance of this method has been evaluated for a period of over two years and involved several different analysts.

EXPERIMENTAL

Sediment samples from various surface waters throughout the state of Florida were sampled using a petite ponar dredge (Wildlife Supply, Saginaw, MI, USA). The dredge contents were placed in a flat enameled pan, mixed with a chrome-plated trowel, and obvious artifacts were removed. The sediment samples were placed in solvent rinsed one-quart glass jars with aluminum foil lined lids and placed on ice for transport to the laboratory.

The extraction procedure combines elements of EPA Method 3550 [32] and a method reported by Marble and Delfino [8]. Initially, 30 g of wet sediment are weighed into a 250-ml glass centrifuge tube and manually mixed with 60 g anhydrous sodium sulfate using a stainless-steel spatula. Sediment samples designated as matrix spikes are mixed with 0.10–0.50 ml of a solution containing OPPs in acetone prior to solvent addition. Following the sodium sulfate addition, 75 ml of acetonitrile (Optima grade, Fisher Scientific, Orlando, FL, USA) is added to the mixture. The mixed sample is then sonicated (Model W-375 Sonicator Ultrasonic Liquid Processor, Heat Systems, Farmingdale, NY, USA) for 3 min at 100% power output and 50% duty cycle. The sonicated sample is centrifuged for 30 min at 160 g (1000 rpm) before the solvent is decanted into a 250-ml erlenmeyer flask. This procedure is repeated twice for each sample. The extracts are combined, dried over anhydrous sodium sulfate and placed in a 250-ml round bottom boiling flask. This solution is then concentrated on a rotary flash evaporator (Rotavapor RE-111, Buchi Laboratoriums-Technik,; Fisher Scientific) to a volume of 8–10 ml and quantitatively transferred to a graduated centrifuge tube for concentration to a final volume of 6 ml under a stream of nitrogen gas (N-Evap Model 111, Organomation Assoc., Berlin, MA, USA). This extract is designated as the crude sample extract. Typically this crude extract is centrifuged to remove any suspended particulates prior to further sample clean-up.

Polar interferences are removed from the crude extracts using 1 g C_{18} solid-phase extraction columns (SPE, Part No. P469R, Fisher Scientific). An amount of 0.5 g of copper powder (purified grade, Fisher Scientific) is added on top of the C_{18} packing to remove any elemental sulfur present in the sediment sample extracts [9]. The C_{18} columns are conditioned with 6 ml of methanol (optima grade, Fisher Scientific) followed by 6 ml of deionized water [8]. After the column is conditioned, 2 ml of the crude extract are combined and mixed with 4 ml of deionized water in the column reservoir. The extract mixture is pulled through the C_{18} SPE column, which is then dried for 30 min under vacuum. The OPP analytes are eluted from the SPE column using 6 ml of a solvent mixture containing dichloromethane–hexane–acetonitrile (50:47:3, v/v) [8]. This final extract is concentrated under a stream of nitrogen to a final volume of 1 ml.

The components in the sediment extracts are separated, identified and quantified using a GC–ITMS system (GC Model 8500, and ITMS model 6210, Perkin Elmer, Norwalk, CT, USA). The mass spectrometer was tuned using perfluorotributylamine, the calibration compound specified by the manufacturer. The GC–ITMS system was calibrated for quantification of the semi-volatile OPPs using solutions prepared in our laboratory from pure primary standards (>96% purity, Supelco, Bellefonte, PA, USA) using an external calibration procedure. Injection standards were analyzed to verify that operating conditions were within acceptable QC limits each day that the system was used for analysis.

Samples were quantified by injecting a 2-μl aliquot of the final extract onto a capillary GC column

(30 m × 0.32 μm I.D., 1 μm film thickness, RX-5, part No. 10254, Restek, Bellefonte, PA, USA) using the splitless injection mode. The GC oven was temperature programmed to separate the desired analytes as follows: 50°C for 1.5 min; increased at 20°C/min to 130°C; held at 130°C for 3 min; increased at 12°C/min to 180°C; then, increased at 7°C/min to 300°C; and finally held at 300°C for 32 min. The analytes eluting from the GC column were identified and quantified using 70 eV electron ionization ITMS.

All samples analyzed using this sediment extraction procedure were spiked with a mixture of surrogate semi-volatile compounds. The surrogate spike was added to each sample before the solvent and anhydrous sodium sulfate were added. A variety of compound classes were represented in the surrogate spike mixture which included: [$^2H_4$]1,4-dichlorobenzene, [$^2H_8$]naphthalene, [$^2H_{10}$]anthracene, 4-bromophenol and 2,6-dibromophenol. These surrogate compounds were used to assess the effect of different sample matrices on OPP recovery.

RESULTS

Analyte recoveries

An initial validation experiment was performed to investigate the capability of the method to recover a wide range of OPP compound classes. For this validation experiment, 25 compounds representing each of the extractable compound classes listed in EPA Method 625 [33] were chosen. The recovery of these compounds from the C_{18} clean-up procedure was determined by directly spiking mixtures of water–acetonitrile (2:1) with OPPs and loading these samples onto C_{18} columns. The results of this experiment, presented in Table I, show excellent clean sample recovery for all analytes except 2,4-dinitrophenol.

TABLE I

RESULTS OF RECOVERY EXPERIMENT FOR ANALYTES FROM SPIKED C_{18} SPE COLUMNS

Analyte	Amount spiked on C_{18} column (μg)	Mean recovery[a] (μg)	Mean recovery (%)	R.S.D. (%)
Phenol	18.4	17.3	94	8.6
[$^2H_4$]1,4-dichlorobenzene	29.3	27.2	93	8.2
Nitrobenzene	13.7	12.7	93	6.8
2,4-Dimethylphenol	15.8	18.0	114	6.0
1,2,4-Trichlorobenzene	22.9	31.3	137	3.2
Naphthalene	18.2	18.9	103	6.8
Hexachlorobutadiene	21.8	22.5	103	12.8
p-Chloro-*m*-cresol	18.7	19.6	105	8.5
Dimethyl phthalate	23.0	25.1	109	3.3
4-Nitrophenol	16.0	13.4	84	45.4
2,4-Dinitrotoluene	14.3	13.8	97	3.6
Fluorene	15.2	15.8	104	0.1
4-Bromophenyl phenyl ether	22.2	20.8	94	28.8
Pentachlorophenol	18.8	23.5	125	5.0
γ-BHC (lindane)	16.6	20.8	125	4.8
Phenanthrene	16.6	17.7	107	1.1
Anthracene	15.2	15.8	104	0.4
Aldrin	15.6	16.0	103	0.6
Heptachlor epoxide	13.2	14.7	112	7.6
Fluoranthene	14.2	15.6	109	1.1
Pyrene	16.9	18.2	108	1.3
4,4'-DDE	18.9	19.0	100	13.2
Endrin	17.8	15.5	87	35.6
Chrysene	15.8	18.7	119	29.9
2,4-Dinitrophenol	15.4	4.0	26	16.7

[a] Mean of 3 replicate experiments.

Once recovery of the analytes through the C_{18} columns had been verified, the mixture of 25 OPPs was spiked onto a sediment which had been previously screened by GC–ITMS and been determined to be free of OPPs. The spiked samples were extracted and quantified by the procedure described above. The results summarized in Table II indicate that this screening method can recover, with varying but generally satisfactory efficiency, many different types of the OPP chemicals spiked into sediment. Only two analytes (endrin and 2,4-dinitrophenol) and one surrogate ([$^2H_4$]1,4-dichlorobenzene) showed very low recoveries. Further method validation was carried out by extracting National Institute of Standards and Technology (NIST) Standard Reference Material 1941. The data collected for duplicate analyses of this sediment sample are presented in Table III, indicating quite satisfactory recoveries of target analytes. Values for anthracene were high for undetermined reasons.

Given these more than adequate recovery results, the method was applied to freshwater and marine sediment samples collected and analyzed over a period of two years. Quality assurance procedures were instituted wherein a standardized sediment, spiked with an analyte mixture, was analyzed with every set of field samples. In addition, every field sample was spiked with a mixture of surrogate compounds to assess sample matrix effects on analyte recovery. The data collected from both types of spiked samples are summarized in Table IV. It should be noted that the data presented in Table IV were collected over an extended period of time and include technique variation contributed by several different analysts. Recoveries of all analytes, except hexachloroethane, met or exceeded EPA Method 8270 acceptance criteria.

TABLE II

RESULTS OF RECOVERY EXPERIMENT FOR ANALYTES FROM SPIKED SEDIMENTS

Analyte	Spike level (mg/kg dry wt.)	Mean recovery[a] (%)	R.S.D. (%)
Phenol	3.07	34	18.5
[$^2H_4$]1,4-dichlorobenzene	4.88	8	61.8
Nitrobenzene	2.28	49	57.4
2,4-Dimethylphenol	2.63	77	17.4
1,2,4-Trichlorobenzene	3.81	45	41.2
Naphthalene	3.04	49	56.4
Hexachlorobutadiene	3.63	31	35.5
p-Chloro-*m*-cresol	3.12	105	41.4
Dimethyl phthalate	3.84	94	32.1
4-Nitrophenol	2.67	70	36.1
2,4-Dinitrotoluene	2.39	87	28.4
Fluorene	2.53	67	22.6
4-Bromophenyl phenyl ether	3.71	99	26.7
Pentachlorophenol	3.13	81	37.2
γ-BHC (lindane)	2.77	76	14.8
Phenanthrene	2.76	77	28.3
Anthracene	2.53	82	25.7
Aldrin	2.60	61	66.2
Heptachlor epoxide	2.20	70	41.9
Fluoranthene	2.37	84	32.0
Pyrene	2.81	88	32.0
4,4'-DDE	3.15	125	29.5
Endrin	2.96	9	129.
Chrysene	2.63	131	18.0
2,4-Dinitrophenol	2.56	24	13.8

[a] Mean of 8 replicate experiments.

TABLE III

RESULTS OF ANALYSES OF STANDARD REFERENCE MATERIAL 1941 (in mg/kg dry wt.)

Analyte	This study		NIST reported values	
	Sample 1	Sample 2	SRM 1941 certified concentration[a]	SRM 1941 GC–MS concentration[b]
Phenanthrene	0.49	0.51	0.58 ± 0.06	0.60 ± 0.01
Anthracene	0.43	0.43	0.20 ± 0.04	0.23 ± 0.01
Pyrene	1.47	1.29	1.08 ± 0.20	1.24 ± 0.02
Fluoranthene	0.97	0.87	1.22 ± 0.24	1.40 ± 0.04
Benz[*a*]anthracene	0.49	0.49	0.55 ± 0.08	0.60 ± 0.01
Benzo[b+*k*]fluoranthene	0.65	0.91	1.22 ± 0.24	nr[c]
Benzo[*a*]pyrene	0.91	0.81	0.67 ± 0.13	0.75 ± 0.05
Benzo[*ghi*]perylene	0.71	0.71	0.52 ± 0.08	0.57 ± 0.06
Indeno[1,2,3-*cd*]pyrene	0.63	0.69	0.57 ± 0.04	0.56 ± 0.02
Chrysene	0.66	0.65	nr[c]	0.70 ± 0.02
			SRM 1941 non-certified conc.[b]	
Acenaphthylene	0.13	0.03	0.12 ± 0.01	
Naphthalene	0.56	0.56	1.32 ± 0.01	

[a] Values are weighted means of two or more analytical techniques ±95% prediction interval with an allowance for systematic error among the methods used.
[b] Values are determined by GC–MS ± one standard deviation of a single measurement.
[c] No concentration reported for this compound and this method by NIST.

Field duplicates

Additional quality assurance samples included field duplicate sampling at the rate of 10% of all field sampling locations. Field duplicate samples were obtained by lowering the petite ponar dredge a second time and collecting another sample as close as possible to the location of the first sample. Data for these field duplicate samples are presented in Table V.

Variation observed in the field duplicate data led to an experiment designed to identify a possible reason for this variation. Two sediment samples from a contaminated site were each homogenized by placing the wet sediment into a 1-l glass beaker. The sediments were manually stirred with a steel spatula for 45 min. Five 30-g subsamples of each homogenized sample were extracted and quantified. The data obtained from this replicate extraction experiment are presented in Table VI.

DISCUSSION

Analyte recoveries

The results of the initial method validation experiment (Tables I and II) indicated that the method recovered a wide variety of compounds from different chemical classes. The initial experiments included acidic (phenolic) as well as neutral and basic organic priority pollutants. Octadecyl bonded phase columns have been demonstrated to be the optimum non-polar phase for recovery of a wide variety of compounds from water, including phenolics, neutral and basic compounds [34]. Acidic organic compounds have a polar and non-polar fraction depending on their pK_a values and the solution pH. The addition of water to the crude acetonitrile extract increases the polarity of the mobile phase relative to the C_{18} column. Thus, the non-polar fraction of the acidic compounds is more strongly retained on the SPE column, increasing the overall

TABLE IV

RECOVERY OF ANALYTES FROM SPIKED SEDIMENTS

Analyte	n[a]	Average sediment spiked conc. (mg/kg dry wt.)	Mean recovery (%)	Standard deviation	EPA Method 8270 recovery criteria (%)
Base/neutral compounds					
1,2,4-Trichlorobenzene	40	3.49	50	19.3	44-142
1,2-Dichlorobenzene	20	3.73	22	18.3	32-129
1,2-Diphenylhydrazine	20	3.13	94	29.9	–[b]
1,3-Dichlorobenzene	20	5.32	9	7.5	D–172[c]
2,4-Dinitrotoluene	40	2.18	83	34.4	39–139
2,6-Dinitrotoluene	20	3.68	82	23.0	50–158
2-Chloronaphthalene	20	3.38	88	23.2	60–118
3,3-Dichlorobenzidine	20	2.84	23	15.9	D–262
4,4'-DDD	20	3.71	98	23.8	D–145
4,4'-DDE	40	2.88	101	37.0	4–136
4-Bromophenyl phenyl ether	40	3.39	92	24.7	53–127
4-Chlorophenyl phenyl ether	20	2.65	83	18.3	25–158
Aldrin	40	2.38	84	29.5	D–166
α-BHC	20	3.48	96	36.6	–
Anthracene	40	2.32	97	33.9	27–133
β-BHC	20	2.06	78	21.3	24–149
Bis(2-chloroethoxy)methane	20	3.26	75	21.8	33–184
Bis(2-chloroisopropyl)ether	20	3.97	71	26.1	36–166
Benzyl butyl phthalate	20	3.81	92	35.6	D–152
Chrysene	40	2.40	84	36.3	17–168
4,4'-DDT	20	2.94	59	31.0	D–203
Dieldrin	20	3.99	94	27.8	29–136
Diethyl phthalate	20	4.88	83	38.4	D–114
Dimethyl phthalate	40	3.51	97	24.0	D–112
Di-*n*-butyl phthalate	20	4.05	95	30.4	1–118
Di-*n*-octyl phthalate	20	5.29	83	14.7	4–146
Endosulfan sulfate	20	3.60	90	32.3	D–107
Endrin	40	2.71	27	17.5	–
Fluoranthene	40	2.17	91	30.7	26–137
Fluorene	40	2.32	83	41.1	59–121
γ-BHC (lindane)	40	2.54	95	25.9	–
Heptachlor	20	3.40	78	31.0	D–192
Heptachlor epoxide	40	2.01	81	34.5	26–155
Hexachlorobenzene	20	3.09	95	17.8	D–152
Hexachlorobutadiene	40	3.32	29	13.3	24–116
Hexachloroethane	20	4.35	9	7.2	40–113
Isophorone	20	4.47	86	32.1	21–196
Naphthalene	40	2.78	53	21.4	21–133
Nitrobenzene	40	2.09	62	23.4	35–180
n-Nitrosodi-*n*-propylamine	20	4.19	72	26.6	D–230
Phenanthrene	40	2.52	80	20.2	54–120
Pyrene	40	2.57	76	25.8	52–115
2,2',4,4',5,5'-Hexachlorobiphenyl	6	3.33	58	17.3	–
2,2',4,5,5'-Pentachlorobiphenyl	6	3.33	74	10.0	–
2,3',4,4',5-Pentachlorobiphenyl	6	3.33	78	10.4	–
3,3',4,4',5-Pentachlorobiphenyl	6	3.33	64	11.3	–
3,3',4,4'-Tetrachlorobiphenyl	6	7.33	103	8.2	–

TABLE IV *(continued)*

Analyte	n^a	Average sediment spiked conc. (mg/kg dry wt.)	Mean recovery (%)	Standard deviation	EPA Method 8270 recovery criteria (%)
Acidic compounds					
2,4,6-Trichlorophenol	20	3.95	81	41.8	37–144
2,4-Dichlorophenol	20	3.29	88	22.9	39–135
2,4-Dimethylphenol	40	2.40	69	24.1	32–119
2,4-Dinitrophenol	8	2.34	75	32.5	D–191
2-Chlorophenol	20	5.12	62	23.7	23–134
2-Methyl-4,6-dinitrophenol	11	4.63	16	9.3	D–181
2-Nitrophenol	20	3.13	60	20.1	29–182
4-Chloro-3-methylphenol	40	2.85	119	37.2	22–147
4-Nitrophenol	40	2.44	46	24.8	D–132
Pentachlorophenol	40	2.87	51	29.3	14–176
Phenol	40	2.80	32	13.0	5–112
Surrogate compounds					
$[^2H_4]$1,4-Dichlorobenzene	306	9.95	15	14.8	–
$[^2H_8]$Naphthalene	306	11.50	69	31.3	–
$[^2H_{10}]$Anthracene	306	9.40	98	28.1	–
4-Bromophenol	306	9.05	76	12.1	–
2,6-Dibromophenol	306	10.00	93	23.8	–

[a] n = Number of replicate spikes during a 24-month period.
[b] –, No QC acceptance criteria given in EPA Method 8270.
[c] D = Detected; result must be greater than zero.

recovery efficiency of the method. This phenomenon, combined with the selection of an intermediate polarity extraction solvent, assists in the simultaneous recovery of acidic compounds along with the basic and neutral compounds without the pH adjustment and re-extraction required in other procedures [21,25]. The recovery of polar analytes by this method agrees with the results of a predecessor method that recovered (at about 50% efficiency) the multifunctional polar pesticide chlorpyrifos from sediments [8].

Non-polar solvents (*e.g.* dichloromethane, hexane and benzene) are often used for the extraction of anthropogenic chemicals from sediments [23,28]. Since these solvents are relatively immiscible with water, such methods often require drying the sediments to insure that the extraction solvent can adequately interact with the sample. Drying sediments, at even the relatively low temperatures of 50–60°C, can result in significant loss of semi-volatile analytes [20,35]. If sediments are not dried prior to extraction, it is questionable whether water immiscible solvents can effectively extract the target analytes. The use of water miscible solvents for sediment extraction allows more intimate mixing of sample and solvent without drying the sample [20].

Comparison with other methods and techniques

Sonication extraction using non-polar solvents for the recovery of polynuclear aromatic hydrocarbons (PAHs) from sediments has been reported to yield lower recoveries than either tumbling [28] or supercritical-fluid extraction [23]. Our recovery values for PAHs in Tables II and IV compare favorably with the values reported for these analytes by the tumbling and supercritical-fluid extraction methods. Satisfactory PAH recovery by our method is further confirmed by the data presented for SRM 1941 in Table III.

The recovery limits for EPA Method 8270 [36] and the recovery ranges obtained for the method we developed and reported here are compared in Table

TABLE V

FIELD DUPLICATE RESULTS FOR VARIOUS SAMPLES AND SELECTED ANALYTES (in mg/kg dry wt.)

Analyte	Sample concentration	Sample duplicate concentration
Anthracene	0.33	1.09
Anthracene	0.88	5.85
Anthracene	0.25	0.74
Benz[*a*]anthracene	0.04	<0.04[a]
Benz[*a*]anthracene	2.02	0.32
Benz[*a*]anthracene	1.15	<0.04
Benz[*a*]pyrene	0.13	0.51
Benz[*a*]pyrene	2.61	2.52
Chrysene	0.70	2.46
Chrysene	3.16	2.79
Fluoranthene	5.96	0.52
Fluoranthene	20.72	18.04
Fluoranthene	0.33	0.31
Fluoranthene	6.62	<0.03
Fluorene	<0:08	<0.08
Fluorene	0.38	7.88
Pyrene	2.14	5.67
Pyrene	2.13	0.30
Pyrene	1.17	1.18
Pyrene	4.02	<0.03
4,4'-DDE	<0.21	1.11
4,4'-DDE	0.50	<0.21
4,4'-DDE	7.23	8.80
4,4'-DDD	0.64	0.77
4,4'-DDD	0.40	<0.11

[a] <, Value indicates the limit of detection.

IV. The precision of our method is comparable with the precision required by EPA Method 8270. However, since the data for our method in Table IV were obtained using external standard calibration, the precision could be improved by using internal standard calibration procedures [37]. Current EPA methods now require analysis by the internal standard procedure [36,38]. The recovery data provided for the OPPs are the result of quality control spike samples analyzed with sets of field samples. The surrogate spike recovery data in Table IV are based on data collected for over 300 analyses of field sample extracts, including both freshwater and marine sediment samples. The recovery values for our method are comparable with data in the literature for various individual classes of compounds [19,21,23,27,28]. The data in Table IV indicate that this method can accurately and reproducibly recover these analytes from sediment matrices.

Poorly recovered compounds

The low recoveries observed for the dichlorobenzenes and some other analytes are likely due to their relatively high volatility. Lopez-Avila *et al.* [19] reported low recovery of dichlorobenzene isomers relative to other chlorinated hydrocarbons in their evaluation of EPA Method 8120. That method is similar to our method and involves the sonication extraction of sediment and soil samples with dichloromethane followed by gas chromatography–elec-

TABLE VI

REPLICATE EXTRACTION OF CONTAMINATED SEDIMENTS

Analyte	Sediment 1		Sediment 2	
	Mean[a] concentration	R.S.D. (%)	Mean[a] concentration	R.S.D. (%)
4,4'-DDD	6.06	9.6	nd[b]	nd
4,4'-DDE	0.68	7.5	nd	nd
Acenaphthalene	nd	nd	0.04	14.5
Acenaphthene	82.9	12.8	6.47	15.6
Anthracene	3.18	35.3	3.68	28.0
Benz[*a*]anthracene	3.52	18.2	0.69	14.3
Benz[*a*]pyrene	9.22	17.0	0.59	35.1
Benzo[*b*+*k*]fluoranthene	29.9	15.8	6.71	15.8
Chrysene	3.14	39.4	0.59	19.0
Fluoranthene	20.7	15.3	5.65	22.6
Fluorene	75.2	14.5	5.22	12.4
Naphthalene	29.6	10.1	0.44	30.2
Phenanthrene	76.5	31.1	11.7	25.8

[a] Mean of 5 replicate experiments expressed in mg/kg dry weight basis.
[b] nd = Analyte not found in sample.

tron-capture detection. Several of the phenolic compounds were also poorly recovered presumably due to their low pK_a values. Many of the compounds with lower recoveries are ones which would be less likely to associate with sediments due to their greater water solubility or higher volatility. The low recovery of endrin by our method is likely due to thermal degradation in the GC injection port [33].

Non-homogeneity of sediments

Normal field QA procedures required that duplicate sediment samples be obtained at the rate of one field duplicate for every nine field sampling locations. The results of analyses of field duplicate samples (Table V) indicate a larger variation between some duplicate samples than can be attributed to the laboratory analytical precision alone (Tables II and IV). This variation was presumed to be related to the non-homogenous nature of the sediments rather than the variation contributed by the sediment extraction method. The results of a replicate extraction experiment, presented in Table VI, in addition to the SRM recovery data in Table III, support this hypothesis. The variation among the different replicate extractions from single homogenized samples is well within the variation of the analytical method. This indicates that the variation observed in Table V for the analyses of field duplicate samples is related to the non-homogeneous spatial distribution of organic contaminants in these field duplicate samples rather than to the sediment extraction analytical method.

The non-homogeneous spatial distribution of organic contaminants in sediments related to direct chemical analysis has received little attention. However, a few reports indicate that sediment toxicity exhibits a high degree of spatial variability [1,39,40]. Stemmer *et al.* [1] reported extreme spatial variation of toxicological bioassay response in creosote contaminated sediment samples taken from a river in Ohio. They noted that toxicological response varied by up to two orders of magnitude among subsamples taken within a given square meter area. The data presented in Table V indicate that some field duplicate sediment samples analyzed during our study appear to be reasonably homogeneous while other field duplicate samples vary in contaminant concentration by over one order of magnitude. Swartz *et al.* [40] reported strong correlations among the spacial distribution of sediment toxicity, total organic carbon and 4,4′-DDE concentration in sediment cores. These findings support a hypothesis that the wide variations we observed in the field duplicate analyses (Table V) may be due in part to a non-homogeneous distribution of natural organic carbon in sediments.

CONCLUSIONS

The method presented in this paper reliably recovers OPPs, representing a variety of chemical classes, from both freshwater and marine sediments. The procedure provides an efficient and rapid method to simultaneously screen sediment samples for both base, neutral and acidic extractable organic priority pollutants in sediments. The use of a water soluble organic solvent (acetonitrile) for sediment extraction improves the recovery of polar compounds without sample pH adjustment or drying.

The method has been used to screen for OPPs in more than 300 freshwater and estuarine sediment samples from throughout the state of Florida. The surrogate spike recovery data, with one exception, indicate the method's broad applicability. The analysis of field duplicate samples indicates that near-scale spatial variation in the distribution of organic pollutants exists in some sediments.

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High-performance liquid chromatographic analysis of carbofuran residues in tomatoes grown in hydroponics

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ABSTRACT

Tomato plants grown in hydroponics were irrigated three times on alternate days with nutrient solutions fortified with carbofuran at levels of 36, 111 and 222 mg l^{-1}, and carbofuran residues were analysed in tomato fruits. Residues were found to be below the maximum residue level set up by Codex Alimentarius Mundi (0.1 mg kg^{-1}) 6, 11 and 18 days after the third irrigation with nutrient solutions fortified with carbofuran. Consequently, the withholding period of 60 days may be reduced to allow continuous harvest of tomatoes under the conditions used in this experiment.

INTRODUCTION

Meloydogyne spp. and *Pratylenchus* spp. are the most damaging nematode pests in tomatoes grown in the Canary Islands [1]. Several methods have been used to control these pests, and applications of the liquid nematicide Furadan 35 P/V have given successful results. Carbofuran (2,3-dihydro-2,2-dimethylbenzofuranyl-N-methyl carbamate), a broad-spectrum pesticide used to control insects, mites and nematodes, is the active ingredient of this formulation, which has been developed for its use in nutrient solutions and irrigation water.

Data concerning the fate of carbofuran in horticultural crops may be of interest, to allow adequate control of a number of pests, and also to assess safe residue levels in the edible organs of crops. Despite scientific and economic interest in these studies, only a small number of papers concerning these topics are available in current literature [2–4].

Several methods have been proposed for the chromatographic determination of carbofuran residues in agricultural and environmental samples. A group of them have been developed for the direct determination of this pesticide by GLC [5–8]. However, direct GLC analysis of carbamate pesticides with aromatic rings is difficult, because these pesticides have a tendency to break down to the corresponding phenol on the column under normal analytical conditions. Therefore, other methods based on the derivatization of carbofuran prior to GLC have been described [9–11]. Unfortunately, these methods have several limitations, which often reduce their sensitivity and versatility.

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The polar nature of carbofuran makes its analysis by HPLC an atractive alternative, and its aromatic moiety assures a reasonable UV response for detection. Several studies on the use of HPLC for quantitative analysis of carbofuran and other carbamate pesticides have been reported, with successful results [12–16].

In this paper, data on residue levels of carbofuran, determined by HPLC, in tomatoes grown in an experimental hydroponic culture are reported, in order to determine if the irrigation schedule which was carried out is compatible with the maximum residue limit of carbofuran for tomatoes set by the Codex Alimentarius Commission (0.1 mg kg^{-1}).

EXPERIMENTAL

Trial design

Virus-free runners of *Lycopersicon esculentum Mill.*, cv. Meltine, were planted in twenty hydroponic beds of 2.88 m^2, using lapilli as an inert support. The plants were grown in double rows down each bed, with rows 0.75 m apart and 0.20 m between plants. The trial was arranged in a randomized block design to eliminate experimental error, with three replicates for each nutrient solution containing carbofuran and two blank beds. Each replicate consisted of twelve plants.

Tomato plants were irrigated with a standard nutrient solution [17] for 10 weeks. Then, three nutrient solutions, fortified with carbofuran at levels of 36, 111 and 222 mg l^{-1} by dissolving appropriate volumes of Furadan liquid emulsion (containing 35%, w/v, carbofuran), were placed in the tanks (0.95 m^3) linked to each set of hydroponic beds. The hydroponic beds were irrigated three times on alternate days with these solutions, which were replaced afterwards by new nutrient solutions without pesticide. Blank beds were irrigated with a carbofuran-free standard nutrient solution.

Fruit samples were ramdomly collected from each set of plants soon after the third irrigation with nutrient solutions fortified with carbofuran, and then after 4, 6, 8, 11, 13, 15, 18, 20 and 22 days.

Reagents and analytical standards

All reagents were HPLC grade. A standard of carbofuran for HPLC analysis (99.6% purity) was provided by FMC, Agricultural Chemicals Division (Middleport, NY, USA).

Extraction and clean-up procedures

The sample extraction was based on the method described by Lawrence and Leduc [18] for the analysis of carbofuran and two non-conjugated metabolites in vegetables and grains. Fruit samples were prepared for analysis by removing the non-edible parts and then chopped and mixed throughly. A 30-g aliquot pulped tomatoes was blended with 100 ml of acetone for 3 min in an Osterizer blender and filtered by suction through a Whatman No. 1 filter paper. The cup and the filter paper were washed with acetone and the washing was filtered. The filtrate was transferred to a 500-ml separation funnel, and 100 ml of hexane–methylene chloride (1:1, v/v) were added. Then the funnel was shaken and the phases were allowed to separate. The aqueous phase was drawn off into a 250-ml separation funnel, 15 ml of saturated sodium chloride solution were added, and the mixture was extracted twice with 70 ml of methylene chloride. The organic extracts from the three partitions were combined and filtered through anhydrous sodium sulphate. The filter cake was rinsed with 10 ml of methylene chloride, and the combined extract was evaporated under vacuum at 30°C.

The organic extracts were cleaned up prior to HPLC analysis using a modification of the procedure described by Ohlim [19]. The residue after evaporatation was transferred quantitatively with hexane to a 10-ml glass syringe, and injected onto a silica Sep-Pak cartridge (Waters, Milford, MA, USA), preconditioned by passing 30 ml of hexane through it. Then the cartridge was washed with 15 ml of 2% acetone in hexane, discarding the eluate, and with 10 ml of 10% acetone in hexane. This eluate was collected in a 25-ml round-bottom flask, evaporated to dryness under vacuum at 30°C, and the residue was dissolved with 1 ml of mobile phase to be used in HPLC analysis.

HPLC analysis

HPLC analyses were carried out in a Waters chromatograph, equipped with an M-510 solvent-delivery system, a Wisp M-710 automatic injector and an M-441 UV detector set at 280 nm, with a sensitivity of 0.02 a.u.f.s. The separation was performed with a μBondapak C_{18} stainless-steel column (30 cm × 4 mm I.D.), using a mobile phase of acetonitrile–water (40:60, v/v) at a flow-rate of 1 ml

min^{-1}. The injection volume was 20 μl. The content of carbofuran residues in tomatoes was determined by an external standard procedure, using multiple-point calibration.

The efficiency of the procedure was checked using a set of four replicates of a control sample fortified with carbofuran at a level of 0.1 mg kg^{-1}. The average recovery was 91%, with a coefficient of variation of 0.15%, and the analytical method allowed for detection of carbofuran at a level of 0.012 mg kg^{-1}, as reported previously [16]. A chromatogram of an extract of tomatoes collected 4 days after the last irrigation with a nutrient solution fortified with carbofuran (111 mg l^{-1}), after the clean-up procedure, is shown in Fig. 1.

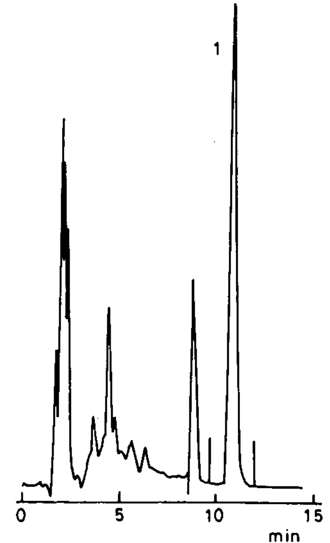

Fig. 1. Chromatogram of an extract of tomatoes collected 4 days after last irrigation with a nutrient solution fortified with carbofuran. Peak 1 corresponds to carbofuran.

RESULTS AND DISCUSSION

Table I shows raw analytical values of the content of carbofuran residues in tomatoes after irrigation of plants with nutrient solutions fortified with carbofuran at three different levels (36, 111 and 222 mg l^{-1}). As can be seen, carbofuran residues in tomatoes decay with time, and levels below the maximum residue limit set by Codex Alimentarius Mundi (0.1 mg kg^{-1}) are reached more quickly the lower the carbofuran concentration in the nutrient solutions. In the case of nutrient solutions fortified with 36 mg l^{-1} carbofuran, carbofuran residues are below 0.1 mg kg^{-1} 6 days after the third irrigation.

TABLE I

DECAY OF CARBOFURAN RESIDUES IN TOMATOES AFTER IRRIGATION WITH NUTRIENT SOLUTIONS FORTIFIED WITH CARBOFURAN

Treatment I: nutrient solution containing 36 mg l^{-1} carbofuran. Treatment II: nutrient solution containing 111 mg l^{-1} carbofuran. Treatment III: nutrient solution containing 222 mg l^{-1} carbofuran. A, B and C are the three different hydroponic beds which were irrigated with each nutrient solution. t = Trace.

Days after third irrigation	Carbofuran residues (mg kg^{-1})								
	Treatment I			Treatment II			Treatment III		
	A	B	C	A	B	C	A	B	C
0	0.233	0.282	0.128	0.654	0.860	0.515	2.612	1.782	1.811
4	0.164	0.138	0.103	0.230	0.563	0.189	2.280	1.827	0.985
6	0.060	0.070	0.025	0.194	0.269	0.089	2.480	1.817	0.783
8	t	t	t	0.150	0.221	0.067	1.393	1.067	0.214
11	–	–	–	0.040	0.070	0.050	0.930	0.705	0.014
13	–	–	–	0.012	t	t	0.278	0.169	0.018
15	–	–	–	–	–	–	0.166	0.012	t
18	–	–	–	–	–	–	0.035	0.015	t
20	–	–	–	–	–	–	0.012	t	t
22	–	–	–	–	–	–	t	t	t

TABLE II

EXPONENTIAL EQUATIONS FOR THE DECAY OF CARBOFURAN RESIDUES IN TOMATO FRUITS OVER TIME, AND HALF-LIFE OF CARBOFURAN RESIDUES

Carbofuran concentration in nutrient solutions (mg l^{-1})	Replicate	Equation for carbofuran decay	Half-life (days)
36	I	$R = 0.259e^{-0.19t}$	3.50
36	II	$R = 0.295e^{-0.22t}$	3.08
36	III	$R = 0.148e^{-0.23t}$	2.90
111	I	$R = 0.852e^{-0.28t}$	2.40
111	II	$R = 1.068e^{-0.22t}$	3.07
111	III	$R = 0.444e^{-0.22t}$	3.10
222	I	$R = 8.049e^{-0.28t}$	2.46
222	II	$R = 6.231e^{-0.21t}$	2.19
222	III	$R = 9.010e^{-0.31t}$	1.34

Tomatoes from plants irrigated with a nutrient solution containing carbofuran at a level of 111 mg l^{-1} show carbofuran residues below 0.1 mg kg^{-1} 11 days after the third irrigation. Finally, residue levels below 0.1 mg kg^{-1} are achieved 18 days after the third irrigation when tomato plants were irrigated with a nutrient solution containing 222 mg l^{-1} of carbofuran.

Some differences were observed between the different replicates of each treatment. To explain these results, physiological differences in pesticide uptake between plants and/or differences in the availability of the pesticide in the inert support have to be assumed.

By plotting carbofuran residue levels in tomatoes against time after the third irrigation, the measured data can be represented in a linear coordinate system by an exponential function:

$$R = R_0 \cdot e^{-kt}$$

where R is the residue content (mg kg^{-1}) at time t (days), R_0 is the theoretical residue content at time t = 0, and k is the decay rate constant. Table II summarizes the equations obtained for the decay of carbofuran residues in tomatoes over time for each treatment and each replicate, and the half life of carbofuran residues in each case. The data show a linear relationship between the logarithms of the content of carbofuran residues and time, and this fact indicates that the decay of carbofuran residues in tomato fruits follows first order kinetics. The decay of carbofuran residues is more intense during the first few days after the third irrigation in the experiment carried out by using a nutrient solution fortified with carbofuran at a level of 222 mg l^{-1} and, as a consequence, half-life of carbofuran residues is less in this case, as shown in Table II.

The results obtained in these experiments show that the use of nutrient solutions fortified with carbofuran for growing tomato plants in hydroponics, using the irrigation schedule described above, results in levels of carbofuran residues in tomato fruits, determined by HPLC, below the maximum residue limit set up by the Codex Alimentarius Commission 18 days after the final irrigation with those nutrient solutions, even if the recommended concentration (111 mg l^{-1}) is doubled. This is much less than the recommended withholding period for this pesticide in horticultural crops (60 days).

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Mobility of cadmium as influenced by soil properties, studied by soil thin-layer chromatography

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ABSTRACT

We studied the mobility of cadmium in various natural soils by soil thin-layer chromatography. The R_F values of the soils varied between 0.14 and 1.00 (mean = 0.64, mode = 0.87). Cadmium was found to be slightly mobile in 27%, moderately mobile in 14%, mobile in 41% and highly mobile in 18% of the soils studied. A statistical analysis of the results obtained revealed a highly significant correlation ($p < 0.001$) between R_F values and pH, the sum of bases and the exchangeable Ca^{2+} and Mg^{2+} contents, as well as a significant negative correlation ($p < 0.05$) between R_F and the clay content and cation-exchange capacity of the soils. The results show the significance of soil properties to the mobility of cadmium wastes from industrial, mining and farming applications.

INTRODUCTION

The rapid expansion of farming, industrial and urban activities has raised serious environmental problems in relation to heavy metals in general and cadmium in particular. This element is considered to be the most hazardous of all heavy metals as it poses serious threats to human health even at very low concentrations in air, water or food [1]. This calls for the environmental control and monitoring of cadmium in order to avoid hazards, particularly in those places where it is bound to occur at high concentrations as a result of human activities [2–4].

Cadmium in soil may in principle be incorporated into the food cycle via vegetables or, alternatively, be washed towards surface or underground waters. In order to minimize environmentals hazards, one should investigate its soil mobility and how it is influenced by the soil properties.

Cadmium mobility in soils has so far usually been measured indirectly by batch adsorption techniques [5–7]. Other authors have used soil-packed columns [8] and, more recently, soil thin-layer chromatography (soil TLC) for this purpose [9]. This last technique, which was developed by Helling and Turner [10], has been widely used to study the mobility of pesticides in soils [11–17] on account of its simplicity, reproducibility and low cost. The soil TLC technique has also been used by Khan *et al.* [9] and by Singhal and Shing [18] to investigate the mobility of heavy metals and trace elements in soil, respectively; these studies did not consider the influence of soil characteristics on the metal mobility.

In this work we studied the influence of soil properties and constituents on the mobility of cadmium by soil TLC.

EXPERIMENTAL

Soil samples

We used 22 samples of natural, uncultivated soils that were collected from the soil surface horizon (0–20 cm deep) at different places in the province of Salamanca (Spain). The samples were sieved through 2-mm mesh, after which they were characterized chemically by using standard soil analysis

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TABLE I

SELECTED PROPERTIES OF 22 SOILS STUDIED

Soil	pH	Organic matter (%)	C/N	Clay (%)	Cation-exchange capacity (10^{-2} mol/kg)	Exchange cations (10^{-2} mol/kg)					Base saturation (%)
						Na^+	K^+	Mg^{2+}	Ca^{2+}	Σ	
1	6.9	6.90	11.1	19.3	23.07	0.41	0.39	3.64	10.42	14.86	64.41
2	6.3	0.60	4.9	59.2	26.90	0.19	0.13	3.87	13.27	17.46	64.90
3	6.0	5.22	12.0	17.7	24.72	0.58	0.23	1.42	11.02	13.25	53.60
4	5.0	8.90	15.3	13.9	18.50	0.08	0.10	0.37	1.38	1.93	10.43
5	5.3	5.95	15.3	11.6	15.25	0.51	0.01	0.20	1.82	2.54	16.65
6	6.7	6.68	11.9	16.8	16.95	0.17	0.43	1.14	5.20	6.94	40.94
7	4.9	5.29	22.7	14.8	12.20	0.17	0.07	0.57	2.24	3.05	25.00
8	5.1	5.52	15.8	22.4	11.95	0.14	0.19	0.69	2.28	3.90	32.63
9	4.9	4.24	20.8	17.8	9.00	0.29	0.14	0.82	2.22	3.47	38.55
10	5.0	6.10	22.6	22.0	14.20	0.26	0.21	0.48	1.03	1.98	13.94
11	5.2	7.28	21.1	19.3	19.30	0.11	0.53	0.84	2.10	3.60	18.65
12	5.6	3.44	13.5	11.8	13.45	0.23	0.19	0.72	1.40	2.54	18.88
13	5.1	4.66	17.3	14.2	13.00	0.61	0.21	0.19	0.67	1.68	12.92
14	5.3	1.13	8.6	8.7	4.50	0.56	0.00	0.31	1.03	1.90	42.22
15	5.4	0.36	6.8	8.9	5.25	0.10	0.06	0.30	0.81	1.27	24.19
16	5.5	1.78	9.2	14.1	9.65	0.25	0.23	1.25	2.90	4.63	47.97
17	6.1	1.15	12.0	10.6	6.60	0.25	0.08	0.79	2.85	3.97	60.15
18	5.7	4.33	14.4	7.0	7.00	0.18	0.08	1.25	2.66	4.17	59.57
19	6.9	1.75	7.9	20.8	13.70	0.30	0.62	1.51	7.46	9.89	72.18
20	5.9	1.55	14.7	14.9	17.40	0.10	0.53	0.82	6.29	7.74	44.48
21	4.8	1.92	16.8	27.1	8.20	0.58	0.21	1.15	2.51	4.45	54.26
22	5.4	5.16	20.0	20.3	12.30	0.09	0.13	1.18	4.25	5.65	45.93

methods [19], the organic matter content (%C × 1.72) and the carbon–nitrogen relationship (C/N) were calculated. The results obtained are shown in Table I.

Reagents

The solutions used included 0.1 *M* cadmium chloride in methanol and 0.05% dithizone in carbon tetrachloride. The orange colour of cadmium–dithizone complex was readily observed on all the soil plates used.

Preparation of the soil plates

The soil samples were ground in a mortar and subsequently sieved through 160 μm mesh, after which 7.5 g of soil and 15 g of distilled water were used to prepare a slurry that was spread as a 0.5-mm-thick layer over each of the 20 × 5 cm plates used with the aid of a TLC soil applicator. The three central plates in each set of five used for each type of soil were chosen for the subsequent experiments. The selected plates were dried in a chamber at room temperature and a relative humidity of 70%.

Soil TLC procedure

The plates were marked with two horizontal lines at distances of 2 and 12 cm, from the base. One drop (*ca.* 5 μl) of the cadmium chloride solution was placed on the baseline of the three plates with the aid of a micropipette. The plates were then allowed to develop in closed individual glass chromatographic chambers that were 22 cm long and 3 cm wide by using distilled water as developer. Next, the plates were washed to a distance of 10 cm from the baseline and allowed to dry at room temperature. Finally, cadmium mobility was determined by spraying the plates with the dithizone solution.

Fig. 1 shows line sketches of some typical chromatograms obtained. All spots showed some tailing. Cadmium mobility was measured as R_F and R_b values by using the following relations:

$$R_F = R_L/10$$

$$R_b = R_t/10$$

where R_L and R_t denote the frontal distance travelled by the metal and the bottom of the spot, respectively.

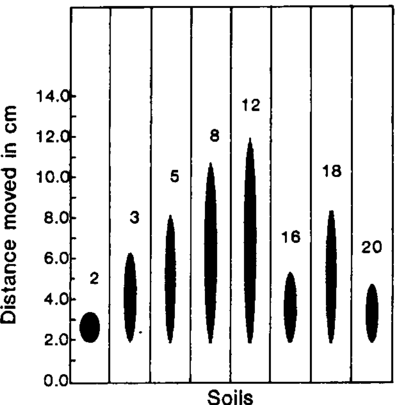

Fig. 1. Line sketches of some typical chromatograms.

RESULTS AND DISCUSSION

The results obtained in the determination of the mobility of cadmium in the 22 soils studied are shown in Table II as R_F values.

TABLE II

R_F OF CADMIUM FOR THE SOILS STUDIED

Soil No.	R_F (mean ± S.D., n = 3)
1	0.15 ± 0.01
2	0.14 ± 0.02
3	0.43 ± 0.01
4	0.85 ± 0.03
5	0.62 ± 0.01
6	0.19 ± 0.02
7	0.69 ± 0.03
8	0.87 ± 0.03
9	0.87 ± 0.02
10	0.93 ± 0.02
11	0.89 ± 0.03
12	1.00 ± 0.01
13	0.93 ± 0.02
14	1.00 ± 0.01
15	0.80 ± 0.01
16	0.34 ± 0.01
17	0.88 ± 0.03
18	0.66 ± 0.03
19	0.33 ± 0.02
20	0.27 ± 0.01
21	0.35 ± 0.01
22	0.80 ± 0.03
Range	0.14 ± 0.02–1.00 ± 001
Average	0.64 ± 0.02
Mode	0.87 ± 0.01

TABLE III

CADMIUM MOBILITY IN SOILS[a]

Class	R_F	Mobility	Soil No.	Soil (%)
1	0.00–0.09	Immobile	–	0
2	0.10–0.34	Slightly mobile	1, 2, 6, 16, 19, 20	27
3	0.35–0.64	Moderately mobile	3, 5, 21	14
4	0.65–0.89	Mobile	4, 7, 8, 9, 11, 15, 17, 18, 22	41
5	0.90–1.00	Very mobile	10, 12, 13, 14	18

[a] Classification according to Helling and Turner [10].

The R_F values for the soils varied from 0.14 to 1.00, which indicates that the mobility of cadmium in them was highly variable. The mean (0.64) and mode (0.87) of these values indicate that cadmium was highly mobile in many of the studied soils.

The R_b values were found to be 0.00 in all the chromatograms, so cadmium was not fully mobile in any soil.

Because the soil TLC technique has only recently begun to be used for determining cation mobility in soils, and since few systematic studies involving large numbers of soils have been carried out so far, no mobility classification according to R_F values has yet been put forward. One the other hand, there is one such classification for pesticides in soils, which was proposed by Helling and Turner [10]. Table III shows the cadmium mobility in the studied soils according to such a classification.

TABLE IV

SIMPLE CORRELATION COEFFICIENTS RELATING SOIL PROPERTIES TO R_F IN 22 SOILS

Soil property	Correlations coefficient (r)
pH	−0.66[a]
Organic matter content	0.16
Clay content	−0.45[b]
Cation-exchange capacity	−0.51[b]
Na^+ content	−0.06
K^+ content	−0.47[b]
Mg^{2+} content	−0.70[a]
Ca^{2+} content	−0.76[a]
Σ bases	−0.78[a]
Base saturation	−0.64[c]

[a] Significant at the < 0.001 level.
[b] Significant at the 0.05–0.01 level.
[c] Significant at the 0.01–0.001 level.

In order to determine the influence of the soil properties on the mobility of cadmium, we determined the simple correlations between the R_F values and soil properties. The correlation coefficients obtained are included in Table IV. As can be seen, there was a highly significant negative correlation ($p < 0.001$) between R_F and pH, the Ca^{2+} and Mg^{2+} contents and the sum of bases. There is also a significant correlation at the 0.01–0.001 level between R_F and percentage base saturation, and another significant correlation at the 0.05-0.01 level between R_F and the cation-exchange capacity, clay and K^+ contents. On the other hand, there is no correlation between R_F and the organic matter content.

The variability in the cadmium mobility is mostly accounted for by the sum of bases (R^2 = 0.61), followed by the contents of the divalent cations Ca^{2+} (R^2 = 0.58) and Mg^{2+} (R^2 = 0.49). This is a result of the proven competition [20–23] between Cd^{2+} and exchangeable cations, divalent calcium and magnesium in particular. Milberg *et al.* [20] found cadmium to be adsorbed preferentially over calcium in soils. Also, McBride *et al.* [21] observed the cadmium retention capacity of soils to depend markedly on the exchangeable calcium content of the soil concerned: the retention capacity increased with increase in the calcium concentration. This author believes the calcium content of a soil is a reliable indicator of its cadmium retention capacity. Kinniburgh *et al.* [22] established a sequence of relative affinity of divalent cations for soil surfaces where the affinity of all alkaline earth elements is always lower than that of cadmium, and García-Miragaya and Page [23] found the competition of

cadmium with exchangeable cations to decrease in the following order: Al < Ca < K < Na.

According to the above findings, the affinity for cadmium varies in the order Ca > Mg > K > Na, which is consistent with the variation of the correlation coefficients of R_F with the contents in these elements.

The pH of soil is considered by some researchers to be an important parameter on affecting the distribution and mobility of cadmium in soil [24,25]. Fuller [26] established a classification according to which cadmium should be fairly mobile in soils of pH 4.6–6.6 and moderately mobile in those of pH 6.7–7.8. The highly significant negative correlation between R_F and pH found in this work is consistent with this classification. Even though major generalizations are precluded by the large number of soil parameters that may influence cadmium mobility, we found high mobility (class 5) in soils whose pH values never exceed 5.6; on the other hand in soils whose pH values is $\geqslant$ 6.3 the mobility is slight (class 2).

We also observed a significant negative correlation between the R_F values and the cation-exchange capacity. Exchangeable cations are known to come from clay, the content in which was also significantly correlated with the cadmium mobility, and from organic matter. However, correlation with this last was insignificant and positive, so the increase in organic matter content should result in an increase in cadmium mobility. In fact, organic matter in soils may influence cadmium mobility by retaining the element through ion exchange, thereby reducing its mobility, and by favouring the formation of soluble complexes with the soluble humic fraction (fulvic acid), thus increasing the cadmium mobility [27]. Therefore cadmium eluviation in soils will be particularly marked in humic soils as the forests soils that possess high C/N ratios (*i.e.* they feature low degrees of organic matter humification and high fulvic acid contents). Soils 4, 9, 11, 12 and 13 were of this type. All of them yielded $R_F \geqslant 0.85$, so they allowed for a high cadmium mobility.

Complex-formation reactions between humic substances and heavy metals have aroused growing interest in the last few years [28–30]. The complex-forming ability of fulvic acids is ascribed to their possessing a number of oxygen-containing groups including–COOH, aromatic, alkyl and enol–OH, and –C=O functions of various types. The stability constant of the metal complex of cadmium with fulvic acid on the assumption of a 1:1 stoichiometry as determined by Mantoura *et al.* [31] varies between 4.57 and 4.95 (log *K*) depending on the acid concerned.

The results obtained in this work show the significance of soil properties to the behaviour of cadmium from industrial, mining and farming activities in soil. In addition, they prompt the need to determine some soil paramaters to be used as critical indicators in controlling the cadmium load of soils.

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CHROMSYMP. 2833

Application of solid-phase partition cartridges in the determination of fungicide residues in vegetable samples

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ABSTRACT

Disposable, ready-to-use cartridges filled with a macroporous diatomaceous material are used to extract in a single step fungicide residues with dichloromethane from aqueous acetone extracts of vegetables. This procedure takes the place of some functions (such as separating funnel partition, drying over anhydrous sodium sulphate and clean-up) usually performed by separate steps in classical schemes. Fourteen fungicides (dichloran, vinclozolin, chlorthalonil, triadimefon, dichlofluanide, procymidone, hexaconazole, captan, folpet, ditalimfos, iprodione, captafol, pyrazophos and fenarimol) were determined using the described procedure with recoveries between 83 and 107% at spiking levels ranging for the different compounds from 0.04 to 0.40 mg/kg. Crops subjected to the described procedure included lettuce, strawberry, apple, yellow pepper and peach, and gave extracts containing a mass of co-extractives between 5 and 30 mg. Compared with classical schemes, the described procedure is simple, less labour intensive, allows parallel handling of several extracts and does not require preparation and maintenance of equipment. Troublesome emulsions such as those frequently observed in separating funnel partitioning do not occur.

INTRODUCTION

Polar, water-miscible solvents, such as acetone [1–6], acetonitrile [7–10] or methanol [11] are the most frequently used solvents for the extraction of pesticide residues from vegetable samples in multiresidue procedures. With these solvents, pesticide residues are usually separated from crude aqueous acetone (or aqueous acetonitrile or aqueous methanol) extracts by dilution with salt solution and multiple separating funnel partitions into dichloromethane to remove hydrophilic unwanted co-extractives. Under these conditions, a wide range of both polar and non-polar pesticide residues can be recovered [2–6]. The dichloromethane is dried by passage through a column of anhydrous sodium sulphate and subjected to clean-up before the final determination. In multi-residue procedures, the clean-up is generally based on one or a combination of basic clean-up steps, such as size-exclusion chromatography [4,5,12–18], sweep co-distillation [19–23], column chromatography on Florisil [24–26], alumina [27,28], silica gel [4,29,30] or charcoal and its mixtures [2,7,31]. For certain crops and/or levels of determination, the crude dichloromethane extracts can be used without further clean-up [6].

In general, the drawbacks of the above-mentioned and other similar procedures are the amounts of solvents and reagents required, the washing and preparation of glassware, the occurrence of troublesome emulsions in the aqueous acetone–dichloromethane partition stage with certain vegetables, the preparation and maintenance of costly apparatus and, most important, the number of handling operations, which strongly affect the throughput of the residue laboratory.

On the basis of our experience in the control of vegetables for pesticide residues, a major part of residues occurring especially on leafy vegetables are

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attributable to fungicides. Therefore, we sought a non-conventional procedure, applicable to several fungicide compounds, for the rapid separation of these residues from crude aqueous acetone extracts, which could avoid the use of a separating funnel and provide at the same time a certain clean-up.

As we have reported previously on the advantages of the use of solid-phase partition cartridges in the field of pesticide residue determination [32–37], we tried a similar approach for the determination of different classes of fungicides in vegetable samples.

EXPERIMENTAL

Reagents

Dichloromethane and acetone were analytical-reagent grade solvents redistilled from an all-glass apparatus. Extrelut-20 cartridges obtained from Merck (Darmstadt, Germany) were washed with 6 *M* HCl until yellow colour was no longer eluted from the column and washed with water until neutrality. The water remaining in the column was removed with 100 ml of acetone and the acetone was removed with an upward stream of nitrogen at 1 l/min. Pesticide reference standards from the collection in this laboratory were kindly supplied by the main manufacturer of the pesticides and were >99% pure.

Apparatus

GC analyses were carried out on a Hewlett-Packard Model 5890 gas chromatograph with electron-capture detection (ECD). An HP 17 wide-bore, fused-silica column (cross-linked 50% phenyl–50% methylsilicone) was used. The gas flow-rates were carrier gas (helium) 7 ml/min, split vent 9 ml/min and septum purge vent 1 ml/min, the column head pressure was 10.5 kPa and the auxiliary gas to the detector was nitrogen at 60 ml/min. The column oven temperature programme was 50°C held for 2 min, increased to 180°C at 10°C/min, then to 270°C at 5°C/min and finally held at 270°C for 20 min. The injector was splitless, temperature 240°C and purge-off time 60 s. The detector temperature was 300°C. Quantification was carried out by the external standard method.

Procedure

Prepare aqueous acetone extracts of fruits and vegetables by homogenizing 100 g of vegetable with 200 ml of acetone, filtering and diluting with acetone washings to 350 ml according to ref. 2. Take an aliquot of 20 ml of the extract equivalent to *ca.* 5.7 g of crop and transfer it on to the top of an Extrelut-20 column. Allow the liquid to drain and wait 10 min to obtain an even distribution on the filling material. Pass through the column, from bottom to top, a nitrogen flow of 1 l/min for 20 min. Disconnect the Extrelut-20 column from the gas line, attach to the column outlet a 32 × 0.70 mm I.D. Luer-lock needle (supplied with the column) as a flow restrictor and elute the column with five 20-ml portions of dichloromethane. Concentrate to a small volume using a rotary evaporator (40°C; reduced pressure), then to dryness by manually rotating the collecting flask. Dilute to a suitable volume with acetone and analyse by GC–ECD.

For recovery experiments, add suitable amounts of standards to the chopped vegetables in the homogenization jar. Allow the solvent to evaporate, then proceed with the extraction.

RESULTS AND DISCUSSION

As discussed in the Introduction, liquid–liquid partitioning is used to separate compounds of interest from the bulk of the crude extract. In conventional procedures the liquid–liquid partitioning is carried out by shaking the two phases in a separating funnel, and repeating this step several times to attain completeness of the transfer process. Some drawbacks are associated with this kind of operation.

Basically the same operation can be performed on solid-phase, ready-to-use, disposable cartridges filled with a macroporous diatomaceous earth, which is used to hold one of the liquid phases (the crude aqueous acetone extract) while the other (the partition solvent) is simply poured in portions on to the cartridge and allowed to drain. This type of cartridge is commercially available from different manufacturers. We used Extrelut-20 cartridges that can hold *ca.* 20 ml of crude aqueous acetone extract, leaving *ca.* 1 cm of the bed at the bottom unwetted.

Before running the dichloromethane (the partitioning solvent) through the column, acetone is partially removed with an upward stream of nitrogen. This reduces the acetone in the partition

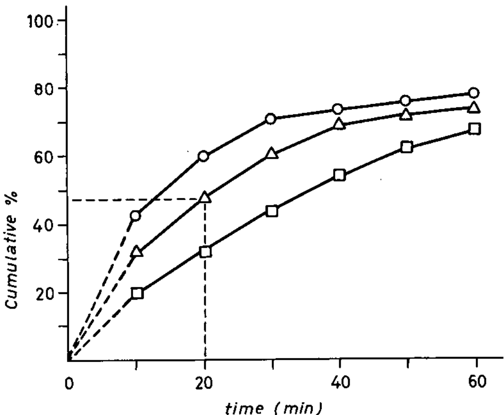

Fig. 1. Cumulative percentage mass loss (y-axis) of Extrelut-20 cartridges loaded with 20 ml of aqueous acetone extract using different nitrogen flow-rates: ○ = 2; △ = 1; □ = 0.5 l/min.

solvent, thus preventing the carryover of water and, by reducing the eluting strength of the draining mixture, improving the clean-up. Different combinations of flow and time parameters have been tried (see Fig. 1). In the first application we reported [32], we used a nitrogen flow of 2 l/min for 30 min, which removed a great proportion of acetone and consequently green pigments of vegetables were retained on the column, when it was eluted with low-polarity solvents (light petroleum or 25% dichloromethane in light petroleum of b.p. 40–60°C). However, as we are interested in a general purpose procedure, we now use conditions (1 l/min for 20 min) that leave more acetone on the column (*ca.* 50% of the original content), thus allowing the recovery of more polar compounds, such as the fungicides under consideration. Under these conditions the mass of material remaining in the extract after the solid-phase partitioning is in the range 5–30 mg, having loaded on to the column the equivalent of 5.7 g of different fruits and vegetables. This mass range is of the same order as that obtained by subjecting the aqueous acetone extract to the classical, time-consuming sequence of separating funnel partitioning into dichloromethane, drying over anhydrous sodium sulphate, solvent exchange and clean-up. However, compared with the classical schemes, the same performance with our method is obtained in a shorter time (*ca.* 60 min), with very simple operations, and by using only one disposable item and a reduced volume of solvent. Crops subjected to the described procedure included lettuce, strawberry, apple, yellow pepper and peach. Although the extract from lettuce contains green pigments, it is amenable to capillary GC with splitless or direct (in liner) injection techniques. Figs. 2–4 show chromatograms obtained from the analysis of representative crops containing incurred fungicide residues, which show a satisfactory clean-up of the crop extract. Fig. 5 shows a chromatogram of the standard mixture of fourteen fungicides.

The fourteen fungicides assayed with the described procedure were dichloran, vinclozolin, chlorthalonil, triadimefon, dichlofluanide, procymidone, hexaconazole, captan, folpet, ditalimfos, iprodione, captafol, pyrazophos and fenarimol. For recovery experiments, the pesticides were added to a vegetable in a homogenization jar, the extraction was carried out with acetone and a portion of the acetone extract was processed according to the described procedure. In Table I are presented the results of the recovery experiments. The recoveries of the fourteen fungicides were satisfactory (between 83 and 107%) when the solid-phase cartridge was eluted with 100 ml of

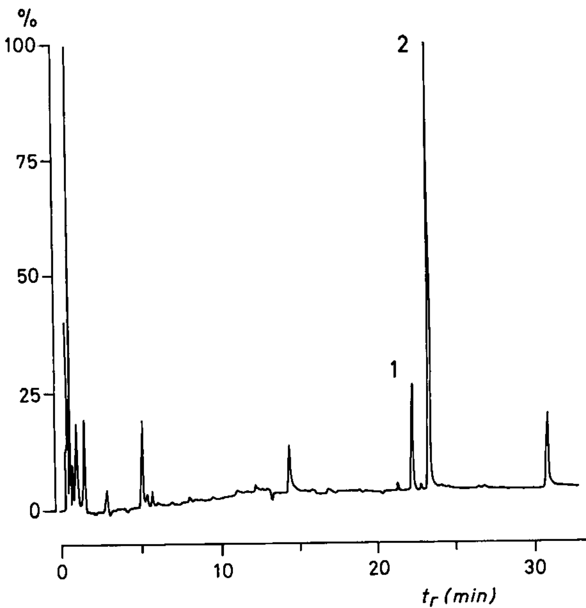

Fig. 2. GC–ECD of lettuce extract with incurred residues: 5.7 g in 250 ml, 1 μl injected. 1 = Vinclozolin (4.3 mg/kg); 2 = chlorthalonil (9.8 mg/kg).

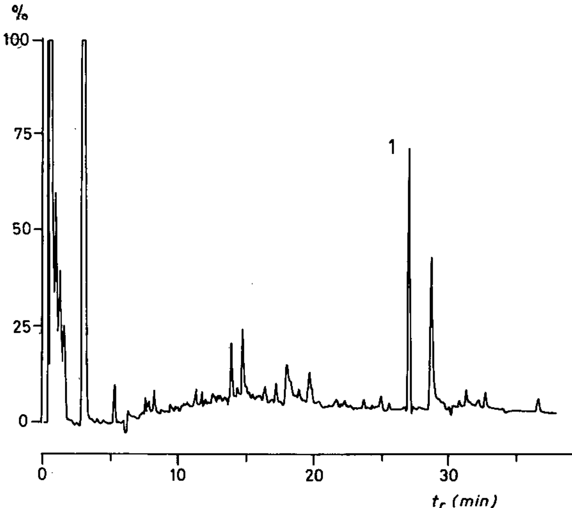

Fig. 3. GC–ECD of apple extract with incurred residues: 5.7 g in 25 ml, 1 μl injected. 1 = Procymidone (2.1 mg/kg).

dichloromethane at spiking levels ranging from 0.04 to 0.40 mg/kg for the different fungicides. In the development of the method consistently low recoveries of captan, folpet and captafol were observed when Extrelut-20 cartridges were used as supplied. The cause was attributed to the cartridges and, when the compounds could not be eluted with high volumes of dichloromethane, a reaction with or

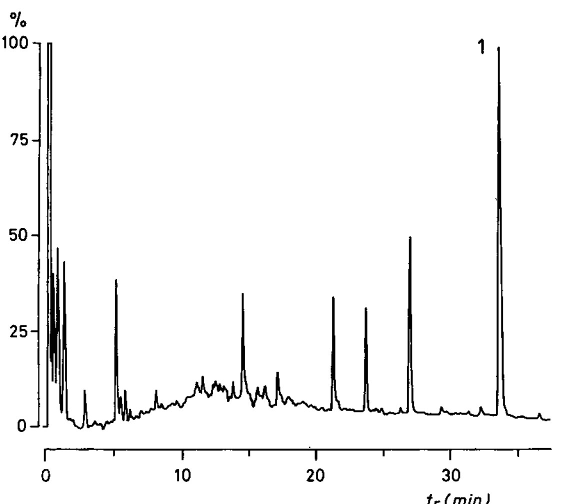

Fig. 4. GC–ECD of strawberry extract with incurred residues: 5.7 g in 40 ml, 1 μl injected. 1 = Iprodione (8.0 mg/kg).

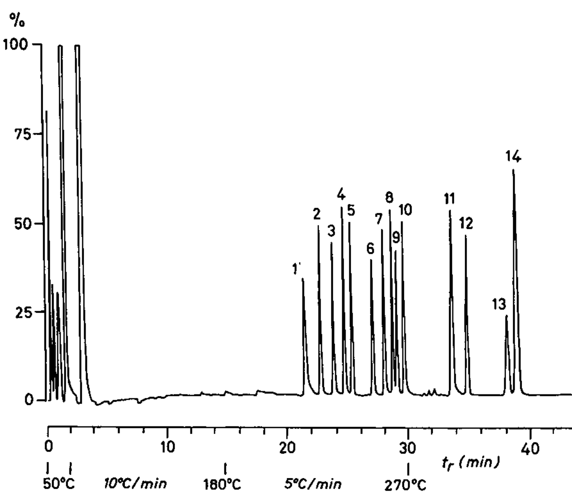

Fig. 5. GC–ECD of the standard mixture of fourteen fungicides. 1 = Dichloran (0.1 ng); 2 = vinclozolin (0.2 ng); 3 = chlorthalonil (0.1 ng); 4 = triadimefon (0.32 ng); 5 = dichlofluanid (0.50 ng); 6 = procymidone (0.7 ng); 7 = hexaconazole (0.30 ng); 8 = captan (0.24 ng); 9 = folpet (0.21 ng); 10 = ditalimfos (0.40 ng); 11 = iprodione (1.0 ng); 12 = captafol (0.39 ng); 13 = pyrazophos (1.00 ng); 14 = fenarimol (0.50 ng).

TABLE I

RECOVERIES OF FOURTEEN FUNGICIDES ADDED TO "BLANK" LETTUCE SAMPLES DETERMINED BY THE DESCRIBED PROCEDURE, AND COMPARISON BETWEEN ACID-WASHED AND NON-ACID-WASHED EXTRELUT-20 COLUMNS

Fungicide	Spiking level (mg/kg)	Mean reovery ± R.S.D. (%) (n = 6)	
		Acid-washed	Non-acid-washed
Dicloran	0.04	94 ± 6	92 ± 5
Vinclozolin	0.08	101 ± 8	107 ± 11
Chlorthalonil	0.04	107 ± 10	89 ± 10
Triadimefon	0.13	91 ± 10	87 ± 11
Dichlofluanid	0.20	88 ± 11	76 ± 12
Procymidone	0.28	93 ± 11	87 ± 12
Hexaconazole	0.12	92 ± 11	87 ± 11
Captan	0.10	85 ± 13	67 ± 13
Folpet	0.08	83 ± 24	67 ± 15
Ditalimfos	0.16	90 ± 19	78 ± 14
Iprodione	0.40	93 ± 17	88 ± 14
Captafol	0.16	92 ± 20	50 ± 17
Pyrazophos	0.40	94 ± 18	90 ± 16
Fenarimol	0.40	92 ± 17	89 ± 16

irreversible adsorption by the filling material was assumed. The results in Table I show that good recoveries for these three compounds could be obtained with acid-washed cartridges. The performance of the method was also tested by analysing crops containing incurred residues with both the described procedure and the conventional procedure of separating funnel partitioning. The results, given in Table II, show satisfactory agreement.

The main feature of the described procedure is that the column appears to perform several functions in a single step, *viz.*, the removal of water and hydrophilic co-extractives, the transfer of pesticide residues into a low-boiling solvent and a low-activity adsorption clean-up. The last function is not easily recognized when the extract is analysed by GC–ECD, but it is more apparent when alkali flame ionization detection (AFID) is used, as can be seen from Fig. 6, where the GC–AFID (the GC conditions were the same as for GC–ECD) of an apple analysed for triazophos (an organophosphorus compound not considered in this work) by acetone extraction and separating funnel partitioning according to ref. 2 is compared with GC–AFID performed after acetone extraction and Extrelut-20 partitioning (the procedure described here). In classical schemes, the same functions are carried out through separate, time-consuming and labour- and glassware-intensive operations. Unlike the classical separating funnel partitioning, with the described procedure the extraction is rapid, emulsions do not occur and addition of salt solution and drying of the extraction solvent with anhydrous sodium sulphate are not necessary. In comparison with the described procedure, the procedure reported by Hopper [38] for partitioning of organophosphorus pesticide residues on a solid-phase partition column requires very large volumes of solvents and reagents to condition the column prior to use and appears lengthy. Compared with instrumental clean-up techniques (*e.g.*, size-exclusion chromatography and sweep co-distillation), the described procedure is very simple,

TABLE II

COMPARISON BETWEEN RESULTS OBTAINED IN THE ANALYSIS OF VEGETABLE SAMPLES WITH INCURRED FUNGICIDE RESIDUES USING THE CONVENTIONAL AND THE PROPOSED PROCEDURES

Sample	Fungicide	Residues (mg/kg)	
		Conventional procedure[a]	Proposed procedure
Apple	Procymidone	1.8	2.1
Celery	Vinclozolin	1.7	1.6
	Vinclozolin	0.06	0.05
	Iprodione	0.1	0.2
Lettuce	Vinclozolin	14.0	13.8
	Vinclozolin	2.6	4.3
	Chlorthalonil	4.6	3.8
	Chlorthalonil	11.6	9.8
	Procymidone	0.8	0.7
Strawberry	Chlothalonil	1.2	0.9
	Procymidone	3.1	2.6
	Procymidone	0.5	1.0
	Vinclozolin	1.1	1.0
	Iprodione	9.6	8.8

[a] Acetone extraction and separating funnel partitioning into dichloromethane according to ref. 2.

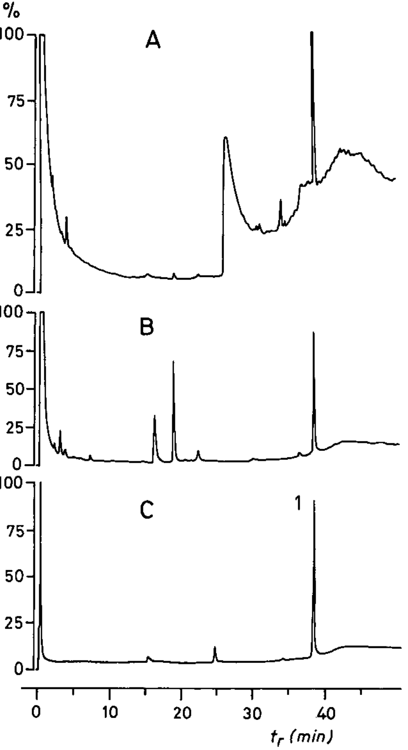

Fig. 6. GC–AFID of an apple extract obtained by (A) acetone extraction and partitioning into dichloromethane according to ref. 2, 25 g in 2 ml, 1 μl injected, and (B) acetone extraction and Etrelut-20 partitioning, 5.7 g in 1 ml, 2 μl injected. (C) Triazophos standard (2 ng). GC conditions as for GC–ECD.

rapid and inexpensive and does not require the preparation and maintenance of costly apparatus or skilled operators.

CONCLUSIONS

Unlike the classical procedures, separation of fungicide residues from hydrophilic co-extractives is carried out in a single step on ready-to-use, disposable cartridges filled with a macroporous diatomaceous earth. The essential features of this procedure include high efficiency of the process, lack of emulsions, reduced consumption of solvents, no reusable glassware, single-step and straightforward operations, low-cost items, reduced time and the possibility of parallel handling of several samples.

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Review

Liquid chromatographic analysis of antibacterial drug residues in food products of animal origin

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ABSTRACT

This paper reviews recent developments in the liquid chromatographic (LC) methods of analysis for the residues of antibiotics (aminoglycosides, chloramphenicol, sulfonamides, tetracyclines, macrolides, β-lactams, etc.) in food products of animal origin. The review also covers clean-up procedures, such as, ultrafiltration, liquid–liquid partition, solid-phase extraction, immunoaffinity, and matrix solid-phase dispersion, for use as extraction, deproteination, and concentration steps. The LC methods offer considerable potential for rapid automated analysis, and some may be used as direct screening for residues in meat and milk.

CONTENTS

1. Introduction . . . 369
2. Aminoglycoside antibiotics . . . 370
3. Chloramphenicol . . . 371
4. Sulfonamides . . . 372
5. Tetracyclines . . . 372
6. Macrolide antibiotics . . . 374
7. β-Lactam antibiotics . . . 375
8. Other atibiotics . . . 375
9. Conclusions . . . 376
References . . . 376

1. INTRODUCTION

Antibiotics are used in food-producing animals not only for treatment of disease, but also subtherapeutically to maintain health and promote growth. The use of unauthorized antibiotics or the failure to follow label directions for approved antibiotics could result in unsafe antibiotic residues in food products. Therefore, monitoring antibiotic residues in food forms part of a general policy to prevent unapproved uses of antibiotics.

Traditionally, most antibiotics have been determined by microbiological assay. However, it is very difficult to distinguish one antibiotic from another

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using microbiological methods. The United States Department of Agricultures's Food Safety Inspection Service frequently finds microbial inhibitors in animal tissues which can not be identified by standard multiresidue procedures. There is increasing recognition of the need for improved procedures for identification and quantitation of suspect residues detected by screening methods. Liquid chromatography (LC) has emerged as the method of choice for determination of antibiotics which are rather polar, non-volatile, and sometimes heat sensitive. Other chromatographic modes such as gas–liquid and supercritical fluid chromatography have had very limited application to the determination of antibiotics. Thin-layer chromatography could be an inexpensive alternative, although it lacks sensitivity and reproducibility and quantitation is more difficult.

Many methods have been described for determination of antibiotics in formulations and in biological fluids for clinical applications. For residue analysis, isolation from more complex substrates and greater sensitivity to meet the established tolerance is required. To be useful, chromatographic methods should equal or exceed the sensitivity of screening tests. Otherwise, doubt will remain as to the identity of residues detected by screening tests if they are not detectable by chromatographic methods.

Development of methods with adequate sensitivity has proven elusive for many compounds. However, significant progress has been made in recent years. This paper discusses recent progress in the applications of LC methods for determination of antibiotic residues in food products of animal origin.

2. AMINOGLYCOSIDE ANTIBIOTICS

In recent years, LC has been increasingly used as a method of choice for the determination of aminoglycoside antibiotic residues in tissues and milk of food-producing animals. A detailed review of physical-chemical methods, including LC methods for aminoglycoside antibiotics in tissues and fluids of food-producing animals was reported in 1985 [1]. The overwhelming majority of procedures for aminoglycoside antibiotics use paired-ion chromatography on reversed-phase columns. Generally, post-column derivatization has been used with the paired-ion technique. In pre-column derivatization, the chromophore is linked to the primary amine group of aminoglycosides, yielding a less polar solute which then is readily separated by reversed-phase partition. Before LC analysis, sample pretreatment is needed to remove endogenous substances so that they do not interfere with the compounds of interest during LC analysis. Shaikh and co-workers [2–4] reported determination of neomycin in tissues and milk by LC using ion-pairing mobile phase, post-column derivatization with *o*-pthalaldehyde (OPA), and fluorometric detection. The limit of determination of neomycin in kidney tissue and milk was 1 mg/kg and 0.15 μg/ml, respectively. Buffer extraction and heat deproteination was used to extract neomycin from the tissues. Direct centrifugation of whole milk at 4°C was used to separate lipid material from the aqueous part of the milk. This was followed by deproteination with trichloroacetic acid before LC analysis. This LC procedure has also been applied to the determination of gentamicin in milk [5]. The above defattening and deproteination procedures were also used for the determination of gentamicin in milk. An additional solid-phase extraction (SPE) step, as reported by D'Souza and Ogilvie [6], was included to concentrate gentamicin on an SPE column to lower the limit of determination to 30 ng/ml. The injection volume was 500 ul and LC condition used were same as reported in ref. 4 above.

Schenck [7] used a matrix solid-phase disperson (MSPD) technique to extract neomycin from bovine kidney tissue and quantitated by using post-column LC, as reported previously [2]. The limit of determination was 2.5 mg/kg.

McLaughlin *et al.* [8] used Schenck's MSPD technique [7] to extract a number of aminoglycosides from bovine kidney tissue. Formic acid instead of sulfuric acid was used to elute the analytes from the MSPD column to obtain improved peak shapes. The LC separation was performed on a minibore YMCbasic (C_8) column using a gradient mobile phase containing acetonitrile and pentafluoropropionic acid as ion-pairing agent. The detection was by mass spectrometry (MS) using ion spray interface. This LC–MS system was used to detect aminoglycosides such as, neomycin, gentamicin, streptomycin and dihydrostreptomycin in fortified bovine kidney tissue below 1 mg/kg. However, no recoveries from fortified kidney were reported for any of the aminoglycosides.

Agarwal used pre-column derivatization for the determination of gentamicin in bovine muscle tissue and milk [9,10] and neomycin in milk [11]. For gentamicin, CM-Sephadex was used to remove endogenous interfering compounds from both tissue and milk, followed by further purification and on-column derivatization with OPA on silica Sep-Pak cartridges. The detection limit for gentamicin in both tissue and milk was 0.2 μg/ml. However, only two of the three major components of gentamicin, C_{1a} and C_2, were resolved from interfering background compounds in tissue. A different weak cation-exchange resin, Amberlite CG-50, was used for isolation of neomycin from milk. This was followed by on-column derivatization with OPA. A HISEP reversed-phase LC column, ion-pairing mobile phase, and fluorometric detection was used for LC analysis. The detection limit was 50 ng/ml. However, the OPA derivative of neomycin formed two peaks and had to be stored in the freezer for 15 min to achieve complete derivatization, before LC analysis.

Okayama *et al.* [12] reported LC determination of streptomycin in meat using ninhydrin as a post-column derivatization reagent. The method included extraction with perchloric acid solution and clean-up using a C_8 pretreatment SPE column. The LC conditions employed were as follows: reversed-phase C_{18} column; mobile phase of water–acetonitrile containing disodium 1,2-ethanesulfonate, sodium 1-octanesulfonate, and ninhydrin; post-column reaction solution of 0.3 *M* sodium hydroxide; and fluorescence detection. The method was used to determine streptomycin in chicken meat, and the recovery of added streptomycin at the 2 μg/g level was about 67%.

Shaikh *et al.* [13] developed LC conditions for the separation and determination of streptomycin and dihydrostreptomycin reference standards. The conditions used were as follows: reversed-phase ODS column (Spherisorb 5 ODS 2, 15 cm × 4.5 mm I.D.) at 50°C; mobile phase, 20 m*M* sodium hexane sulfonate, 25 m*M* tribasic sodium phosphate, 5 m*M* ninhydrin in acetonitrile–water (8:92), pH adjusted to 3.0 with phosphoric acid; post-column reagent, 0.5 *M* sodium hydroxide; and fluorescence detection at 400 and 495 nm excitation and emission wavelengths, respectively. A number of clean-up systems were also evaluated to isolate streptomycin and dihydrostreptomycin from bovine kidney tissue. The most promising was the extraction procedure of Okayama *et al.* [12] followed by additional clean-up using polymeric materials, Polysorb MP-1 solid-phase extraction cartridges (Interaction Chromatography, San Jose, CA, USA). However, the overall determinative procedure is not completed.

Recently, Gerhardt *et al.* [14] reported determination of streptomycin in porcine and bovine tissue by reversed-phase LC. Streptomycin is extracted with 3.6% perchloric acid as reported by Okayama *et al.* [12]. The extract is further purified on cation-exchange SPE column and analyzed using an inline column enrichment–post-column derivatization LC system with fluorescent detection. The limit of detection was 20 μg/kg, and the mean recovery from fortified tissue was 61.5%. However, the standard curve was prepared in tissue extracts for quantitative analysis.

3. CHLORAMPHENICOL

Allen [15] reviewed chromatographic methods, including LC, for the determination of chloramphenicol (CAP) in food products of animal origin. All methods used ethylacetate extraction followed by liquid–liquid partition and in some cases purification on SPE cartridges before HPLC analysis. Allen provided a detailed review of LC methods; therefore, only additional developments since then will be reviewed.

Sanders *et al.* [16] reported LC determination of CAP in calf tissue. The method employs ethylacetate for extraction followed by liquid–liquid partition with hexane–chloroform–water. The LC analysis consists of a reversed-phase column, acetonitrile–phosphate buffer mobile phase, and UV detection at 275 nm. The detection limit in muscle was 1 μg/kg. CAP was also found to be stable in muscle at −20°C for 180 days.

Van de Water and Haagsma [17] reported analysis of CAP residues in swine tissues and milk. The authors used silica gel SPE cartridges and antibody-mediated clean-up (AMC) as sample pretreatment procedures. Originally, the SPE procedure was developed for isolation of CAP from swine tissue [18] and later modified for use in milk. The milk method employs ethylacetate extraction, SPE clean-up, and LC analysis. The AMC procedure is based on a very specific clean-up and concentration of CAP from

aqueous meat extracts and defatted milk using immobilized monoclonal antibodies directed against CAP [16,18]. The monoclonal antibodies are covalently bound to immunoaffinity gel (carbonyldiimidazole-activated trisacryl GF-2000). The sample solutions are passed through these immunoaffinity columns by means of a peristaltic pump. The columns are washed with phosphate-buffered saline (PBS). The antibody-bound CAP was eluted with 20 ml of a solution containing 0.2 *M* glycine and 0.5 *M* NaCl (pH 2.8). The HPLC consisted of reversed-phase column, acetonitrile–0.01 *M* sodium acetate buffer (1:3, v/v), and UV detection at 280 nm. The limit of determination was 1 μg/kg in milk and 10 μg/kg in swine tissue. The results of LC procedures compared well with two enzyme-linked immunosorbent assay (ELISA) screening procedures.

4. SULFONAMIDES

Recently Agarwal [19] provided an exhaustive and updated review of LC methods for the determination of sulfonamides in tissues, milk, and eggs. Therefore, only general approaches to the LC determination of sulfonamides will be discussed here. Traditionally, the extraction of sulfonamides from various matrices has involved use of organic solvents such as chloroform, acetonitrile, or acetone followed by extraction with hexane to remove lipids. For example, Weber and Smedley [20] quantitated 10 ng/ml and above sulfamethazine in milk using a simple chloroform extraction followed by partitioning between potassium phosphate buffer and hexane to remove lipids. They further extended this work to the determination of ten sulfonamides in milk [21] by using a chloroform–acetone extraction. In many cases, an additional [19] liquid–liquid extraction is also carried out to further purify the sample extracts before LC analysis. However, in the recent past, use of SPE columns has been introduced to replace liquid–liquid extraction steps. The SPE columns used include Cyclobond-1, where β-cyclodextrin is bonded to silica [22], C_{18} [23–25] and cation-exchange resins [26]. The use of SPE columns has not only provided cleaner extracts but significantly reduced use of organic solvents and hence contributed to the reduction of amounts of hazardous waste generated. Long *et al.* [27] extracted sulfonamides from tissue or milk using MSPD techniques, where the sample is directly blended with C_{18} material. This was followed by LC analysis with a limit of detection of 31 μg/kg. The LC analysis in most cases was carried out on C_{18} columns. However, in some cases, C_8 and C_2 columns were also used. Detection in most cases was UV with a limit of determination of 5–10 μg/kg. However, fluorescence derivatization with *p*-dimethylaminobenzyldehyde (DMAB) [23] and electrochemical detection [28] were also employed. Fluorescence detection provided reduced background levels and was more discriminatory for sulfonamides resulting in increased sensitivity. Electrochemical detection was comparable to UV detection.

5. TETRACYCLINES

Significant progress has been reported in recent years on development of HPLC methods for determination of the tetracycline group of antibiotics in food substrates including honey, milk, tissues and eggs. A variety of approaches to extraction, clean-up, and HPLC analysis have been used.

Honey has been analyzed directly for oxytetracycline with no sample preparation other than dilution and filtration [29,30]. In this case, the LC method is comparable in speed and simplicity to screening methods. Others used a preliminary extraction and clean-up for determination of oxytetracycline in honey [31,32].

Other food substrates require some type of extraction procedure. For determination of residues in milk, Thomas [33] used ultrafiltration followed by direct injection of the filtrate. Recoveries were near 100% but separation from interferences was less satisfactory, limiting sensitivity. Kijak [34] used Thomas' ultrafiltration [33] in combination with a modification of Oka's *et al.* [35] C_{18} SPE procedure to obtain cleaner extracts for use in LC–MS. Fletouris *et al.* [36] and White *et al.* [37] used extraction with HCl–acetonitrile. Fletouris *et al.* [36] used a partitioning clean-up. White *et al.* [37] injected the water layer formed by adding methylene chloride and hexane to the filtrate. Farrington *et al.* [38] described a method using extraction with pH 4.0 buffer followed by clean-up on chelating sepharose and XAD-2 resin. This procedure was further modified by Carson and co-workers [39,40] for determination of seven tetracyclines in milk. For analysis, Thomas

[33] used a bonded ODS column with oxalate buffer at pH 2.0. Fletouris *et al.* [36] used an ODS column with phosphoric acid. They found it necessary to saturate the column with chlortetracycline for satisfactory results. White *et al.* [37] used a polymeric PLRP-S column (Polymer Labs, Amherst, MA, USA) with pH 2.0 oxalate buffer containing sodium decanesulfonate as an ion-pair to improve separation from interferences. Carson [40] also found that use of a polymeric column for analysis was advantageous. The method of Thomas [33] will detect residues at 10–20 ng/ml and the other procedures can determine tetracyclines at levels of less than 10 ng/ml in milk. This is well under official levels of concern which are 80, 30 and 30 ng/ml for tetracycline, oxytetracycline, and chlortetracycline, respectively, in the USA [41] and also below the maximum levels of 100 ng/ml in milk recommended by the World Health Organization (WHO) [42].

Long *et al.* [43,44] used MSPD to extract oxytetracycline, tetracycline, and chlorotetracycline from milk and oxytetracycline from catfish. For analysis, a reversed-phase ODS column was used with a mobile phase of 0.01 *M* oxalic acid–acetonitrile (70:30) for milk and 0.02 *M* oxalic acid–acetonitrile–methanol (70:27.5:2.5) for catfish. The limit of determination was 100 ng/ml for milk and 50 μg/kg for catfish.

Sharma and Bevill [45] described a procedure for extraction of tetracyclines from tissues into methylene chloride using the complexing agents phenylbutazone, calcium chloride, and sodium barbital. The tetracyclines were then extracted with 0.33 *M* phosphoric acid prior to analysis. The recoveries were good with a sensitivity limit of 0.5 mg/kg in tissues. The authors noted the need for extensive conditioning of the bonded ODS column prior to use.

Bocker and Estler [46] found that 0.03 *M* H_3PO_4–acetonitrile was superior to extraction with acid alone for recovery of residues from tissues. Aliquots of the extract were filtered and analyzed using a C_8 bonded column with NaH_2PO_4 buffer adjusted to pH 2.4 with 0.1 *M* HNO_3 as described by Sharma *et al.* [47].

Onji *et al.* [48] used extraction with 1 *M* HCl to recover tetracyclines from meat and tissue. Residues were concentrated by clean-up on an XAD-2 column. They noted losses during evaporation, especially of chlortetracycline. Analysis was on either dimethyl silica or polystyrene columns.

Ashworth [49] used acid and heat to convert tetracyclines in tissues to the anhydro forms which could then be extracted with chloroform. He described problems encountered with the use of silica-based reversed-phase columns.

Nelis and De Leenheer [50] extracted doxycycline from human tissue with 0.1 *M* HCl followed by partitioning into ethyl acetate from phosphate–sulfite buffer. For analysis, a LiChrosorb-RP-8 column was used with 0.1 *M* citric acid–acetonitrile (75:25) as the mobile phase.

Oka and co-workers [35,51] extracted tetracycline from tissues using pH 4.0 Na_2EDTA–McIlvaine buffer. For clean-up a Baker C_{18} SPE cartridge was used. They found that retention of tetracyclines on commercial C_{18} SPE cartridges differed considerably. For analysis, a LiChrosorb RP-8 column was used with a mobile phase of methanol–acetonitrile–0.01 *M* pH 2.0 oxalic acid (2:3:5). This procedure was applied to a number of substrates including milk and eggs. Ikai *et al.* [52] reported further studies with this procedure which has reported detection limits of 0.01 mg/kg in tissues.

Moats [53] found that optimum extractions from tissues were obtained using 1 *M* HCl–acetonitrile. The tetracycline could be recovered in the water layer formed when hexane and methylene chloride were added to the filtrates. Multiple injections were used to concentrate the tetracycline on the HPLC column. They were then eluted with an acetonitrile gradient. A polymeric PLRP-S column was used for analysis which avoided the problems reported with silica-based columns. For analysis, a mobile phase of 0.01 *M* H_3PO_4–methanol–acetonitrile was used with a gradient of 80:20:0 (0.2 min)–30:20:50 (25 min).

Mulders and Van de Lagemaat [54] used the extraction system described by Oka *et al.* [35] for animal tissues. A Sep-Pak C_{18} cartridge was used for clean-up after silylation of the cartridge. For analysis a NovaPak Phenyl Radial-Pak column was used with a Resolve CN guard cartridge. The mobile phase was acetonitrile–0.02 *M* oxalic acid–methanol 15:80:5 (0 min)–27:60:13 (23 min).

Nordlander *et al.* [55], extracted fish tissue with 1 *M* HCl–trichloracetic acid. For clean-up, a Sep-Pak

C_{18} cartridge was used. For analysis, a Shandon ODS Hypersil column was used. The mobile phase was pH 2.5 phosphate buffer with diethanol amine–acetonitrile–dimethylformamide (81:19:6).

Kondo *et al.* [56] extracted tetracycline from bovine tissue into ethyl acetate using the procedure described above [45]. The compounds were then extracted into phosphoric acid. For analysis, a μBondapak C_{18} column was used with a mobile phase similar to that described by Nordlander *et al.* [55].

Reimer and Young [57] used an extraction and clean-up procedure for fish similar to that described by Oka *et al.* [35]. For analysis, a Merck Hibar LiChro CART RP-18 column was used with a mobile phase of 0.01 *M* oxalic acid–acetonitrile–methanol (73:17:10).

Rogstad *et al.* [58] extracted fish tissue with 0.1 *M* Na_2EDTA in pH 4.2 phosphate buffer. Clean-up was on C_8 SPE column. For analysis, a Supelcosil LC-18-DB column was used with pH 2 phosphate buffer–acetonitrile–tetrahydrofuran (81:10:9).

Murray *et al.* [59] extracted fish tissue with HCl and $HClO_4$ mixture followed by clean-up on an XAD-2 column. For analysis, a Hypersil SAS column was used with a mobile phase consisting of citrate buffer and acetonitrile (70:30) with added Na_2EDTA.

Farrington *et al.* [38] used pH 4.0 succinate buffer containing EDTA for extraction of residues from tissues. As with milk, a chelating Sepharose column was used. A second clean-up step on an XAD-2 column was included. For analysis, a LiChrosorb RP-8 column was used with 0.01 *M* oxalic acid–acetonitrile (1:1) as the mobile phase.

Botsoglou *et al.* [60] described a procedure for determination of tetracyclines in eggs using extraction into methylene chloride with the aid of complexing agents. The tetracyclines were then recovered in acid. Analysis was on an ODS reversed-phase column using pH 2.6 phosphate buffer–acetonitrile.

Blanchflower *et al.* [61] described a procedure for determination of chlortetracycline in tissues in which tissues were extracted with 1 *M* HCl–glycine. For clean-up, the extract was passed through Bond-Elut cyclohexyl cartridges. The chlortetracycline was converted to a fluorescent derivative in pH 12 glycine buffer. For analysis, a polymer PLRP-S column was used with pH 12 glycine buffer.

Walsh *et al.* [62] determined tetracyclines in beef and pork muscle by HPLC. The tissues were homogenized in EDTA–McIlvaine buffer, centrifuged, and precipitated with trichloroacetic acid. For clean-up, Sep-Pak cartridges were used. For analysis, a NovaPak C_{18} column was used with phosphate–citrate–acetonitrile buffer.

Riond *et al.* [63] described a procedure for determining doxycycline in bovine tissues and body fluids. Ultrafiltration was used for clean-up of extracts.

Tolerances for tetracycline in edible tissues vary from 0.1–4 mg/kg in the USA [49]; and in Canada, tolerances have been set at 1, 2 and 0.25 mg/kg in edible tissue for chlorotetracycline, oxytetracycline, and tetracycline, respectively [64]. Thus, extremely high sensitivity may not be required for regulatory purposes.

6. MACROLIDE ANTIBIOTICS

There are relatively few reports of the application of LC methods to the determination of macrolide antibiotic residues in milk and tissues. However, methods have been described for determination of tylosin [65–67], spiramycin [68,69], and sedecamycin and its metabolites [70].

Moats *et al.* [65,66] described a method for determination of tylosin (tylosin A) in blood serum and tissues. Tissues were blended with 3 volumes (v/w) of water or 0.2 *M* pH 2.2 buffer for liver and kidney. Then 4 volumes of acetonitrile (v/v) were added to the homogenate, and the supernatant was filtered. Tylosin was extracted with methylene chloride, evaporated to dryness and taken up in acetonitrile. Analysis was on reversed-phase LC column using 0.005 *M* $NH_4H_2PO_4$–acetonitrile–methanol (10:60:30). The proportions were varied to improve separations depending on the matrix. As with many basic compounds on silica-based reversed-phase columns, tylosin interacts with the silica support as well as the bonded phase. As the organic solvent concentration was increased to more than 50%, the effect of the silica support became dominant as was observed with other basic compounds [71,72]. Tylosin was loaded in acetonitrile as for normal-phase chromatography on silica rather than in water as would be normal practice for reversed-phase chromatography. The interaction with the silica support

aided separation from interferences [72]. Detection limits were about 0.05 mg/kg in serum and tissues.

Horie *et al.* [67], described a procedure for tylosin A, B, C, and D in tissues using an extraction and clean-up procedure similar to that of Moats *et al.* [65,66]. Analysis was on a bonded ODS column using NaH_2PO_4–acetonitrile (65:35). Detection limits were about 0.05 mg/kg.

Horie *et al.* [68], described a procedure for determination of spiramycin in tissues using extraction with 0.5% H_3PO_4–methanol and clean-up by partitioning into methylene chloride. For analysis, a Nucleosil 5 C_{18} column was used with a mobile phase of 0.05 *M* NaH_2PO_4–acetonitrile (72:38) with UV detection at 232 nm.

Nagata and Saeki [69] extracted spiramycin residues from chicken muscle with acetonitrile. Cleanup was by partitioning into $CHCl_3$. For analysis, a Zorbax DB-C8 column was used with methanol–0.4%–H_3PO_4, 0.2% sodium heptanesulfonate (7:3) with UV detection at 231 nm. The detection limit was about 0.05 mg/kg.

Okada and Kondo [70] described a procedure for determination of sedecamycin and metabolites in swine plasma and tissues. Residues were extracted with ethyl acetate. Florisil and silica columns were used for clean-up. For analysis, two types of columns, silica (μPorasil) and bonded reversed-phase (μBondapak C_{18}) and three mobile phases were used: (a) *n*-hexane–isopropanol (80:20), (b) *n*-hexane–isopropanol–acetic acid (75:25:0.2) for normal-phase chromatography, and (c) 0.01 *M* pH 8.2 phosphate buffer–acetonitrile (60:40) for reversed-phase chromatography. Detection limits were about 0.05 mg/kg.

7. β-LACTAM ANTIBIOTICS

LC methods for determination of β-lactam antibiotics were recently reviewed by Moats [73] and by Petz [74]. As with other antibiotics, LC methods are generally the procedures of choice although other chromatographic methods have been used [73,74]. For β-lactam antibiotics, several screening tests will detect residues at levels of 2–5 μg/kg [75]. Regulatory requirements are also quite stringent, especially for milk. In the USA, the levels of concern in milk are 5 ng/ml for penicillin G; 10 ng/ml for amoxicillin, ampicillin, and cloxacillin; and 20 ng/ml for cephapirin [76]. The Food and Agriculture Organization (FAO)/WHO Expert Committee has recommended a limit of 4 ng/ml for penicillin G in milk [74]. A level of 50 μg/kg of penicillin G in tissues is widely accepted as the international standard [74]. Recently, several sensitive LC methods have been described for determination of penicillins with neutral side-chains at levels of <10 ng/ml in milk [77–81] and tissues [82,83]. Development of methods of comparable sensitivity for determination of amphoteric β-lactams has proven elusive. Except for a method recently described by Moats for cephapirin [84] using LC fractionation, none will detect <10 μg/kg of amphoteric β-lactams [85–88]. The only procedure reported for determination of amphoteric compounds in tissues is one described for ampicillin in fish [89].

All the procedures used require some type of extraction from the matrix. A variety of approaches have been used including extraction/deproteinization with acetonitrile [77–80,82,84,90], tungstic acid [83,91], methanol [89], ultrafiltration (milk only [85,87]), direct solid-phase extraction (of milk [84,88]), and partitioning into dichlormethane at acid pH [81]. Generally, further clean-up was required. Methods used for clean-up included solid-phase extraction [83,88,89,91,92], partitioning between buffers and organic solvents [77,78,81,82,90], and HPLC fractionation [79,80,84]. Detection methods included direct UV absorbance [79–82, 84–89,91,92], and derivatization with either UV [78–83] or fluorometric detection [77,90].

8. OTHER ANTIBIOTICS

Virginiamycin is added to feed as a growth promotor. It is actually a mixture; the principle components have been designated M_1 and S. The antimicrobial activity is dependent on synergism between the two components and is affected by the ratio of the two. The M_1 component is the predominant component of the mixture.

Nogase and Fukamachi [93] described a procedure for determination of both the S and M_1 components in muscle. Virginiamycin was extracted from tissues with acetonitrile. Clean-up was by partitioning into $CHCl_3$ and then into the HPLC mobile phase. Analysis was on an ODS column with fluorescence detection. Moats and Leskinen [94]

blended tissue with 0.2 M $NH_4H_2PO_4$, added methanol at a volume equal to that of the blend, and homogenized again. The filtrate was extracted into methylene chloride–petroleum ether and then into HPLC mobile phase. For analysis, a Supelco LC-18 column was used with 0.01 M $NH_4H_2PO_4$–acetonitrile gradient. Detection was based on the M_1 component. Saito *et al.* [95] extracted virginiamycin from tissues using methanol-phosphotungstic acid. Clean-up was based on partitioning followed by solid-phase extraction. For analysis, an ODS column was used with acetonitrile–water. Detection was based on the M_1 component. Further studies by Moats and Leskinen [94] demonstrated that only traces of virginiamycin were found in tissue of swine fed very high levels. Since this is the only mode of administration to farm animals, it is therefore unlikely that violative residue will be found in animal tissues.

Moats and Leskinen [96] described a rapid procedure for determination of novobiocin in milk and tissues. Milk or tissue were homogenized with 0.2 M $(NH_4)H_2PO_4$, methanol added at a volume equal to that of the blend, and homogenized again (twice the volume with liver and kidney). The filtrates (diluted with water if necessary) were concentrated on-line on an ODS column and eluted with an acetonitrile gradient for analysis with UV detection at 320 nm.

Moats [97] described a procedure for lincomycin in milk and tissues. Milk and tissue was blended in 0.1 M $(NH_4)H_2PO_4$, methanol added at a volume equal to that of the blend, and homogenized again. The resulting filtrate was mixed with 1.5 volumes of acetonitrile and refiltered. The filtrate was evaporated to remove acetonitrile. For clean-up, solid-phase extraction and fractionation using the HPLC system was used. Detection was UV at 210 nm.

9. CONCLUSIONS

In the last decade many LC methods have been developed for the determination of antibiotic residues in meat and milk. Many of these methods are relatively simple, specific, and able to analyze at tolerance levels. However, in order for them to be practical and rugged for residue monitoring, they must be subjected to collaborative studies or validated in various other laboratories.

Some LC methods require minimal sample preparation and can be completed in one h or less (*e.g.* refs. 29, 37 and 96). They are therefore comparable in speed and cost with some screening tests. The LC methods can provide a more specific result in a short time while most conventional screening tests can only establish the need for further testing.

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Organic micropollutants in Swiss sewage sludge

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ABSTRACT

Sludges from sixteen municipal and eleven industrial Swiss sewage treatment plants were analysed for adsorbable organic compounds (AOX), chlorinated pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. Except for the AOX values, which were significantly higher ($p < 0.05$) in industrial sludges, there was no significant difference between industrial and municipal sludges. The AOX values did not correlate with any of the measured pollutants. The methods employed (capillary GC–MS and GC–electron-capture detection) proved to be suitable for monitoring organic micropollutants in industrial and municipal sewage sludges.

INTRODUCTION

In Switzerland, about 40% of the sewage sludge produced is used as a fertilizer in agriculture [1]. The heavy metal and enteric bacteria contents are monitored on a routine basis before use, in contrast to organic micropollutants [2]. However, it is not known if the load of organic micropollutants needs to be measured frequently, as its significance is still not well understood. The number of different organic compounds in sludge may be extremely high. Therefore, a selection of relevant xenobiotics has to be made. Selection criteria may include high production volume, widespread use, toxicity, carcinogenicity, persistence and accumulation. Chlorinated pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are relevant groups of compounds [3–11], which have been selected for analysis, in addition to the overall value of "adsorbable organic halogen compounds" (AOX) [12–14].

Only a few European countries have proposed or established tolerance or limit values of organic micropollutants, *e.g.*, Netherlands for soil [15], Germany for soil [16] and sewage sludge [17] (values for PCBs and AOX) and Switzerland for sewage sludge (value for AOX). Various results have been published for organic micropollutants in sewage sludges in Switzerland [18–23] (mainly PCBs) and elsewhere [24–32].

The methods used in this work are not new.

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However, it was the aim of this study to test if they are suitable for monitoring. Further, this study was intended to compare sludges from industrial and municipal treatment plants in relation to the concentration of organic micropollutants listed in Table I, to check AOX for correlation with specific micropollutants and to establish whether the ban on PCBs and the mentioned pesticides have had an effect on sludge contamination.

EXPERIMENTAL

Selection of sewage treatment plants

In April 1989, samples of digested sludge were taken from 27 Swiss sewage treatment plants. These plants are located in various areas of Switzerland (in parentheses are given abbreviations as in Tables II and III):

16 samples from municipal plants:
- 2 plants from unpopulated areas (UNP)
- 3 plants from little populated areas (LOWP)
- 5 plants from large agglomerations with incinerators (BIGIN)
- 4 plants from small agglomerations with incinerators (SMAIN)
- 2 plants from large agglomerations without incinerator (BIG)

11 samples from industrial plants:
- 6 plants from chemical industry (CHEM)
- 2 plants from paper industry (PAP)
- 3 plants from textile industry (TEX)

The capacities of municipal sewage treatment plants were in the range 130–620 000 biological equivalents of inhabitants. One equivalent corresponds to 25 kg of dry matter of sewage sludge per year. The capacities of industrial sewage treatment plants were in the range 22 000–415 000 equivalents. The number of inhabitants linked to municipal sewage treatment plants ranged from > 100 to about 290 000; the number of inhabitants linked to industrial sewage treatment plants did not exceed 26 000. The wide size range of the treatment plants and the widespread geographic location throughout Switzerland represent a good coverage.

Sample storage

Wet samples of 3 l of digested sludge were collected on April 10th, 1989, and stored at 4°C until express delivery the next day. Immediately upon receipt, the samples were homogenized, divided into 500-ml portions and stored at − 20°C. The origin of the samples was known only to the Federal Research Station for Agricultural Chemistry until the end of the measurements ("blind analysis").

Analysis

For the measurement of AOX an official method [33] was used. For the other organic micropollutants, published methods [34–36] were modified for sewage sludge. Recovery data are listed in Table I. No values have been corrected for losses during extraction.

Determination of adsorbable organic halogen compounds (AOX)

The wet frozen sewage sludge samples were thawed to room temperature in a desiccator, cut into small pieces and homogenized. The dried sludge (10–100 mg) was suspended in 25 ml of nitrate solution (0.2 *M* $NaNO_3$–0.15 *M* HNO_3). Charcoal (20 mg) were added and the mixture was shaken for 1 h and filtered through a polycarbonate membrane filter (0.4-μm pores). The solid residue in the filter was washed with 25 ml of nitrate washing solution (0.01 *M* $NaNO_3$–0.008 *M* HNO_3). The residue was combusted in a combustion apparatus at a temperature of at least 950°C in a stream of oxygen. The combustion gases were passed through an adsorber filled with concentrated sulphuric acid and were then delivered into a microcoulometer for measurement of halogens [33].

Determination of chlorinated pesticides

Doubly distilled water (20 ml) was added to 5 g of wet homogenized sewage sludge and the mixture was left at room temperature for at least 12 h. After addition of 1 ml of acetic acid, 20 ml of methanol, 0.4 g of potassium oxalate and 20 ml of light petroleum, the suspension was homogenized for 5 min with an electrical mixer and centrifuged for 5 min at 2500 rpm (radius = 21 cm). Subsequently the supernatant was separated. The aqueous layer was extracted twice more with light petroleum ether. The combined organic layers were dried over sodium sulphate, concentrated to a volume of 3–4 ml and mixed with cyclohexane–ethyl acetate (1:1, v/v) to a final volume of 10 ml.

The solution was passed through an Acrodisc fil-

TABLE I

MEAN RECOVERY OF MICROPOLLUTANTS IN SEWAGE SLUDGE SAMPLES

Compound[a]	*Chemical Abstracts* Registry Number	Abbreviation	Recovery (%) (n = 3)[b]			
			Rec_1	SD_1	Rec_2	SD_2
Organochlorinated pesticides						
o,p'-DDD	53-19-0	DDT&MET	88	11	65	4
p,p'-DDD	72-54-8	DDT&MET	95	22	111	10
o,p'-DDE	3424-82-6	DDT&MET	89	9	86	4
p,p'-DDE	72-55-9	DDT&MET	87	8	98	3
o,p'-DDT	789-02-6	DDT&MET	97	1	83	11
p,p'-DDT	50-29-3	DDT&MET	102	6	113	2
Hexachlorobenzene (HCB)	18-74-1	HCB	88	11	71	5
γ-Hexachlorocyclohexane (γ-HCH)	58-89-9	Lindane	90	11	121	5
PAHs						
Benzo[*b*]fluoranthene	205-99-2	Σ6PAH	98	6	78	8
Benzo[*k*]fluoranthene	206-44-0	Σ6PAH	88	9	90	4
Benzo[*g,h,i*]perylene	191-24-2	Σ6PAH	103	13	100	4
Benzo[*a*]pyrene	50-32-8	Σ6PAH/BaP	93	13	88	7
Fluoranthene	206-44-0	Σ6PAH	87	16	102	8
Indeno[1,2,3-*cd*]pyrene	193-39-5	Σ6PAH	82	27	96	3
PCBs						
PCB congener No. 28 (IUPAC: 2,4,4'-trichlorbiphenyl)	7012-37-5	K28	100	15	91	4
PCB congener No. 52 (IUPAC: 2,2',5,5'-tetrachlorbiphenyl)	35693-99-3	K52	90	7	85	13
PCB congener No. 101 (IUPAC: 2,2',4,5,5'-pentachlorbiphenyl)	37680-73-2	K101	105	7	88	7
PCB congener No. 138 (IUPAC: 2,2',3,4,4',5'-hexachlorbiphenyl)		K138	92	7	103	5
PCB congener No. 153 (IUPAC: 2,2',4,4',5,5'-hexachlorbiphenyl)	35065-27-1	K153	113	15	94	15
PCB congener No. 180 (IUPAC: 2,2',3,4,4',5,5'-heptachlorbiphenyl)	35065-29-3	K180	115	7	99	8

[a] Limits of determination: organochlorinated pesticides, 2 μg/kg wet matter; PAHs, 1 μg/kg wet matter; PCBs, 1 μg/kg wet matter.
[b] Rec_1: organochlorinated pesticides, addition of 10 μg/kg wet matter; PAHs, addition of 3 μg/kg wet matter; PCBs, addition of 2 μg/kg wet matter. SD_1: standard deviation 1 (n = 3). Rec_2: organochlorinated pesticides, addition of 50 μg/kg wet matter; PAHs, addition of 50 μg/kg wet matter; PCBs, addition of 17 μg/kg wet matter. SD_2: standard deviation 2 (n = 3).

ter (type Chromafil; 0.2-μm pore diameter; Macherey–Nagel, Düren, Germany). Purification by gel permeation chromatography was performed on a column (30 × 6 cm I.D.) filled with 33 g of Bio-Beads S-X3 (200–400 mesh) (Bio-Rad Labs., Richmond, CA, USA) and with a Model ABC Autorep 1002 B apparatus (Analytical Biochemistry Labs., Columbia, MO, USA) (carrier gas, 99.996% nitrogen; pressure 5 p.s.i.; 1 p.s.i. = 6894.76 Pa). Organochlorinated pesticides were eluted with cyclohexane–ethyl acetate (1:1, v/v) (load time 10 s, dump time 14 min, collection time 20 min, wash time 6 min). The eluate was condensed to a volume of 1–2 ml and treated with 2 g of Celite.

The extract was further purified on a dual-column system. The precolumn (10 cm × 2.5 cm I.D.) was filled with 0.5 g of Celite and the eluate–Celite suspension and covered with sand. The main column (25 cm × 2.5 cm I.D.) was filled with 5 g of sodium sulphate, 10 g of aluminium oxide, 20 g of Florisil and 2 g of sand. Three 5-ml volumes or propylene carbonate were passed under slight pressure

through the precolumn into the main column, where it remained for 1 h. Chlorinated pesticides were eluted from the second column with 130 ml of cyclohexane. The eluate was condensed to 1–2 ml, diluted with cyclohexane to a final amount of 2 g (corresponding to 2.55 ml) and filtered through a Florisil–Sep-Pak cartridge (100 mg) (Waters, Milford, MA, USA) into a sample vial for analysis.

Capillary gas chromatography with electron-capture detection (cGC–ECD) was performed on a Hewlett-Packard Model 5890 gas chromatograph equipped with a Model 7673A autosampler (Figs. 1 and 2). The injection volume was 1 μl (splitless mode) at an injector temperature of 250°C. A DB-1 and DB-17 capillary column (30 m × 0.25 mm I.D.) was used (J&W Scientific; film layer 0.25 μm; carrier gas, nitrogen at 5 ml/min). The temperature was programmed from 60°C to 280 °C at 20°C/min with a final hold time of 30 min and a total run time of 41 min. The temperature of the electron-capture detector was set at 300°C. Peak measurement was performed with a Hewlett-Packard Pascal workstation (revision 4.0). The system was calibrated by four-point linear regression and peak heights were compared with an external standard. The limit of determination was 2 μg/kg and the range of measurement was 2–500 μg/kg [34,35].

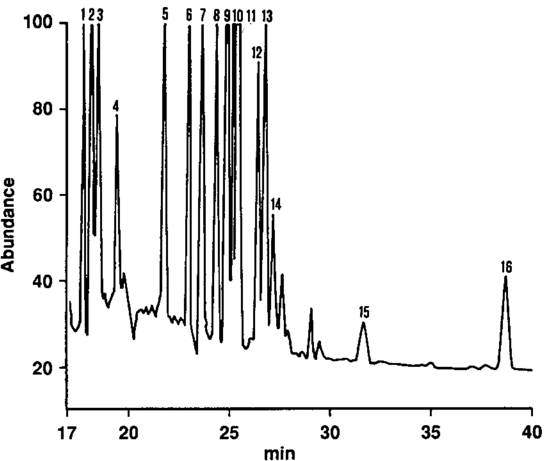

Fig. 1. GC–ECD of chlorinated pesticides for a spiked sewage sludge (recovery experiment, 50 μg/kg). Peaks: 1 = α-HCH; 2 = HCB; 3 = lindane; 4 = δ-HCH, 5 = aldrin; 6 = HC epoxide; 7 = *o,p*-DDE; 8 = endosulfan; 9 = *p,p'*-DDE; 10 = *o,p*-DDD; 11 = dieldrin; 12 = endrin/*p,p'*-DDD; 13 = *o,p*-DDT; 14 = *p,p'*-DDT; 15 = methoxychlor; 16 = mirex.

Determination of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)

Wet homogenous sewage sludge (30 g), 60 ml of saturated sodium chloride solution and 100 ml of cyclohexane were shaken (upside down) for 45 min. The phases were separated (after centrifugation, if necessary). The aqueous phase was extracted once more with 100 ml of fresh cyclohexane for 45 min. The combined organic layers were dryed with sodium sulphate, evaporated to dryness and the residue dissolved in 5 ml of toluene–hexane (1:3, v/v).

Subsequent solid-phase extraction was performed with an ICT Bond-Elut bonded-phase SI cartridge (1000 mg; 3 ml; AL 601 406) (ICT, Harbor City, CA, USA). The cartridge was first washed with 5 ml of hexane and 3 ml of toluene–hexane (1:3, v/v) with the help of slight vacuum suction. The extract was passed trough the cartridge, which was washed with two portions of 3 ml of toluene–hexane (1:3, v/v). The combined eluates were evaporated to dryness and the residue dissolved in 1 ml of cyclohexane [36].

PAHs and PCBs were measured with different aliquots from the same extracts by cGC–MS (electron impact ionization) (Fig. 3). A Hewlett-Packard system was used with a Model 5890 gas chromatograph equipped with a Model 7673A autosampler and an MSD 5971B mass spectrometer. The transfer line temperature was 280°C. The injection volume was 1 μl (splitless mode) at 270°C. Helium

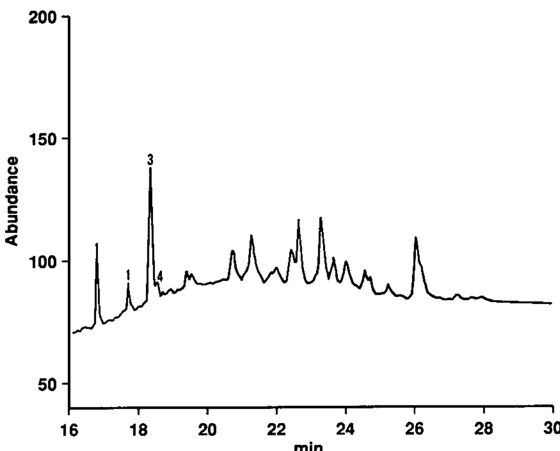

Fig. 2. GC–ECD of chlorinated pesticides in sewage sludge. Peak numbers as in Fig. 1.

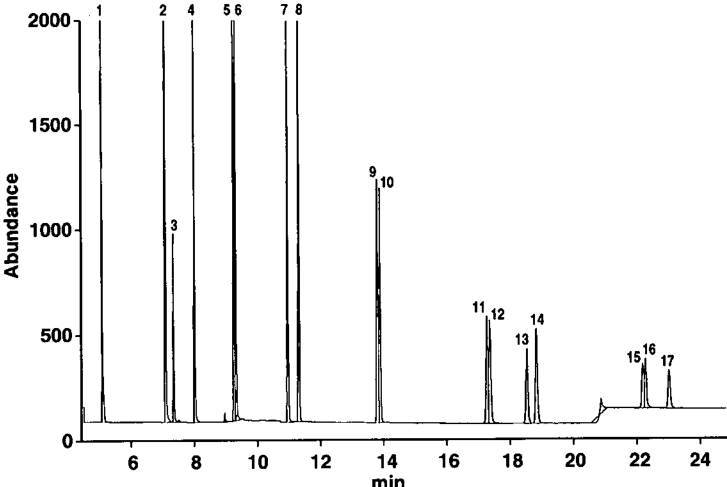

Fig. 3. GC–MS (SIM mode) of PAHs for a spiked sewage sludge (recovery experiment, 50 μg/kg). Peaks: 1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = perylene; 15 = indeno[1,2,3-*cd*]pyrene; 16 = dibenz[*a,h*]anthracene; 17 = benzo[*ghi*]perylene.

(99.996%) was used as the carrier gas at a flow-rate of 40 ml/min and a head pressure of 12 p.s.i. A 30 m × 0.25 mm I.D. glass capillary column coated with DB-1 (methylsilicone; J&W Scientific; layer thickness 0.25 μm) was used with the following temperature programme: 4 min at 60°C (solvent delay), increased at 20°C/min to 250°C, then at 5°C/min to 270°C, held for 5 min at 270°C, increased at 25°C/min to 300°C, with a final hold for 16 min at 300°C. Quantification was achieved by single-ion monitoring (SIM) during a run time of 35.7 min. For PAHs, ions at *m/z* 128, 152, 166, 178, 202, 228, 252, 276, 278 were monitored, and PCBs were monitored in the second analysis with ions at *m/z* 256, 291, 326, 360, 394. The system was calibrated automatically by three-point linear regression. Quantification was done by comparison of peak heights with that of an external standard. The limit of determination was 1 μg/kg, the detection limit was 0.5 μg/kg and the range of determination was 1–100 μg/kg for each compound.

Statistical analysis

The water content of the samples was determined with an aliquot of the sewage sludge and the results were calculated with respect to dry matter.

It was not possible to calculate an arithmetic mean, because some values were below the limit of determination. Instead, two means were calculated: mean 1, values below the limit of determination were set equal to zero; mean 2, values below the limit of determination were set equal to the limit of determination. Mean 2 was not calculated for sum parameters.

The signed rank test (two-sided Wilcoxon test) was used for comparison of values within this study and for comparison of values obtained in this study with those given elsewhere [37]. $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Adsorbable organic halogen compounds (AOX)

AOX is an overall parameter for the content of non-volatile, halogenated organic hydrocarbons in sewage sludge and other environmental samples. It is now frequently used for an initial ecological assessment of sewage sludge in relation to halogenated organic compounds [31]. Germany has adopted a limit of 500 mg/kg AOX (500 mg Cl/kg dry matter) in the amendment of the ordinance on sewage sludge, and Switzerland has established a guide value for the same limit [38].

AOX were found in all samples in this study (Tables II and III). The AOX concentrations were significantly higher ($p < 0.05$) in industrial sludge

TABLE II

ORGANIC MICROPOLLUTANTS IN SWISS MUNICIPAL SEWAGE SLUDGE

No.	Origin[a]	AOX (mg/kg)	Lindane (μg/kg)	HCB (μg/kg)	DDT&MET[b] (μg/kg)	PCB congeners (μg/kg)						PAHs (μg/kg)		Water content (%)
						C28	C52	C101	C138	C153	C180	Σ6PAH[c]	BaP	
1	UNP	220	nd	nd	340	nd	nd	nd	20	11	nd	118	18	90.7
2	UNP	240	144	25	149	nd	nd	nd	nd	nd	nd	nd	nd	92.0
3	LOWP	210	nd	47	nd	nd	nd	16	32	26	nd	258	40	84.9
4	LOWP	270	nd	nd	nd	nd	18	nd	15	nd	nd	467	74	92.5
5	LOWP	400	nd	13	376	16	41	nd	89	12	nd	1833	16	87.4
6	BIGIN	360	93	73	nd	nd	19	nd	nd	nd	nd	747	128	90.1
7	BIGIN	410	nd	nd	nd	nd	nd	19	29	25	nd	233	20	92.7
8	BIGIN	2000	nd	nd	nd	nd	17	29	42	48	15	347	47	92.5
9	BIGIN	400	nd	60	nd	25	66	nd	25	15	nd	537	105	91.8
10	BIGIN	150	nd	nd	nd	16	86	nd	73	41	26	862	nd	91.3
11	SMAIN	280	95	nd	nd	14	43	nd	37	14	nd	202	31	90.1
12	SMAIN	360	nd	118	nd	nd	nd	nd	38	24	nd	39	nd	92.4
13	SMAIN	220	nd	nd	nd	nd	14	nd	13	nd	nd	114	nd	91.2
14	SMAIN	230	nd	nd	89	25	46	nd	18	nd	nd	767	nd	88.4
15	BIG	130	nd	nd	159	17	41	37	66	56	17	338	nd	92.0
16	BIG	300	nd	nd	nd	9	24	nd	41	26	12	583	101	88.5
Min.		130	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	84.9
Max.		2000	144	118	376	25	86	37	89	56	26	1833	128	92.7
Median		275	nd	nd	nd	nd	19	nd	31	15	nd	343	19	91.3
MV_1[d]		390	21	21	70	8	26	6	34	19	4	465	36	90.5
SD_1[d]		440	46	35	125	9	25	12	24	17	8	451	43	2.2
MV_2[e]		390	39	35		14	29	14	35	22	13		40	90.5
SD_2[e]		440	37	27		5	23	8	22	14	4		40	2.2

[a] For abbreviations of sewage treatment plants, see Experimental.

[b] Sum of *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT. Single values below the limits of determination were set equal to zero before addition.

[c] Sum of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, fluoranthene and indeno[1,2,3-*cd*]pyrene. Single values below the limits of determination were set equal to zero before addition.

[d] Single values below the limits of determination were set equal to zero for calculation of mean value 1 (MV_1) and standard deviation 1 (SD_1); nd = 0.

[e] Single values below the limit of determination were set equal to the limit of determination for calculation of mean value 2 (MV_2) and standard deviation 2 (SD_2): nd = limit of determination.

TABLE III

ORGANIC MICROPOLLUTANTS IN SWISS INDUSTRIAL SEWAGE SLUDGE

No.	Origin[a]	AOX (mg/kg)	Lindane (μg/kg)	HCB (μg/kg)	DDT& MET[b] (μg/kg)	PCB congeners (μg/kg)						PAHs (μg/kg)		Water content (%)
						C28	C52	C101	C138	C153	C180	Σ6PAH[c]	BaP	
17	CHEM	810	nd[f]	nd	nd	nd	17	nd	nd	nd	nd	184	45	92.4
18	CHEM	2200	177	nd	164	15	nd	nd	7	nd	nd	336	nd	86.6
19	CHEM	110	nd	11	119	nd	10	nd	11	8	nd	105	20	82.8
20	CHEM	420	nd	46	nd	nd	34	nd	16	nd	nd	897	154	93.2
21	CHEM	310	nd	nd	125	nd	nd	nd	nd	nd	nd	1031	nd	90.2
22	CHEM	1000	25	28	1030	nd	27	nd	nd	nd	nd	nd	nd	92.7
23	PAP	540	nd	29	118	nd	nd	20	38	31	nd	nd	nd	94.5
24	PAP	7600	19	nd	159	nd	21	23	54	39	nd	nd	nd	92.4
25	TEX	2700	188	303	579	nd	nd	nd	nd	nd	nd	377	nd	92.3
26	TEX	420	nd	33	472	nd	26	nd	32	17	nd	802	153	90.9
27	TEX	750	nd	nd	nd	10	32	nd	25	13	nd	412	63	90.3
Min.		110	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	82.8
Max.		7600	188	303	1030	15	34	23	54	39	nd	1031	154	94.5
Median		750	nd	11	125	nd	17	nd	11	nd	nd	336	nd	92.3
MV_1[d]		1500	37	41	251	2	15	4	17	10	0	377	40	90.8
SD_1[d]		2200	72	89	319	5	13	8	18	13	0	377	60	3.4
MV_2[e]		1500	52	51		13	20	13	21	15	12		46	90.8
SD_2[e]		2200	65	84		3	9	5	14	10	3		56	3.4

[a–e] See Table II.

[f] nd = Not detected (below the limit of determination).

(median 750 mg Cl/kg dry matter; mean 1533 mg/kg) than in municipal sludge (median 275 mg/kg; mean 386 mg/kg). Only one out of sixteen municipal samples yielded an AOX value higher than 500 mg/kg, in contrast to seven out of eleven industrial sludges. PCBs and organochlorinated pesticides did not correlate with the AOX concentrations. This is not surprising, as there is a difference of three orders of magnitude between their concentrations.

Leschber *et al.* [32] measured a median value of 280 mg/kg in 170 samples of German sewage sludge (suitable for agricultural use). This is in the range of the present study and is almost identical with our median value (275 mg/kg) in municipal sludges.

Chlorinated pesticides

The concentrations of aldrin, dieldrin, endosulfan, endrin and heptachlorepoxide were all below the limit of determination of 2 μg/kg (wet matter) and are not discussed further. Values for other chlorinated pesticides are given in Tables II and III. Mean value 1 and mean value 2 showed a difference only at low values (of the order of one standard deviation). None of the compounds determined were found in all the samples tested. In municipal sewage sludges all median values were below the limit of determination. In contrast, the mean concentration of lindane was the only one below the limit of determination in industrial sewage sludges. There was no significant difference between municipal and industrial sludges.

Lindane was detected in three out of sixteen municipal and in four out of eleven samples of industrial sewage sludge. Mean value 2 for the municipal samples (39 μg/kg dry matter) was similar to corresponding concentrations in 57 German samples (27 and 56 μg/kg dry matter [28]) and the values for the industrial samples of this study (52 μg/kg).

HCB was found in six municipal and six industrial sewage sludge samples at concentrations comparable to values found by Witte *et al.* [28]. The measured values are significantly lower than those found by Müller [19] in nine Swiss sludges in 1982 (mean value 50 μg/kg).

DDT and metabolites were measured in five municipal (70 μg/kg, mean value 1) and eight industrial (251 μg/kg) sludge samples. Witte *et al.* [28] found 80 μg/kg dry matter for DDT (without measuring its metabolites). Clevenger *et al.* [29] reported a mean value of 100 μg/kg dry matter for DDT. Drescher-Kaden *et al.* [39] and Witte *et al.* [28] considered the contamination of sewage sludge in Germany by chlorinated pesticides not to be important compared with other sources of contamination.

The chlorinated pesticides measured in this study are banned in Switzerland [40], with the exception of lindane, which used for seed dressings. The concentrations of lindane and HCB in the River Rhine (16 and 219 ng/l in 1974) and in the ground water (9 and 6 ng/l in 1974) near Basle have been falling since 1980 (river, 5 and 3 ng/l; ground water, 0.5 and 2 ng/l in 1989) [41–43].

Polychlorinated biphenyls (PCBs)

The concentrations of PCB congeners 28, 52, 101, 138, 153 and 180 are given in Tables II and III. There was no significant difference between municipal and industrial sludges for any PCB congener. The values obtained in this study are lower than data published by Kampe *et al.* [27] and Witte *et al.* [28] (median values for the sequence of the above mentioned congeners = 22, 20, 40, 70, 70 and 43 μg/kg).

Drescher-Kaden *et al.* [39] mentioned a mean overall PCB concentration of 500 μg/kg dry matter in German sewage sludge, which is comparable to our results if it is taken into consideration that the six congeners measured correspond to about 20% of all PCB congeners found in sewage sludge [44].

In 1980, Burgermeister *et al.* [23] reported a median value of 1200 μg/kg dry matter as Aroclor in nine Swiss samples. Müller [19] found 2900 μg/kg "PCBs" as a median value for nine samples in 1982. Although it is difficult to compare these values, a decrease in PCB contamination can be discerned.

Germany introduced a limit value of 200 μg/kg dry matter for each of the PCB congeners 28, 52, 101, 138, 153 and 180 in an amendment of its ordinance for sewage sludge [17]. In this study no PCB congener exceeded a concentration of 200 μg/kg in any sample.

Kampe *et al.* [27] estimated that a maximum application rate of sewage sludge of 5 tons/hectare and within 3 years (with PCB concentrations markedly higher than measured in this study) will not raise the PCB concentration within 100 years in the treated soils. Losses were shown to be due to biodegradation and volatilization [45]. In another

study, decomposition of higher chlorinated PCB congeners could not be observed within the error of analysis (about 20%) in 1 year [22]. Therefore, no decomposition rate can be calculated from this study.

Polycyclic aromatic hydrocarbons (PAHs)

The concentration of the marker xenobiotic benzo[*a*]pyrene (BaP) and the summed concentrations (Σ6PAH) of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, fluoranthene and indeno[1,2,3-*cd*]pyrene are given in Tables II and III.

Benzo[*a*]pyrene was found in 15 of 27 sewage sludge samples, with similar concentrations in municipal and industrial sludge. The concentrations of benzo[*a*]pyrene found in our study are significantly (one order of magnitude) lower than the values published by Witte *et al.* [28]. Switzerland has no coal-fired electric plants, thermal energy production by burning coal or coke production, which may be one reason for the low concentrations of PAHs found in this study.

Degradation of PAHs has been demonstrated [46]. However, especially if concentrations are low, adaptation of microorganisms may not be possible. In one instance no measurable degradation at all was found [47].

CONCLUSIONS

The extraction procedures and the chromatographic methods used in this study proved to be suitable for monitoring organic micropollutants in industrial and municipal sewage sludges.

Except for AOX values there are no significant differences between industrial and municipal sludges. The elevated AOX values are an indicator of xenobiotic contamination and support the present ban on the use of industrial sludges in agriculture owing to high concentrations of heavy metals [48]. The AOX values do not correlate with any of the other measured micropollutants and therefore cannot be used to assess contamination by these compounds.

It is encouraging that the PCB and HCB levels have decreased in the last decade and that PAH concentrations are relatively low. However, further studies of organic micropollutants in selected samples of sewage sludge should be carried out in the near future; hazardous ecological effects cannot be fully excluded. Other groups of xenobiotics not analysed in this study may become relevant. A better knowledge of the concentrations of organic micropollutants in selected samples of sewage sludge is necessary in order to assess further the risk of xenobiotics in the environment.

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Review

Chromatographic determination of volatile solvents and their metabolites in urine for monitoring occupational exposure

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ABSTRACT

The determination of volatile solvents and their metabolites in biological materials such as expired air, blood or urine allows the estimation of the degree of exposure of these chemicals. Chromatographic methods are now universally employed for this purpose and numerous analytical procedures are available for the determination of the most commonly used volatile solvents and their metabolites in urine. GC methods appear well adapted to the determination of the parent volatile solvents in blood and urine and may be used for the determination of their urinary metabolites, but these methods often require several prechromatographic steps. However, HPLC is becoming a powerful tool for the accurate and easy determination of urinary metabolites of volatile solvents, considering its decisive advantages for routine monitoring. Further, recent developments in HPLC could widen the usefulness of this method for most complex analytical problems that could be encountered during this measurement. However, despite the relative neglect of planar chromatography in this area of concern and considering the great interest in methods that could permit the simultaneous assay of numerous samples often required by routine monitoring, new approach using improved methods such as overpressured TLC could be very fruitful in the future.

CONTENTS

1. Introduction . . . 389
2. Monitoring of volatile solvent exposure —the analytical problem . . . 390
3. Methods . . . 392
   3.1. Gas chromatography . . . 392
   3.2. HPLC . . . 394
   3.3. Planar chromatography . . . 395
4. Conclusions . . . 395
References . . . 396

1. INTRODUCTION

Organic solvents and their vapours are widely distributed in the modern environment, both in the home and at work. Although the occupational exposure to these solvents is generally well monitored in developed countries, the domestic exposure may be greatly underestimated (dry-cleaning fluids, paint diluents, nail-polish removers, etc.). Moreover, the problem of solvent abuse ("glue sniffing") among teenagers has become a subject of growing concern for health practitioners and toxicologists [1]. Most of these solvents are very toxic.

Vapours of volatile or volatilizable liquids are

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often readily absorbed by the lungs but the skin may be also an important route of absorption. Since the vapour in the alveoli equilibrates almost instantaneously with blood passing through the pulmonary capillary bed, the rate of absorption is variable and strongly dependent on the toxicant's blood/gas solubility. If the vapour has a low blood solubility, the rate of transfer is blood-flow dependent (perfusion limited), whereas for vapours with higher solubility it is strongly dependent of the respiratory process (ventilation limited) [2].

Many of the early toxic effects of inhaled solvents after an acute exposure are observed on the central nervour system. In general, these effects are directly related to the unchanged substance and resolved well after the cessation of exposure. However, during exposure, most of these solvents follow metabolization pathways that can generate highly toxic short-lived intermediates such as free radicals or arene epoxides (Fig. 1). As volatile solvents are generally highly hydrophobic, the metabolization allows initially conversion into a more hydrophilic compound by a phase-I reaction such as hydroxylation of an aromatic ring to phenol. This reaction is then followed by a phase-II conjugation process (acetylation, glucuroconjugation, glycinoconjugation, etc.) leading to very soluble conjugated derivatives that are easily excreted by the kidneys.

2. MONITORING OF VOLATILE SOLVENT EXPOSURE —THE ANALYTCAL PROBLEM

The determination of volatile solvents and their metabolites in biological materials such as expired

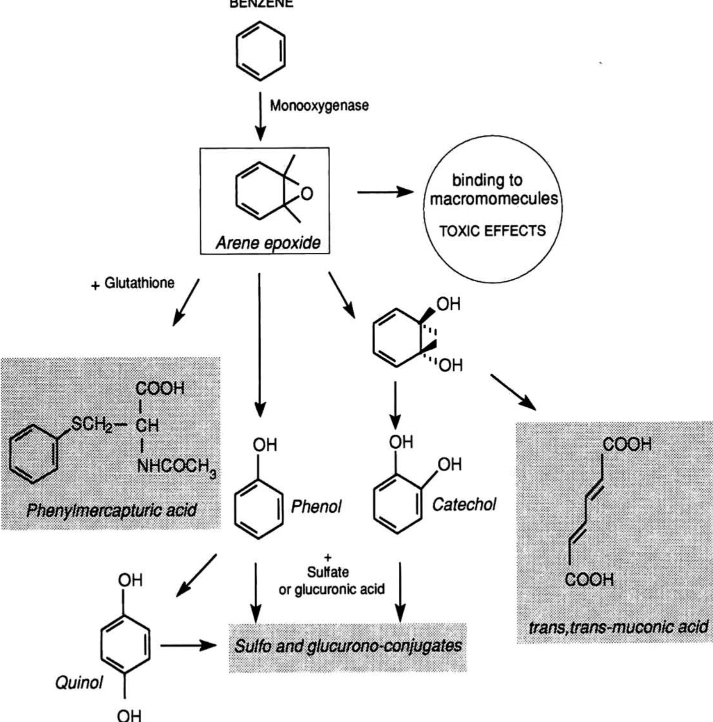

Fig. 1. Metabolization profile of benzene. Blood benzene is oxidized by liver or bone-marrow mixed-function oxidase systems, generating the highly reactive short-lived intermediate benzene epoxide. This toxic metabolite is able to bind to macromolecules such as proteins or nucleic acids leading to deleterious effects (benzene-induced haematological abnormalities). Various detoxification pathways lead to several derivatives, which are subsequently conjugated and excreted in urine. Shaded areas show the major urinary metabolites that are currently determined for monitoring purposes.

air, blood or urine allows the estimation of the degree of exposure of these chemicals [3–5]. The definition of permissible levels of exposure to chemical agents can be expressed in terms of allowable atmospheric concentrations [5] or permissible biological levels for these chemicals or their metabolites expressed as the biological "threshold limit values" (TLV), representing a biological marker of the ambient conditions under which it is considered that nearly all workers may be exposed for 8 h a day without significant adverse effects [6].

In occupational laboratory medicine, if the problem in question is the routine monitoring of workers exposed to ambient chemicals, the analytical performance of a suitable method has to answer the following question: had the workers been exposed to higher levels of chemicals than those accepted on the basis of their biological TLVs? As the biological TLVs of the studied chemicals are relatively high, the sensitivity is not a real problem. However, an acceptable specificity, a low cost and a rapid and possibly automated method are required. Indeed, high specificity is less critical for routine monitoring than for pharmacokinetic or forensic studies, as a more detailed and specific analysis can be performed if a higher metabolite concentration is found than its biological TLV.

The determination of metabolites in urine offers important and decisive advantages over other monitoring methods: (1) it takes into account absorption by all the possible routes (skin, oral ingestion, lungs), (2) it can consider indvidual variations in the toxicokinetics and biotransformations of the solvent, (3) it may also reflect the total exposure (amounts excreted during a given period) and (4) it is easily performed for routine purposes.

However, the nature of the metabolite to be determined can be critical and may influence the choice of the analytical method. For example, urinary phenol determinations have classically been used to monitor high levels of occupational benzene exposure. However, the same technique cannot be used to monitor low levels of exposure (*e.g.*, environmental exposure) because of the possible high excretion of phenol resulting from the metabolism of alimentary and endogenous aromatic amino acids. Thus, alternative biological indices for exposure, such as urinary muconic acid, may be useful [7]. As phenol may be present at high levels in urine, relatively simple analytical methods can be sufficient. However, for muconic acid determination, a more sophisticated method (GC–MS) is required. Similarly, hippuric acid is the glycine conjugate of benzoic acid, the oxidative metabolite of the toxic solvent toluene, but benzoic acid may also be present as an additive in foods and beverages.

Moreover, urinary excretion of unchanged solvents may be also closely related to the degree of exposure. For example, a close relationship between benzene exposure level and urinary concentrations was found in a group of workers exposed to low environmental benzene concentrations (mean value 1.2 mg/m^3) [7]. Consequently, the choice of an appropriate analytical method must be an acceptable compromise between several conflicting requirements.

In toxicological analysis, chromatographic methods such as GC, HPLC and planar chromatographic techniques such as TLC are now extensively used. Thus, for the urinary determination of volatile solvents and their metabolites, these methods are now almost universally employed. For this purpose, elution chromatographic methods exhibit decisive advantages over global methods such as spectrophotometry because they can often separate a toxic compound and its metabolites from the biological matrix materials. However, the level of specificity, governing the choice of an analytical method, is an important (but not always resolved) problem in practice.

In a clinical situation (*e.g.*, acute poisoning) a simple answer ("yes/no") may be sufficient because other clinical or biochemical data must be also considered in order to confirm the possible diagnosis. However, in a medicolegal situation or for monitoring exposed workers when legal and socioeconomic consequences may be important, more precise identification and determination are essential. Consequently, other physical parameters, such as IR or mass spectra, may be required. These considerations may explain the growing use of coupled techniques such as GC–MS in this area.

For the collection and handling of specimens containing solvents, special care is required. Lipophilic organic solvents may be readily absorbed into plastic and therefore blood samples should be collected in glass tubes. Moreover, contamination during sampling (venepuncture swabs containing

alcohols) or during handling (laboratory environment) should be guarded against.

For the determination of the urinary metabolites of solvents, untimed or random specimens are generally unsuitable because of the wide circadian variations of urine volume and dilution. Thus, urine specimens for quantitative analysis must be collected over an interval of time, such as 24 h, providing an "integrated" picture of the metabolite production (*e.g.*, amount excreted per day) which is more clinically relevant than its concentration. However, the urine concentration of metabolites is currently corrected for dilution using the expression grams of excreted metabolite per gram of urinary creatinine.

3. METHODS

3.1. Gas chromatography

The advantages of GC for the determination of volatile substances in complex mixtures such as biological materials are well known. First, the separation of volatile solvents from the biological matrix can easily be achieved by the headspace method [8,9], exploiting their favourable gas/liquid distribution ratio. Second, temperature programming leads to a good chromatographic pattern of high-boiling-point solvents such as glycols or branched-chain aromatic hydrocarbons. Third, the use of sensitive detectors permits the determination of minor components such as various isomers in the presence of important amounts of the major metabolite and a precise determination of trace concentrations of the parent solvent. Among these, flame-ionization detector is a widely used device that is an excellent general-purpose detector, particularly with capillary columns. This detector is extensively used to the determination of aliphatic or aromatic solvents in biological materials [10–12]. For submicrogram amounts of halogenated solvents or carboxylic metabolites, the electron-capture detector is very useful. However, GC–MS appears very suitable for the identification and determination of most volatile solvents and their metabolites [13–16] and a growing use of this coupled method can be observed [7,17]. In addition, the use of stable isotope tracer techniques permits more precise metabolic studies of solvents in humans and animals.

Unfortunately, not all compounds of interest are amenable to GC study because of poor thermal stability or volatility. Thus, polar compounds are often analysed by GC after a derivatization process such as methylation or silylation. Consequently, GC remains the method of choice for the determination of volatile solvents in biological samples but appears less adapted for the routine determination of their polar urinary metabolites.

GC has been used for the blood determination of almost all volatile solvents, including aliphatic and halogenated hydrocarbons, ketones, aromatic hydrocarbons, nitro and amino derivatives, carbon disulphide, nitriles and complex mixtures of these compounds. In particular, headspace GC appears very suitable for the simultaneous blood determination of several solvents [8,9,18]. The analytical diagnosis of solvents abuse has been reviewed by Oliver [1,19], who gave useful procedures for the extraction of solvents from biological materials.

GC has been less used for urinary than for blood determinations but appears to be the method of choice for the determination of the unchanged form of some volatile toxic compounds, such as ketones, aliphatic and halogenated hydrocarbons and the non-conjugated hydroxylated metabolites of several solvents [11,20].

In general, published methods use a classical headspace procedure to isolate volatile compounds from the normal components of urine [21,22], but direct injection of the biological material may be sometimes employed, especially when Porapak or polyethylene glycol (PEG) columns are used [23–26]. Alternatively, microdiffusion techniques are often used [27]. Various sample-handling procedures and chromatographic conditions have been proposed by Duboswski [12] for the determination of 52 volatile substances in biological materials including urine. For headspace analysis of a wide range of volatile substances in biological materials, a Carbopak B column coated with 5% Carbowax 20M permits an acceptable separation [22]. However, a Tenax GC column appears well adapted for the analysis of substances involved in solvent abuse.

For the detection of low levels of volatile solvents in blood samples, a precise and rigorous degassing procedure using a cold trap has been described by Anderson *et al.* [28]. In addition, the sensitivity of the simple headspace procedure can be improved by

degassing the blood using helium in a double-syringe arrangement [1].

Recently, high-precision sampling of trace gas-borne volatiles by a dynamic solvent effect was described [29]. In addition, a new method was described by Fiorentino *et al.* [30] for determining low concentrations of benzene in urine samples by means of a dynamic headspace method. The urine (50 ml in a 120-ml vial) is saturated with anhydrous sodium sulphate and the volatile substances are extracted and concentrated on an adsorbent substrate (Carbotrap 100) by means of a suction pump with simultaneous entry of charcoal-filtered air. Benzene is then thermally desorbed and injected into a capillary column. A detection limit of 50 ng/l (R.S.D. 4.7%) was claimed. This method could be very useful for the trace determination of several other solvents in urine samples. Moreover, the blood/urine and urine/air partition coefficients for 43 commonly employed industrial substances have been determined by GC using a multiple equilibration method [31]. Although packed columns are still used, a growing use of capillary column GC in toxicology, especially for trace-level determinations, can be observed [1]. Moreover, for volatile solvents and their metabolites, the use of capillary columns combined with MS is becoming popular.

The biological monitoring of occupational exposure to methyl ethyl ketone (MEK) by means of urinary determination of MEK itself and its metabolites (2-butanone, 2-butanol and 3-hydroxy-2-butanone) has been described using a capillary headspace GC method [11,32–34]. Acetone can also be easily determined in urine after exposure by using GC [11,34]. Capillary GC has also been extensively used for the determination of the neurotoxic 2,5-hexanedione, the main urinary metabolite of hexane [11,15,35–41]. Similarly, cyclohexanol, the urinary metabolite of cyclohexane, and several urinary metabolites of *n*-heptane, including 2,5-heptanedione, have been determined by GC and GC–MS [14,42].

Halogenated solvents are mainly excreted in urine as hydroxylated and acidic metabolites such as trichloroethanol and trichloroacetic acid. Thus, several GC methods have been used for the accurate determination of urinary metabolites of trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane and propylene dichloride [11,20].

Simple aliphatic alcohols, such as methanol or ethanol, are sparingly determined in urine because of the lack of correlation between urinary levels and toxicity but, if required, these determinations can be easily performed by simple injection on to packed columns. However, the determination of urinary metabolites of 2-ethoxy- or 2-methoxyethanol, methoxyacetic and ethoxyacetic acid, has been performed by capillary GC [43] and with electron-capture detection [44].

For the urinary determination of low-volatility conjugated metabolites, GC has been lesser widely employed than other chromatographic methods such as HPLC or TLC, perhaps because of the necessity for a derivatization step. Several organic acids have been isolated and determined for routine metabolic screening by GC–MS [45]. Hippuric and methylhippuric acid, metabolic derivatives of toluene and xylene, have been determined in urine by capillary GC after derivatization with diazomethane [46–49]. Styrene and its metabolites (mandelic acid, phenylglyoxylic acid, phenylethylene glycol and phenylethanol) have been determined by capillary GC [50–53] and a comparison between HPLC and GC has been performed. Diazomethane derivatization has been used by some workers for the simultaneous determination of mandelic and phenylglyoxylic acids [54].

Separation of the isopropyl esters and isopropylisopropylurethane esters of the diastereoisomers of mandelic acid has been described by Korn *et al.* [55] using GC capillary columns coated with Chirasil-Val.

Phenols are important urinary metabolites of aromatic solvents such as benzene, toluene, xylene and halo, nitro and amino aromatic compounds. Phenolic compounds are highly sulpho- and glucurono-conjugated and require acid [56] or enzymatic [57,58] hydrolysis of urine samples before chromatography. The relative volatility of simple phenols such as phenol and cresols has permitted the development of several GC methods after simple steam distillation and without derivatization [10,59,60]. However, derivatization of phenols is often required, using diazomethane, heptafluorobutyryl anhydride [61] or hexamethyldisilazane [62]. The simultaneous determination of phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and 2,3,5,6-tetrachlorophenol in urine of exposed workers has been reported [63].

In conclusion, GC methods appear well adapted to the determination of the parent volatile solvents in blood and urine and may be used for the determination of their urinary metabolites. However, these methods often require several prechromatographic steps.

3.2. HPLC

The introduction of HPLC into occupational medicine represents a valuable tool for the routine monitoring of workers exposed to volatile solvents. As previously indicated, a wide range of lipophilic volatile solvents are metabolized and then excreted by the kidneys in more hydrophilic forms such as phenols or carboxylic acids, in the free or conjugated form. The well known versatility of HPLC methods increased the range of compounds that can be measured, complementing GC and also proving more suitable for the determination of low-volatility or thermally labile substances. Thus, for the urinary determination of solvent metabolites, especially for routine monitoring, HPLC is becoming a method of choice. Indeed, this method offers decisive advantages in this area.

First, the wide range of separation mechanisms now permits the determination of almost all urinary solvent metabolites, regardless of their polarity, molecular mass and thermal stability. However, reversed-phase chromatography is probably the most widely used mode of chromatography. Second, HPLC permits the simultaneous determination of several metabolites in an isocratic mode or by gradient analysis if needed, leading a complete metabolic profile, and can be easily automated. Moreover, separation of substances of interest from structurally similar compounds or isomers is feasible. Third, urine samples do not usually require extraction or derivatization steps and pretreatment is generally limited to sample filtration and acidic or enzymatic hydrolysis of conjugates.

The major inconvenience of HPLC for the routine urinary determination of solvent metabolites is a lower sensitivity than GC. However, as previously indicated, the urinary TLVs of many common volatile solvents are high, causing no real difficulties despite the direct injection of unprocessed urine samples (except for a simple filtration step) used in many "improved" methods. The detector most widely used in this context depends on UV absorption as many solvent metabolites, especially from aromatic solvents, absorb strongly in the UV–visible region. For non-UV-absorbing metabolites, derivatization with chromophore-adding reactants could be performed using a pre- or postcolumn mode and has been widely used in toxicology [64]. However, this approach had not been sufficiently explored for low-UV-absorbing solvents and their metabolites except for the HPLC determination of ethylene glycol [65]. Similarly, the use of a more sensitive detector such as a spectrofluorimeter with or without derivatization has not been investigated. Indeed, this approach could be an attractive alternative to capillary GC for the determination of trace levels of hydroxylated or acidic metabolites from haloalkanes or phenolic derivatives from polyhalogenated phenyls.

The HPLC determination of acidic and phenolic urinary metabolites of aromatic solvents (*e.g.*, benzene, toluene, xylene, ethylbenzene, styrene) has been extensively studied. Several papers were focused on the HPLC determination of metabolites from a specific solvent such as catechol and quinol from benzene [66], hippuric acid and cresols from toluene [59,67–69] or mandelic acid and phenylglyoxylic acid from ethylbenzene or styrene [70,71]. However, the simultaneous determination of acidic and phenolic metabolites from these aromatic solvents for screening purpose has been widely performed [72–83]. A simple reversed-phase method, using a mixture of acetonitrile or methanol and water at acidic pH, was generally sufficient for separating the acidic metabolites, but some workers preferred an ion-pair method [79]. Conjugated phenolic compounds were hydrolysed by both acidic or enzymatic methods and the free phenols (phenol, *o*-cresol) were then separated using a specific mobile phase.

However, the simultaneous determination of phenol and four urinary acidic metabolites (hippuric, methylhippuric, mandelic and phenylglyoxylic acid) has been achieved using acetonitrile–1% phosphoric acid (10:90) in an isocratic mode [83] and by an automated method [78]. Further, urinary metabolites of halo or nitro aromatic solvents such as 4-chlorocatechol and chlorophenols from chlorobenzene [84], bromophenols from bromobenzene [85] and 4-nitrophenol from nitrobenzene [83] have

also been determined using HPLC.

HPLC has been also employed for a more sophisticated approach to volatile solvent exposure than the simple monitoring. The simultaneous determination of styrene and acetaminophen metabolites was described by Colin *et al.* [86], as acetaminophen, which is a widely used drug, and its metabolites can interfere during routine monitoring, allowing to a false interpretation of results. HPLC also permits the accurate determination of cresol isomers, as toluene exposure markers [87,88]. An improvement in the HPLC of *trans,trans*-muconic acid, a substitute for phenol in the biological monitoring of benzene exposure, was recently described by Ducos *et al.* [89]. As HPLC is a simpler and cheaper approach than the GC–MS method used by others [7,16,87,90], this method appears to be a powerful tool for a more sophisticated approach to the routine monitoring of solvent exposure, separation of isomers, trace-level determination or toxicokinetics [91–93]. The determination of *p*-chloronitrobenzene and its complex metabolic by-products in urine has been studied by both GC–MS and HPLC by Yoshida and co-workers [94–96] in rats treated by this aromatic compound and in patients suffering from acute poisoning. Evaluation of occupational exposure to carbon disulphide in biological materials including urine has been performed by an HPLC method [97]. S-Phenyl-N-acetylcysteine, as a marker of benzene exposure, has also been determined in urine by Jongeneelen *et al.* [98]. Moreover, some papers indicate that HPLC and GC give similar results [72].

In conclusion, it now appears evident that HPLC is becoming a powerful tool for the accurate and easy determination of urinary metabolites of volatile solvents.

3.3. Planar chromatography

In the past, classical planar chromatography (*i.e.*, TLC) has been widely used for the biological monitoring of solvent exposure. Compared with GC and HPLC, this method presents clear advantages in terms of simplicity, rapidity, low cost and the possibility of the simultaneous determination of several samples. Urinary metabolites of aromatic solvents have mostly been measured by this method [57,99,100]. TLC has also permitted the determination of *p*-aminophenol after mixed exposure to aniline and toluene [101] and a simple method for detecting ethylene glycol in urine by TLC has been proposed [102]. In these papers, TLC was used mostly for mass screening and semi-quantitative determination of urinary levels.

A major drawback to TLC remains the difficulty of the quantitative assessment of the spots but the use of suitable densitometers can allow a more accurate determination. However, for routine monitoring or detection of intoxication, classical TLC could be sufficient, as urinary levels will be high in cases of massive exposure such as solvent abuse or solvent handling without protective devices.

HPTLC could be an attractive improvement of classical TLC [103], but this method has not been employed in the biological monitoring of solvent exposure. Similarly, overpressured thin-layer chromatography (OTLC) could be a promising and powerful tool in this area [104–106]. As one-line detection can be coupled to OTLC, this method could combine the major advantage of OTLC (simultaneous assay of 50–100 samples) and the specific and quantitative determination furnished by appropriate detectors such as a UV spectrophotometer.

4. CONCLUSIONS

The biological monitoring of solvent exposure is a growing field of interest in toxicological and occupational laboratories. As always in biomedical analysis, the choice of the most appropriate analytical method remains a major problem. As an ideal method does not exist, the best method will be a compromise between several conflicting requirements.

For research purposes or for an accurate diagnosis of a rare and complicated intoxication, sophisticated methods such as GC–MS and a specialized laboratory will be required. Indeed, in these situations, cost, length, complexity and workload problems generated by the analytical method are not necessarily major concerns. In contrast, for the routine monitoring of several hundred individuals exposed to volatile solvents in an industrial environment, the cost per determination, simplicity and automation will be important considerations.

HPLC appears to be the primary choice for the

determination of urinary metabolites. The apparatus is relatively economical, automatable and sufficiently versatile to be used for other determinations in related areas such as drug monitoring or clinical toxicology that are currently of interest in the same laboratory. As previously indicated, the analyst now has a choice between several methods, well adapted to routine and simultaneous determinations of the urinary metabolites of some common volatiles solvents that require biological monitoring. Moreover, HPLC is also becoming an attractive tool in the growing field of more elaborate approaches with new urinary markers such as specific conjugates or isomers.

Whereas capillary GC and its coupled methods remain currently of major interest for the determination of parent volatile solvents in blood and urine [107], HPLC appears to be the best choice for the measurement of their urinary metabolites. Further, recent developments in HPLC such as high-efficiency columns, chiral phases, diode-array or electrochemical detectors and HPLC–MS, which are seldom used in this domain, could widen the usefulness of this method for most complex analytical problems that may be encountered during this measurement. However, despite the relative neglect of planar chromatography in this area and considering the great interest in methods that could permit the simultaneous assay of numerous samples, as often required in routine monitoring, a new approach using improved methods such as OTLC is urgently required.

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Environmental analysis of polychlorinated terphenyls: distribution in shellfish from the Ebro Delta (Mediterranean)

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ABSTRACT

Polychlorinated terphenyls (PCTs) have characteristics almost identical with those of polychlorinated biphenyls (PCBs) and have been used for analogous applications, but only sporadic reports of the occurrence of PCTs in the environment have been published. High-resolution gas chromatography with electron-capture detection (HRGC–ECD) and mass spectrometric detection in the selected ion monitoring mode was used to analyse samples for PCTs. The homologue distribution of Aroclor 5432, 5460, Leromoll 141 and the PCTs in samples of shellfish from the Ebro Delta (Catalonia, Spain) was established, taking into account the contribution of the $[M-Cl_2]^+$ fragments. Quantification was achieved by HRGC–ECD. Concentrations were between 790 and 3 ng/g (dry mass).

INTRODUCTION

In the last two decades, there has been considerable public concern about the presence of halogenated anthropogenic compounds in the environment, because of their persistence, bioaccumulation potential and health risks [1–4]. Many studies have been conducted to determine the extent and significance of polychlorinated biphenyl (PCB) residues, and the occurrence of these compounds in the environment has been extensively documented [5–10]. In contrast, little attention has been paid to the environmental distribution of polychlorinated terphenyls (PCTs), which are similar to PCBs in chemical characteristics and uses.

PCBs and PCTs have been used in hydraulic fluids, electrical equipment, sealants, plasticizers, paints, adhesives and casting agents because of their electrical and flame-retardant properties [1]. From an environmental protection point of view, these applications have been divided into open (from which PCBs cannot be recovered) and closed systems [1, 11], the latter having traditionally been the most important applications of PCBs. Indeed, closed systems are the only applications of PCBs to be covered by legislation since 1973 in the USA and 1976 in EEC countries. Similar regulatory attention has not been paid to PCTs but it has been argued that their usage is controlled by regulations restricting PCBs.

Commercial formulations of PCTs could be expected to be much more complex than those of PCBs. There are not only more positions and combinations of positions available for chlorination, but the phenyl rings could also have three possible arrangements. Hence the number of isomers and homologues should be higher than the theoretical

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209 PCB congeners, although the exact number has never been calculated.

Analysis for PCTs has proved to be difficult because of the complexity of the mixtures, the high boiling points of the heavily chlorinated congeners, the coincidence of the gas chromatographic retentions of the lower chlorinated PCTs with those of some PCBs and the presence of interferences in the analysis of molecular ions by mass spectrometry.

Little information is available about the distribution, fate and effects of PCTs in the environment, but as highly chlorinated aromatic compounds, PCTs might be expected to have a high resistance to biodegradation and photodegradation and to be capable of being accumulated in living organisms and through food chains. PCTs are not so widely and heavily dispersed in the environment as PCBs, but they have nevertheless been detected in river water [12], soil and sediment samples [13–16], a variety of biological samples, including shellfish, eels, seals, birds [15,17–19] and bovine milk [20], food packaging materials [21,22] and human tissue [17,23–25].

In this work, the distribution of the different chlorinated congeners in two Aroclors, 5432 and 5460, and Leromoll 141, was studied and the presence of PCTs in samples of shellfish from the Ebro Delta (Catalonia, Spain) was investigated.

EXPERIMENTAL

Chemicals

Aroclor 1254 and 1260 PCB standard mixtures were purchased from Promochem (Wesel, Germany) and Aroclor 5432 and 5460 PCT mixtures from Chem Service (West Chester, PA, USA); the commercial product Leromoll 141 was a gift from Dr. U. A. Th. Brinkman (Amsterdam, Netherlands). The internal standards 2,4-dichlorobenzyl hexyl ether (DCBE-6) and 2,4-dichlorobenzyl hexadecyl ether (DCBE-16) were a gift from Dr. D. E. Wells (Aberdeen, UK). Florisil for residue analysis (60–100 mesh), from Merck (Darmstadt, Germany) was used as a chromatographic adsorbent. It was activated at 675°C for 2 h and then stored at 130°C before use. Granular anhydrous sodium sulphate for residue analysis from Merck was dried at 450°C for 2 h and kept at 130°C. The solvents *n*-hexane and dichloromethane were redistilled in glass to obtain residue analysis grade materials. The purity of the solvents was determined by concentration of 150 ml to 0.5 ml and injection of the extract into the gas chromatographic–electron capture detection (GC–ECD) system. All glass materials were cleaned with AP-13 Extran alkaline soap (Merck) for 24 h and dried overnight at 180°C. The chromatographic clean-up column (30 cm × 1 cm I.D.) was packed with 7 *g* of activated Florisil.

Standard solutions of PCB and PCT mixtures containing 10 mg/l of individual Aroclors 1254, 1260, 5432, 5460 and Leromoll 141 were prepared in isooctane for residue analysis (Carlo Erba, Milan, Italy).

Apparatus

Gas chromatography was carried out on a Carlo Erba (Milan, Italy) Model 5300 Mega Series gas chromatograph equipped with a ^{63}Ni electron-capture detector using nitrogen as make-up gas. A DB-5 fused-silica (J&W Scientific, Folsom, CA, USA) capillary column (60 m × 0.25 mm I.D.) with a 0.25-μm film thickness, was used with helium as carrier gas at a linear velocity of 30 cm/s. The temperature was held isothermally at 90°C for 3 min, programmed to 150°C at 25°C/min and maintained at 150°C for 1 min, then programmed to 310°C at 2.5°C/min, and mantained at this temperature for 30 min. The injector and detector temperatures were 270 and 330°C, respectively. The chromatographic data were analysed using an Merck–Hitachi Model D-2000 integrator.

For HRGC–MS a Konik (Barcelona, Spain) Model 3000 gas chromatograph with a VG TS-250 (VG Instruments, Manchester, UK) mass spectrometer and a VG 11-250 data system was used. A DB-5 fused-silica (J&W Scientific) capillary column (30 m × 0.25 mm I.D.), with a 0.25-μm film thickness, was used with helium as carrier gas at a linear velocity of 25 cm/s. The temperature programme for GC–MS (full scan) was from 70°C (held for 1 min) to 200°C (held for 1 min) at 2°C/min and then to 300°C (held for 30 min) at 4°C/min. For HRGC–MS–[selected ion monitoring (SIM) mode] the temperature programme was from 70°C (held for 0.7 min) to 150°C (held for 1 min) at 20°C/min and then from 150 to 280°C (held for 15 min) at 3°C/min. The injector was kept at 250°C. The MS operating conditions were as follows: ion source and interface temperatures, 225 and 290°C, respectively; ioniza-

tion energy, 70 eV (electron impact mode); resolving power, 500; and mass range, 40–625 a.m.u. at 2 s per decade when the full-scan mode was used. In the HRGC–MS–SIM mode the ions at *m/z* 298, 300, 332, 334, 366, 368, 402, 404, 434, 436, 470, 472, 504, 506, 538, 540, 574, 576, 608 and 610 were monitored and *m/z* 207 was used as lock mass. Other conditions were dwell time 50 ms and trap current 700 μA.

Sampling and analysis

A field sampling programme was conducted over 2 years for the determination of PCBs and chlorinated pesticides in mussels and oysters from the Fangar and Alfacs bays in the Ebro Delta. Sample sites were selected to give overall information about the quality of the cultivated bivalves. The samples were collected at the breeding site and frozen at -18°C upon collection.

Whole fish were ground, homogenized and dried with a vacuum glass desiccator at 60°C for 24 h. Samples (*ca.* 8 g, dry tissue) were mixed with 200 g of anhydrous sodium sulphate and then Soxhlet extracted with 300 ml of hexane–dichloromethane (1:1) for 3 h. The extract was reduced in volume by rotary evaporation and solvent-exchanged to *n*-hexane (5 ml). Sample clean-up was carried out by elution of 2 ml of the extract through a Florisil column using 50 ml of *n*-hexane and 50 ml of hexane–dichloromethane (1:1). PCBs, PCTs and *p,p'*-DDE were eluted in the first fraction, whereas the second fraction contained the rest of the chlorinated pesticides. Internal standards were added and the final volume of the extract was adjusted to 0.5 ml. Samples were analysed by gas chromatography GC–ECD and GC–MS.

For the determination of PCBs in the shelfish samples, the congener specific method with Aroclor 1254 as a standard was used. The chromatographic peaks from the sample were compared with those of the Aroclor using DCBE-6 and DCBE-16 as internal standards. Total PCBs are expressed as the sum of the single congeners present. For the determination of PCTs the standard Aroclor 5460 was used; in this case the internal standards were compared with the total area of the PCT peaks because single congeners could not be identified; results were expressed on a dry mass basis. The recoveries of the PCBs and PCTs were calculated by spiking the samples with careful mixing of standard solutions. Differents amounts of PCB congeners and Aroclor 5460 standard were used in order to obtain concentrations around 50, 100, 150 and 200% (n = 3) of the actual concentration in the real samples. The efficiency was evaluated by studying the recoveries of PCBs and PCTs from spiked samples. The means of the analytical recoveries observed for PCBs and PCTs were 98.8% and 91.2% with relative standard deviations of 9.7 and 11.6%, respectively.

RESULTS AND DISCUSSION

Chromatography of PCT mixtures

The PCT mixtures Aroclor 5460 and 5432 and Leromoll 141 were analysed using capillary GC with ECD and MS detection. The non-polar DB-5 stationary phase was especially suitable because of its stability, allowing temperatures of up to 310°C to be used, at which the highly chlorinated PCTs are eluted.

Fig. 1 shows the TIC profiles of HRGC–MS for Leromoll 141 and Aroclor 5460. The retention of PCT congeners tends to increase with increasing degree of chlorination, although co-elution between congeners containing fewer or more chlorine atoms than a given isomer group is observed. The TIC profiles show that Aroclor 5460 is mainly composed of PCT having six to eleven chlorine substituents, whereas Leromoll 141 has five to nine. These values agree with literature data, which indicate that Aroclor 5460 is mainly constituted of terphenyls substituted with seven to ten or eleven chlorine atoms [26–28] and Leromoll from six to nine [28]. Our results show a contribution from the five substituted terphenyls in the latter, which has not been reported previously.

The mass ranges covered in the TIC profiles in Fig. 1 include the major ions in the isotope clusters for molecular ions of the terphenyls. Co-elution of the PCT congeners containing fewer chlorines did not interfere with the detection and measurement of the $M^{+\cdot}$ ions produced by a member of a given isomer group, although there may be interference from the $[M-Cl_2]^+$ fragment ions of homologues containing two additional chlorine atoms [24]. This effect is seen in Fig. 2, in which the selected ion $M^{+\cdot}$ monitoring register for the homologues of Aroclor 5460 is given. Comparison of Figs. 2b, d and/or a, c

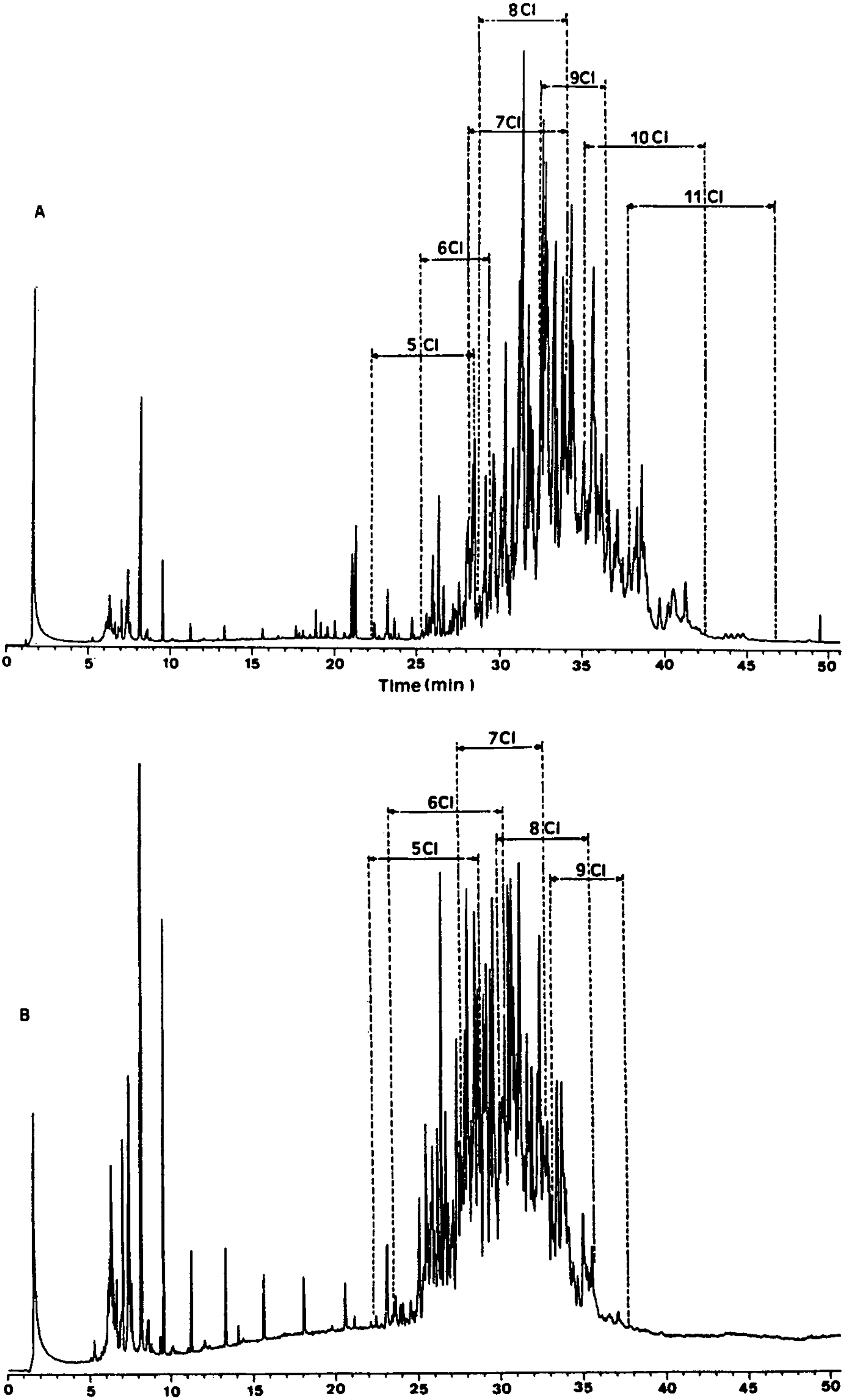


Fig. 1. Total ion current register for (A) Aroclor 5460 and (B) Leromoll 141. Numbers above the peaks designate the number of chlorine sustituents.

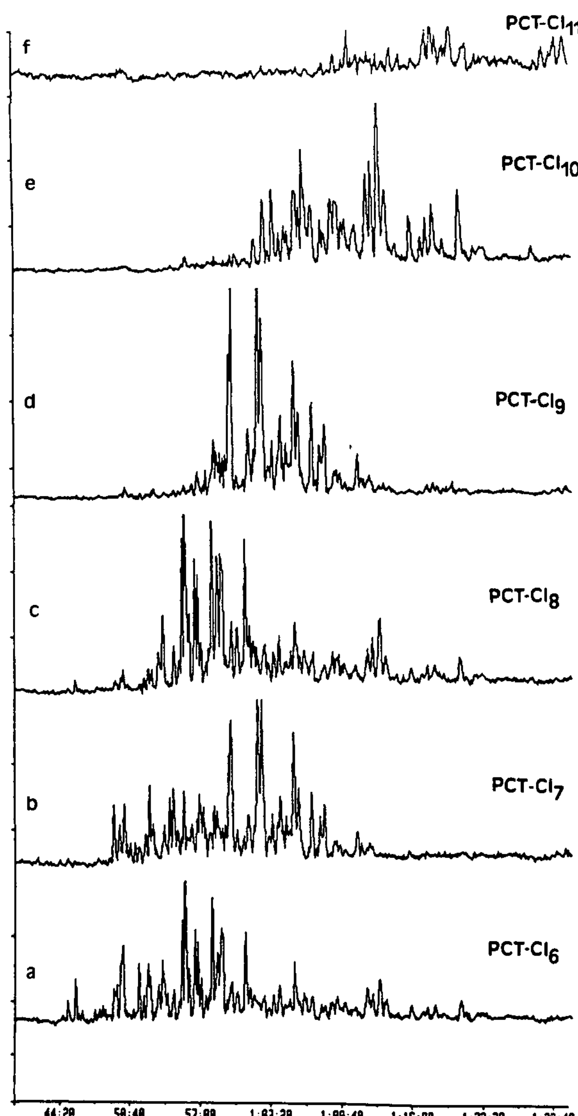


Fig. 2. Selected ion monitoring for the homologues of Aroclor 5460. (a) 6-Chloro-PCT; (b) 7-chloro-PCT; (c) 8-chloro-PCT; (d) 9-chloro-PCT; (e) 10-chloro-PCT; (f) 11-chloro-PCT. x-axis: time.

and e shows that the $[M-Cl_2]^+$ fragments of the nonachloroterphenyls are major contributors to the heptachloroterphenyls and fragments of the decachloroterphenyls contribute to the octachloroterphenyls, as occurs with the fragments of the latter with the hexachloroterphenyls. Another potential interference may be produced by the $[M-Cl]^+$ fragment ions, although this does not seem to be important in our case because the signal is weak as can be seen in Fig. 2.

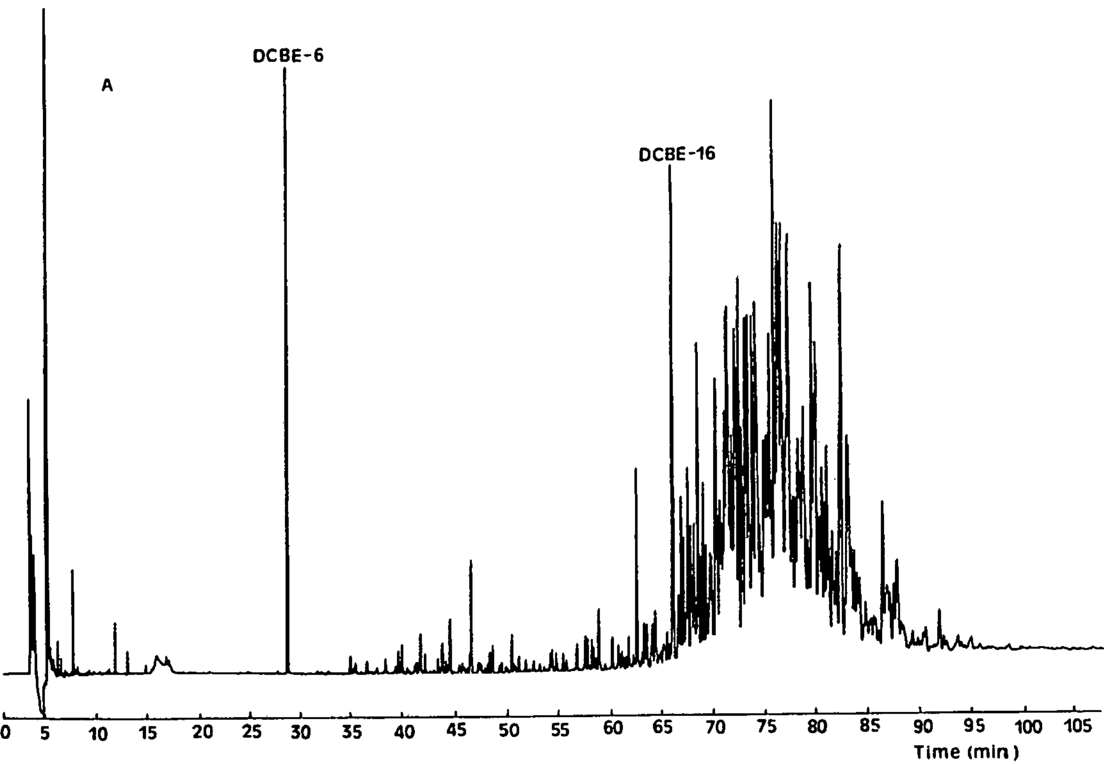
The assigment of the level of chlorination of the homologues was calculated taking the retention times and the effect of the contribution of the $[M-Cl_2]^+$ fragments into account. Hence the contribution of the $[M-Cl_2]^+$ ions was eliminated from the selected ion register for each homologue and the approximate distribution in the original compound was calculated assuming that virtually no co-elution between homologues containing two additional chlorine atoms, as can be seen in Figs. 1 and 2. To discriminate between these two species, high-resolution mass spectrometry would be necessary (higher than 20 000). The values obtained are given in Table I, showing that Aroclor 5432 was mainly composed of PCTs having two, three and four chlorine sustituents, with minor amounts of one and five, and Aroclor 5460 had terphenyls with eight, nine and ten chlorine substituents with minor amounts of six, seven and eleven. On the other hand, Leromoll 141 was mainly composed of PCTs having five, six, seven and eight chlorine substituents with minor amounts of nine.

TABLE I

PERCENTAGE HOMOLOGUE DISTRIBUTIONS FOR AROCLOR 5432, 5460, LEROMOLL 141 AND SAMPLE 1

Compounds	Percentage of homologues[a]			
	Aroclor 5432	Aroclor 5460	Leromoll 141	Sample 1
Mono-CTs	<1	–	–	–
Di-CTs	31	–	–	–
Tri-CTs	42	–	–	–
Tetra-CTs	23	–	–	–
Penta-CTs	3	–	14	9
Hexa-CTs	–	<1	28	22
Hepta-CTs	–	6	30	40
Octa-CTs	–	17	27	27
Nona-CTs	–	42	1	2
Deca-CTs	–	28	–	<1
Undeca-CTs	–	6	–	<1

[a] Dashes indicate not detected.



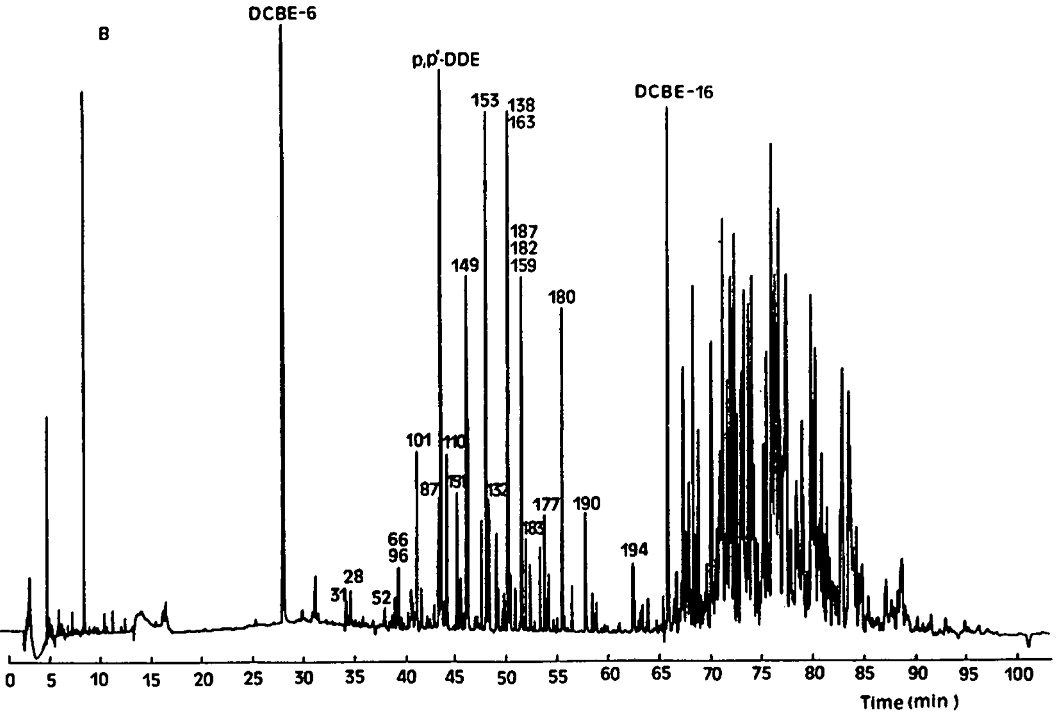
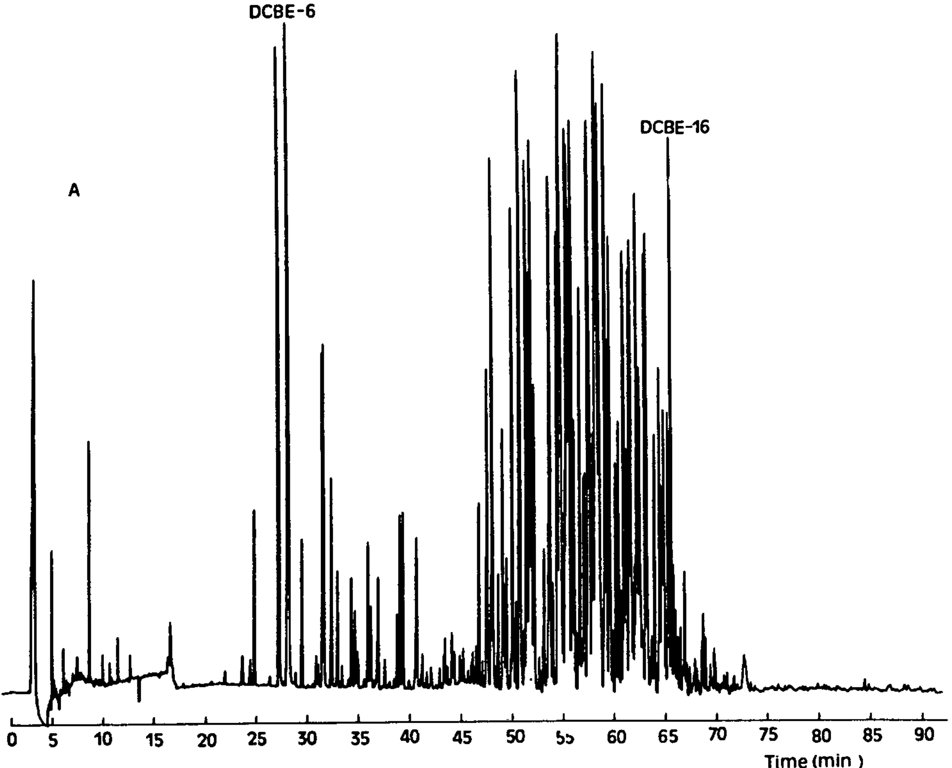


Fig. 3. HRGC–ECD (60-m DB-5 column) of (A) Leromoll 141 and (B) sample 1. PCB congener numbers and internal standards are indicated.



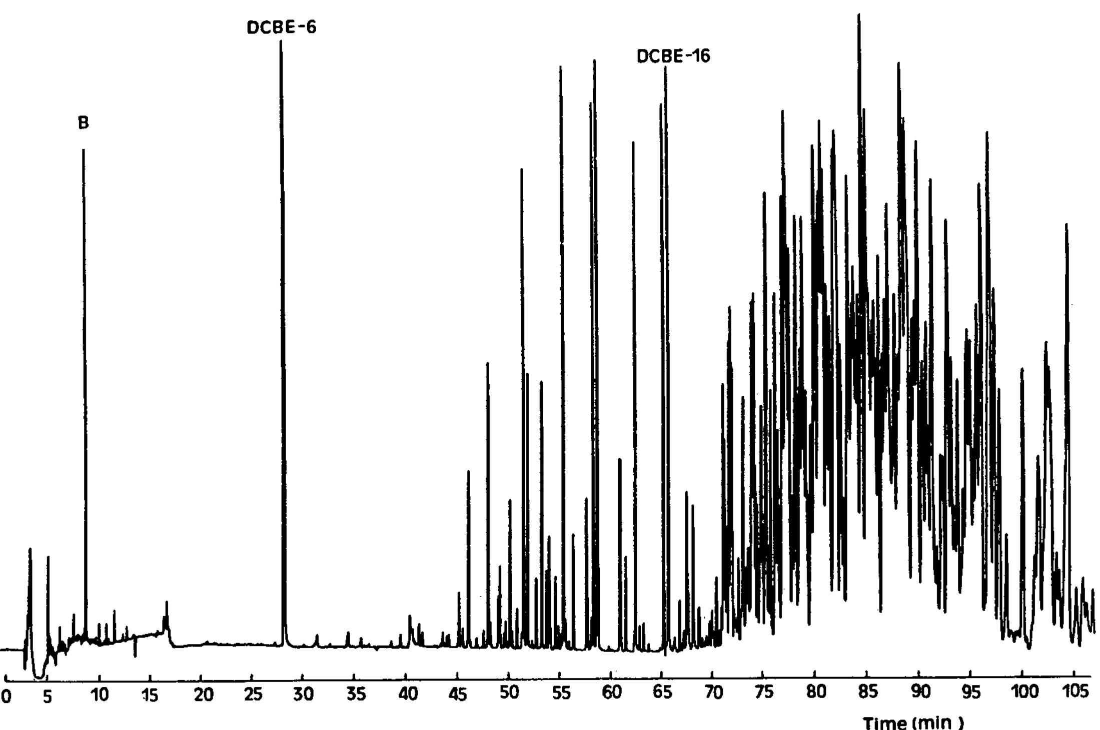


Fig. 4. HRGC–ECD (60-m DB-5 column) of (A) Aroclor 5432 and (B) Aroclor 5460.

PCT in shellfish samples

A monitoring programme of anthropogenic halogenated compounds in shellfish from the Ebro Delta was carried out in 1989 and 1990. The PCB content in these samples from mussels (*Mitylus galloprovincialis*), clams (*Tapes decussata* and *Tapes semidecussata*) and oysters (*Ostrea edulis* and *Crasostrea gigas*) lay between 2950 and 4 ng/g dry mass (600 and 1 ng/g wet mass).

The chromatograms obtained from the extracts of the samples collected in February 1989 showed a series of compounds with retention times higher than the PCBs, which were identified by HRGC–MS as PCTs. Figs. 3 and 4 show, as an example, a comparison of an HRGC–ECD trace of the extract of one sample and those of the Leromoll 141 and Aroclor 5460 and 5432 standards. It is apparent from these profiles that the PCTs in the sample are very similar to those of Leromoll 141.The peaks eluted between 34 and 65 min were identified as PCBs by HRGC–MS.

Fig. 5 shows the HRGC–MS–SIM profiles for the sample extract. PCTs in the sample are mainly isomers of between five and ten chlorine atoms. The homologue distribution of the sample is shown in Table I. Hexa-, hepta- and octachloroterphenyls are the major contributors to the PCT content in the sample, and the distribution obtained is very similar to that of Leromoll 141, so the presence in the sample of the terphenyls from this formulation or a similar one was suggested.

The amount of PCTs in the sample was calculated from the HRGC–ECD data using Aroclor 5460 as standard and DCBE-16 as internal standard. Leromoll 141 was not used because it has a relatively low content of PCTs, as can be seen in Fig. 6, where the full-scan chromatogram of this compound is shown. PCBs were not detected in this mixture. Levels of PCT in Leromoll 141 calculated from this chromatogram gave a value of about 17%, in agreement with the data reported by De Kok *et al.* [28] calculated from the chlorine content of the commercial mixture. Purification procedures and certification analysis of the PCT content in Leromoll 141 are not available. The concentrations of PCTs in the samples are given in Table II. PCTs were detected at high levels in samples collected in February 1989 (790 ng/g dry mass) but the concentrations were much lower in April (198 ng/g). All the samples collected during 1989 contained PCTs, but at low levels, whereas in 1990 PCTs were not observed.

Although the exact use of PCTs in industrial formulations is unknown, especially as the production of PCBs has been banned, and little information about their fate in the environment is available, we must point out that in all samples collected in 1989 we found PCTs in decreasing amounts from February to November. Further, we found PCBs in all the samples collected over the 2 years, but no correlation between PCBs and PCTs was observed, so it does not seem that the PCT content could be related

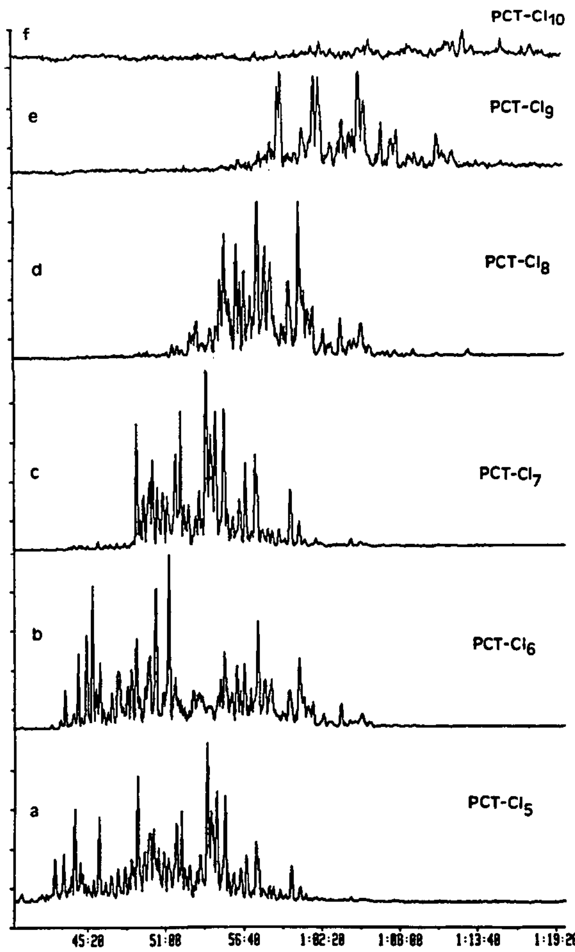


Fig. 5. Selected ion monitoring for the sample 1. (a) 5-Chloro-PCT; (b) 6-chloro-PCT; (c) 7-chloro-PCT; (d) 8-chloro-PCT; (e) 9-chloro-PCT; (f) 10-chloro-PCT. x-axis: time.

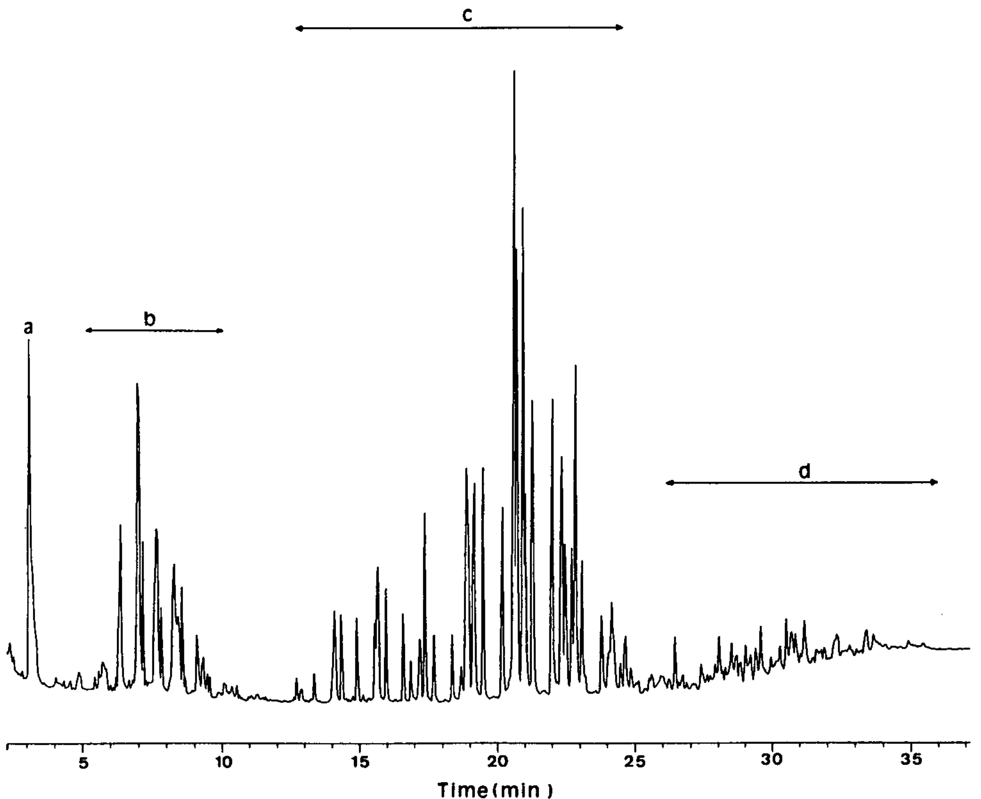


Fig. 6. Total ion current register for Leromoll 141. (a) Phenol; (b) aliphatic hydrocarbons; (c) tentative assigment phenolic resins, base peak mass 94; (d) terphenyls.

TABLE II

CONCENTRATION OF PCB AND PCT COMPOUNDS IN SHELLFISH

Sample No.	Shellfish	Date (1989)	Concentration (ng/g dry mass)	
			PCBs[a]	PCTs[b]
1	Mussels	February	131	790
2	Mussels	April	435	198
3	Mussels	July	690	10
4	Clams	July	128	7
5	Mussels	July	2000	21
6	Clams	July	730	3
7	Mussels	September	2950	115
8	Clams	September	745	118
9	Clams	October	528	97
10	Mussels	November	1797	100
11	Clams	November	215	105

[a] Whole mass between 597 and 18 ng/g.
[b] Whole mass between 180 and 1 ng/g.

to the PCBs. The fact that the Ebro River crosses an industrial zone *ca.* 60 km upstream from the delta, where there is an important chemical complex, appears to suggest a sporadic release into the river water of Leromoll 141 or other industrial formulations of similar terphenyl composition.

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Rapid isolation of polychlorinated biphenyls from milk by a combination of supercritical-fluid extraction and supercritical-fluid chromatography

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ABSTRACT

The extraction and isolation of polychlorinated biphenyls (PCBs) from sample matrices such as freeze-dried milk is a lengthy and expensive process that conventionally requires the isolation of microgram amounts of PCBs from large volumes of flammable organic solvents used in the extraction and chromatographic stages. Supercritical-fluid extraction (SFE) and chromatography (SFC) using carbon dioxide has been investigated as a replacement procedure using a Simplex optimisation approach to optimise the working conditions. The SFE conditions required to extract PCBs and milk fat from freeze-dried milk were investigated, and it was found that although extraction was rapid (10 min), the conditions required were identical so that there was no separation of PCBs and fat. However, SFC conditions have been developed that permit the resolution of PCBs from milk fat in 15 min. Thus, a combination of SFE and SFC have the potential to replace the existing methods of extraction and isolation with procedures that are quicker, cheaper, and avoid the use of flammable organic solvents.

INTRODUCTION

The high solvating power of supercritical fluids was reported in the last century [1], but practical applications, such as supercritical-fluid extraction (SFE) [2] and supercritical-fluid chromatography (SFC) [3], were first reported in 1962 and 1969, respectively. These papers have led to the application of supercritical fluids being investigated for the improvement of analytical methods by a large number of researchers [4–11]. A variety of organic compounds have been extracted from different matrices using carbon dioxide. Extraction using supercritical fluids can achieve better efficiencies than conventional Soxhlet extraction in a much shorter time period for some analytes and matrices [9]. This has been demonstrated for the extraction of polycyclic aromatic hydrocarbons from environmental solids by Hawthorne and Miller [10]. SFE also minimises the use of toxic organic solvents, and the need for concentration steps before analysis of the extracts. SFE can be optimised for a particular extraction because the solvent strength of a supercritical fluid is directly related to its density which can be easily modified by changing the extraction pressure, and to a lesser extent, the temperature.

SFC has also been widely investigated, because of the high efficiency that is possible, and the ability of SFC to separate types of compounds which cannot be analysed by traditional gas chromatography [5]. Relatively non-volatile, thermally unstable and high-molecular-mass solutes can all be analysed by SFC. One such example is the analysis of thermally labile sulphonylureas reported by McNally and Wheeler [11]. Separations by SFC can be achieved and enhanced by varying the pressure, temperature, and mobile phase composition. The mobile phase composition can be varied by the addition of organic compounds as modifiers which change the eluting power of a supercritical mobile phase such as carbon dioxide. A range of organic solvents, including methanol and acetonitrile, have been investigated

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as supercritical fluid modifiers by Levy and Ritchey [7].

Current literature methods that are employed for the extraction of polychlorinated biphenyls (PCBs) from solid matrices and from liquids that have been either absorbed on porous inert solids, or freeze-dried, are usually lengthy. A typical procedure would require Soxhlet extraction (3–8 h or overnight) with an organic solvent followed by evaporation to a small volume (Kuderna–Danish apparatus), followed by a chromatographic clean-up step to isolate PCBs (either with organochlorine pesticides, or separated from them), from fats. All these steps require uncontaminated, clean glassware, and ensure that sample preparation for GC analysis is the most lengthy and expensive part of the analytical process. This report describes the application of SFE and SFC to replace the traditional extraction and isolation steps respectively, with considerable savings in time and cost.

EXPERIMENTAL

Supercritical-fluid extraction

SFE conditions were investigated and optimised for the extraction of PCBs from a typical matrix (*e.g.* freeze-dried milk + Florisil) using supercritical carbon dioxide (CO_2). A Simplex optimisation procedure was undertaken [12], with variations in the extraction temperature and back pressure used as the significant factors for the extraction.

A Jasco SFE system was kindly provided by Ciba-Corning Analytical (Halstead, UK). An ethylene glycol-filled cooler was used to maintain the head of the carbon dioxide pump, Model 880-PU at −10°C, with the flow-rate controlled from the electronic keypad. The extraction vessel, consisting of an empty 13 cm × 10 mm I.D. HPLC column with two screw-capped ends, was housed in a Model 860-CO column oven at a set temperature. The material to be extracted was placed in the HPLC column and the two ends screwed on tightly by hand. A Rheodyne switching valve was used to switch the flow of supercritical CO_2 through the extraction vessel once the sample had been loaded. A Model 875-UV ultraviolet detector with a high-pressure flow-cell was used to continuously monitor the extract at a specific wavelength. A Model 880-81 back pressure regulator kept the entire extraction system at a constant back pressure, via an electronic feedback regulator that was flow independent. This arrangement provides greater stability throughout the system than the more frequently used capillary restrictors. The temperature of the back pressure regulator was also controlled to avoid the problem of the extract becoming plugged in the exit tubing. The extract was vented to the atmosphere through the back pressure regulator and collected in 1 ml of heptane in an ice-cooled 120 × 14 mm I.D. test tube. Throughout the optimisation of the extraction pressure and temperature, the liquid CO_2 flow-rate was 3.0 ml min^{-1}, and the UV detector was used at a wavelength of 254 nm and a range of 0.08 AUFS.

Supercritical-fluid chromatography

SFC was investigated for the separation of PCBs from fat. The PCBs and fat were both extracted from cow's milk by SFE. The same SFE system was used for the SFC work, with a few modifications. A second Model 880-PU HPLC pump was used to allow the addition of an organic modifier to the supercritical CO_2 mobile phase. The two phases were mixed in a Model 880-30 mixer module. Three different HPLC columns were investigated by replacement of the extraction vessel used in the SFE work, a 30-cm PLRP-S column (Polymer Labs., Church Stretton, UK), a 15-cm Hamilton PRP-1 column (Jones Chromatography, Hengoed, UK), and a 10-cm Brownlee Labs. RP-8 Spheri-5 column (Anachem, Luton, UK).

A second Rheodyne valve was fitted in series with the first Rheodyne valve to allow a 20-μl injection loop to be used to introduce the sample onto the column. A syringe was used to inject the samples onto the column, and the analytes were collected in the same way as described for SFE.

Gas chromatography–mass spectrometry

All the extracts were analysed by a Hewlett-Packard (Bracknell, UK) HP 5890 gas chromatograph with a 5970 MSD mass spectrometer on a 50-m OV-1 column (Hewlett-Packard). A selected ion monitoring (SIM) programme designed specifically for the analysis of PCBs according to their chlorination level was used [13]. A 5-μl aliquot of each extract was injected onto the column in the splitless mode. The injector temperature was set at 250°C, and the flow-rate of hydrogen carrier gas set at 1 ml

min^{-1}. A temperature programme with an initial temperature of 75°C for 2 min, followed by ramps of 30°C min^{-1} to 120°C, and then 10°C min^{-1} to 270°C, and a final temperature of 270°C for 35 min was used. The SIM programme meant that at any given time during a run, the mass spectrometer was monitoring for four mass ions specific to two levels of PCB chlorination.

Every peak found by GC–MS was mathematically tested to prove whether or not it was due to a PCB by the method of Erickson *et al.* [13]. The peaks that failed this test were ignored, and only those peaks that passed were used to assess the levels of PCBs extracted [13].

Gas chromatography–electron-capture detection (ECD)

A Perkin-Elmer (Beaconsfield, UK) 8320B capillary GC–ECD system with a 25-m HT-5 column (SGE Pty., Milton Keynes, UK) was also used to analyse some of the extracts. The injector and detector temperatures were set at 250°C, and hydrogen was used as the carrier gas with nitrogen as the make-up gas. A temperature programme was used for the analyses, commencing at 75°C for 2 min, followed by ramps of 30°C min^{-1} to 120°C and then 1.5°C min^{-1} to 210°C, and a final temperature of 210°C for 10 min.

RESULTS AND DISCUSSION

Supercritical-fluid extraction

PCBs from cow's milk. The solvent strength of a supercritical fluid is determined by a number of factors, one of which is its density, so that the solvating ability of a particular supercritical fluid towards a particular species (*i.e.* PCBs) can be modified by changing the extraction pressure [10]. Also for carbon dioxide SFE, increasing temperature (at constant pressure) can enhance extraction efficiencies even though density is lower at the higher temperature.

A mixture of freeze-dried skimmed milk, equivalent to 10 ml of the original milk (fat content 0.1%, w/w) and Florisil was used as the initial extraction medium. The low level of fat from the milk ensured that the UV signal for extracted PCBs would be easily identified, and the Florisil aided the rapid penetration of the freeze-dried milk by the supercritical CO_2.

Initially, the level of the PCB spike required for the Simplex optimisation experiment was investigated. The spike was introduced into the powder contained in the extraction chamber with a syringe. Moderate extraction conditions were used and the concentration of PCBs added was increased gradually until a suitable response was found on the chart recorder. A 50-μl spike of 100 μg ml^{-1} Aroclor 1242 was found to give the required response, and this level of spike was used throughout the optimisation procedure.

An optimisation procedure is usually commenced at a set of conditions removed from the anticipated optimum. The variables investigated here were the extraction pressure and temperature, and the response measured was peak height. Peak areas gave inaccurate estimates of extraction efficiency due to the fluctuating nature of the baseline. The optimisation procedure was started at a high extraction temperature and a low back pressure as this was expected to give a poor extraction of PCBs from cow's milk, because the extracting strength of supercritical CO_2 usually increases with increasing pressure and usually decreases with increasing temperature, although solvation is not the only limiting process of SFE with CO_2. Matrix interactions are also an important consideration.

Initial studies at 65°C and 120 kgf cm^{-2} and at 55°C and 120 kgf cm^{-2} (1 kgf/cm^2 = 0.098 MPa) resulted in no PCB peaks being observed during the extraction although subsequent GC–MS analysis of the two collected extracts showed the typical Aroclor 1242 pattern. This indicated that the extraction of the PCBs from the cow's milk had occurred, but

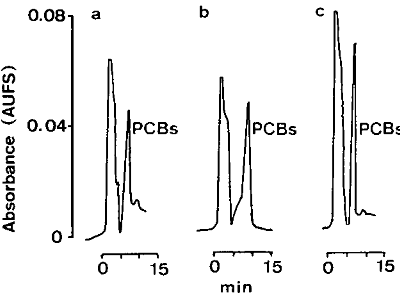


Fig. 1. SFE of PCBs from freeze-dried, skimmed milk with a spike of 50 μl of 100 μg ml^{-1} Aroclor 1242. Conditions: (a) 55°C and 160 kgf/cm^2; (b) 45°C and 240 kgf/cm^2; (c) 47°C and 220 kgf/cm^2.

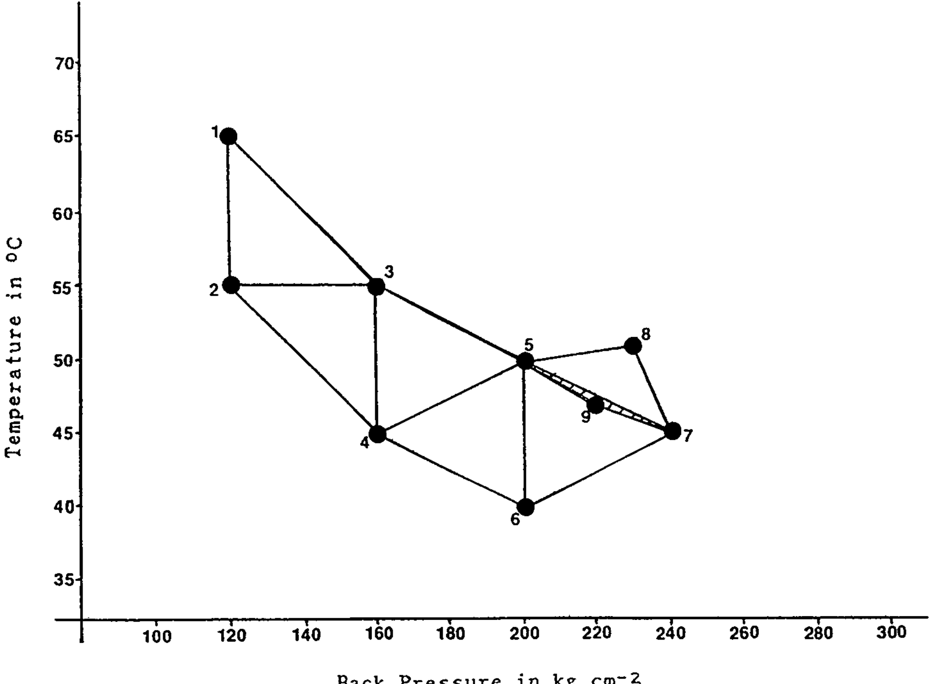


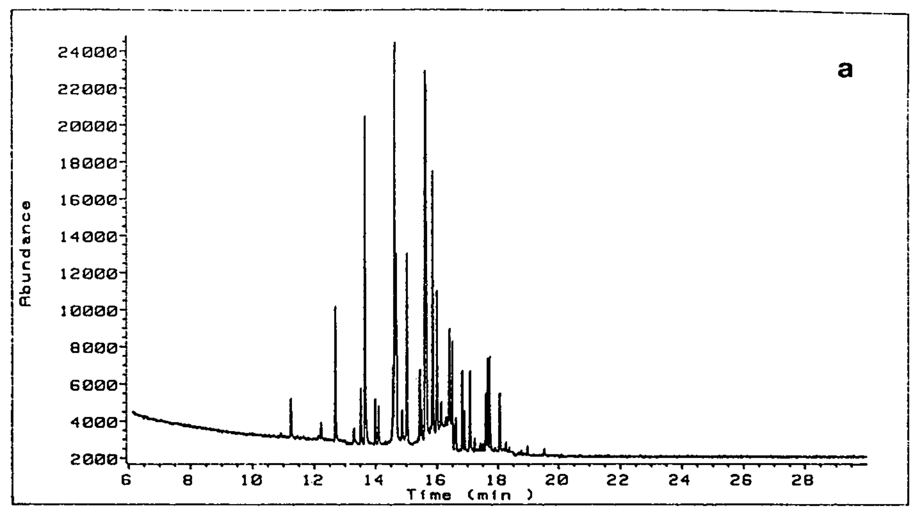
Fig. 2. Diagram of the Simplex optimisation procedure for the extraction of PCBs from freeze-dried, skimmed milk.

that the rate of extraction was too slow for a peak to be detected by the UV detector.

When conditions of 55°C and 160 kgf cm^{-2} were selected a peak was obtained on the chart recorder (Fig. 1a), and the first triangle of the Simplex optimisation was set up by measuring the peak height under these conditions, and at 45°C and 160 kgf cm^{-2} and at 50°C and 200 kgf cm^{-2}. The conditions giving the smallest peak height response were rejected, and the next set of experimental conditions was calculated according to Miller and Miller [14]. Fig. 1 shows three of the UV traces obtained during the optimisation. The Simplex optimisation was continued until a triangle was constructed where each of the three points gave a similar peak height response. This final triangle was made up of points 5, 7 and 9 and is shaded in Fig. 2. The optimum extraction conditions for the extraction of PCBs from cow's milk using supercritical CO_2 lie within this final triangle. The last point to be investigated, 47°C and 220 kgf m^{-2}, achieved an extraction of Aroclor 1242 from cow's milk in approximately 10 min, (Fig. 1c).

All of the extracts were collected, analysed by GC–MS, and all the extracts showed the characteristic Aroclor 1242 pattern, proving that the peak monitored by the UV detector was due to the Aroclor 1242 spike. No discrimination was seen in the extraction of PCBs according to the level of chlorination, *i.e.* all the different PCB congeners in Aroclor 1242 (monochlorinated to pentachlorinated) were extracted under each set of conditions. Fig. 3 shows a comparison of the chromatogram obtained from the extraction at 45°C and 240 kgf cm^{-2} and the chromatogram of Aroclor 1242 standard. Some very minor differences in the pattern of peaks are evident in Fig. 3a compared with Fig. 3b but these were not considered to be important at this stage of the study. A sample of Aroclor 1260 (pentachlorinated to nonachlorinated) was also shown to have been extracted under the conditions of 47°C and 220 kgf cm^{-2}

Fat from cow's milk. The Simplex optimisation of the extraction of fat from cow's milk followed the same procedure (Fig. 2) as that for the extraction of PCBs. A UV detector wavelength of 230 nm was



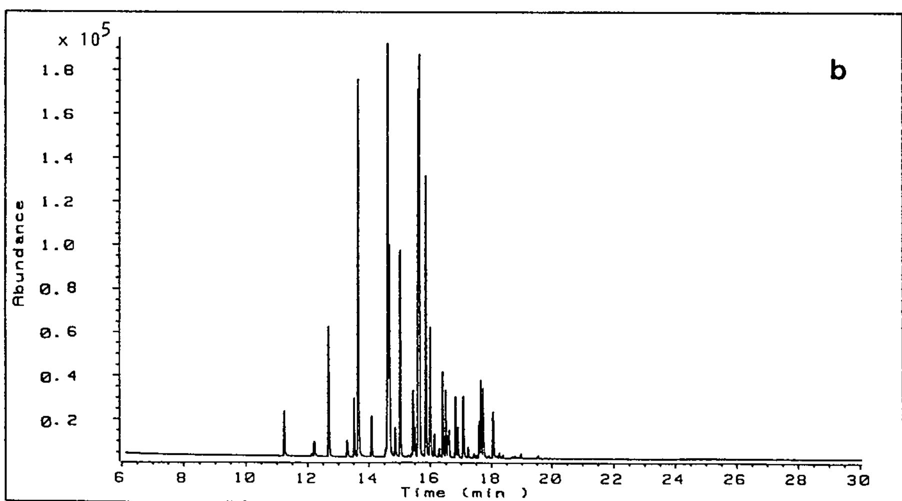


Fig. 3. Comparison of the GC–MS chromatogram of one of the SFE extracts from milk and an Aroclor 1242 standard. (a) GC–MS of extract at 45°C and 240 kgf cm^{-2}; (b) GC–MS of Aroclor 1242 standard.

used to monitor the fat. A mixture of freeze-dried unskimmed milk (fat content 4%, w/w) and florisil was used as the extraction medium.

Two features were immediately recognised as the optimisation procedure was carried out. Firstly, the peaks obtained for fat were not as sharp as previously found for the PCB peaks. Secondly, the differences in peak height recorded during the optimisation of fat extraction were smaller than for the PCB optimisation. Fig. 4 shows two of the UV traces obtained for the extraction of fat from cow's milk.

The extraction temperature and pressure conditions found to be optimal for the extraction of PCBs from cow's milk and fat from cow's milk were shown to be essentially the same. This illustrated that although SFE using carbon dioxide can readily extract both fat and PCBs from cow's milk, this approach is not selective enough to allow the extraction of PCBs, the analyte of interest, from cow's milk free of fat. A second step was, therefore, required to separate the PCBs from the fat.

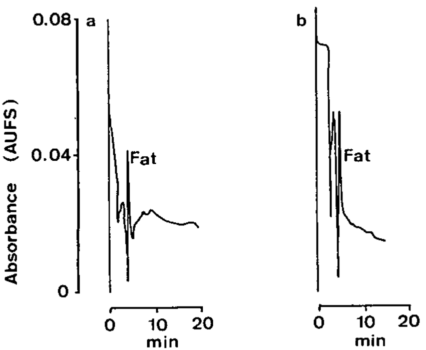


Fig. 4. SFE of fat from freeze-dried milk with a spike of 50 μl of 100 mg ml^{-1} Witepsol S55. Conditions: (a) 55°C and 160 kgf cm^{-2}; (b) 47°C and 220 kgf cm^{-2}.

Supercritical-fluid chromatography

SFC of PCBs. For a number of years a polymeric PLRP-S column (300 × 7.5 mm I.D.) has been routinely used in this laboratory as a semi-preparative step to isolate PCBs (+ pesticides) from milk fat using heptane–2-propanol (35:65) as the mobile phase [15]. This column was selected for this work to provide a direct comparison between HPLC and SFC, by using the same flow-rate (2.3 ml min^{-1}). It was noted that this column could not be used at pressures greater than 3000 p.s.i. (= 211 kgf cm^{-2}).

A 20-μl sample of 100 μg ml^{-1} Aroclor 1242 in heptane was injected onto the column at 45°C and 160 kgf cm^{-2} with a mobile phase of CO_2–2-propanol (90:10). Several peaks were observed on the chart recorder, but no PCBs were detected when the extract was analysed by GC–MS and GC–ECD. An injection of blank heptane gave the same set of peaks on the chart recorder, indicating that the PCBs were probably not being extracted. Therefore, stronger extraction conditions were employed to try to elute the PCBs from the PLRP-S column. The back pressure was increased to 200 kgf cm^{-2} and the mobile phase changed to 100% CO_2, but no PCBs were detected by GC–MS or GC–ECD. Under HPLC conditions, 100% heptane would elute PCBs with the solvent front. This implies that heptane is more non-polar than supercritical CO_2 under the conditions stated. This contrasts with the statement of Mourier *et al.* [16] that the polarity of supercritical CO_2 is close to that of hexane. The conclusion drawn from these results was that the Aroclor was not eluting from the column under SFC conditions and therefore, a column expected to show less retention of PCBs (10-cm RP-8 Spheri-5 column) was selected.

When a 20-μl injection of 100 μg ml^{-1} Aroclor 1242 in heptane was made at 50°C and 180 kgf cm^{-2}, with a mobile phase of 100% CO_2 at 1 ml min^{-1}, a peak was detected which GC–MS analysis proved was due to the Aroclor 1242. However, although a number of different chromatographic conditions were tested, including a mobile phase of CO_2–2-propanol (40:60) at 50°C and 140 kgf cm^{-2} and a flow-rate of 0.5 ml min^{-1}, it was not possible to increase the retention of the Aroclor 1242 to any degree. Therefore, separation of Aroclor 1242 from fat using this column would not be possible.

These results showed that an HPLC column with more retention for PCBs than the RP-8 column, but less retention than the 30-cm PLRP-S column was needed. In view of previous experience with the polymeric PRP-1 material, a 15-cm column was tested under a range of experimental conditions. At 50°C and 160 kgf cm^{-2} with a mobile phase of CO_2–2-propanol (80:20), flow-rate 1 ml min^{-1}, a set of peaks were detected when 20 μl of 100 μg ml^{-1} Aroclor 1242 in heptane were injected (Fig. 5). The extract was collected as two fractions, (i and ii, Fig. 5). When analysed by GC–ECD, fraction i was shown not to contain PCBs, while fraction ii showed the Aroclor 1242 pattern.

The back pressure was reduced to 140 kgf cm^{-2}, and several different mobile phase compositions were investigated to improve the results chromatographically. A mobile phase of CO_2–2-propanol

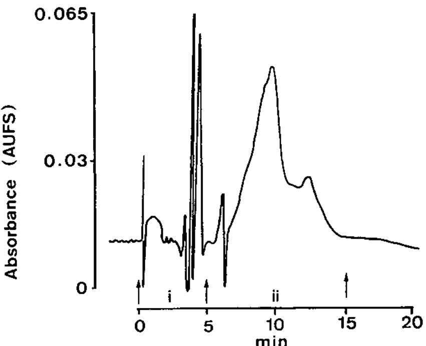


Fig. 5. SFC of a 20-μl spike of 100 μg ml^{-1} Aroclor 1242 on a 15-cm PRP-1 column with a mobile phase of CO_2–2-propanol (80:20) at 50°C and 160 kgf cm^{-2}.

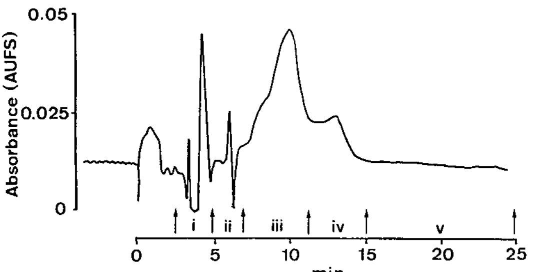


Fig. 6. SFC of Aroclor 1242; fraction collection with mobile phase of CO_2–2-propanol (80:20) at 50°C and 140 kgf cm^{-2}

(80:20) gave the best peaks shapes. This mobile phase showed a chromatogram with a series of peaks between 6 and 20 min. The exact location of the PCBs in the elution pattern was identified by the collection of five fractions during a run (Fig. 6). These fractions were analysed by both GC–ECD and GC–MS, and PCBs were found to be present in fractions iii and iv; fractions i, ii and v did not contain any PCBs.

SFC of fat. Witepsol S55 is a typical lipid material similar in composition to milk fat [15], and was used to investigate the SFC behaviour of fat. The 15-cm PRP-1 column was again used for the SFC of fat because the results for PCBs indicated that this column showed the most promise for achieving the required separation of PCBs from fat. The level of Witepsol S55 spiked needed was investigated, and a spike of 20 μl of 100 mg ml^{-1} Witepsol S55 in heptane was adopted. The chromatographic conditions optimised for PCBs [CO_2–2-propanol (80:20), 1 ml min^{-1}, 50°C, 140 kgf cm^{-2}), were then used for the fat in order to provide a direct comparison with the PCB results. When the fat spike was injected, a peak was detected at about 4 min, the same elution time as a blank injection of heptane. The identity of the fat peak was confirmed by injecting fat samples of different concentrations, and peaks of different heights were observed. The fat peak eluted at about 4 min, which was before the PCBs eluted under the same chromatographic conditions, and this indicated that the separation of fat from PCBs was possible on this column.

SFC of fat + PCBs. To confirm the above findings a combined sample containing fat and PCBs was prepared by mixing 50 μl of 100 μg ml^{-1} Aroclor 1242 with 50 μl of 100 mg ml^{-1} Witepsol S55.

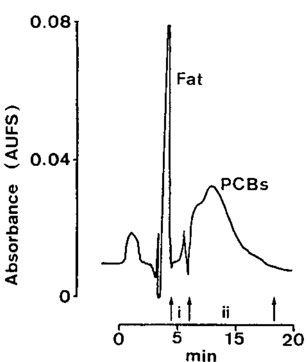


Fig. 7. SFC of fat and PCBs on a 15-cm PRP-1 column with a mobile phase of CO_2–2-propanol (80:20) at 50°C and 140 kgf cm^{-2}.

This was injected onto the 15-cm PRP-1 column at 50°C and 140 kgf cm^{-2}. Two mobile phase compositions were investigated to achieve the best possible separation of fat from PCBs, namely CO_2–2-propanol (80:20) and (60:40).

With each of the mobile phases, the mixture of fat and PCBs showed one large peak at about 4 min, followed by a series of peaks between 6 and 15 min. The previous work indicated that the first peak was due to the solvent and fat, and that the later peaks were due to the Aroclor 1242. The "fat" and "PCB" fractions were collected for each mobile phase, (Figs. 7 and 8). The identity of the peak at 4 min was confirmed by varying the fat concentrations injected onto the column. Two "PCB" fractions, (i, ii, Fig. 7) were collected with the CO_2–2-propanol

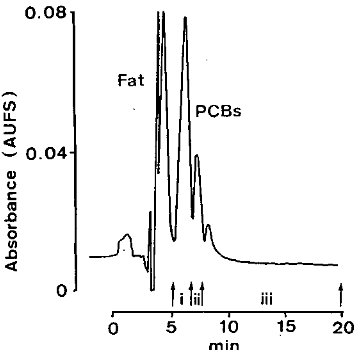
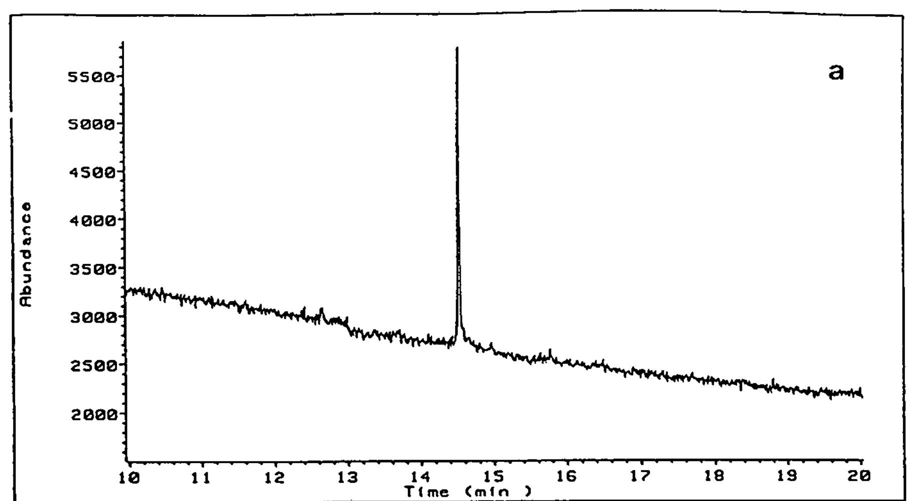


Fig. 8. SFC of fat and PCBs on a 15-cm PRP-1 column with a mobile phase of CO_2–2-propanol (60:40) at 50°C and 140 kgf cm^{-2}.

(80:20) mobile phase, and these were analysed by both GC–MS and GC–ECD. Three "PCB" fractions (i–iii, Fig. 8) were collected and analysed with the CO_2-2-propanol (60:40) mobile phase. The GC–MS traces obtained are shown in Figs. 9 and 10. The extract from using CO_2-2-propanol (80:20) mobile phase showed PCBs only in fraction ii (Figs. 7 and 9b), whereas the extracts from using CO_2-2-propanol (60:40) mobile phase showed PCBs in fractions (i–iii), (Figs. 8 and 10). The separation of the fat from the PCBs was achieved with both mobile phases, with the CO_2-2-propanol (80:20) mobile phase giving the better separation of the fat from the PCBs. This was because the fat peak with the 80:20 mobile phase was sharper than with the 60:40 mobile phase. This is extremely important because in our experience, the presence of trace levels of fat in PCB extracts has a deleterious effect on the column performance of the GC–MS. This leads to an increase in baseline noise and a reduction in MS sensitivity. The 60:40 mobile phase also shows some chromatographic resolution occurring between PCBs, probably based upon differences in levels of biphenyl chlorination. In Fig. 10, an increase in the



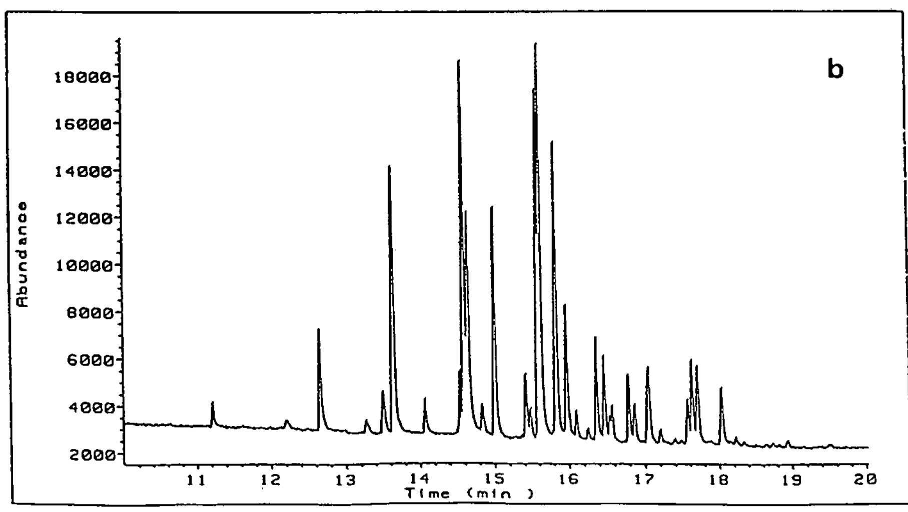
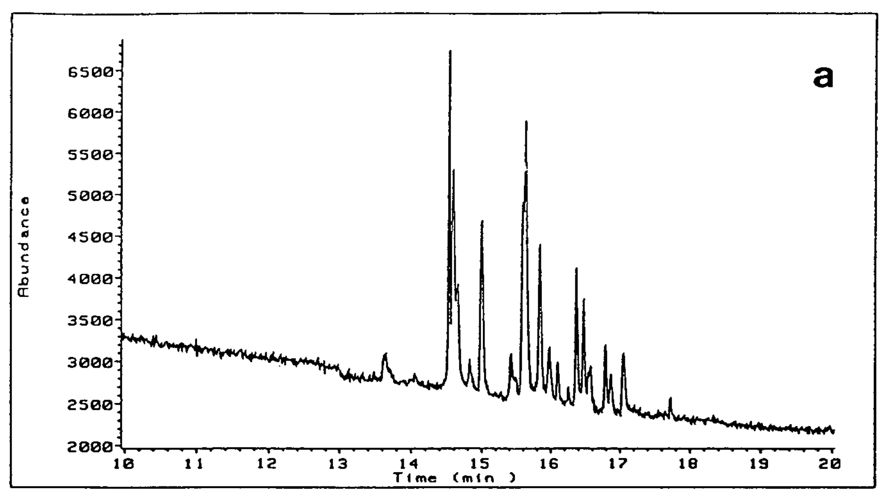
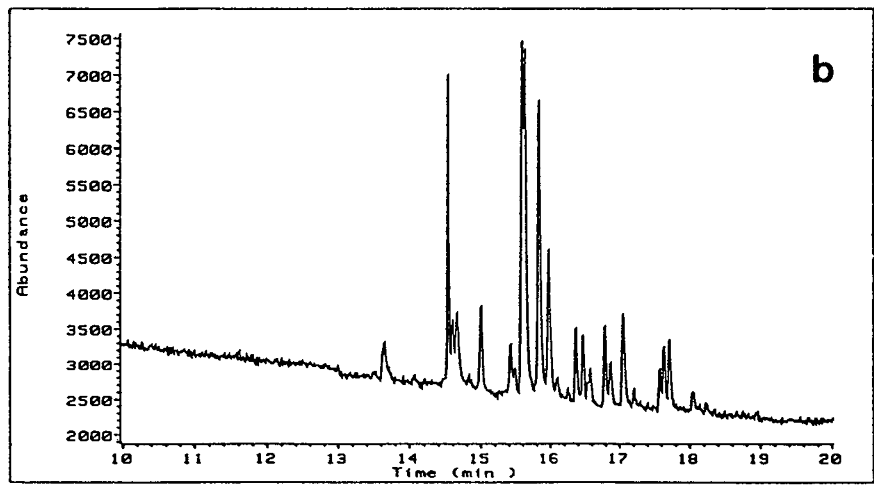


Fig. 9. GC–MS chromatograms of the "PCB" fractions collected in Fig. 7. (a) "PCB" fraction i; (b) "PCB" fraction II.





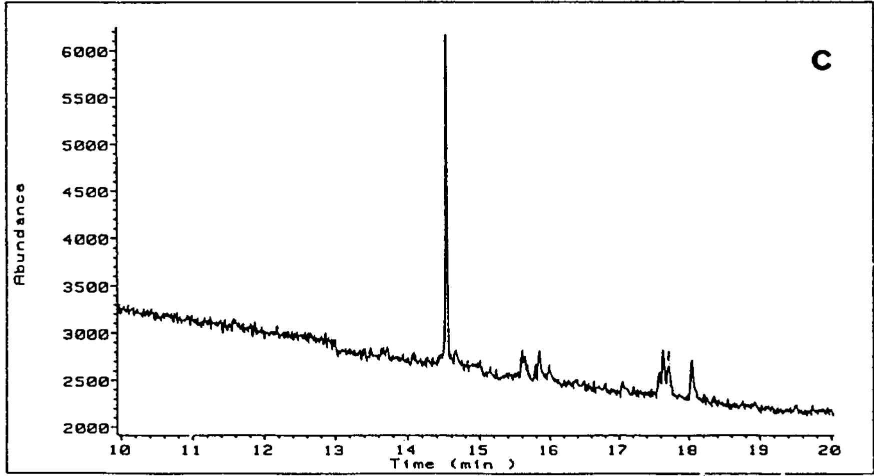


Fig. 10. GC–MS chromatograms of the "PCB" fractions collected in Fig. 8. (a) "PCB" fraction i; (b) "PCB" fraction ii; (c) "PCB" fraction iii.

occurrence of higher-molecular-mass PCBs can be seen in (c) and (b) compared to (a). The peak at 14.5 min in Figs. 9 and 10 is not a PCB, but probably a silyl fragment from the stationary phase of the capillary column.

These experimental results proved that it was possible to separate fat from PCBs by SFC on a 15-cm PRP-1 column. SFC should be a preferable separation method to HPLC for PCBs in fatty foods because SFC does not require the use of large amounts of hazardous, toxic organic solvents or the further concentration of samples before analysis that are inherent drawbacks of HPLC methodology. Studies are continuing to develop the method into a routine combined SFE–SFC procedure, optimised for the maximum percentage recovery of PCBs. In this report, recoveries were not accurately determined because a combined SFE–SFC procedure will almost certainly require different conditions to be developed. On a semi-quantitative basis, recoveries were typically about 60%.

CONCLUSIONS

The study shows the effectiveness of supercritical fluids for the extraction of PCBs and fat from cow's milk, and for the chromatographic separation of the extracted PCBs and fat. The current methods for the extraction of PCBs from cow's milk in the literature usually employ a Soxhlet extraction step, followed by a chromatography step to separate the PCBs from fat and other co-extractants.

This traditional approach has three main disadvantages when compared to methods employing supercritical fluids: (i) a large volume of toxic organic solvents is needed; (ii) the time taken for the extraction is lengthy; (iii) additional pre-concentration steps are needed prior to the final analysis, increasing the possibility of sample loss or contamination.

The proven speed, efficiency, and ease of use of supercritical fluids for extraction and chromatographic separation, coupled with the increased availability of supercritical-fluid equipment points to the increased utilisation of supercritical fluids on a routine basis for the analysis of trace organics in milk and, probably, a wide range of other matrices.

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Determination of paraquat in rat brain by high-performance liquid chromatography

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ABSTRACT

The applications of a method based on ion-pair solid-phase extraction and reversed-phase HPLC are reported. The method was used to measure paraquat concentrations in discrete brain areas at different times after its systemic administration in rats. In addition, the method was employed in the determination of paraquat levels in whole-brain samples from rats of various ages systemically treated with several doses of the herbicide.

INTRODUCTION

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a potent non-selective foliage-applied contact herbicide. It was discovered in 1955 and introduced in agriculture and horticulture for weed control in 1962. Paraquat is inactivated rapidly in sunlight and on contact with most soils so that no biologically active residues remain in the soil and almost immediate sowing or planting is allowed. The lack of biologically active residues, together with its speed of action, rainfastness and lack of selectivity, makes paraquat an essential agent in chemical weed control and, indeed, it is widely used in many countries of the world [1].

The herbicide, however, is toxic to man, and since its marketing in 1962 hundreds of cases of human death have been attributed to paraquat poisoning [2]. Almost all of the cases resulted from suicidal or accidental ingestion of the herbicide, although there are some reports of deaths caused by dermal exposure (see ref. 3).

The herbicide displays a peculiar toxicity for the lungs, where it accumulates, in an energy-dependent manner, by an uptake system shared by polyamines, leading to acute alveolitis, widespread fibrosis and fatal hypoxia [4–6]. The mechanism of paraquat toxicity involves the cyclic reduction/reoxidation of the herbicide with production of reactive oxygen species, consumption of NADPH and induction of lipid peroxidation, resulting in cell death [7–9]. Although paraquat poisoning produces mainly lung injury, toxic effects on heart [10], liver [11], kidney [12] and brain [13,14] have also been described.

Recently, the discovery that 1-methyl-4-phenylpiridinium ion (MPP^+), a compound exhibiting a striking chemical analogy with paraquat and marketed as herbicide under the name Cyperquat, induces a parkinsonian-like state in humans and primates [15,16] has renewed interest about the possibility that environmental chemicals, including paraquat, may be related to the development of Parkinson's disease [17–19].

Several epidemiological studies indicate a strong correlation between exposure at an early age to rural environment and drinking well water and development of idiopathic Parkinson's disease [20,21]. In addition, a higher prevalence of Parkinson's disease

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has been found in those rural areas of Quebec specializing in market gardening and pulp milling and utilizing higher levels of herbicides and pesticides [22].

Bocchetta and Corsini [23] reported the case of early-onset Parkinson's disease in two men exposed to herbicides and pesticides. However, for several reasons, including the poor penetration of paraquat into the central nervous system (CNS) due to its chemical structure, doubt has been expressed about the ability of paraquat to cause Parkinson's disease (see ref. 24).

Rigidity and akinesia have been reported in frogs after intraperitoneal injection of paraquat [25]. In addition, following systemic administration of [^{14}C]paraquat in frogs it was demonstrated that, in spite of its poor penetration into the brain, a high concentration of radioactivity can be observed in the neuromelanin-containing nerve cells [26].

Until recently, no analytical techniques had been established for accurate detection of paraquat in the brain. We have developed a method based on the use of ion-pair reversed-phase high-performance liquid chromatography (HPLC) with UV detection that is reliable and easy to perform [27]. This technique allowed the detection of whole-brain paraquat concentrations as low as 0.030 μg/g wet brain mass in rats systemically treated with the herbicide (1 mg/kg subcutaneously). Owing to the sensitivity of the method, we have now studied the concentrations of paraquat in specific brain regions after its systemic injection in rats.

Evidence exists in the literature indicating that the permeability of the blood-brain barrier (BBB) can be affected by ageing processes (see ref. 28). Therefore, we studied brain paraquat concentrations in rats of different ages after systemic injection of the herbicide.

EXPERIMENTAL

Experimental animals, collection and preparation of samples

Two-week-old and adult (3, 12 and 24 months old) male Wistar rats, housed in stable conditions of humidity (65%) and temperature (22 ± 2°C), were used. They were fed with a standard diet and water *ad libitum*. Paraquat dichloride (1,1′-dimethyl-4,4′-bipyridinium dichloride; Sigma, St. Louis, MO, USA), dissolved in bidistilled pyrogen-free water, was administered by subcutaneous (s.c.) injection (1, 2.5, 5, 20 or 100 mg/kg) in a volume of 1 ml/kg body mass. After 1, 3 or 24 h, the animals were sacrificed and the brain rapidly removed, weighed and stored at −20°C until analysis. To measure paraquat levels in specific areas of the CNS, the brain was placed on an ice-chilled Petri dish and the hypothalamus, prefrontal cortex, pyriform cortex, mesencephalon, hippocampus, caudate, pons and medulla oblongata were rapidly dissected out, weighed and stored as for whole-brain samples.

Paraquat was determined in whole-brain samples using ion-pair solid-phase extraction and reversed-phase HPLC with UV detection as recently described [27]. The same extraction procedure and HPLC method were employed with only minor modifications in the measurement of paraquat concentrations in discrete brain areas. Briefly, tissue samples were homogenized with a Branson Model 250 ultrasonic cell disruptor in 2–4 ml of 0.10 *M* perchloric acid containing known amounts (0.375–7.5 μg/g wet tissue mass) of internal standard (1,1′-diethyl-4,4′-bipyridinium diodide). Following centrifugation (15 min at 15 000 *g*), the supernatants were transferred to polypropylene tubes containing 250–500 μl of ammonium hydroxide. After vortexing (30 s) and subsequent centrifugation (15 000 *g*; 15 min), the resulting supernatants were passed through disposable Sep-Pak C_{18} cartridges (Water), previously prepared for ion-pair extraction by eluting with 10 ml of alkaline sodium heptanesulphonate. The cartridges were washed with water (5 ml) and methanol (5 ml). Paraquat and internal standard were then eluted with 5 ml of acidic methanol. The eluates were evaporated to dryness at 40°C under a stream of air and reconstituted in the HPLC mobile phase (0.4–8 ml/g wet tissue mass). The injection volume was 20 μl.

Chromatography and mobile phase

The HPLC system consisted of a Beckman Model 110B pump with an Altex Model 210A injection valve connected to a 45 mm × 4.6 mm I.D. Beckman Ultrasphere ODS guard column (C_{18} reversed-phase, particle size 5 μm) followed by a 25 cm × 4.6 mm I.D. Altex Ultrasphere ODS analytical column (C_{18} reversed-phase, particle size 5 μm). A Beckman Model 163 variable-wavelength UV detector

monitored the column effluent at 258 nm. The mobile phase consisted of 7.5 mM sodium heptanesulphonate and 0.10 M orthophosphoric acid (pH adjusted to 3.00 with triethylamine) plus acetonitrile to yield a 10% (v/v) mixture. The flow-rate was 1.0 ml/min at room temperature.

Calibration and statistical analysis

To construct the calibration curves, aliquots of brain tissue from untreated animals were spiked with known amounts of paraquat and internal standard to give final paraquat concentrations ranging from 0.015 to 0.960 or from 0.300 to 9.600 μg/g wet tissue weight and taking the samples through the entire procedure. Calibration curves for whole-brain sample analysis ranged from 0.015 to 0.360 μg/g wet brain mass. Results were plotted as the paraquat/internal standard peak-height ratio *versus* concentration of paraquat, and the ratio for an unknown sample was converted into concentration by interpolation.

The results are expressed as mean ± S.E.M. Differences between means were assessed by unpaired Student's t-test and were considered significant when p was <0.05. In addition, the differences in the mean regional brain paraquat concentrations at 1-, 3- or 24-h intervals were evaluated by one-way analysis of variance (ANOVA). To verify whether herbicide levels in discrete brain areas were dose-related, analysis of the regression line was performed by using the mean concentration values obtained 24 h after 5, 20 and 100 mg/kg paraquat; the correlation was considered significant when the correlation coefficient (r) yielded a values of $p < 0.05$.

RESULTS AND DISCUSSION

The systemic administration of paraquat (5.0 mg/kg s.c.) yielded different brain regional distributions at 1, 3 and 24 h following treatment (Table I). In particular, 1 h after the acute administration, the lowest levels of herbicide were observed in the caudate nucleus (0.073 ± 0.016 μg/g wet tissue mass), highest concentrations being obtained in the prefrontal cortex (1.047 ± 0.151 μg/g); paraquat concentrations ranging from 0.177 ± 0.020 (hippocampus) to 0.721 ± 0.052 μg/g (hypothalamus) were obtained in the other regions studied (Table I).

The rate of paraquat elimination was time dependent in the prefrontal cortex and the hypothalamus, where the lowest levels were detected 24 h after treatment; by contrast, in the other brain areas the

TABLE I

PARAQUAT CONCENTRATIONS (μg/g WET TISSUE MASS) IN DISCRETE AREAS OF RAT BRAIN AS DETERMINED 1, 3 OR 24 h AFTER ADMINISTRATION OF 5.0 mg/kg s.c. HERBICIDE

Values are the mean ± S.E.M. of 3–9 determinations. Each sample of discrete area was the pool from six brains. Significant regional differences in paraquat concentration: $^{*}p < 0.01$, $F = 17.87$; $^{**}p < 0.01$, $F = 79.35$; $^{***}p < 0.01$, $F = 8.85$ (one-way ANOVA).

Brain area	Time after administration		
	1 h*	3 h**	24 h***
Hypothalamus	0.721 ± 0.052	0.573 ± 0.030[a]	0.328 ± 0.023[a]
Prefrontal cortex	1.047 ± 0.151	0.280 ± 0.030[c]	0.166 ± 0.031[c]
Mesencephalon	0.241 ± 0.021	0.118 ± 0.010[c]	0.176 ± 0.030
Hippocampus	0.177 ± 0.020	0.108 ± 0.005[c]	0.137 ± 0.009[d]
Caudate	0.073 ± 0.016	0.046 ± 0.005	0.085 ± 0.009[e]
Pons	0.491 ± 0.110	0.115 ± 0.010[c]	0.283 ± 0.042[e]
Medulla oblongata	0.376 ± 0.080	0.116 ± 0.006[c]	0.159 ± 0.036

[a] $p < 0.05$ *vs.* 1-h concentrations.
[b] $p < 0.01$ *vs.* 1- and 3-h concentrations.
[c] $p < 0.01$ *vs.* 1-h concentrations.
[d] $d < 0.05$ *vs.* 3-h concentrations.
[e] $p < 0.01$ *vs.* 3-h concentrations (unpaired Student's t-test).

TABLE II

PARAQUAT CONCENTRATIONS (μg/g WET TISSUE MASS) IN DISCRETE AREAS OF RAT BRAIN AS DETERMINED 24 h AFTER ADMINISTRATION OF 5.0, 20 OR 100 mg/kg HERBICIDE

Values are the mean ± S.E.M. of 3–5 determinations. The data concerning 5.0 mg/kg dose were obtained in samples of discrete areas consisting of pools from six brains. Analysis of the regression line: * significant correlation at the $p < 0.01$ level; ** significant correlation at the $p < 0.05$ level. r = correlation coefficient.

Brain area	Treatment (mg/kg s.c.)			r
	5.0	20	100	
Hypothalamus	0.328 ± 0.023	1.610 ± 0.370	7.746 ± 0.959	0.9998*
Prefrontal cortex	0.166 ± 0.031	1.223 ± 0.594	7.398 ± 1.484	0.9999*
Pyriform cortex	0.208 ± 0.020	0.870 ± 0.389	4.006 ± 0.930	0.9998*
Mesencephalon	0.176 ± 0.030	1.208 ± 0.486	4.206 ± 0.548	0.9948
Hippocampus	0.137 ± 0.009	0.700 ± 0.184	2.740 ± 0.349	0.9982**
Caudate	0.085 ± 0.009	0.483 ± 0.181	1.944 ± 0.298	0.9983**
Pons	0.283 ± 0.042	1.033 ± 0.384	5.726 ± 1.232	0.9998*
Medulla oblongata	0.159 ± 0.036	0.792 ± 0.292	3.370 ± 0.486	0.9992**

initial drop observed at 3 h was followed by a second phase of significant accumulation, which in some regions yielded concentrations of paraquat similar to those seen 1 h after administration.

Compared with rats receiving 5.0 mg/kg, animals treated with doses of 20 and 100 mg/kg showed a similar pattern of paraquat distribution in the brain as observed 24 h following administration; in addi-

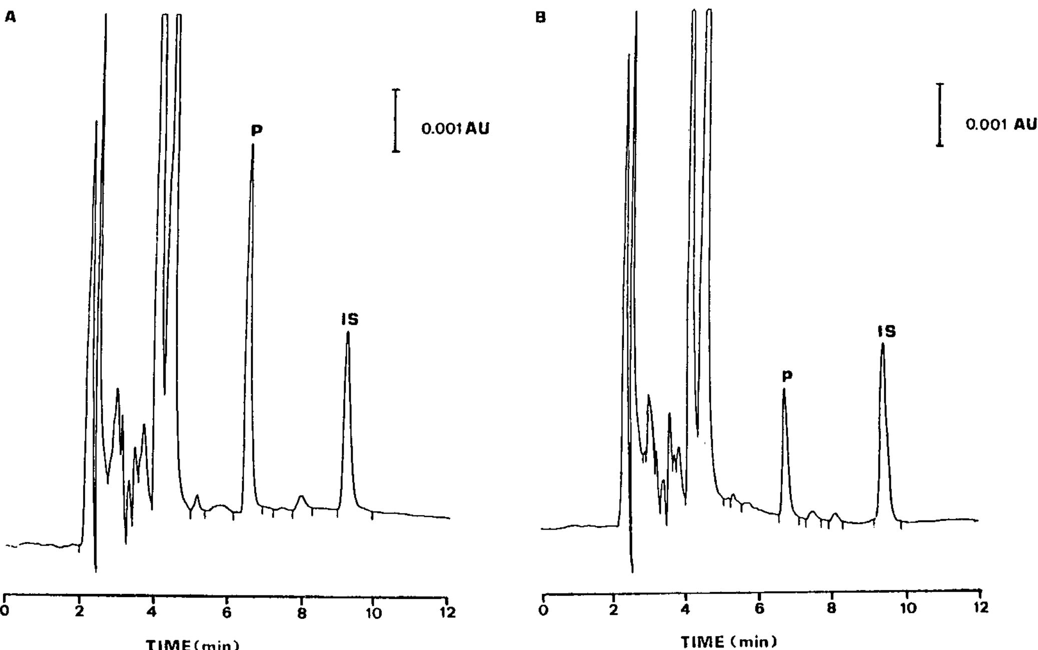

Fig. 1. HPLC elution profiles of discrete rat brain area extracts: (A) hypothalamus, (B) hippocampus. Each extract was the pool of six brains of rats treated 24 h earlier with paraquat. P = paraquat; IS = internal standard (0.375 μg/g wet tissue mass). Paraquat concentrations: (A) = 0.378 and (B) = 0.131 μg/g wet tissue mass.

tion, a dose-dependent increase in the regional levels of the herbicide was observed (Table II). Representative chromatograms of extracts from discrete brain areas are given in Fig. 1.

In the hypothalamus and medulla oblongata, two brain regions known to contain sites lacking BBB [29], the concentrations of paraquat were significantly different. This would indicate that the distribution of the herbicide in the brain does not depend solely on the ease with which it reaches a specific area via the bloodstream. The possibility that paraquat damages the capillary wall [14], facilitating its penetration into the CNS, cannot be excluded.

In rats, the microinfusion of paraquat into several brain areas produces locomotor disorders accompanied by electrocortical (ECoG) epileptic discharges and neuronal cell death, regardless of the site of injection [30,31]. The lack of selective neurotoxicity can be explained by its proposed mechanism of toxicity, *i.e.* the ability to generate oxygen free radicals and lipid peroxides with consequent cell damage and death. Paraquat can also be neurotoxic after systemic administration in rats [32,33]; in fact, it evokes limbic motor seizures, ECoG epileptic discharges and, of greater interest, selective neuronal cell death in the pyriform cortex, the last effect being observed 24 h after treatment (20 mg/kg s.c.); a lower dose (5 mg/kg s.c.) is ineffective.

Paraquat concentrations in the pyriform cortex, measured 24 h following administration of doses of 5 and 20 mg/kg, were 0.208 ± 0.020 and 0.870 ± 0.389 μg/g wet tissue mass, respectively.

These data indicate that in the pyriform cortex a paraquat concentration of about 0.200 μg/g is not neurotoxic, at least at 24 h after acute treatment with the herbicide, whereas paraquat levels of 0.870 ± 0.389 μg/g are associated with neuronal cell death.

Different areas of the CNS seem to exibit different sensitivities to the toxic effects elicited by paraquat. In contrast to the pyriform cortex, no neuronal damage was observed 24 h after treatment in other brain areas with higher or almost similar paraquat levels, *i.e.* the mesencephalon and hippocampus. The greater vulnerability of pyriform cortex to neuropathological insults has been well documented [34,35], and it could account for the selective neuronal cell death observed in this area 1 day after systemic administration of paraquat.

However, we cannot exclude the possibility that neuronal damage could also occur in brain areas other than pyriform cortex after longer exposure to paraquat.

The concentrations of paraquat detected in the brain of 3-, 12- and 24-month-old rats 1 h after the injection of the herbicide were dose-related and age-dependent, as shown in Table III.

In comparison with 3-month-old rats, higher paraquat levels were found in the brain of older animals (12 and 24 months old) following each dose of herbicide used (1.0, 2.5 and 5.0 mg/kg). In particular, the differences in brain paraquat concentrations were statistically significant over the range of doses administered when the data obtained in 3- and 24-month-old animals were compared (see Table III). In addition, in comparison to 3-month-old rats, statistically significant ($p < 0.05$) higher levels of paraquat were also obtained in the brain of 12-

TABLE III

PARAQUAT CONCENTRATIONS (μg/g WET BRAIN WEIGHT) AS MEASURED 1 h AFTER ITS SYSTEMIC (s.c.) ADMINISTRATION IN RATS OF DIFFERENT AGES

Values are the mean ± S.E.M. of 5–8 determinations. * $p < 0.05$ and ** $p < 0.01$ *vs.* the data obtained in 3-month-old rats (unpaired Student's *t*-test)

Dose (mg/kg)	Age (months)			
	0.5	3	12	24
1.0	0.064 ± 0.011	0.039 ± 0.006	0.047 ± 0.005	0.079 ± 0.016*
2.5	0.117 ± 0.005*	0.082 ± 0.012	0.132 ± 0.018*	0.170 ± 0.013**
5.0	0.334 ± 0.019*	0.185 ± 0.017	0.216 ± 0.022	0.243 ± 0.016*

month-old animals following the 2.5 mg/kg dose. The comparison of data obtained in 12- and 24-month-old rats revealed no statistically significant differences between these two groups of animals.

These data, whilst confirming the ability of paraquat to cross the BBB and to give rise to dose-related brain concentrations following systemic administration in rats [27], show that the diffusion of paraquat across the BBB is age-related. In fact, 1 h after the treatment, higher brain levels of paraquat are found in aged rats than in young animals, thus supporting the hypothesis of some degree of BBB leakage during ageing due to alterations in cerebral microvasculature (see ref. 28).

In addition, in comparison with 3-month-old rats, higher levels of paraquat were also detected in the brains of very young animals (2 weeks old) 1 h after acute administration of the herbicide (1.0, 2.5 and 5.0 mg/kg s.c.) (Table III). These results support data reported previously in the literature [36] indicating that full maturity of BBB is not completely reached in very young animals. Taken together, these data emphasize the possibility that the vulnerability of the CNS to environmental chemicals may be greater in the early stages of life and during ageing.

CONCLUSIONS

The availability of a specific and sensitive analytical technique for the determination of paraquat brain concentrations has allowed us to obtain more, and interesting, information on kinetic profile of paraquat after its systemic administration in rats.

The ion-pair solid-phase extraction and reversed-phase HPLC method previously described for the determination of paraquat concentrations in whole-brain samples [27] has been employed to detect herbicide levels in discrete rat brain areas. This has allowed us to demonstrate that a differential regional distribution of paraquat occurs in the brain 1, 3 and 24 h after systemic injection in rats, and that a different pattern of paraquat elimination exists among the brain regions studied. In addition, the concentrations of the herbicide in discrete brain areas were dose-related.

Age-dependent paraquat brain concentrations were detected in rats with the highest herbicide brain levels being obtained in very young and older animals.

In conclusion, the present results, whilst adding new data on paraquat kinetic profile, confirm the use of HPLC as a reliable analytical technique for the detection of environmental chemicals in biological samples.

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Ion chromatographic investigation of brown algae (*Fucus vesiculosus*) of the German Environmental Specimen Bank

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ABSTRACT

An investigation of the inorganic anions and cations Cl^-, HPO_4^{2-}, NO_3^-, SO_4^{2-}, NH_4^+, K^+ and Na^+ in brown algae collected from various locations in the North Sea and Baltic Sea is presented. Ion chromatography was effectively used for the simultaneous determination of anions and cations after extraction of the homogenized algae samples with deionized water following the standard preparation technique of the German Environmental Specimen Bank (ESB). Acidic and enzymatic digestion was applied to investigate the completeness of cell disintegration in the homogenized algae samples. Results obtained with brown algae clearly indicate the influence of environmental pollution in the North Sea and the Baltic Sea.

INTRODUCTION

With increased application in a variety of areas, ion chromatography (IC) is today a well-established technique in analytical chemistry. The number of articles published in various scientific journals indicates the reliability of this technique [1]. Ion chromatography is not only a versatile multicomponent analytical technique, it also possesses a relatively high sensitivity, enabling the determination of specific trace elements at the sub-part-per-billion level (w/w) [2,3]. A wide variety of environmental problems, such as climatic change, forest decline and increasing enviromental pollution of the North and Baltic Sea, can be addressed, and consequently an intensive effort is devoted to the determination of anions and cations by ion chromatography.

Nitrite can cause serious health problems in humans, such as methaemoglobinaemia in infants, and under certain conditions carcinogenic nitrosamines can be formed in the human body. Fluorine is an essential substance for humans and animals, but increased fluorine uptake can lead to serious health problems such as fluorosis of bones and teeth [4,5].

In various fields of environmental research, ion chromatography plays a key role, mainly in investigations of aqueous samples. A review of the environmental applications of ion chromatography is given elsewhere [6]. The IC analysis of anionic and cationic species in plant materials has already been reported [7–10] using different sample preparation techniques and detection methodologies. Expanding the field of application for IC is particularly attractive for the environmental sciences, since the enormous potential of the technique can help to elucidate the uptake rates, accumulation capacities or transfer routes of heavy metals and anionic species in biological materials. The German Environmental Specimen Bank (ESB) is devoted to the real-time monitoring and archiving of authentic material for retrospective analysis and is greatly interested in additional information on sample constituents less readily obtainable by other techniques. In the course of a screening programme within the ESB for the monitoring of environmental pollutants in the North Sea and Baltic Sea, investigations of the inorganic anions and cations in brown algae were performed using IC.

The purpose of this work was to establish the suitability of brown algae as possible indicator or-

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ganisms for coastal pollution by phosphate, nitrate, ammonium and other components.

EXPERIMENTAL

Materials and chemicals

Brown algae were collected in October 1991 from the Baltic and North Seas. Sampling and preparation techniques following a standard protocol laid down by the ESB were applied for the collection and preparation of the algae samples and are described elsewhere [11,12]. After cleaning and removal of adhering materials at the sampling site using sea water, the brown algal samples were deep frozen in stainless-steel containers immersed in liquid nitrogen (123K) for transport to the ESB. In the ESB laboratories, the samples were precrushed by a crusher at a temperature of 273–280 K and freeze-dried in appropriate batches, finely ground in zirconium dioxide ball mills and passed through 200-μm sieves.

Standard solutions of 1000 mg/l chloride, phosphate, nitrate, sulphate, ammonium, potassium and sodium were prepared with analytical-grade chemicals (Merck, Germany). Bakerbond octadecyl (C_{18}) columns, (Bakerbond, USA) were used to remove aromates, fatty acids, hydrocarbons and tensides from the supernatant solution. For the disintegration of the cell wall material of brown algae, a mixture of three enzymes was used: cellulase Onozuka R-10 from *Trichoderma viride* (EC 3.2.1.4), macerozyme R-10 from *Rhizopus* sp. and pectinase from *Aspergillus niger* (EC 3.2.1.15) (Merck, Germany). The blank values of Cl^-, HPO_4^{2-}, NO_3^-, SO_4^{2-}, NH_4^+, K^+ and Na^+ contained in 600 mg enzyme mix were 0.15 mg, 17.9 mg, 3.30 mg, 0.73 mg, 0.62 mg and 7.29 mg, respectively.

Methods and instrumentation

A modified Dionex ion chromatograph, system 12 (Dionex, USA), with HPIC-AG3 and HPIC-CG3 guard columns, HPIC-AS3 and HPIC-CS3 separation columns and AMMS-1 and CMMS-1 suppressor columns was used for the determination of anions and cations. Algal sap pH values were measured with a WTW pH-meter, Model pH 192, with a WTW gelatin-type E50 electrode (Weilheim, Germany). Samples were mixed with a Vortex Genie 2 TM mixer (Bender & Hobein, Zürich, Switzerland) and kept at 333 K in a water bath (a glass container with water and an MGW Lauda RC6 Thermostat, Germany). Algal suspensions were separated with a Megafuge 1.0 centrifuge (Heraeus Sepatech, Germany). The Baker spe-10 Column Processing System (J.T. Baker, USA) was used for solid phase extraction with Bakerbond octadecyl (C_{18}) columns.

Digestion technique

For the investigation of the complete removal of ions from the materials, two digestion techniques were used.

Digestion with hydrochloric acid. Samples of 200 mg of homogenized algae were weighed into 14-ml polypropylene centrifuge tubes. After the addition of 1.5 ml of 5% hydrochloric acid, the samples were kept at room temperature and shaken for 1 h. A further 3.5 ml of deionized water were added to the polypropylene tubes. The samples were shaken in a water bath at 333 K for 2 h. After 10 min centrifugation at 6000 rpm (6240 *g*), the supernatant solution was diluted with 250 ml of deionized water. The cations and anions released from the algal solid phase into the supernatant solution were measured by ion chromatography.

Enzymatic digestion. A mixture of three enzymes (200 mg of pectinase, 200 mg macerozyme and 200 mg of cellulase) was used for the enzymatic digestion of algal samples [13–15]. After the addition of 5 ml of deionized water into the 14-ml polypropylene centrifuge tube containing 200 mg of algae and enzyme, the samples were shaken at room temperature (293 K) for 1 h and separated with a centrifuge at 6000 rpm (6240 *g*). The liquid phase was diluted with 250 ml of deionized water for the determination of ions. Because of a high blank value of anions and cations, the ion levels of the enzymes were checked several times.

Determination of cations and anions released from algae samples from the Baltic Sea and North Sea

Samples of 200 mg of homogenized algae samples were weighed into 14-ml polypropylene centrifuge tubes. After the addition of 5 ml of deionized water, the samples were kept in a water bath at 333 K for 3 h and then separated by centrifugation at 6000 rpm (6240 *g*) for 10 min. Solid-phase extraction with Bakerbond octadecyl (C_{18}) columns was used for to

remove aromates, fatty acids, hydrocarbons and tensides from the clear supernatant solution. After dilution with 250 ml of deionized water, the cations and anions were determined using a modified ion chromatography Dionex system 12 [16]. The standard solutions were processed in appropriate concentrations to identify the retention time and detector response. Owing to lower blank values, the detection limits are considerably lower (1 μg/l for chloride, 2 μg/l for nitrate, phosphate and sodium, 3 μg/l for sulphate and 5 μg/l for ammonium and potassium) when no digestion at all is used and the samples are extracted with deionized water only.

RESULTS AND DISCUSSIONS

Methodological aspects

In contrast to animal cells, plant cells have a rather robust cell wall containing cellulose, polysaccharides and protopectin, which can be taken to influence the investigation of inorganic anions and cations of plant materials. Therefore, the complete removal of the ions from the homogenized algae samples using deionized water was checked using an acidic digestion technique and an enzymatic digestion technique. A comparison of the released Cl^-, HPO_4^{2-}, SO_4^{2-}, NH_4^+, K^+ and Na^+ from the algae samples after extraction with deionized water and after digestion with 5% hydrochloric acid is presented in Table I. The results show that only HPO_4^{2-}, SO_4^{2-}, NH_4^+ and Na^+ could be detected, and errors introduced by interference from the high Cl^- concentration were observed. The injection of stronger acidic solutions into the ion chromatography system resulted in a large Cl^- peak hampering the measurement of other components. It is obvious that the inorganic anions and cations were completely released from the homogenized algae by extraction with deionized water alone. This can be additionally confirmed by the data given in Table II. An enzyme mix of pectinase, macerozyme and cellulase was used to check the completeness of cell disintegration in comparison with the ESB preparation technique and the extraction of the algae samples with deionized water. Following the enzymatic attack, the rest of the robust cell structure and macromolecules in the samples are destroyed. Additional investigations of the total protein content of enzymatically treated and untreated samples show no evidence of additional protein release from the cell structure in the treated samples, confirming the finding that homogenized algae samples can be readily extracted by deionized water and analysed without additional digestion steps. The results clearly show that the anion and cation levels obtained by both techniques are similar and indicate complete cell disintegration as well as complete extraction with deionized water from the homogenized algae. It can be deduced that the cellular structure of brown algae is less stable than that of other plants and is easily decomposed by the application of the ESB preparation technique. It has still to be proved whether the ESB preparation is as complete for other plant materials as it has been

TABLE I

ION CHROMATOGRAPHIC DETERMINATION OF IONS IN HOMOGENIZED BROWN ALGAE SAMPLES AFTER EXTRACTION WITH DEIONIZED WATER (A) AND 5% HYDROCHLORIC ACID (B)

Average values (in g per kg dry mass) were calculated from means of three brown algae samples. The mass of each sample was 200 mg.

Ion	North Sea samples		Baltic Sea samples	
	A	B	A	B
Cl^-	55.8 ± 2.25	–	27.5 ± 0.70	–
HPO_4^{2-}	6.14 ± 0.55	4.34 ± 0.37	5.46 ± 0.21	5.02 ± 0.35
SO_4^{2-}	7.07 ± 0.47	6.55 ± 0.71	4.54 ± 0.11	4.96 ± 0.13
NH_4^+	18.1 ± 0.23	23.7 ± 0.32	37.7 ± 0.44	39.2 ± 1.53
K^+	0.13 ± 0.05	–	0.45 ± 0.01	–
Na^+	22.0 ± 0.39	25.2 ± 0.17	31.6 ± 0.33	27.1 ± 0.29

TABLE II

COMPARISON OF ION LEVELS IN HOMOGENIZED BROWN ALGAE SAMPLES AFTER EXTRACTION WITH DEIONIZED WATER (A) AND AFTER ENZYMATIC DIGESTION WITH A MIXTURE OF ENZYMES (B)

Average values (in g per kg dry mass) were calculated from means of three brown algae samples. The mass of each sample was 200 mg.

Ion	North Sea samples		Baltic Sea samples	
	A	B	A	B
Cl^-	55.8 ± 2.25	54.7 ± 2.33	27.5 ± 0.70	27.5 ± 0.26
HPO_4^{2-}	6.14 ± 0.55	6.60 ± 0.28	5.46 ± 0.21	5.54 ± 0.10
SO_4^{2-}	7.07 ± 0.47	6.87 ± 0.51	4.54 ± 0.11	3.40 ± 0.09
NH_4^+	18.1 ± 0.23	20.9 ± 0.14	37.7 ± 0.44	34.3 ± 2.05
K^+	0.13 ± 0.05	0.10 ± 0.06	0.45 ± 0.01	0.32 ± 0.04
Na^+	22.0 ± 0.39	20.1 ± 0.09	31.6 ± 0.33	26.1 ± 0.09

shown to be for algae or whether additional enzymatic digestion is necessary before IC analysis of anions and cations can be carried out. The distribution of cation and anion levels in the homogenized brown algae samples after three successive extractions with 5 ml of deionized water is illustrated in Table III. The results show that 75–90% of K^+, NH_4^+, Na^+, Cl^-, HPO_4^{2-} and SO_4^{2-} could be removed from the algae at the first extraction step with deionzed water (200 mg in 5 ml of deionized water). A further extraction step resulted in an additional total removal of 1–17.3%.

Investigation of the inorganic anions and cations in brown algae collected from the North Sea and Baltic Sea

The data on the average concentrations of chloride, phosphate, sulphate, ammonium, sodium and potassium for the brown algae samples from the North Sea and the Baltic Sea are summarized in Tables IV and V. The average values were calculated from means of three measurements and are given in g per kg dry weight of algae. A comparison of chloride and sodium levels in Tables IV and V shows that higher chloride levels were observed in the North Sea algae. The average concentration of chloride ranged from 40.0 g/kg to 72.0 g/kg for the North Sea algae and from 29.2 g/kg to 53.9 g/kg for the Baltic Sea algae. It indicated a difference in the salinity of sea water between North and Baltic Seas. The results obtained in Tables IV and V show that chloride accumulation in brown algae decreases from the North Sea to the Baltic Sea. This observation is generally in good agreement with the salinity measurements of sea water in the North and Baltic Seas [17]. Only a slight decrease in sodium levels in brown algae from the North Sea to the Baltic Sea was observed. The average sodium concentration varied from 25.7 g/kg to 39.1 g/kg in the North Sea and from 19.3 g/kg to 35.9 g/kg in the Baltic Sea. Environmental pollution due to anthropogenic influences on ecosystem species in the North and Baltic Sea areas is clearly confirmed in comparison with phosphate levels. Two significant results can be drawn from the data on phosphate in Tables IV and V: (a) the average phosphate values of the Baltic Sea algae were generally higher than those of the North Sea algae; and (b) a significant increase in phosphate levels in algae samples was found from the sampling locations 11 (Reddewitz), 12 (Zudar

TABLE III

DISTRIBUTION OF ION LEVELS IN g PER kg DRY MASS AND AS A PERCENTAGE (%) OF THE HOMOGENIZED BROWN ALGAE SAMPLES AFTER THREE SUCCESSIVE EXTRACTIONS WITH DEIONIZED WATER

Ion	First extraction	Second extraction	Third extraction
Cl^-	51.8 (86.4%)	7.55 (12.6%)	0.59 (1.00%)
HPO_4^{2-}	9.23 (85.5%)	1.43 (13.2%)	0.11 (1.30%)
SO_4^{2-}	8.83 (85.7%)	1.22 (11.8%)	0.26 (2.50%)
NH_4^+	29.5 (86.3%)	3.55 (10.4%)	1.13 (3.30%)
K^+	0.34 (75.0%)	0.08 (17.3%)	0.03 (7.70%)
Na^+	32.7 (90.0%)	2.99 (8.24%)	0.61 (1.76%)

TABLE IV

AVERAGE CONCENTRATION OF CHLORIDE, SODIUM, PHOSPHATE, AMMONIUM, SULPHATE AND POTASSIUM IN g PER kg DRY MASS OF THE HOMOGENIZED BROWN ALGAE SAMPLES COLLECTED FROM THE NORTH SEA

Ion	Sampling location[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Cl^-	59.9	40.0	60.8	58.4	72.0	68.7	56.2	53.2	59.5	52.5	62.2	58.0
Na^+	36.3	25.7	32.8	32.9	27.4	32.4	35.3	30.9	39.1	28.1	37.9	31.4
HPO_4^{2-}	10.3	10.8	8.15	6.56	4.80	7.70	7.11	7.10	7.33	6.09	9.20	9.52
NH_4^+	34.2	35.0	31.1	33.1	40.6	28.5	26.5	37.7	29.8	27.0	29.3	29.3
SO_4^{2-}	10.3	6.66	10.8	10.6	11.8	11.2	9.93	9.59	12.4	8.42	12.7	10.4
K^+	0.45	0.17	0.21	0.09	0.32	0.15	0.10	0.08	0.18	0.32	0.10	0.15

[a] Sampling locations in the North Sea: 1 = Eckwarderhörne 07302; 2 = Cuxhaven Kugelbake 10402; 3 = Altenbruch 10401; 4 = Trischendamm 07270; 5 = Meldorfer Bucht 07271; 6 = Sylt/List 07102; 7 = Sylt/Königshafen 07151; 8 = Sylt/Königshafen 07152; 9 = Sylt/Königshafen 07161; 10 = Sylt/Königshafen 07163; 11 = Sylt/Königshafen 07181; 12 = Sylt/Königshafen 07182.

gelbes Ufer), 8 (Boiensdorf) and 13 (Drigge) in the Baltic Sea. At the Baltic coast near to Zudar, gelbes Ufer and Drigge, the concentration of phosphate was measured to be 21 g per kg dry mass of brown algae. This corresponds to a 2.9-fold increase compared with the average phosphate level of 7.36 g per kg dry mass in algae from Sylt in the North Sea. This effect can be explained by the influence of higher environmental pollution in the Baltic Sea [18]. A high input of nutrients via the rivers from fertilizers and via municipal and industrial waste water generally causes a very large increase in phosphate and nitrogen in the sea water. This can be confirmed by the data given in Tables IV, V and VI. The highest concentration of 21 g/kg for phosphate (Table V) and 2.13 g/kg for nitrate (Table VI) in these experiments was measured in brown algae from Zudar gelbes Ufer in the Baltic Sea. Phosphorus is an essential nutrient for the cell structure composition of plants. But excessive quantities of nutrients in sea and lake water cause a massive growth of algae and change the life areas and life conditions of several animals in the aquatic system. Moreover, the algae samples collected in the Cuxhaven estuary contained a higher level of phosphate and nitrate. In this aquatic system, a mixing of highly polluted

TABLE V

AVERAGE CONCENTRATION OF CHLORIDE, SODIUM, PHOSPHATE, AMMONIUM, SULPHATE AND POTASSIUM IN g PER kg DRY MASS OF THE HOMOGENIZED BROWN ALGAE SAMPLES COLLECTED FROM THE BALTIC SEA

Ion	Sampling location[a]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Cl^-	53.0	48.1	46.5	53.5	53.9	46.4	38.3	50.5	30.4	35.4	44.3	29.2	42.0
Na^+	29.9	35.9	35.6	30.7	19.3	25.7	25.3	28.6	23.7	26.6	27.5	26.0	30.7
HPO_4^{2-}	9.44	6.68	5.12	8.06	8.81	7.34	5.88	13.8	8.63	6.33	12.4	20.5	21.0
NH_4^+	26.3	30.8	39.5	27.1	20.4	23.3	22.9	24.1	18.6	21.0	23.1	19.6	21.2
SO_4^{2-}	8.16	7.33	5.89	8.47	9.39	7.03	5.81	8.11	4.71	5.17	7.71	6.09	6.49
K^+	0.10	0.33	0.64	0.26	0.21	0.15	0.16	0.16	0.17	0.27	0.36	0.35	0.10

[a] Sampling locations in the Baltic Sea: 1 = Glücksburg 08069; 2 = Gut-Öhe 08071; 3 = Langholz 08072; 4 = Kiel Strande 08073; 5 = Strukkamphuk 08074; 6 = Katharinenhof 08075; 7 = Dahme 08076; 8 = Boiensdorf 08078; 9 = Kap Arcona 08084; 10 = Lome 08085; 11 = Reddewitz 08087; 12 = Zudar gelbes Ufer 08088; 13 = Drigge 08083.

TABLE VI

AVERAGE NITRATE CONCENTRATION IN HOMOGENIZED BROWN ALGAE SAMPLES COLLECTED FROM THE NORTH AND BALTIC SEAS

Levels in other samples were below the determination limits (≤ 0.20 g/kg).

Sampling location	Location	Level (g per kg dry mass)
No. 2 (North Sea)	Cuxhaven–Kugelbake	1.16 ± 0.10
No. 3 (North Sea)	Cuxhaven–Altenbruch	2.02 ± 0.18
No. 3 (Baltic Sea)	Langholz	0.28 ± 0.06
No. 4 (Baltic Sea)	Kiel Strande	1.35 ± 0.11
No. 9 (Baltic Sea)	Kap Arcona	1.38 ± 0.08
No. 12 (Baltic Sea)	Zudar gelbes Ufer	2.13 ± 0.10

fresh water and sea water occurs. The data shown in Table IV and VI show that the brown algae collected from Cuxhaven contained more phosphate (sampling location 2, Cuxhaven Kugelbake) and nitrate (sampling location 3, Cuxhaven Altenbruch) than in brown algae from other sampling sites in the North Sea. Furthermore, in the estuary areas Cuxhaven–Kugelbake (the Elbe and the North Sea), a lower chloride and sodium level (Table IV) was observed. With regard to the ammonium and sulphate levels in brown algae from the North and Baltic Seas, the trend is similar to that of chloride. The average concentration of ammonium and sulphate in the North Sea algae is higher than in the Baltic Sea algae. Ammonium ranged from 26.5 g/kg to 40.6 g/kg in North Sea algae and from 18.6 g/kg to 39.5 g/kg in Baltic Sea algae, and the average levels of sulphate ranged from 6.66 g/kg to 12.7 g/kg in the North Sea algae and from 4.71 g/kg to 9.39 g/kg in the Baltic Sea algae. Comparing sulphate data from the algae samples from the North and Baltic Seas, one can see an increase in the average concentration by a factor of 1.5. The concentration of ammonium also varied by a factor of 1.3 between the algae samples from North and Baltic Seas. Moreover, the results presented in Tables IV and V show a significant increase in ammonium levels in algae collected from Langholz in the Baltic Sea (sampling location 3) and Meldorfer Bucht in the North Sea (sampling location 5). Active agricultural production in these areas may influence this effect. The values for potassium in Tables IV and V show no significant trend except a high fluctuation of low potassium amounts. Potassium is an essential and mobile nutritive substance for plants. The simultaneous determination of low potassium concentrations and a high amount of ammonium and sodium by this measuring method may cause this fluctuation.

CONCLUSIONS

The ESB preparation technique and extraction with deionized water can be effectively used for routine ion chromatographic analysis of inorganic anions and cations in algae samples. Between 75 and 90% of K^+, NH_4^+, Na^+, Cl^-, HPO_4^{2-} and SO_4^{2-} could be removed from the algae by the first extraction with deionized water. Complete removal of the inorganic anions and cations from brown algae is guaranteed by use of the ESB standard preparation technique and extraction with deionized water. Enzymatic digestion with a mixture of pectinase, macerozyme and cellulase can be used to investigate the completeness of the cell disintegration of other plants following the ESB preparation technique and extraction with deionized water for ion chromatographic analysis. Because of a possible high blank value of anions and cations in the enzymes, their ion levels should be monitored. The results of the investigation of the inorganic anions and cations in brown algae samples from the North Sea and Baltic Sea have futher confirmed the use of brown algae as environmental monitors of local pollution in marine areas.

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Plane tree bark as a passive sampler of polycyclic aromatic hydrocarbons in an urban environment

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ABSTRACT

The use of plane tree bark as a natural and passive sampler allowed the detection of nineteen polycyclic aromatic hydrocarbons in the molecular mass range 128–202. The compounds extracted using a Soxtec system were separated by gas chromatography, characterized by mass spectrometry and confirmed by comparison with reference product data. The investigation emphasized the amount of pollution in the urban and rural environments, the kind of traffic and the concentrations of pollutants at various heights in the trunk of the plane trees. The accuracy and precision of the method were also studied.

INTRODUCTION

The sampling of air pollutants is a fundamental phase in the whole analytical process. In fact, the most efficient collection system applied for a suitable time ensures the accuracy of the measurements performed on the species under investigation [1–3].

The choice of the sampler may be difficult because its adsorption efficiency is not constant and uniform over a wide range of compounds, even though these may belong to the same family, because of the different physical characteristics of the substances to be collected [4]. Further, the monitoring of a large zone needs a large number of pumping systems, for a long time, so as to obtain data that correspond to an experimental integration of the pollutants in a particular period. Hence passive samplers are of interest because no power supply and operator presence are necessary [5,6]. The only limitation may be the vulnerability of the apparatus with reference to intentional damage if the collection time in the areas under investigation must be prolonged for runs of several days or weeks.

An index of the pollution level produced by vehicles is represented by the presence of the polycyclic aromatic hydrocarbons (PAHs) in the air [7–9]. Reduced monitoring, like sampling numbers, may be performed according to the NIOSH method using a PTFE filter followed, on-line, by an XAD-2 sorbent tube [10]. In this case, double or triple analytical runs with the three components of the adsorption system (filter and first and second load of sorbent tube) must be carried out.

On the basis of these considerations, the study of a wide zone for a number of days becomes complex and large numbers of samplings and partial analyses must be accurately managed. On the other hand, the sampling phase may be simple and immediate, reducing at least the events which occur outside laboratory if the bark of the trees is used. This paper shows that such a biological matrix is polluted by PAHs and that it represents a natural and passive sampler, constantly present in the investigated zone. The PAH concentration, compared with that detected in a reference zone, is of interest in order to establish not only how the traffic contributes to the level of pollution, but also which kind of traffic flow (slow, fast, queuing) is most responsible for polluting the passive sampler.

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EXPERIMENTAL

Chemicals

All the reference compounds and HPLC-grade solvents (dichloromethane, *n*-hexane and acetone) were purchased from Aldrich Chemie (Steinheim, Germany).

Collection of the samples

Bark was collected as chips in the form in which they left plane trees (*Platanus hybrida*) situated in urban and rural environments. Samples collected at three different heights (0.3–0.8, 0.8–1.2 and 1.2–2 m) up the trunk facing north-east were studied with the aim of identifying and determining the PAHs present. The trees skirted urban roads (2 m from car traffic) with normal traffic in a town of 300 000 inhabitants. The samplings in the urban area corresponded to two zones with different types of traffic flow. The data for each zone investigated were obtained by the averaged analyses of three trees.

Preparation of samples

Bark samples (100 cm^2, corresponding to about 12 g) were broken into small pieces and placed in a Soxtec system (Tecator, Höganäs, Sweden) for extraction with 70 ml of dichloromethane for 3 h in rinsing conditions [10,11]. The organic solution was dried and the residue was successively treated with five 2-ml portions of *n*-hexane so as to obtain a quantitative recovery of the pollutants. This organic phase was evaporated to dryness under a flow of nitrogen and the residue was dissolved in 1 ml of hexane–acetone (1:1, v/v). A 1-μl volume of each sample was injected into a GC–MS instrument after the addition of 10 μl of acetophenone solution (0.3 g l^{-1}) as an internal standard.

GC–MS apparatus and conditions

The measurements were performed using a Hewlett-Packard GC–MS system, consisting of a Model 5890 gas chromatograph equipped with a 25 m × 0.31 mm I.D. fused-silica capillary column coated with Ultra-2 and a Model 5971 A quadrupole. An HP 59970 C data system was used for data acquisition and editing.

For GC separations the column temperature was programmed from 100°C (isothermal for 3 min) at 10°C min^{-1} to 250°C (maintained for 10 min). The injector and transfer line temperatures were 260 and 280°C, respectively.

The MS conditions were electron energy 70 eV, emission current 300 μA and ion source temperature 176°C. Mass spectra were recorded by cyclically scanning from 50 to 250 mass units with a total cycle time of 0.49 s and a solvent delay of 2.5 min. The injection volume was 1 μl in splitless conditions (0.2 min). Quantitative analysis was performed by integration of the chromatographic peaks corresponding only to the current of the molecular ions of the compounds examined.

RESULTS AND DISCUSSION

The bark of fast-growing trees such as plane trees constitutes a superficial layer which is, on average, replaced within 2 years (half-life *ca.* 1.5 years) owing to the annual development of the trunk. The permanence of the bark in the same environment for a sufficiently long time as dead biological material allows the cumulative deposition and adsorption of the pollutants without their transformation during contact. Therefore, suitable extraction and subsequent analysis may indicate the compounds which characterize some particular kinds of environmental pollution. In this work, the use of the Soxtec system with dichloromethane as an extraction solvent allowed the recovery of the PAHs from the bark. These compounds are known as pyrolysis compounds and are present in vehicle particulate emissions [10–12].

The Soxtec thermal treatment and the evaporation to dryness were verified on two target compounds, acenaphthene and pyrene (2.0 μg of each in injectable solution) of different molecular masses, 154 and 202, added to the thimble, to have no destructive or loss effect on the analytes, leading to their quantitative recovery.

GC–MS analysis of the bark extract, obtained according to the experimental conditions, led to the unequivocable detection of nineteen PAHs, some of which were methyl and dimethyl isomers of naphthalene and methyl derivatives of phenanthrene, as reported in Table I and Fig. 1. Such structures were confirmed by verifying the complete correspondence with the GC and MS data for standards under the same experimental conditions.

The average data obtained in duplicate analyses

TABLE I

AVERAGE CONCENTRATIONS (μg PER 100 cm^2) OF PAH COMPOUNDS DETECTED ON PLANE TREE BARKS AT THREE DIFFERENT HEIGHTS

Sample height: A = 0.3–0.8, B = 0.8–1.2 and C = 1.2–2 m. Columns D show the average concentrations calculated in the 0.3–2 m range.

Peak No.	Compound	M_r	Urban 1 samples				Urban 2 samples				Rural sample
			A	B	C	D	A	B	C	D	
1	Naphthalene	128	2.76	2.44	1.56	2.25	1.40	1.47	1.50	1.45	0.33
2	1-Methylnaphthalene	142	3.35	2.92	2.19	2.82	1.87	1.59	1.44	1.63	0.28
3	2-Methylnaphthalene	142	1.61	1.35	1.08	1.34	0.85	0.70	0.70	0.75	0.10
4	Biphenyl	154	0.99	0.63	0.48	0.70	0.27	0.26	0.30	0.27	0.15
5	Dimethylnaphthalene (five isomers)	156	4.91	3.74	4.14	4.26	2.85	2.29	2.04	2.39	N.D.
6	Acenaphthylene	152	0.75	0.58	0.56	0.63	0.32	0.28	0.33	0.31	0.20
7	Fluorene	166	2.03	1.30	1.30	1.54	1.27	1.05	0.99	1.10	0.27
8	Phenanthrene	178	3.74	2.36	2.46	2.85	5.35	3.60	3.87	4.27	0.71
9	Anthracene	178	0.52	0.40	0.33	0.41	0.66	0.42	0.44	0.50	N.D.
10	Methylphenanthrene (four isomers)	192	3.17	1.84	1.84	2.28	7.34	3.75	4.32	5.13	0.25
11	Fluoranthene	202	1.14	0.77	0.61	0.84	5.48	2.51	3.14	3.71	N.D.
12	Pyrene	202	0.79	0.57	0.42	0.59	4.47	1.96	2.43	2.95	N.D.
Mean						20.51				24.46	2.29

for each compound of samples from the same tree (μg per 100 cm^2) are given in Table I. It was interesting that the data seemed to be influenced by three variables, the height of the bark from to the ground, rural or urban environment and the type of urban area investigated.

With the reference to the first parameter, the results demonstrated that the maximum concentration of each compound was found in the lowest band, 0.3–0.8 m from the ground, and decreased as the sampling height increased (Table I, columns A, B and C). This behaviour may be explained by the direct contamination from any vehicle exhaust gases.

The density of the traffic and the pollution sources differed between urban and rural areas. This difference was evident also from the analysis of the bark sampled in the country. The heaviest com-

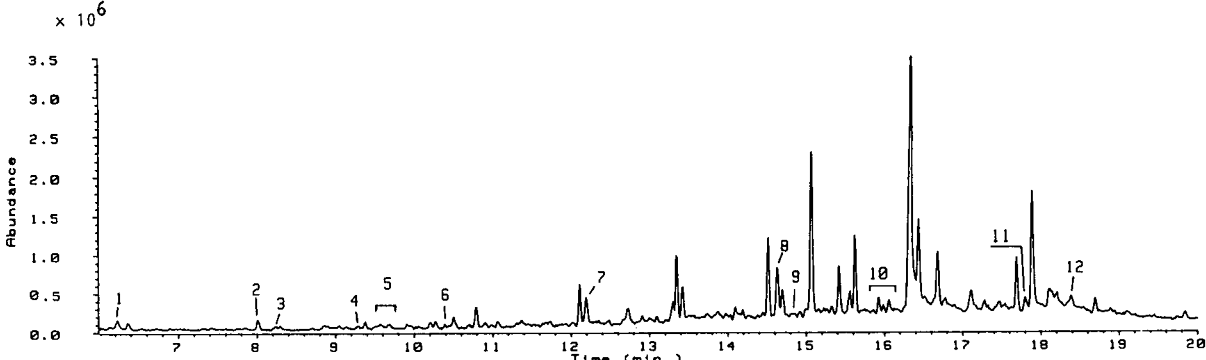

Fig. 1. GC–MS of a typical extract of plane tree bark. For peak identification, see Table I.

pounds such as fluoranthene and pyrene were not detected, whereas other compounds were present, on average, at a total concentration of 2.29 vs. 20.51 and 24.46 μg per 100 cm^2 in the two urban areas (Table I). The ratio of the PAH concentrations in urban and rural environments was thus about 10 [13]. In the country, the presence of the species responsible for pollution could also be due to the atmospheric precipitation, which might transfer the PAHs from polluted areas [8].

This investigation also emphasized a quantitative difference in PAHs within the same urban area. Table I, column D, shows an inversion of the concentration of the lighter with respect to the heavier PAHs. This phenomenon may be attributed to different kinds of traffic. In fact, the sample defined as urban 1 corresponded to bark near to a road with steady traffic whereas the second sample, urban 2, represented a road with slow and irregular traffic, often with queuing. Probably the different working conditions of the car engines might influence the distribution of molecular masses of the PAHs given out. The total concentration of PAHs was, on average, of the same order of magnitude in the two cases studied.

The accuracy of the method was verified by determining the percentage recovery of the examined compounds. Some rural bark samples were spiked with 100 μl of a solution containing 20.0 and 23.4 μg ml^{-1} of acenaphthene and pyrene and extracted successively for periods of 1, 2 or 3 h, according to the usual procedure. These tests on aromatics were chosen because the former compound had never been detected in real samples whereas the latter was present only in urban samples, so the behaviour of the two meant that valid information about the efficiency of the overall Soxtec extraction process could be obtained. The recoveries of the two compounds after 1, 2 and 3 h were acenaphthene 35, 64 and 88% and pyrene 36, 67 and 91%, respectively. The explanation for this behaviour might be attributed to the difficulty in extracting the compounds because of the sample volume and its granulometry. Repeated measurements gave very similar values (relative standard deviation = 3%), demonstrating the good precision of the method used.

Tests carried out on pulverized bark demonstrated better PAH recoveries (almost 100%), but also the excessive presence of species of vegetable origin which interfered with the recovery from the dried organic phase and with the GC–MS analysis. On the basis of these results, it was more convenient to consider the use of bark broken into small pieces because the extraction of the matrix compounds was reduced, but about 90% of the PAHs distributed on the external surface of bark were recovered.

The efficiency of the Soxtec process allowed the detection of low levels of pollution and minor pollutants, as the GC–MS instrument reached equivalent determination limits of 0.02 μg per 100 cm^2 for naphthalene, 0.03 μg per 100 cm^2 for phenanthrene, anthracene and fluoranthene, 0.04 per μg per 100 cm^2 for methylnaphthalenes, acenaphthylene and pyrene, and 0.05 μg per 100 cm^2 for biphenyl, dimethylnaphthalenes, fluorene and methylphenanthrenes.

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Identification and quantification of 18-nor- and 19-norditerpenes and their chlorinated analogues in samples of sediment and fish

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ABSTRACT

The norditerpenes 18-norabieta-8,11,13-triene and 12,14-dichloro-18-norabieta-8,11,13-triene, corresponding to decarboxylation of dehydroabietic acid and 12,14-dichlorodehydroabietic acid, respectively, were isolated from environmental samples of sediment and fish from the Gulf of Bothnia. Identification was unambiguously accomplished by comparison with synthetic reference compounds whose structures were established by NMR. The synthetic product was a mixture of the nor epimers at C-4 in the ratio of *ca.* 1:3, and NMR showed that the 18-nor epimer was the major component. Whereas the 18-nor epimer was the dominant C_{19} hydrocarbon in the sediment samples, the 19-nor compound dominated the C_{19} hydrocarbon fraction in the samples of fish. These C_{19} terpenes are probably environmental transformation products of the corresponding dehydroabietic acids. The 18-nor hydrocarbon was quantified in sediment samples, which were also analysed for a number of other compounds representative of alicyclic and aliphatic components of bleachery effluents. The 19-nor hydrocarbon was conclusively identified, although not quantified in samples of fish. 12,14-Dichloro-18-norabieta-8,11,13-triene was identified in samples of sediment and fish which also contained the 19-nor compound. The presence of both hydrocarbons in fish was consistent with the experimentally determined estimates of their bioconcentration potential by reversed-phase HPLC. A number of other norabietanes and bisnorabietanes were tentatively identified in both sediment and fish samples, together with their dehydrogenation products, and a hypothetical scheme relating these to dehydroabietic acid is proposed. Attention is directed to the value of procedures including open-column chromatography on silica gel, gel permeation chromatography and mild chemical treatment for preparing and pretreatment of samples before identification. It is emphasized that analytical procedures should be directed to the specific structure of the analyte and will depend on the nature of interfering compounds in the samples. The search for universal methods that are applicable to structurally diverse analytes may be unrealistic.

INTRODUCTION

There are several problems in determining the environmental impact of effluents from industrial processes which contain a large number of components with diverse structures. In the specific case of bleachery effluents, the problem is particularly severe. The large number of compounds encountered has resulted in extensive efforts being directed to their identification and quantification. Ideally, all of these compounds should be identified individually so that their persistence, their biological effect and their distribution can be rationally evaluated [1].

Analysis of cyclohexane-extractable organically bound chlorine (EOCl) has been used to monitor the distribution of compounds putatively originating from bleachery effluents in sediments and biota [2]. A previous study [3] was directed to identifying

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the components of these extracts from contaminated sediment samples, but resulted in identification of only *ca.* 10% of the total organochlorine content in terms of specific compounds. Further effort has now been devoted to this problem and attention directed to procedures for fractionating the extracts prior to attempted identification of their components.

In this investigation, a hitherto unidentified group of chlorinated hydrocarbons was isolated and identified by comparison with authentic synthetic compounds. The assessment of the distribution of these compounds in environmental samples has therefore now become possible: their concentration in sediment samples was compared with those of other alicyclic and aliphatic compounds originating in bleachery effluents. As these compounds appear not to have been described previously as environmental contaminants, their octanol–water partition coefficients were measured by a surrogate HPLC procedure.

EXPERIMENTAL

Source of samples

Effluent samples were chlorination-stage (C-stage) and extraction-stage (E-stage) bleach liquors, and were kept at 4°C after collection. Samples of sediment and perch (*Perca fluviatilis*) were collected from the Gulf of Bothnia within *ca.* 5 km of the discharge of bleachery effluents. The sediment was kept in filled screw-capped jars at 4°C before use; the moisture content was determined from the mass loss after heating at 105°C for 12 h and the organic carbon from the mass loss from acidified dried samples after ignition at 550°C for 5 h. The fish samples were wrapped in aluminium foil and kept frozen at −20°C. They were cut into fillets before analysis.

Chemicals

Dehydroabietic acid was purchased from Helix (Richmond, BC, Canada) and retene from ICN Biomedicals (Costa Mesa, CA, USA). 12,14-Dichlorodehydroabietic acid was synthesized by modification [3] of a published procedure. The following reagents were purchased from the sources given in parentheses: phosgene (20% solution in toluene) (Fluka, Buchs, Switzerland), 2-thiopyridone N-oxide, 4-dimethylaminopyridine and tri-*n*-butylstannane (Aldrich, Steinheim, Germany) and α,α′-azoisobutyronitrile (Jansen, Beerse, Belgium). Solvents were obtained from Burdick and Jackson (Muskegon, MI, USA).

Synthesis of reference compounds

The structures of the 18{19}-norditerpenes investigated in this study are shown in Fig. 1. It should be noted that the prefix "*z*-nor" implies replacement of the original substituent at position *z* (in this case CO_2H) with H. We have used the standard terpene numbering system throughout, and designated the compounds as derivatives of abietane. In *Chemical Abstracts*, however, these compounds are designated as octahydrophenanthrenes with a numbering system different from that used for sterols and terpenes; for example, 18{19}-norabieta-8-11,13-triene would be 1,4a-dimethyl-7-(1-methylethyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene.

The synthesis of 18{19}-norabieta-8,11,13-triene and 12,14-dichloro-18{19}-norabieta-8,11,13-triene was carried out by the decarboxylation of the corresponding dehydroabietic acids using a published procedure [4]. Briefly, this consisted of the preparation of the acid chlorides from the corresponding carboxylic acids with phosgene in benzene at room temperature, reaction with 2-thiopyridone N-oxide to form the ester, followed by reduction with tributyltin hydride in the presence of the radical initiator α,α′-azoisobutyronitrile. The products were separated from small amounts of remaining organotin compounds by chromatography, first by elution from silica gel with hexane followed by elution from

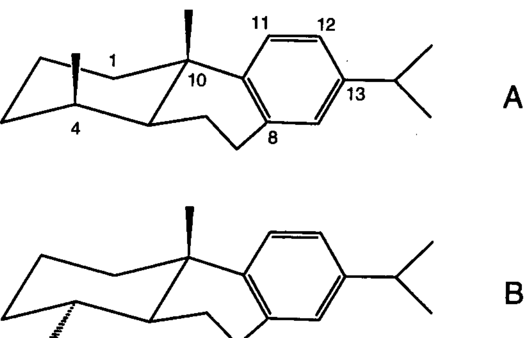

Fig. 1. Structural formulae of (A) 18-norabieta-8,11,13-triene and (B) 19-norabieta-8,11,13-triene.

neutral alumina with hexane. All reactions were carried out in a stream of dry nitrogen. It should be noted that all the reagents are either toxic, potentially explosive or both. The products were initially characterized by GC–MS analysis and consisted of a mixture of the diastereoisomers at C-4 (18-nor and 19-nor compounds). These could readily be separated on a 15-m DB-5 capillary column, but no attempt was made to separate them on a preparative scale. For quantification, the GC response was calculated from the concentrations of the respective epimers.

Identification of 18{19}-norabieta-8,11,13-trienes in sediment cyclohexane-extractable organic chlorine (EOCl) extracts

Cyclohexane-EOCl extracts were prepared from contaminated sediment samples as described previously [3]. Fractionation was carried out on silica gel (Kieselgel 60, 70–230; mesh; Merck). Elution with hexane yielded a neutral fraction in which the 19-norabieta-8,11,13-triene was initially identified.

Extraction of water samples

Samples of water (1 l) were treated as follows: 50 μl of a solution of 5-α-cholestane (1 mg/ml) in benzene were added as a surrogate, the pH of the solution was adjusted to pH 12 and the mixture was extracted twice with hexane–*tert*.-butyl methyl ether (7:3, v/v) (100 ml). The organic phase was washed twice with a saturated solution of NaCl (50 ml), the organic phase removed, dried (Na_2SO_4), the solvent removed and the residue dissolved in hexane (1 ml). The hexane solution was applied to a Kieselgel 60 (70–230 mesh) silica gel column (30 × 6 mm I.D.) in a Pasteur pipette. The hexane eluate was evaporated to dryness and the residue was dissolved in hexane (1 ml) containing biphenyl (25 μg/ml) as internal standard. Analysis was carried out by GC using a Hewlett-Packard HP 5890 instrument and with splitless injection (the split closed for 45 s) and flame ionization detection. The following temperature programme was used: 45°C for 1 min isothermal, increased at 15°C/min to 300°C, which was held for 10 min. The injector temperature was 240°C and the detector temperature 300°C.

Extraction of sediment samples

Extraction with water-miscible solvents. Samples of sediment (*ca.* 5 g wet mass) were weighed into 10-ml glass tubes fitted with PTFE-lined screw-caps and 5 ml of each of the following solvents were added: tetrahydrofuran, acetonitrile, 2-propanol or dimethylformamide. The samples were kept in an ultrasonic bath for 10 min, shaken gently overnight by inversion, centrifuged (1000 *g*) and the organic phase removed. The extraction was repeated with a further 5-ml portion of solvent, the extracts were combined and 50 μg of the following were added as surrogate standards: for neutral compounds, cholestane; for diterpenes, podocarpic acid O-ethyl ether (prepared from the corresponding acid with diethyl sulphate in alkaline medium and crystallized from acetonitrile); for alkanoic acids, 9,10-dibromooctadecanoic acid (prepared by bromination of 9-octadecenoic acid in CCl_4); and for sterols and triterpenes, 5-α-cholestan-3-β-ol.

The extracts were then treated as follows: the extracts from tetrahydrofuran, acetonitrile or 2-propanol were concentrated under a stream of nitrogen to a volume of *ca.* 1 ml, diluted to 7 ml with saturated NaCl solution and extracted three times with 1.5 ml of hexane–*tert*.-butyl methyl ether (7:3, v/v). The combined extracts were washed with saturated NaCl solution, the organic phase dried (Na_2SO_4) and the solvent removed under a stream of nitrogen.

The dimethylformamide extract was diluted to 80 ml with saturated NaCl solution and extracted twice with 25 ml of hexane–*tert*.-butyl methyl ether (7:3, v/v), the combined organic phases were washed twice with saturated NaCl solution, the organic phase was removed and dried (Na_2SO_4) and the solvent removed under a stream of nitrogen.

Extraction with water-immiscible solvents. Extraction with benzene was carried out by two methods: (i) Soxhlet extraction of freeze-dried samples (*ca.* 10 g) with benzene for 3 h and (ii) Dean and Stark extraction of wet samples with benzene; when all the water had been removed, extraction was continued for 2 h. Surrogate standards were added to the benzene extracts, which were then concentrated in vacuum and chromatographed as follows.

The concentrates prepared above were dissolved in 2 ml of cyclohexane and chromatographed on a column of silica gel (Kieselgel 60, 70–230 mesh).

Neutral compounds were eluted with hexane [fraction (a)] and other compounds with *tert.*-butyl methyl ether [fraction (b)]. Fraction (a) was concentrated under a stream of nitrogen to a volume of 2 ml. Elemental sulphur was removed by shaking with an aqueous solution of tetrabutylammonium sulphite (1 ml) and 2-propanol (1 ml) [5]. The organic phase was dried (Na_2SO_4) and used for quantification by the GC procedure described above. Fraction (b) was concentrated under a stream of nitrogen, methylated with a solution of diazomethane in diethyl ether and concentrated. The concentrate was acetylated overnight at room temperature with equal volumes of acetic anhydride and pyridine (50 μl). The reaction mixture was dissolved in hexane, pyridine was removed by washing with 0.5 *M* HCl (4 ml) and acetic anhydride was hydrolysed by shaking with 0.8 *M* K_2CO_3 solution (4 ml). The hexane solution was dried (Na_2SO_4) and chromatographed on a column (4 × 1 cm I.D.) of silica gel (Kieselgel 60, 70–230 mesh). Elution was carried out with hexane–*tert.*-butyl methyl ether (7:3, v/v), the solvent removed under a stream of nitrogen and a solution of biphenyl in hexane (25 μg) was added as internal standard. GC analysis was carried out as described above.

For mass spectrometric identification, the extracts were further purified by gel-permeation chromatography (GPC) as follows: the solvent was removed under a stream of nitrogen and the residue was dissolved in tetrahydrofuran (70 μl) and applied to a column of PL-gel (particle size 10 μm, porosity 500 Å, 600 × 10 mm I.D.). The column was eluted with tetrahydrofuran at a flow-rate of 1 ml/min. Detection was carried out at 280 nm and the fraction eluting at the same time as the authentic standard was collected. The sample was concentrated, dissolved in hexane, chromatographed on a short column of neutral alumina (Merck), eluted with hexane or benzene and the solutions obtained were used for GC–MS analysis.

Extraction of fish samples

Muscle tissue was removed from fish and samples (*ca.* 35 g) were treated by two different procedures. (i) Samples were freeze-dried and the residue was Soxhlet extracted with benzene. (ii) Samples were mixed with portions of solid carbon dioxide and mixed in a blender to obtain a fine flour, then the CO_2 was removed by sublimation at −20°C [6]. The product was either freeze-dried and Soxhlet extracted with benzene, or the wet sample Dean and Stark extracted with benzene.

The benzene extracts containing *ca.* 250 mg of lipid material were treated as follows. Benzene was removed in a stream of nitrogen and the residue was dissolved in *tert.*-butyl methyl ether (1 ml). A 2 *M* solution of sodium methoxide in methanol (300 μl) was added and the mixture was shaken for 5 min at room temperature. The reaction was terminated by adding 0.5 *M* HCl (5 ml) and extracted twice with 1.5-ml portions of hexane–*tert.*-butyl methyl ether (7:3, v/v). The combined organic extracts were washed with water (3 ml), dried (Na_2SO_4) and the solvent was removed. The residue was dissolved in hexane (2 ml) and chromatographed on a column of silica gel (5 × 1 cm I.D.); the desired hydrocarbons were eluted with hexane and purified further by the methods used for the sediment samples, *i.e.*, GPC using tetrahydrofuran followed by chromatography on neutral alumina and elution with hexane. The resulting eluate was concentrated and used for GC–MS analysis.

Gas chromatographic–mass spectrometric and nuclear magnetic resonance analysis

GC–MS analysis was carried out as described previously [7].

NMR spectra were recorded on a JEOL GX400 spectrometer (399.65 MHz for ^1H and 100.40 MHz for ^{13}C) equipped with a standard 5-mm diameter C/H probe. The samples were prepared in $C_6{}^2H_6$ or C^2HCl_3 at a concentration of 20 mg/ml. All spectra were acquired using standard pulse sequences supplied by JEOL. The ^1H spectra were referenced to internal tetramethylsilane and the ^{13}C spectra referenced to the central line of the $C_6{}^2H_6$ signal (δ 128.00) or C^2HCl_3 (δ 77.00).

The ^1H spectra were acquired using the following conditions: spectral width 8.8 kHz, 32K data points, a pulse duration of 10 μs (30°) and a cycle time of 6 s. After sixteen scans the data were zero filled once and processed with a Gaussian window (−0.23 Hz line broadening). The unoptimized NOE difference spectra were acquired using the following conditions: spectral width 8.8 kHz, 32K data points, a pulse duration of 10 μs (30°), an irradiation time of 5 s with a total cycle time for the

NOE–normal spectrum pair of 20 s. After 64 scans the data were zero filled once and processed using an exponential window (1.0 Hz line broadening).

The $\{^1H\}^{13}C$ spectra were acquired using the following conditions: spectral width 25 kHz, 32K data points, a pulse duration of 4.7 μs (25°) and a cycle time of 2 s. After 1000 scans the data were zero filled once and processed with an exponential window (1.0 Hz line broadening). Accepting the differences in pulse durations required, the DEPT-90 and DEPT-135 spectra (256 scans each) were acquired and processed in the same way as the $\{^1H\}^{13}C$ spectrum.

Octanol–water partition coefficient

The octanol–water partition coefficient (P_{ow}) was estimated by a surrogate procedure using HPLC [8]. A Nucleosil C_8 column (150 × 10 mm I.D., 5 μm particle size) and a mobile phase of phosphoric acid (0.05 mol/l)–methanol (1:3, v/v) at a flow-rate of 1 ml/min were used; the UV detector was set at 280 nm. The following compounds were used as calibration standards: formamide, benzene, toluene, naphthalene, biphenyl, 1,2,4-trichlorobenzene, phenanthrene, fluoranthrene and DDT. Values for the capacity factors were calculated and from them values of P_{ow}; these values were then used to estimate bioconcentration factors (*BCF*) using the equation of Mackay [9].

RESULTS

Structure of authentic compounds

The mass spectrum of the synthetic 18{19}-norabieta-8,11,13-triene had a parent ion at *m/z* 256 corresponding to $C_{19}H_{28}$ and that of 12,14-dichloro-18{19}-norabieta-8,11,13-triene had a parent ion at *m/z* 324 corresponding to $C_{19}H_{26}Cl_2$ and with a ratio in the intensity of the peaks at 324 and 326 of 100:70. In both products, the two epimers were present in the ratio of *ca.* 3:1 (the minor product eluting first), but no attempt was made to separate these on a preparative scale.

The signals in the ^{13}C NMR spectrum of the synthetic mixture of 18-nor- and 19-norabieta-8,11,13-trienes were assigned using $\{^1H\}^{13}C$, DEPT-90 and DEPT-135 spectra to determine the chemical shifts and the number of protons attached to each carbon. The reported spectra are those observed in $C_6{}^2H_6$ solution.

18-Norabieta-8,11,13-triene (major component): δ 147.14 (C-9), 145.39 (C-13), 135.04 (C-8), 126.94 (C-14), 124.29 (C-11), 123.77 (C-12), 44.79 (C-5), 39.00 (C-3), 37.53 (C-10), 34.25 (C-4), 34.00 (C-15), 33.40 (C-1), 30.68 (C-7), 25.74 (C-20), 24.94 (C-2), 24.30 (C-16,17), 18.37 (C-6), 15.28 (C-19).

19-Norabieta-8,11,13-triene (minor component): δ 145.75 (C-9), 145.44 (C-13), 135.23 (C-8), 126.89 (C-14), 124.60 (C-11), 123.64 (C-12), 49.31 (C-5), 38.55 (C-3), 37.27 (C-10), 36.47 (C-1), 34.00 (C-15), 31.80 (C-4), 30.03 (C-7), 24.30 (C-16,17), 22.93 (C-18), 22.43 (C-2), 21.70 (C-6), 20.64 (C-20).

The δ 1.23–0.86 region of the ^1H spectrum of the $C_6{}^2H_6$ solution of this mixture contained only methyl groups. The methyl group at C-10 of 18{19}-norabieta-8,11,13-triene is a singlet. Irradiation of the major component singlet methyl produced a 3.5% NOE on the corresponding C-4 methyl signal (δ 0.94), indicating that the two groups are spatially close, and thereby confirming the major components as the 18-nor epimer. Irradiation of the minor singlet methyl produced no measurable NOE interaction with the corresponding C-4 methyl signal (δ 0.85) which is consistent with the structure of the 19-nor epimer. These spectra are shown in Fig. 2.

The NMR spectrum for the dichloronorabietatriene was significantly more complex than that of the parent hydrocarbon owing to signals from unseparated impurities, and no attempt was made to provide a complete interpretation on the basis of ^{13}C NMR spectrum. In the critical region of the ^1H spectrum where the methyl resonances occurred, there was sufficient similarity to those of the nonchlorinated compound to support the structural assignments and to show that the major component was the 18-nor epimer. This similarity is illustrated in Fig. 3 for the spectra of the compounds in C^2HCl_3.

Identification and quantification in environmental matrices

For conclusive identification of the hydrocarbons in the sediment samples, it was necessary to remove interfering compounds as far as possible. A combination of silica gel and gel permeation chromatography was used and the value of these is illustrated in Fig. 4. For biota, the additional transesterification step with sodium methoxide was valuable and in addition enabled larger amounts of material to

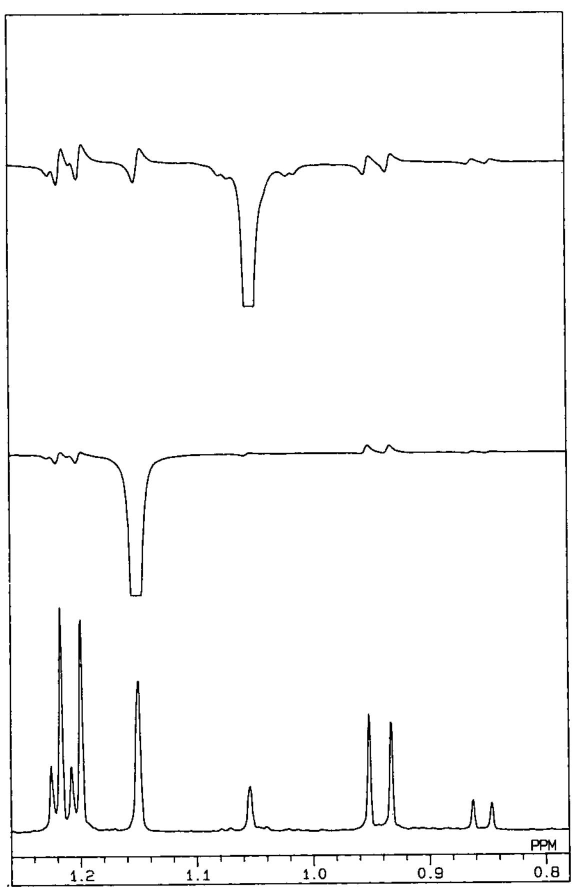

Fig. 2. ^1H normal NMR spectrum in $C_6{}^2H_6$ solution of mixture of synthetic 18-nor and 19-nor epimers (bottom) and NOE on the C-10-methyl of 18-norabieta-8,11,13-triene (middle) and 19-norabieta-8,11,13-triene (top).

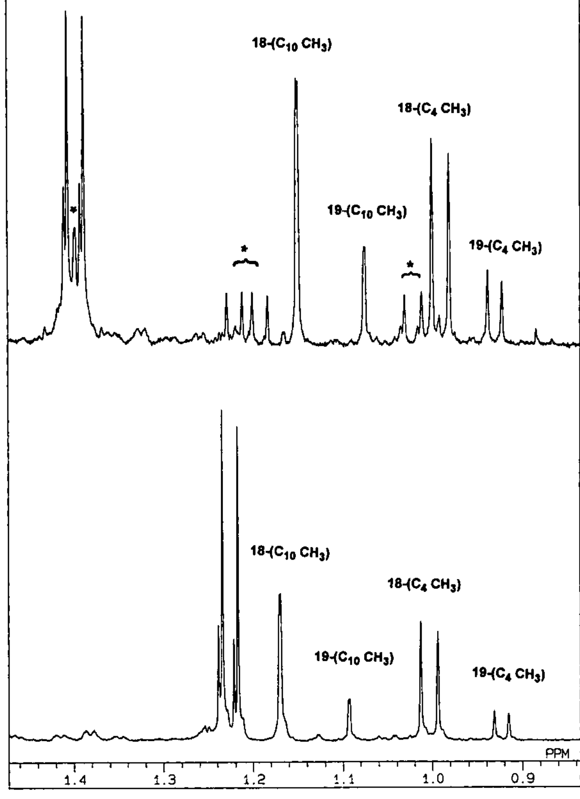

Fig. 3. ^1H normal NMR spectra in C^2HCl_3 solution of 18-norabieta-8,11,13-triene (bottom) and 12,14-dichloro-18-norabieta-8,11,13-triene (top). The peaks marked with asterisks correspond to unknown impurities.

be analysed. A comparison of the mass spectra of authentic 18-norabieta-8,11,13-triene and the compounds isolated from the sediment and fish samples is shown in Fig. 5. A similar comparison for 12,14-dichloro-18-norabieta-8,11,13-triene is shown in Fig. 6.

Both epimers were found in environmental samples, and for quantification it was assumed that the GC response of both epimers was equal. The concentrations of 18-nor-abieta-8,11,13-triene in samples of extraction stage bleachery effluents ranged from 3 to 6 μg/l, whereas levels of the corresponding 12,14-dichloro-18{19}-nor compound were undectable. The extracts from the wet sediment samples were generally dark coloured, presumably owing to the presence of humic substances, whereas the benzene extracts were light yellow. The concentrations of 18-norabieta-8,11,13-triene and its 12,14-analogue are given in Table I together with those of a number of compounds traditionally associated with bleachery effluents; these values were obtained using surrogate standards and their accuracy is calculated to be *ca.* 10%.

Monochloro compounds analogous to 12,14-dichloro-18{19}-norabieta-8,11,13-triene were found in sediment samples, although as reference compounds were not available quantification was not possible. The two isomers could be distinguished on

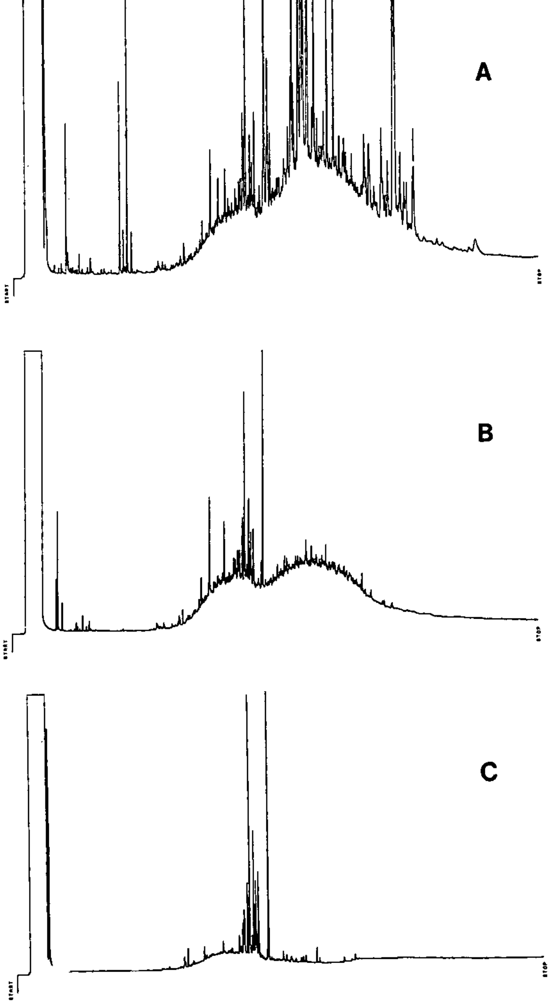

Fig. 4. Illustration of the value of silica gel chromatography (B) followed by gel permeation chromatography (C) on a crude sediment extract (A).

the basis of their different GC retention times whereas the configuration at C-4 could not be determined; the mass spectra of the two compounds, arbitrarily designated A and B, are shown in Fig. 7. In addition, a number of other hydrocarbons were isolated from sediment and fish samples and were tentatively identified from their mass spectra presented in Fig. 8. A comparison of the mass spectrum of authentic retene with that of the compound isolated from sediment samples is given in Fig. 9.

Octanol–water partition coefficient

The logarithms of the octanol–water partition coefficients for 18-norabieta-8,11,13-triene and 12,14-dichloro-19-norabieta-8,11,13-triene determined by HPLC were greater than that for DDT (6.2) and were estimated to have approximate values of 8.1 and 9.4, from which bioconcentration factors (log *BCF*) of 6.8 and 8.1 were calculated.

DISCUSSION

To simplify the discussion, the structures of the diterpene-related hydrocarbons derived from dehydroabietic acid and structurally related to the 18{19}-norabieta-8,11,13-trienes are shown in Fig. 10.

The structures of the 18{19}-nor hydrocarbons were supported by their mass spectra, and the configurations at C-4 were confirmed by the results of the NOE experiment after irradiation of the singlet methyl groups at δ (0.94 (18-nor epimer) and at δ 0.85 (19-nor epimer) in $C_6{}^2H_6$ solution (Fig. 2). Comparison of the ^{13}C chemical shifts of the methyl groups at C-4 and C-10 with those of abieta-8,11,13-trienes reported in the literature [10] strongly supports the assigned stereochemistry. The configuration of the 12,14-dichloro-18(19)-norabieta-8,11,13-trienes was based on the similarity of the ^1H spectra in C^2HCl_3 (Fig. 3) to those of the nonchlorinated hydrocarbons.

Identification of the C-19 nor hydrocarbons and their dichlorinated analogues in the environmental matrices was confirmed by comparison of the mass spectra with that of the authentic compound (Figs. 5 and 6). Conclusive identification was critically dependent on the preparation of samples free from interfering impurities. Whereas the analysis of water samples was essentially straightforward, the other two environmental samples illustrated the need to take into consideration both the nature of the analyte and the type of matrix. Analysis of samples of sediment and biota illustrated the need for different clean-up procedures. Sediment samples were contaminated with large amounts of elemental sulphur, which was readily removed with tetrabutylammonium sulphite, and silica gel was effective in removing the more polar fatty acids and sterols. It was the subsequent application of GPC, however, that effectively removed interfering hydrocarbon

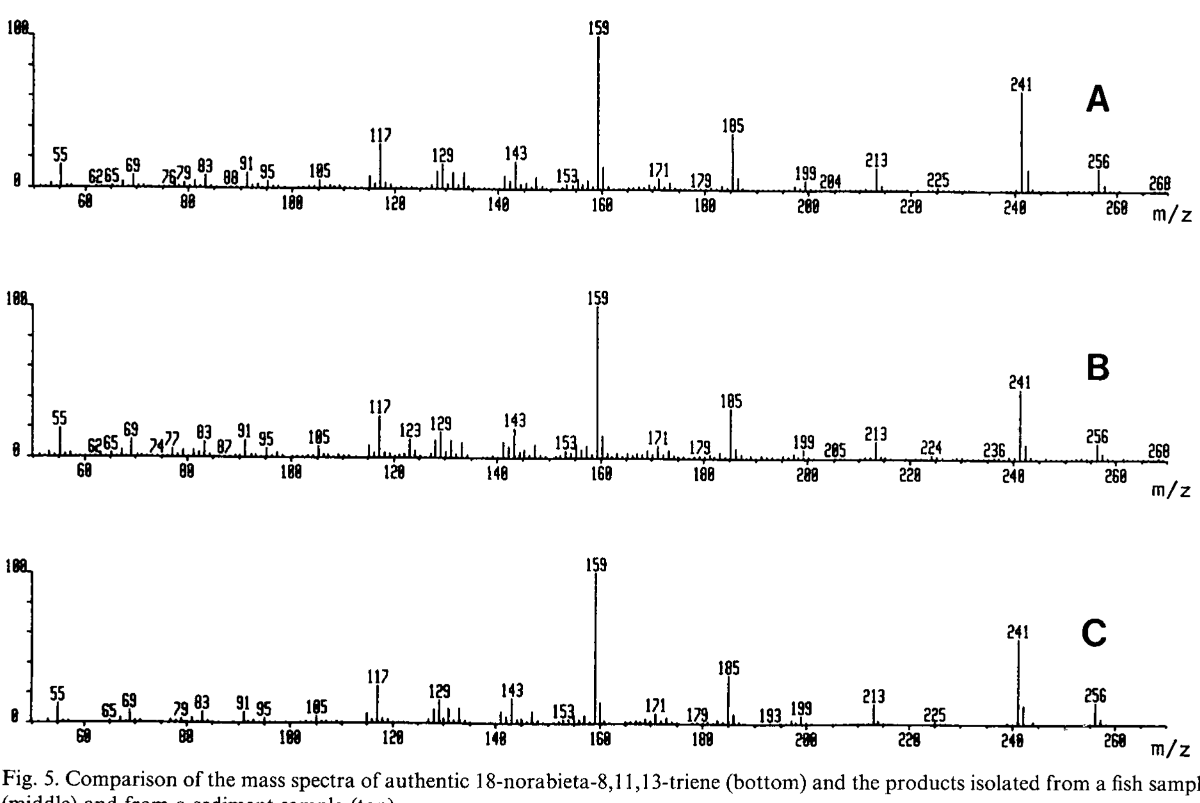

Fig. 5. Comparison of the mass spectra of authentic 18-norabieta-8,11,13-triene (bottom) and the products isolated from a fish sample (middle) and from a sediment sample (top).

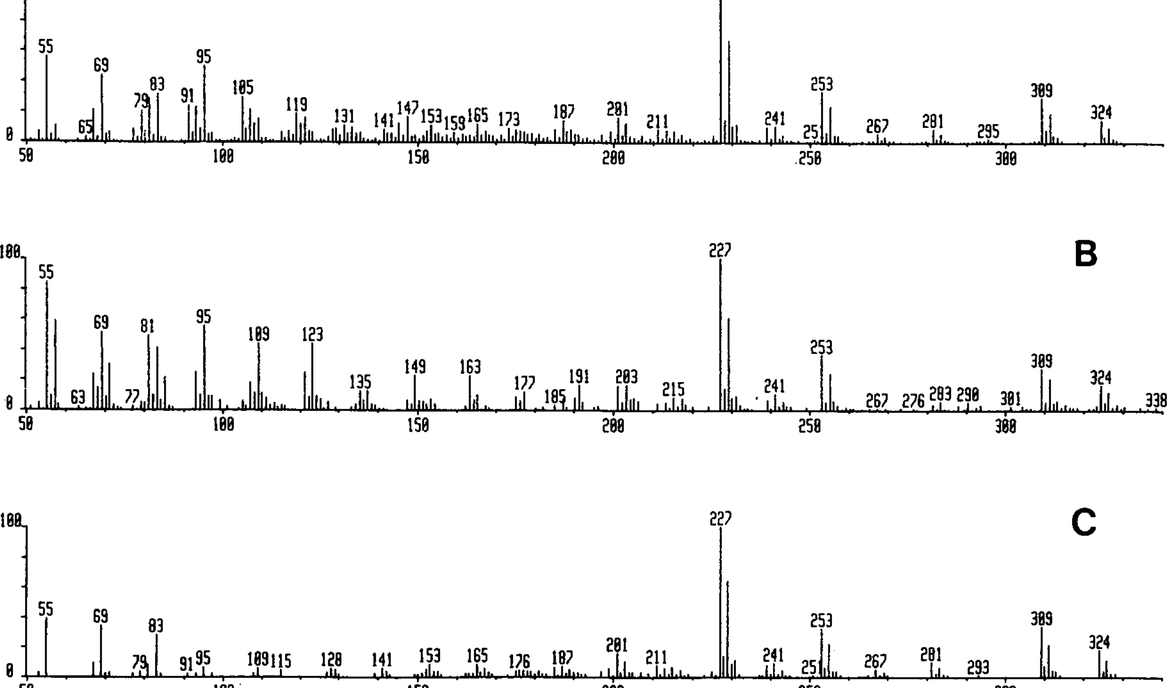

Fig. 6. Comparison of the mass spectra of authentic 12,14-dichloro-18-norabieta-8,11,13-triene (bottom) and the products isolated from a fish sample (middle) and from a sediment sample (top).

TABLE I

CONCENTRATIONS OF SELECTED ORGANIC COMPONENTS OF SEDIMENT SAMPLES FROM TWO LOCALITIES

Sample	Extraction	Concentration (mg/g organic C)[a]							
		nor	Cl_2-nor	DHA	Cl_2-DHA	C_{18}	Cl_2-C_{18}	β-sito	betulin
A	Acetonitrile	40	4.1	160	24	19	17	690	280
	Dimethylformamide	30	2.4	90	17	22	17	420	200
	2-Propanol	50	4.6	250	61	46	36	1400	260
	Tetrahydrofuran	120	5.3	530	130	51	39	1500	280
	Dean-Stark:benzene	50	4.6	200	55	22	14	1150	150
	Soxhlet:benzene	50	4.6	300	61	35	24	1000	180
	Soxhlet:tetrahydrofuran	20	2.5	480	170	75	46	730	85
B	Acetonitrile	650	6.1	580	18	67	43	2300	5500
	Dimethylformamide	450	5.0	160	6	49	18	2000	5700
	2-Propanol	780	15	780	36	128	55	3900	8100
	Tetrahydrofuran	1000	17	2200	120	274	130	4400	8000
	Dean-Stark:benzene	870	11	690	41	62	27	3300	5500
	Soxhlet:benzene	840	11	760	43	135	78	2900	5200

[a] nor = 18-Norabieta-8,11,13-triene; Cl_2-nor = 12,14-dichloro-18-norabieta-8,11,13-triene; DHA = dehydroabietic acid; Cl_2-DHA = 12,14-dichlorodehydroabietic acid; C_{18} = octadecanoic acid; Cl_2-C_{18} = 9,10-dichlorooctadecanoic acid; β-sito = β-sitosterol.

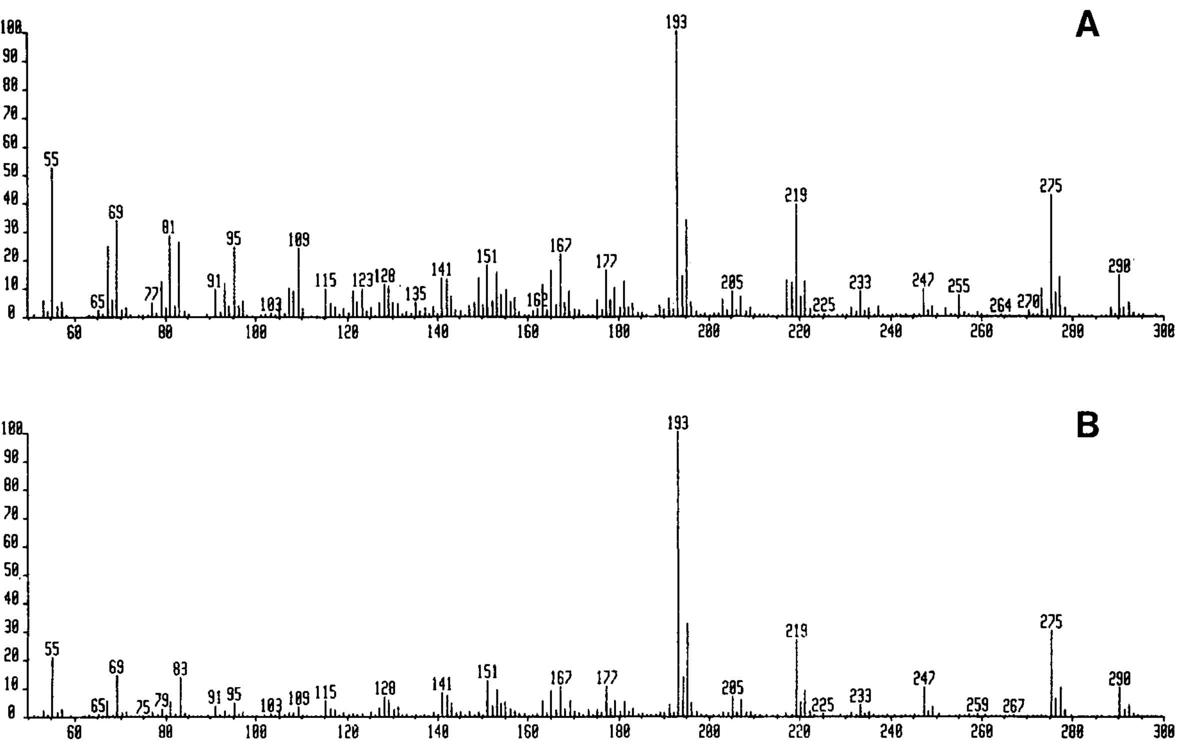

Fig. 7. Mass spectra of the isomeric 12{14}-monochloro-18{19}-norabieta-8,11,13-trienes isolated from sediment samples and arbitrarily designated A and B.

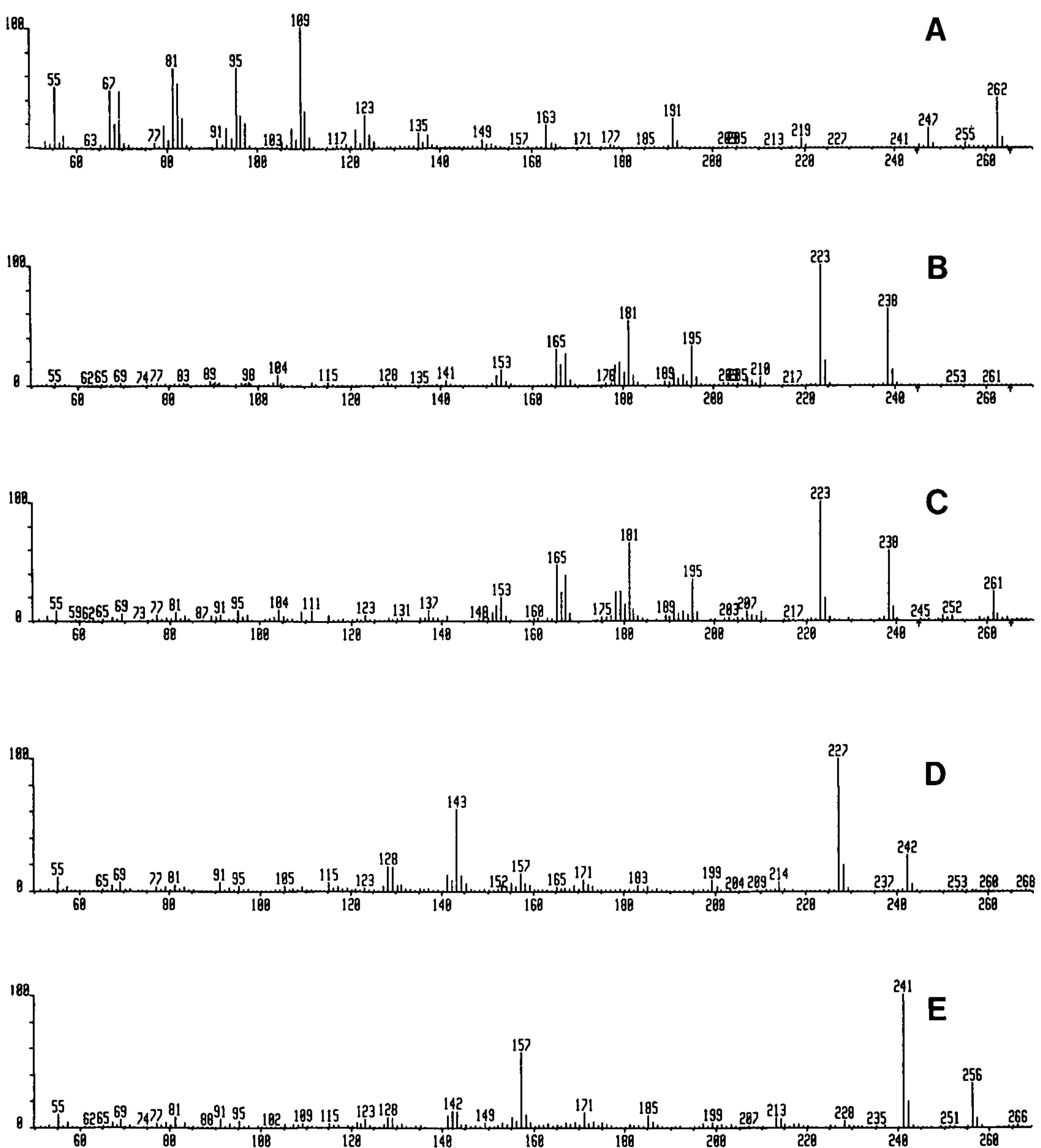

Fig. 8. Mass spectra of diterpene-related hydrocarbons isolated from sediment samples, (A) 18{19}-norabietane (IV) and (B) 10,18{19}-bisnorabieta-5,7,9(10),11,13-pentaene (V) and from fish, (C) 10,18{19}-bisnorabieta-5,7,9(10),11,13-pentaene (V), (D) 10,18{19}-bisnorabieta-8,11,13-triene (VI) and (E) 1-methyl-10,18{19}-bisnorabieta-8,11,13-triene (VII).

components and allowed the unambiguous mass spectrometric identification of the norabietatrienes (Fig. 4). Whereas in the present study no systematic effort was made to evaluate the extraction procedures for biota, as that involving homogenenization with solid CO_2 was both expedient and effective, clean-up of extracts from biota presented an entirely different problem from that of sediments. The major interfering compounds were lipids and a variety of procedures have been proposed for their removal [11–13]. Although no systematic examination was carried out in the present investigations, it

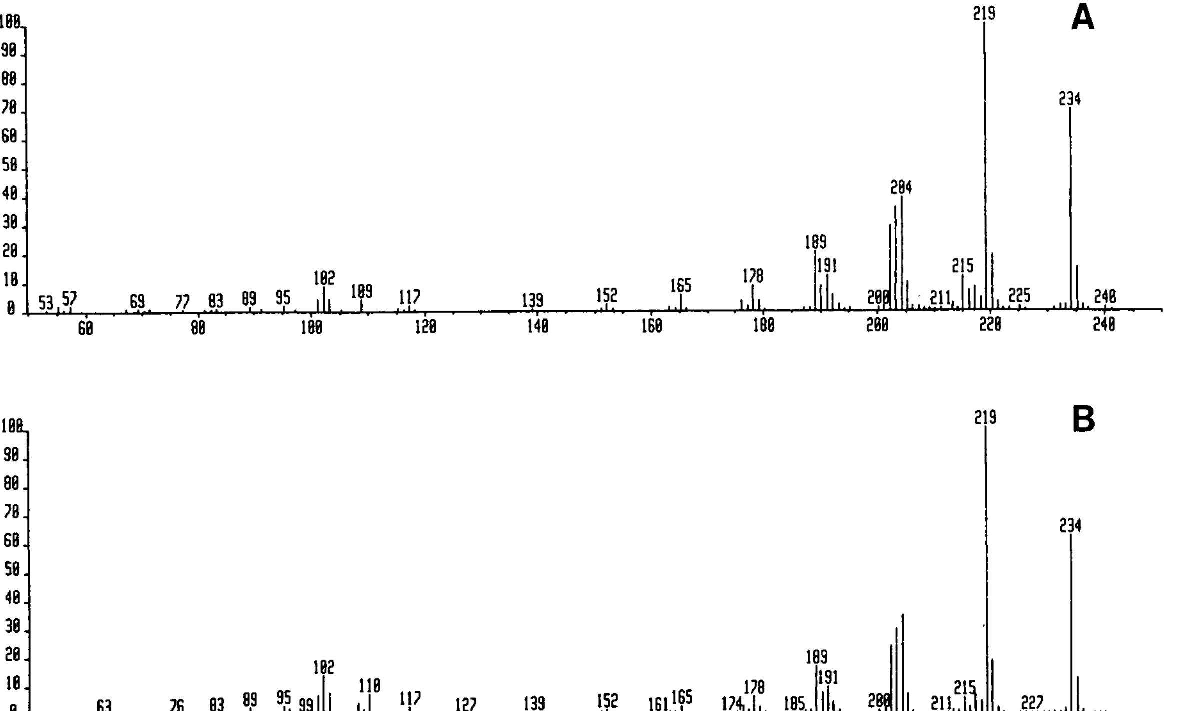

Fig. 9. Comparison of the mass spectrum of retene (**VIII**) (bottom) with that of the compound isolated from sediment samples (top).

Fig. 10. Hypothetical scheme showing the relationship to dehydroabietic acid of the hydrocarbons isolated from sediment and fish samples: compounds **II**, **IV**, **V** and **VIII** were isolated from sediments and **II**, **III**, **V**, **VI** and **VII** from fish.

was found that transesterification of the lipids with sodium methoxide was effective in forming long-chain carboxylic acid esters which were retained on silica gel columns while the hydrocarbons were effectively eluted with hexane. This procedure reduced the level of interfering compounds, even in relatively large fish samples, sufficiently to facilitate the use of GPC at a later stage without overloading the column.

For analysis of the nor hydrocarbons, benzene was chosen for Soxhlet extraction of dried samples of both sediments and fish on account of its successful application to the extraction of polycyclic aromatic hydrocarbons from air particulates [14]. On the other hand, it was clear that the extraction of wet sediment samples with water-miscible solvents was superior (Table I). This is not necessarily true in all circumstances, however, as the use of tetrahydrofuran for Soxhlet extraction was almost twice as effective as that of benzene for the acidic components (chlorinated and unchlorinated dehydroabietic acids and octadecanoic acids), whereas it was clearly less effective for the hydrocarbons. Tetrahydrofuran, which was used advantageously with wet sediment samples, was not suitable for samples of biota, however, as this solvent simultaneously extracted large amounts of undesirable compounds, particularly lipids. Benzene extracts prepared from biota were therefore used throughout this investigation. Although extraction of 18-norabieta-8,11,13-triene and the corresponding dichloro compound from the sediment samples was most effectively accomplished with tetrahydrofuran, it should be emphasized that no single solvent or procedure is likely to be optimum for more than a limited structural range of compounds. Efforts to apply a single methodology to the analysis of a wide range of analytes may therefore encounter serious difficulties, although attempts using spiking to assess recovery have achieved moderate success [15].

The fact that only 18-norabieta-8,11,13-triene, and not the chlorinated analogues, was present in the effluent sample suggested that the chlorinated compounds were produced in the environment by stereospecific decarboxylation of the precursor 12,14-dichlorodehydroabietic acid, and that this reaction was probably mediated by anaerobic bacteria. This is consistent with the observation that the concentration of a compound tentatively identified as 18-norabieta-8,11,13-triene acid increased after anaerobic biological treatment [16]. Presumably the microbial reaction is stereospecific so that only the 18-nor compound, with the same configuration as dehydroabietic acid itself, is found in environmental samples in contrast to the radical decarboxylation used for chemical synthesis, which produced *ca.* 25% of the 19-nor epimer. The 18-nor configuration is also consistent with the structure of the fossil resin hydrocarbon fichtelite that has been established as 18-norabietane [17]. The situation with fish samples was more complex as the principal non-chlorinated nor hydrocarbon was the 19-nor epimer, whereas for the dichlorinated analogue the 18-nor epimer was dominant. Further speculation on the reasons for this is not merited on account of the interacting factors of uptake by fish and metabolism before or after ingestion.

The other compounds that were determined in the sediment samples were chosen to represent a range of alicyclic and aliphatic compounds known to be present in bleachery effluents, and therefore putatively present in contaminated sediments. Particularly for sample B, the concentration of 18-norabieta-8,11,13-triene was comparable to that of dehydroabietic acid itself, although the concentrations of 12,14-dichloro-18-norabieta-8,11,13-triene were lower than those of 12,14-dichlorodehydroabietic acid.

The quantification of the nor hydrocarbons in fish samples presented severe problems because a suitable surrogate standard was not avilable; cholestane clearly cannot be used in sterol-rich extracts from fish. On the other hand, the samples were sufficiently free from interfering impurities to permit unambiguous mass spectrometric identification. To avoid possible misinterpretation, it should be pointed out that the total concentrations of these chlorinated nor compounds found in the environmental samples were not sufficiently high for them to make a numerically significant contribution to the total organic chlorine in the samples, maximally comparable to that of the chlorinated dehydroabietic acids [3].

At present, no data on the toxicity of these neutral compounds is avilable, although their potential for significant bioconcentration is suggested by the estimated values of P_{ow} and hence BCF. The possi-

bility of bioconcentration is unequivocally supported by the identification of both 18(19)-norabieta-8,11,18-triene and the 12,14-dichloro-18-nor compound in muscle samples of fish captured from areas subject to contamination of bleachery effluents. As data on persistence and toxicity are not currently avaible, and require access to pure samples of both enantiomers, it is not possible to provide any estimate of the environmental hazard of these compounds. It is clear, however, that their concentrations in sediment samples are at least as significant as those of dehycroabietic acid and its chlorinated analogues.

A number of other neutral hydrocarbons were isolated for which no authentic samples were available and quantification was therefore not possible. The structures of these compounds were tentatively determined, however, by interpretation of their mass spectra and by comparison with some spectra published in the literature. The following groups of compounds were isolated.

(i) Monochloro compounds corresponding to 12,14-dichloro-18{19}-norabieta-8,11,13-triene were isolated from sediment samples (Fig. 7); these were presumably the 12- and 14-chloro compounds, although it could not be established in this study which isomer was present.

(ii) A range of non-chlorinated hydrocarbons was isolated from both fish and sediment samples and all of these are presumably derived from dehydroabietic acid; a hypothetical scheme illustrating their structural relation is given in Fig. 10.

(a) The totally reduced 18{19}-nor compound (**IV**) was isolated from sediment samples and on the basis of its mass spectrum (Fig. 8A) [18] was identified as fichtelite or its C-4 epimer.

(b) Compounds in which ring B is aromatic with loss of the methyl group at C-10 and decarboxylation of the carboxylic acid group from C-4 (**V**) were isolated both from sediment (mass spectrum in Fig. 8B) and fish samples (mass spectrum in Fig. 8C). These compounds have been isolated previously from sediment samples [19,20] and may plausibly be formed from the diterpenes which dominate such samples.

(c) The completely dehydrogenated compound retene (**VIII**) 1-methyl-7-(1-methylethyl)phenanthrene (mass spectrum in Fig. 9) was unambiguously identified in sediment samples.

(d) The dominant hydrocarbon in fish was a C_{18} compound (**VI**) corresponding to the loss of one additional methyl group from the 18{19}-norabietanes (mass spectrum in Fig. 8D).

(e) A C_{19} hydrocarbon isomeric with the 18{19}-norabietatrienes was isolated from fish samples (mass spectrum in Fig. 8E) and was tentatively assigned the structure **VII**.

In addition, two other groups of non-chlorinated hydrocarbons were isolated. Substantial amounts of compounds corresponding to reduction of the carboxyl group of abietic acid to methyl, and with unsaturation at various positions in rings B and C, were found in sediment samples. These presumably originate from pimaric acid; the mass spectra were identical with those published [21] although the structures appear not to have been unambiguously established. The sesquiterpene calamenen, which has already been identified in bleachery effluents [22], was a significant component in all sediment samples.

The chlorinated compounds identified in these investigations clearly arise from precursors formed during the production of pulp by conventional technologies using molecular chlorine. In a wider context, however, it should be appreciated that a structurally diverse range of non-chlorinated hydrocarbons including many of those isolated in this study are widely distributed in fossil wood, ambers, coal and amber [23] and in deep-sea sediments [19,20]. In all instances, these diterpene-related hydrocarbons originate from plant material during fossilization by a number of reactions, including decarboxylation, reduction and dehydrogenation. This appears, however, to be the first time such compounds have been isolated from biota. It should be clearly appreciated, however, that the chlorinated compounds are the result of industrial activity and that their ultimate fate will depend on the degree to which they may be dechlorinated, degraded or transformed by microbial reactions. In a wider perspective, it may be noted that 24-nor- and 28-nor-triterpenes which have been isolated from marine sediments [24] may be derived from plant triterpenoids by decarboxylation of the corresponding carboxylic acids by reactions formally analogous to those resulting in the formation of the 18-nor- and 19-nor-diterpenes.

CONCLUSIONS

The investigation has illuminated and provided further illustration of three important principles of environmental analysis.

(i) The value of effective clean-up procedures for the preparation of samples before attempted identification of new compounds in environmental samples, and the need for these to be adapted to the structure of the specific compounds to be examined. Even for the neutral hydrocarbons examined in this investigation, the combination of various types of chromatography was more flexible than use of drastic procedures involving, for example, strong acids and alkalis, and avoided the formation of artefacts resulting from chemical reactions with the analytes.

(ii) Access to synthetic reference compounds played an important role in this investigation by providing authentic samples for identification and making possible their quantification. In this case, identification required the use of both MS and NMR as the two epimers had identical mass spectra. The availability of the synthetic compounds also made possible the determination of their physico-chemical properties. A programme in synthetic chemistry was therefore a valuable adjunct to the purely analytical activity in this laboratory.

(iii) The application of the procedures to samples of sediment and fish resulted in the unambiguous identification of a novel group of chlorinated aromatic hydrocarbons which are presumably transformation products of compounds formed during production of bleached pulp by conventional procedures using molecular chlorine.

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Author Index Vols. 642 and 643

Abdel-Hamid, M. S., see Donnelly, J. R. 642(1993)409
Abrahamsson, K. and Ekdahl, A.
Gas chromatographic determination of halogenated organic compounds in water and sediment in the Skagerrak 643(1993)239
Ahmad, J., see Mohammad, A. 642(1993)445
Akimoto, H., see Yokouchi, Y. 642(1993)401
Alcaraz, J., see Zaromb, S. 643(1993)107
Alexander, R., see Fisher, S. J. 642(1993)205
Andersson, K., see Lindahl, R. 643(1993)35
Asakura, T., see Onodera, S. 642(1993)185
Ashbolt, N. J., see Nichols, P. D. 643(1993)189
Astier, A.
Chromatographic determination of volatile solvents and their metabolites in urine for monitoring occupational exposure (Review) 643(1993)389
Attard Barbini, D., see Di Muccio, A. 643(1993)363
Atwood, E. S., see Driscoll. J. N. 642(1993)435
Ausili, A., see Di Muccio, A. 643(1993)363
Bächmann, K., Haag, I., Prokop, T., Röder, A. and Wagner, P.
Chromatographic methods for the analysis of size-classified and individual raindrops 643(1993)181
Balinova, A.
Solid-phase extraction followed by high-performance liquid chromatographic analysis for monitoring herbicides in drinking water 643(1993)203
Bandow, H., see Yokouchi, Y. 642(1993)401
Barceló, D.
Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water (Review) 643(1993)117
Barker, S. A., see Walker, C. C. 642(1993)225
Barkley, D. J., Charbonneau, J. R., Chenier, M. J., Glasgow, C. C. and Graham, J. A.
Study of permanently coated columns for the high-performance liquid chromatographic determination of sulphur anions in environmental samples from metallurgical processes 642(1993)371
Barrefors, G. and Petersson, G.
Assessment of ambient volatile hydrocarbons from tobacco smoke and from vehicle emissions 643(1993)71
Bartle, K. D., see Janda, V. 642(1993)283
Beckett, R., see Murphy, D. M. 642(1993)459
Begum, T., see Rathore, H. S. 643(1993)271
Begum, T., see Rathore, H. S. 643(1993)321
Bianchi, A. P. and Varney, M. S.
Sampling and analysis of volatile organic compounds in estuarine air by gas chromatography and mass spectrometry 643(1992)11
Biggs, W. R., see Fetzer, J. C. 642(1993)319
Bonjour, R., see Frost, P. 643(1993)379
Brancaleoni, E., see Ciccioli, P. 643(1993)55
Brinkman, U. A. T., see Slobodnik, J. 642(1993)359
Brouwer, E. R., see Slobodnik, J. 642(1993)359
Burford, M. D., see Hawthorne, S. B. 642(1993)301
Burger, B. V. and Le Roux, M.
Trace determination of volatile organic compounds in water by enrichment in ultra-thick-film capillary traps and gas chromatography 642(1993)117
Butz, S. and Stan, H.-J.
Determination of chlorophenoxy and other acidic herbicide residues in ground water by capillary gas chromatography of their alkyl esters formed by rapid derivatization using various chloroformates 643(1993)227
Buzitis, J., see Krahn, M. M. 642(1993)15
Caixach, J., see Galceran, M. T. 643(1993)399
Camel, V., Tambuté, A. and Caude, M.
Analytical-scale supercritical fluid extraction: a promising technique for the determination of pollutants in environmental matrices (Review) 642(1993)263
Camenzind, R., see Frost, P. 643(1993)379
Castello, G. and Gerbino, T. C.
Analysis of polycyclic aromatic hydrocarbons with an ion-trap mass detector and comparison with other gas chromatographic and high-performance liquid chromatographic techniques 642(1993)351
Caude, M., see Camel, V. 642(1993)263
Cecinato, A., see Ciccioli, P. 643(1993)55
Cessna, A. J. and Kerr, L. A.
Use of an automated thermal desorption system for gas chromatographic analysis of the herbicides trifluralin and triallate in air samples 642(1993)417
Chan, S.-L., see Krahn, M. M. 642(1993)15
Charbonneau, J. R., see Barkley, D. J. 642(1993)371
Chenier, M. J., see Barkley, D. J. 642(1993)371
Chiba, M., see Singh, R. P. 643(1993)249
Churáček, J., see Ventura, K. 642(1993)379
Ciavatta, C. and Govi, M.
Use of insoluble polyvinylpyrrolidone and isoelectric focusing in the study of humic substances in soils and organic wastes (Review) 643(1993)261
Ciccioli, P., Brancaleoni, E., Cecinato, A., Sparapani, R. and Frattoni, M.
Identification and determination of biogenic and anthropogenic volatile organic compounds in forest areas of Northern and Southern Europe and a remote site of the Himalaya region by high-resolution gas chromatography–mass spectrometry 643(1993)55
Clifford, A. A., see Janda, V. 642(1993)283
Coates, J. A., see Davis, W. M. 643(1993)341
Colli, M., Zabarini, L., Melzi d'Eril, G. V. and Marchetti, R.
Evaluation of a modified Marcali technique with high-performance liquid chromatography–ultraviolet detection for the determination of 2,4-toluene diisocyanate in air 643(1993)51
Coquart, V., see Hennion, M.-C. 642(1993)211
Corasaniti, M. T. and Nisticò, G.
Determination of paraquat in rat brain by high-performance liquid chromatography 643(1993)419

Cserháti, T. and Forgács, E.
Separation of some chlorophenoxyacetic acid congeners on a porous graphitized carbon column 643(1993)331
Davis, W. M., Coates, J. A., Garcia, K. L., Signorella, L. L. and Delfino, J. J.
Efficient screening method for determining base/neutral and acidic semi-volatile organic priority pollutants in sediments 643(1993)341
De Jong, A. P. J. M., Liem, A. K. D. and Hoogerbrugge, R.
Study of polychlorinated dibenzodioxins and furans from municipal waste incinerator emissions in the Netherlands: analytical methods and levels in the environment and human food chain (Review) 643(1993)91
Delfino, J. J., see Davis, W. M. 643(1993)341
De Wilde, O., see Van der Poll, J. M. 643(1993)163
Di Corcia, A., Marchese, S. and Samperi, R.
Evaluation of graphitized carbon black as a selective adsorbent for extracting acidic organic compounds from water 642(1993)163
Di Corcia, A., Marchese, S. and Samperi, R.
Selective determination of phenols in water by a two-trap tandem extraction system followed by liquid chromatography 642(1993)175
Di Muccio, A., Dommarco, R., Attard Barbini, D., Santilio, A., Girolimetti, S., Ausili, A., Ventriglia, M., Generali, T. and Vergori, L.
Application of solid-phase partition cartridges in the determination of fungicide residues in vegetable samples 643(1993)363
Dommarco, R., see Di Muccio, A. 643(1993)363
Dong, M. W., see Grosser, Z. A. 642(1993)75
Donnelly, J. R., Abdel-Hamid, M. S., Jeter, J. L. and Gurka, D. F.
Application of gas chromatographic retention properties to the identification of environmental contaminants 642(1993)409
Doretti, L., see Sturaro, A. 643(1993)435
Dostál, M., see Ventura, K. 642(1993)379
Driscoll, J. N. and Atwood, E. S.
Application of gas chromatography with photoionization and electron-capture detectors for field screening of semi-volatiles in soil and water 642(1993)435
Duinker, J. C., see Kannan, N. 642(1993)425
Eckert-Tilotta, S., see Hawthorne, S. B. 642(1993)301
Ekdahl, A., see Abrahamsson, K. 643(1993)239
Elkins, J. W., see Sturges, W. T. 642(1993)123
Fatima, N., see Mohammad, A. 642(1993)445
Fetzer, J. C. and Biggs, W. R.
Use of full-spectrum absorbance and emission detectors in environmental analyses (Review) 642(1993)319
Fisher, S. J., Alexander, R. and Kagi, R. I.
Size-exclusion chromatography on zeolites in the trace analysis of polyaromatic hydrocarbons and organochlorine pesticides 642(1993)205
Font, G., see Mañes, J. 642(1993)195
Font, G., Mañes, J., Moltó, J. C. and Picó, Y.
Solid-phase extraction in multi-residue pesticide analysis of water (Review) 642(1993)135
Forgács, E., see Cserháti, T. 643(1993)331
Frattoni, M., see Ciccioli, P. 643(1993)55
Frost, P., Camenzind, R., Mägert, A., Bonjour, R. and Karlaganis, G.
Organic micropollutants in Swiss sewage sludge 643(1993)379
Fu, P. P., Zhang, Y., Mao, Y.-L., Von Tungeln, L. S., Kim, Y., Jung, H. and Jun, M.-J.
Relationships of structures of nitro-polycyclic aromatic hydrocarbons with high-performance liquid chromatography retention order 642(1993)107
Fukui, S., see Ogawa, S. 643(1993)221
Furton, K. G., Jolly, E. and Pentzke, G.
Recent advances in the analysis of polycyclic aromatic hydrocarbons and fullerenes (Reviews) 642(1993)33
Galceran, M. T., Santos, F. J., Caixach, J., Ventura, F. and Rivera, J.
Environmental analysis of polychlorinated terphenyls: distribution in shellfish from the Ebro Delta (Mediterranean) 643(1993)399
Garbarino, J. R., see Murphy, D. M. 642(1993)459
Garcia, K. L., see Davis, W. M. 643(1993)341
Generali, T., see Di Muccio, A. 643(1993)363
Gerbino, T. C., see Castello, G. 642(1993)351
Ghaoui, L.
Analysis of semivolatile organic compounds by headspace gas chromatography 642(1993)389
Girolimetti, S., see Di Muccio, A. 643(1993)363
Glasgow, C. C., see Barkley, D. J. 642(1993)371
Goto, S., see Kobayashi, H. 643(1993)197
Govi, M., see Ciavatta, C. 643(1993)261
Graham, J. A., see Barkley, D. J. 642(1993)371
Groenewegen, M. G. M., see Slobodnik, J. 642(1993)359
Grosser, Z. A., Ryan, J. F. and Dong, M. W.
Environmental chromatographic methods and regulations in the United States of America (Review) 642(1993)75
Gurka, D. F., see Donnelly, J. R. 642(1993)409
Haag, I., see Bächmann, K. 643(1993)181
Hanasaki, Y., see Ogawa, S. 643(1993)221
Hart, B. T., see Murphy, D. M. 642(1993)459
Hawthorne, S. B., Miller, D. J., Burford, M. D., Langenfeld, J. J., Eckert-Tilotta, S. and Louie, P. K.
Factors controlling quantitative supercritical fluid extraction of environmental samples 642(1993)301
He, D., see Lai, J. Y. K. 643(1993)77
Heftmann, E.
Preface 642(1993)1
Hennion, M.-C. and Coquart, V.
Comparison of reversed-phase extraction sorbents for the on-line trace enrichment of polar organic compounds in environmental aqueous samples 642(1993)211
Hirayama, N., see Onodera, S. 642(1993)185
Holdsworth, D. G., see Volkman, J. K. 643(1993)209
Hoogerbrugge, R., see De Jong, A. P. J. M. 643(1993)91
Hynning, P.-Å., Remberger, M., Neilson, A. H. and Stanley, P.
Identification and quantification of 18-nor- and 19-norditerpenes and their chlorinated analogues in samples of sediment and fish 643(1993)439
Janda, V., Bartle, K. D. and Clifford, A. A.
Supercritical fluid extraction in environmental analysis (Review) 642(1993)283

Jefferies, T. M., see Mills, A. G. 643(1993)409
Jeter, J. L., see Donnelly, J. R. 642(1993)409
Jimbo, Y., see Kobayashi, H. 643(1993)197
Jiminez-Conde, F., see Ling, C. F. 643(1993)351
Jolly, E., see Furton, K. G. 642(1993)33
Jun, M.-J., see Fu, P. P. 642(1993)107
Jung, H., see Fu, P. P. 642(1993)107
Kagi, R. I., see Fisher, S. J. 642(1993)205
Kaljurand, M., see Smit, H. C. 642(1993)53
Kamata, K., see Motohashi, N. 643(1993)1
Kami, H., see Ogawa, S. 643(1993)221
Kannan, N., Petrick, G., Schultz-Bull, D. E. and Duinker, J. C.
Chromatographic techniques in accurate analysis of chlorobiphenyls 642(1993)425
Kano, I., see Suzuki, T. 643(1993)173
Karlaganis, G., see Frost, P. 643(1993)379
Kerr, L. A., see Cessna, A. J. 642(1993)417
Khan, M. A. M., see Mohammad, A. 642(1993)455
Kim, Y., see Fu, P. P. 642(1993)107
Kita, H., see Ogawa, S. 643(1993)221
Kobayashi, H., Ohyama, K., Tomiyama, N., Jimbo, Y., Matano, O. and Goto, S.
Determination of pesticides in river water by gas chromatography–mass spectometry–selected-ion monitoring 643(1993)197
Kohno, T., see Kuwata, K. 643(1993)25
Kondo, M., see Yokoyama, Y. 643(1993)169
Kovačičová, J., see Tekel', J. 643(1993)291
Krahn, M. M., Ylitalo, G. M., Buzitis, J., Chan, S.-L. and Varanasi, U.
Rapid high-performance liquid chromatographic methods that screen for aromatic compounds in environmental samples (Review) 642(1993)15
Kuwata, K., see Tsujino, Y. 642(1993)383
Kuwata, K., Yamashita, Y., Nakashima, S., Nakato, Y., Kohno, T., Tanaka, S., Okumura, T. and Yamaguchi, Y.
Practical method for monitoring polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in the atmosphere 643(1993)25
Kuwatsuka, S., see Spitzer, T. 643(1993)305
Lai, J. Y. K. , Matisová, E., He, D., Singer, E. and Niki, H.
Evaluation of capillary gas chromatography for the measurement of C_2–C_{10} hydrocarbons in urban air samples for air pollution research 643(1993)77
Langenfeld, J. J., see Hawthorne, S. B. 642(1993)301
Latham, V., see Nichols, P. D. 643(1993)189
Lawson, D., see Zaromb, S. 643(1993)107
Leeming, R., see Nichols, P. D. 643(1993)189
Le Roux, M., see Burger, B. V. 642(1993)117
Lesage, S.
Methods for the analysis of hazardous wastes (Review) 642(1993)65
Levin, J.-O., see Lindahl, R. 643(1993)35
Liem, A. K. D., see De Jong, A. P. J. M. 643(1993)91
Lindahl, R., Levin, J.-O. and Andersson, K.
Determination of volatile amines in air by diffusive sampling, thiourea formation and high-performance liquid chromatography 643(1993)35
Lindner, W., see Seidel, V. 642(1993)253
Ling, C. F., Melian, G. P., Jiminez-Conde, F. and Revilla, E.
High-performance liquid chromatographic analysis of carbofuran residues in tomatoes grown in hydroponics 643(1993)351
Lingeman, H., see Slobodnik, J. 642(1993)359
Lott, H. M., see Walker, C. C. 642(1993)225
Louie, P. K., see Hawthorne, S. B. 642(1993)301
Mägert, A., see Frost, P. 643(1993)379
Majid Khan, M. A., see Mohammad, A. 642(1993)445
Mañes, J., see Font, G. 642(1993)135
Mañes, J., Font, G. and Picó, Y.
Evaluation of a solid-phase extraction system for determining pesticide residues in milk 642(1993)195
Mao, Y.-L., see Fu, P. P. 642(1993)107
Marchese, S., see Di Corcia, A. 642(1993)163
Marchese, S., see Di Corcia, A. 642(1993)175
Marchetti, R., see Colli, M. 643(1993)51
Matano, O., see Kobayashi, H. 643(1993)197
Matano, O., see Shiga, N. 643(1993)311
Matisová, E., see Lai, J. Y. K. 643(1993)77
May, W. E., see Wise, S. A. 642(1993)329
McGarvey, B. D.
High-performance liquid chromatographic methods for the determination of N-methylcarbamate pesticides in water, soil, plants and air (Review) 642(1993)89
Melian, G. P., see Ling, C. F. 643(1993)351
Melzi d'Eril, G. V., see Colli, M. 643(1993)51
Meyer, R., see Motohashi, N. 643(1993)1
Miller, D. J., see Hawthorne, S. B. 642(1993)301
Mills, A. G. and Jefferies, T. M.
Rapid isolation of polychlorinated biphenyls from milk by a combination of supercritical-fluid extraction and supercritical-fluid chromatography 643(1993)409
Moats, W. A., see Shaikh, B. 643(1993)369
Mohammad, A., Fatima, N., Ahmad, J. and Majid Khan, M. A.
Planar layer chromatography in the analysis of inorganic pollutants (Review) 642(1993)445
Mohammad, A. and Khan, M. A. M.
New surface-modified sorbent layer for the analysis of toxic metals in seawater and industrial wastewater 642(1993)455
Moltó, J. C., see Font, G. 642(1993)135
Motohashi, N., Kamata, K. and Meyer, R.
Chromatographic techniques used to determine benz[*c*]acridines in environmental samples (Review) 643(1993)1
Murakami, T., see Tsukioka, T. 642(1993)395
Murphy, D. M., Garbarino, J. R., Taylor, H. E., Hart, B. T. and Beckett, R.
Determination of size and element composition distributions of complex colloids by sedimentation field-flow fractionation–inductively coupled plasma mass spectrometry 642(1993)459
Nagatsuka, A., see Onodera, S. 642(1993)185
Nakashima, S., see Kuwata, K. 643(1993)25
Nakato, Y., see Kuwata, K. 643(1993)25
Neilson, A. H., see Hynning, P.-Å. 643(1993)439

Nguyen, V. D. and Rossbach, M.
Ion chromatographic investigation of brown algae (*Fucus vesiculosus*) of the German Environmental Specimen Bank 643(1993)427
Nichols, P. D., Leeming, R., Rayner, M. S., Latham, V., Ashbolt, N. J. and Turner, C.
Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia 643(1993)189
Niki, H., see Lai, J. Y. K. 643(1993)77
Nisticò, G., see Corasaniti, M. T. 643(1993)419
Noble, A.
Partition coefficients (*n*-octanol–water) for pesticides (Review) 642(1993)3
Norén, K. and Sjövall, J.
Liquid–gel partitioning and enrichment in the analysis of organochlorine contaminants (Review) 642(1993)243
Ogawa, S., Kita, H., Hanasaki, Y., Fukui, S. and Kami, H.
Determination of the potent mutagen 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX) in water by gas chromatography with electron-capture detection 643(1993)221
Ohyama, K., see Kobayashi, H. 643(1993)197
Okumura, T., see Kuwata, K. 643(1993)25
Onodera, S., Nagatsuka, A., Rokuhara, T., Asakura, T., Hirayama, N. and Suzuki, S.
Re-evaluation of solid-phase adsorption and desorption techniques for isolation of trace organic pollutants from chlorinated water 642(1993)185
Ozawa, H., see Tsukioka, T. 642(1993)395
Parvoli, G., see Sturaro, A. 643(1993)435
Pearson, K. S., see Simon, V. A. 643(1993)317
Pentzke, G., see Furton, K. G. 642(1993)33
Petersson, G., see Barrefors, G. 643(1993)71
Petrick, G., see Kannan, N. 642(1993)425
Picó, Y., see Font, G. 642(1993)135
Picó, Y., see Mañes, J. 642(1993)195
Prokop, T., see Bächmann, K. 643(1993)181
Rathore, H. S. and Begum, T.
Thin-layer chromatographic methods for use in pesticide residue analysis (Review) 643(1993)271
Rathore, H. S. and Begum, T.
Thin-layer chromatographic behaviour of carbamate pesticides and related compounds 643(1993)321
Rayner, M. S., see Nichols, P. D. 643(1993)189
Remberger, M., see Hynning, P.-Å. 643(1993)439
Revilla, E., see Ling, C. F. 643(1993)351
Richardson, D. E., see Volkman, J. K. 643(1993)209
Rivera, J., see Galceran, M. T. 643(1993)399
Röder, A., see Bächmann, K. 643(1993)181
Rokuhara, T., see Onodera, S. 642(1993)185
Rolfe, C., see Sherma, J. 643(1993)337
Rossbach, M., see Nguyen, V. D. 643(1993)427
Ryan, J. F., see Grosser, Z. A. 642(1993)75
Samperi, R., see Di Corcia, A. 642(1993)163
Samperi, R., see Di Corcia, A. 642(1993)175
Sánchez-Camazano, M. and Sánchez-Martín, M. J.
Mobility of cadmium as influenced by soil properties, studied by soil thin-layer chromatography 643(1993)357
Sánchez-Martín, M. J., see Sánchez-Camazano, M. 643(1993)357
Sander, L. C., see Wise, S. A. 642(1993)329
Santilio, A., see Di Muccio, A. 643(1993)363
Santos, F. J., see Galceran, M. T. 643(1993)399
Sato, H., see Yokoyama, Y. 643(1993)169
Schröder, H. F.
Pollutants in drinking water and waste water 643(1993)145
Schultz-Bull, D. E., see Kannan, N. 642(1993)425
Seidel, V., Tschernuter-Meixner, I. and Lindner, W.
Sandwich-type extraction column with on-line sulphuric acid treatment for the determination of organochlorine compounds in vegetable oil or oil seeds by gas chromatography with electron-capture detection 642(1993)253
Shaikh, B. and Moats, W. A.
Liquid chromatographic analysis of antibacterial drug residues in food products of animal origin (Review) 643(1993)369
Sherma, J. and Rolfe, C.
Determination of diflubenzuron residues in water by solid-phase extraction and quantitative high-performance thin-layer chromatography 643(1993)337
Shiga, N. and Matano, O.
High-performance liquid chromatographic method for the determination of oxolinic acid residues in crops 643(1993)311
Signorella, L. L., see Davis, W. M. 643(1993)341
Simon, V. A., Pearson, K. S. and Taylor, A.
Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high-performance liquid chromatography with the use of fluorescence detection and a single *o*-phthalaldehyde post-column reaction 643(1993)317
Singer, E., see Lai, J. Y. K. 643(1993)77
Singh, R. P. and Chiba, M.
Determination of benomyl and its degradation products by chromatographic methods in water, wettable powder formulations, and crops (Review) 643(1993)249
Sinkkonen, S.
Environmental analysis of chlorinated aromatic thioethers, sulphoxides and sulphones (Review) 642(1993)47
Sjövall, J., see Norén, K. 642(1993)243
Slobodnik, J., Groenewegen, M. G. M., Brouwer, E. R., Lingeman, H. and Brinkman, U. A. T.
Fully automated multi-residue method for trace level monitoring of polar pesticides by liquid chromatography 642(1993)359
Smit, H. C. and Kaljurand, M.
Different approaches for improving the precision in chromatographic analysis of environmental samples by optimum signal processing and correlation techniques (Review) 642(1993)53
Sparapani, R., see Ciccioli, P. 643(1993)55
Spitzer, T.
Selective clean-up for polynuclear aromatic compounds in airborne particles and soil 643(1993)43

Spitzer, T. and Kuwatsuka, S.
Residue levels of polynuclear aromatic compounds in urban surface soil from Japan 643(1993)305
Stan, H.-J., see Butz, S. 643(1993)227
Stanley, P., see Hynning, P.-Å. 643(1993)439
Sturaro, A., Parvoli, G. and Doretti, L.
Plane tree bark as a passive sampler of polycyclic aromatic hydrocarbons in an urban environment 643(1993)435
Sturges, W. T. and Elkins, J. W.
Use of adsorbents to collect selected halocarbons and hydrohalocarbons of environmental interest from large air volumes 642(1993)123
Suzuki, S., see Onodera, S. 642(1993)185
Suzuki, T., Yaguchi, K. and Kano, I.
Screening methods for asulam, oxine-copper and thiram in water by high-performance liquid chromatography after enrichment with a minicolumn 643(1993)173
Tambuté, A., see Camel, V. 642(1993)263
Tanaka, S., see Kuwata, K. 643(1993)25
Taylor, A., see Simon, V. A. 643(1993)317
Taylor, H. E., see Murphy, D. M. 642(1993)459
Tekel', J. and Kovačičová, J.
Chromatographic methods in the determination of herbicide residues in crops, food and environmental samples (Review) 643(1993)291
Tomiyama, N., see Kobayashi, H. 643(1993)197
Tschernuter-Meixner, I., see Seidel, V. 642(1993)253
Tsujino, Y. and Kuwata, K.
Sensitive flame ionization detector for the determination of traces of atmospheric hydrocarbons by capillary column gas chromatography 642(1993)383
Tsukioka, T., Ozawa, H. and Murakami, T.
Gas chromatographic–mass spectrometric determination of lower aliphatic tertiary amines in environmental samples 642(1993)395
Turner, C., see Nichols, P. D. 643(1993)189
Van der Poll, J. M., Versluis-de Haan, G. G. and De Wilde, O.
Determination of ethylenethiourea in water samples by gas chromatography with alkali flame ionization detection and mass spectrometric confirmation 643(1993)163
Varanasi, U., see Krahn, M. M. 642(1993)15
Varney, M. S., see Bianchi, A. P. 643(1992)11
Ventriglia, M., see Di Muccio, A. 643(1993)363
Ventura, F., see Galceran, M. T. 643(1993)399
Ventura, K., Dostál, M. and Churáček, J.
Retention characteristics of some volatile compounds on Tenax GR 642(1993)379
Vergori, L., see Di Muccio, A. 643(1993)363
Versluis-de Haan, G. G., see Van der Poll, J. M. 643(1993)163
Volkman, J. K., Holdsworth, D. G. and Richardson, D. E.
Determination of resin acids by gas chromatography and high-performance liquid chromatography in paper mill effluent, river waters and sediments from the upper Derwent Estuary, Tasmania 643(1993)209
Von Tungeln, L. S., see Fu, P. P. 642(1993)107
Wagner, P., see Bächmann, K. 643(1993)181
Walker, C. C., Lott, H. M. and Barker, S. A.
Matrix solid-phase dispersion extraction and the analysis of drugs and environmental pollutants in aquatic species (Review) 642(1993)225
Wise, S. A., Sander, L. C. and May, W. E.
Determination of polycyclic aromatic hydrocarbons by liquid chromatography 642(1993)329
Woo, C. S., see Zaromb, S. 643(1993)107
Yaguchi, K., see Suzuki, T. 643(1993)173
Yamaguchi, Y., see Kuwata, K. 643(1993)25
Yamashita, Y., see Kuwata, K. 643(1993)25
Ylitalo, G. M., see Krahn, M. M. 642(1993)15
Yokouchi, Y., Bandow, H. and Akimoto, H.
Development of automated gas chromatographic–mass spectrometric analysis for natural volatile organic compounds in the atmosphere 642(1993)401
Yokoyama, Y., Kondo, M. and Sato, H.
Determination of alkylbenzenesulphonates in environmental water by anion-exchange chromatography 643(1993)169
Zabarini, L., see Colli, M. 643(1993)51
Zaromb, S., Alcaraz, J., Lawson, D. and Woo, C. S.
Detection of airborne cocaine and heroin by high-throughput liquid-absorption preconcentration and liquid chromatography–electrochemical detection 643(1993)107
Zhang, Y., see Fu, P. P. 642(1993)107

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Journal of Chromatography and *Journal of Chromatography, Biomedical Applications*

MONTH	1992	J	F	M	A	M	J	J	
Journal of Chromatography	Vols. 623–627	628/1 628/2 629/1 629/2	630/1+2 631/1+2 632/1+2 633/1+2	634/1 634/2	635/1 635/2 636/1 636/2	637/1 637/2 638/1 638/2	639/1 639/2 640/1+2	641/1 641/2 642/1+2 643/1+2 644/1	The publication schedule for further issues will be published later
Cumulative Indexes, Vols. 601–650									
Bibliography Section				649/1			649/2		
Biomedical Applications		612/1	612/2	613/1	613/2 614/1	614/2 615/1	615/2 616/1	616/2 617/1	

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