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# CHROMATOGRAPHIC REVIEWS (Vol. 20, No. 3)

edited by

Michael Lederer

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# CONTENTS



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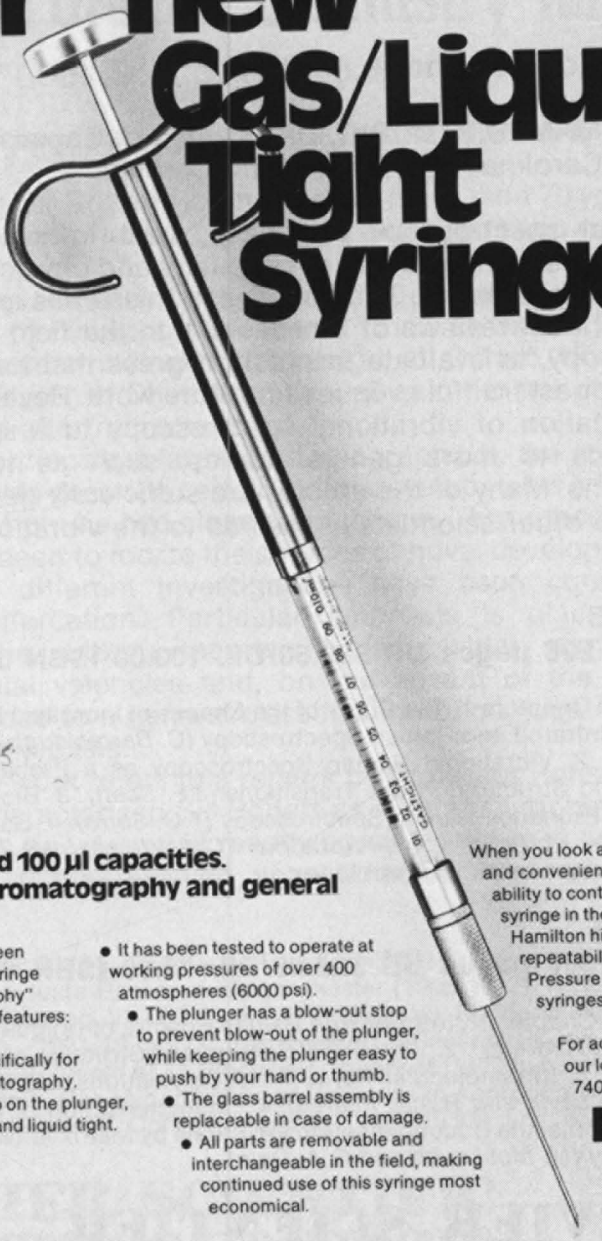

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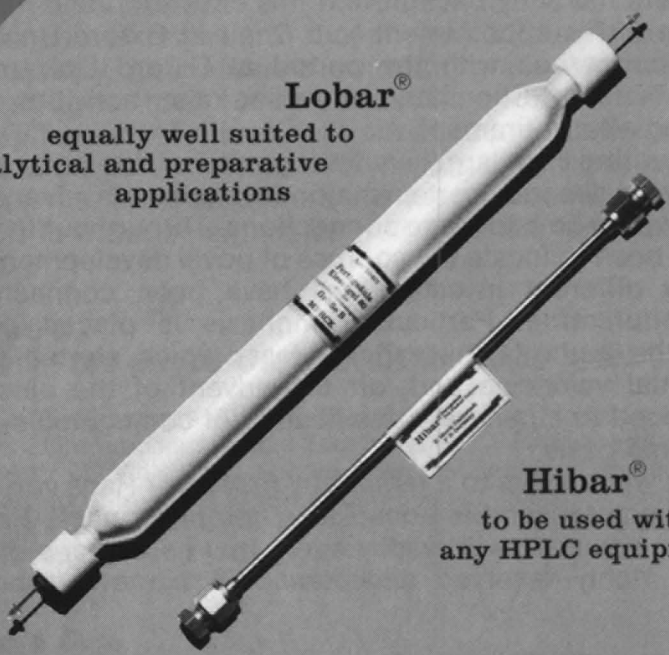

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CHREV. 96

# GAS CHROMATOGRAPHY OF $^{3}H$- AND $^{14}C$-LABELLED COMPOUNDS

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(Received September 7th, 1976)

CONTENTS



## 1. INTRODUCTION

The application and production of labelled compounds are nowadays closely related to the use of chromatographic methods. Gas chromatography (GC) and the preparation and application of labelled compounds are relatively new fields of research; the first basic paper on GC appeared in 1952[185], while the preparation of labelled

compounds began seriously after the second world war, although tritium and carbon-14 were discovered in the 1930s and their convenience for biochemical investigations was recognized at that time[124,197]. GC has contributed to modern research as a result of its separation efficiency and high sensitivity, while the use of labelled organic compounds has made possible investigations of transformations of substances during such complicated biochemical processes as photosynthesis. GC was used for the first time in the analysis of labelled compounds ($^{14}$C-labelled hydrocarbons) in 1955 by Kokes *et al.*[223], but soon afterwards many papers appeared on "hot-atom" chemistry and on biochemical research, accompanied by many others on methodological developments. The advantages of "radio-gas chromatography" (RGC), the GC of radioactive substances combined with a radioassay, have been described in more than 1000 papers on applications in the fields of biochemistry, clinical biochemistry, organic chemistry, chemical processing, "hot-atom" chemistry and the production of labelled compounds. This review attempts to give a complete survey of RGC methods used for analysis of $^{3}$H- and $^{14}$C-labelled compounds; special attention is paid to the chemical problems involved in RGC. Methods of detection of radioactivity form the main part of the review, and types of records and their evaluation and a survey of important applications of RGC are included.

## 2. GENERAL

### *2.1. Labelled compounds and their analysis*

Organic molecules in which one of the atoms, larger structural units or all of the atoms are substituted partially or wholly by radioactive isotopes are called labelled compounds (the use of stable isotopes, because other types of detection are involved, will not be considered here). The methods used for their preparation[71,124,130], the incorporation of radioactive isotopes by chemical synthesis, biosynthesis[71,389], chemical exchange or by physical methods, determine the type of labelling: specific, general (non-specific, random) or uniform. The physical properties of $^{14}$C and $^{3}$H are similar: their long half-lives are more advantageous than the low energy of the beta-radiation (Table 1). The low energy of the beta-particles emitted by $^{14}$C and $^{3}$H has led to the same or a similar manner of detection of the radionuclides themselves and also in the GC of their compounds.

In recent years, increasing attention has been paid to the analysis of labelled compounds[71–73,124,160,262,343], including the determination of total activity, radionuclidic, radiochemical and chemical purity, specific activity (from the content of the labelled compound) and, in certain instances, the pattern of labelling[73,346]. Chroma-

TABLE 1

BASIC RADIOCHEMICAL CHARACTERISTICS OF CARBON-14 AND TRITIUM

| *Isotope* | *Main nuclear production process* | *Half-life (years)* | *Max. β-energy (keV)* | *Max. attainable specific activity (mCi/milliatom)* |
|---|---|---|---|---|
| $^{14}$C | $^{14}$N(n,p)$^{14}$C | 5730 | 159 | 62.6 |
| $^{3}$H | $^{6}$Li(n,α)$^{3}$H | 12.35 | 18 | 29,120 |

tographic methods play a particularly important role in checking purity[72,160,262,274], they are often suitable for the determination of contents by using a convenient mass detector and, in connection with degradation methods, they are sometimes appropriate for the control of the pattern of labelling. Another major use of RGC is in chromatographic methods for the separation of individual species in practical applications of labelled compounds, the distribution of radioactivity after chemical, biochemical or nuclear reactions being followed.

### *2.2. Gas chromatography of labelled compounds*

The role of GC in the analysis of compounds labelled with $^{14}C$ and $^{3}H$ has been surveyed several years ago[72,152,262]. GC is virtually the only convenient method for the analysis of radioactive gases and volatile substances such as hydrocarbons, but the development of derivatization methods has introduced applications for polar substances, including carboxylic acids, steroids, sugars, nucleotides and amino acid enantiomers. In connection with chemical reactions and pyrolysis, GC can also be helpful in questions of specificity of labelling[107,110,191,346,391]. The recently developed liquid chromatography and isotachophoresis have overlapped the applications of some GC methods, but there is one property of GC that can hardly be matched, namely its sensitivity in mass detection, and in many instances also in activity measurements because of the high counting efficiency of the weak $\beta$-emitters $^{3}H$ and $^{14}C$ in the gas phase.

The application of GC to the analysis of labelled compounds enables several types of information to be obtained at the same time: it is possible to identify the compounds and to determine their content, specific activity and radiochemical and chemical purity. RGC was often used only for qualitative or semi-quantitative work in metabolic studies for tracing pathways via the label, and applications of RGC for quantitative purposes in chemical processing and in investigations of chemical and recoil reactions are well known. Suppliers of labelled compounds use RGC during production processes and in the final quantitative analysis of preparations. Relatively few papers have described all aspects of quantitative work and little attention has been paid to chemical problems in RGC.

The technique of RGC and its range of applications have been reviewed many times[5,51,83,90,108,168,182,204,205,208,210,246,270,279,292,321,326,337,345,385–387,401,414], and applications of RGC for special purposes such as in lipid analysis[80,116,192,228,275,356], "hot-atom" chemistry[6,421] and the analysis of pesticides[244] and organometallic compounds[388] have also been surveyed.

### *2.3. Methods used in radio-gas chromatography*

The methods used to monitor the effluent stream from a GC column for radioactivity can be divided into two main groups: those which use the intermittent trapping of the effluent and subsequent separate measurement of the radioactivity of the fractions and those which measure the radioactivity continuously by means of a flow-through detector. A survey of systems used in RGC is given in Table 2.

The simplest and probably the most commonly used approach to assaying the radioactivity of labelled compounds separated by GC is a combination of a trapping

TABLE 2

TYPICAL SYSTEMS USED FOR THE RGC OF $^{3}H$- AND $^{14}C$-LABELLED COMPOUNDS

| *No.* | *Part* | *Reference* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *223 (1955)* | *186 (1956)* | *418 (1957)* | *302 (1959)* | *54 (1960)* | *187 (1951)* | *211 (1962)* | *108 (1953)* | *372 (1966)* | *347 (1965)* | *251 (1968)* | *336 (1973)* | *8 (1974)* |
| 1 | Column outlet* | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | Splitter* | – | – | – | – | – | – | + | – | + | + | – | + | + |
| 3 | Mass detector* | TCD | GDB | TCD | GDB | TCD | TCD | AID | TCD | FID | FID | TCD | FID | FID |
| 4 | Effluent conversion* | – | – | – | – | CMB | CMB | CMB | HC | CMB | HC | CMB | – | – |
| 5 | Counting* | GM | TR | IC | SC | IC | PC | SC | PC | PC | PC | PC | PC | SC + TR |
| | Flow scheme** | 1,3,5 | 1,3,5 | 1,3,5 | 1,3,5 | 1,4,5,3 | 1,4,5,3 | $1,2<^{3}_{4,5}$ | 1,3,4,5 | $1,2<^{3}_{4,5}$ | $1,2<^{3}_{4,5}$ | 1,3,4,5 | $1,2<^{3}_{4,5}$ | $1,2<^{3}_{5}$ |
| | Used for | $^{14}C$ | $^{14}C$ | $^{3}H$ | $^{14}C$ | $^{3}H$, $^{14}C$ | $^{3}H$, $^{14}C$ | $^{3}H$, $^{14}C$ | $^{14}C$ | $^{3}H$, $^{14}C$ | $^{3}H$, $^{14}C$ | $^{14}C$ | $^{14}C$ | $^{3}H$, $^{14}C$ |

* Abbreviations: + = used; – = avoided; TCD = thermal conductivity detector or microthermistor; FID = flame-ionization detector; GDB = gas-density balance; AID = argon ionization detector; CMB = combustion; GM = Geiger–Müller tube; HC = hydrocracking; IC = ionization chamber; SC = scintillator; PC = proportional counter; TR = trapping.

** The code system used is directly connected with the numbers in the first column of the table.

procedure for the collection of the separated components and subsequent counting of the collected fractions. In the first application of this discontinuous method for labelled compounds, described by James *et al.*[186], $^{14}C$-labelled fatty acid methyl esters were detected with a gas-density balance detector and then collected in tubes filled with cotton-wool moistened with methanol, but "off-line" detection has also been used for non-radioactive substances in connection with other types of detection, such as mass spectrometry[12,70,95,203] and ultraviolet[417], infrared[48,87,88,221,393,417] and nuclear magnetic resonance[393] spectroscopy; a review[240] has also been published.

There are two principial modes of fraction collection: (1) the use of an automatic fraction collector (*e.g.*, a Packard Model 830) permits trapping at regular intervals; (2) when the mass detector indicates the appearance of a desired substance, the trap is inserted and the labelled compound collected. Mass detectors include non-destructive thermal conductivity detectors (TCD), argon ionization detectors (AID) and gas-density balances (GDB) and, for the smaller portion of a split effluent, flame-ionization detectors (FID). The use of radioactivity detectors for the preparative GC of $^{14}C$-labelled compounds has been reported[397]. The often discussed risk of not detecting of substances of high specific activity when using a less sensitive mass detector[205,208,213,401] seems to be more of theoretical than of practical value, and the problem studied by means of RGC is usually known to some extent. The reported[251] sensitivity of a microthermistor of about 100 ng is sufficient for $^{14}C$-labelled substances in many instances, while $^{3}H$-labelled compounds of high specific activity (1–25 Ci/mmole or higher) can usually be detected only with a radioactivity detector (sub-nanogram sample sizes). The convenience of the discontinuous method for low total and low specific activities is apparent.

Many systems have been described for the continuous monitoring of radioactivity in the effluent. Early approaches involved systems with mass and heated radioactivity detectors in series[223,281,312,418]; one of these systems[281] is demonstrated in Fig. 1. They are used nowadays only in exceptional circumstances, when the counting efficiency is not influenced by the composition of the substance passing through the counter[66]. This factor, applicable to flow-through proportional counters[108,233,238], ionization chambers[212] and liquid scintillation instruments[345], led to the development

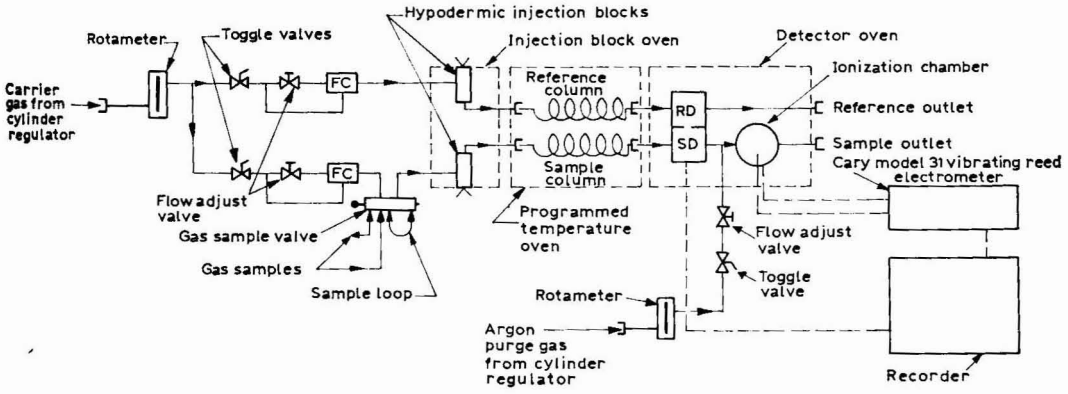



Fig. 1. Flow diagram of RGC system using heated ionization chamber. Reproduced from *Anal. Chem.*, 35 (1963) 1576 (ref. 281).

of more sophisticated systems involving combustion[54,133,142,167,187], as used previously in non-radioactive GC[150,250] and hydrocracking, applied by Zlatkis and Ridgway[431] for detection with TCD. Combustion of the effluent also permits the absorption of $^{14}CO_2$ in ethanolamine[40,122,163], hyamine[170,390] or sodium hydroxide solution[84,91,92] for discontinuous counting. Most flow-through systems use the more sensitive FID after splitting of the effluent (the AID has low linearity). The larger part (usually 80–90%) is led into the radioactive branch. The use of an additive gas for the accurate functioning of the splitter has been reported[211]; because of the non-stability of the splitter operation[135], the exact splitting achieved was determined by comparison of the mass responses of the runs with and without the splitter[372]. Several applications of the FID as a combustion element have been described[91,92,135,310]; in this way, the use of a splitter is avoided. The electron-capture detector (ECD) has probably not yet been used in RGC.

Another possible combination in RGC is absorption of the effluent in a flowing liquid scintillator[336] (Fig. 2), which permits the collection of fractions. Several further methods combine flow-through and "off-line" detection: interrupted-elution GC in conjunction with a static ionization chamber has been reported[62,63,65]; radioactive substances issuing from a column have been collected on a moving strip of paper[429] for TLC plates[100a] for subsequent radioassay[429]; and "buffer storage" (absorption of $^{14}CO_2$ in a flow of sodium hydroxide solution, which is then led through PTFE tubing, the combusted effluent is divided into sections by gas bubbles and these fractions are stored for counting) has been used[209,214]. All of the combined methods increase the time available for counting and enhance the precision and sensitivity of the radioassay, which are the advantages of discontinuous methods.

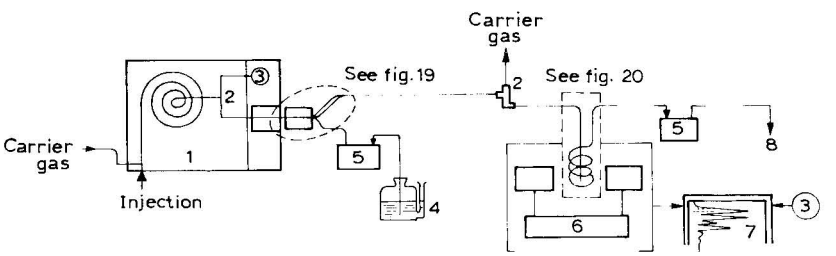



Fig. 2. Scheme of RGC system with continuous detection of radioactivity by liquid scintillation. 1 = oven of gas chromatograph; 2 = splitter; 3 = flame-ionization detector; 4 = container with scintillator solution; 5 = peristaltic pump; 6 = liquid scintillation spectrometer; 7 = two-pen recorder; 8 = fraction collector. Reproduced from *J. Chromatogr.*, 76 (1973) 14 (ref. 336).

It is obvious that many methods are possible in RGC. Those given in Table 2 are selected methods from existing combinations, depending on the type of problem to be solved, on the experience of the analyst and on the instrumental capabilities.

## 3. CHEMICAL PROBLEMS

### *3.1. Preparation of the sample for radio-gas chromatography*

Only a few applications of RGC do not require prior preparation of the sample,

which can be directly injected; examples are the analysis of radioactive gases ($H_2$, CO, $CO_2$ (refs. 104 and 105), $CH_4$, $C_2H_2$, $C_2H_4$ (ref. 3), etc.), hydrocarbons[142] and other volatile substances that do not contain non-volatile components. If the latter are present they are retained on the column and can interact with the solutes, thus changing the retention characteristics[389a]. Also in the preparation of polar substances for GC by derivatization is it desirable and advantageous to remove substances that do not form volatile derivatives and to prevent the column packing from contamination, *e.g.*, by sugars from lipid extracts of algae and their decomposition products after methylation in the case of the analysis of higher fatty acids[260] (vacuum sublimation of methyl esters). The purification step is often accomplished by liquid chromatography[194a] or thin-layer chromatography. The sample clean-up prevents unknown radioactivity or background enhancement being recorded.

Many reviews on derivatization in GC[47a,113,311a], silylation[266], GC of amino acids[176] and fatty acids[228,80], etc., have appeared. The following summarized criteria for the derivatization step were given[139a]: (1) the derivative should be formed simply, without rearrangement or structural changes; (2) the derivatization reaction should go 95–100% to completion; (3) the derivative should have a suitable volatility, retention time and ability for concentration; (4) the derivative must be stable with respect to time, temperature and column packing. The yield of the reaction is a critical point, depending on the purpose of the analysis, *e.g.*, for checking radiochemical and chemical purity a 100% yield is desirable and no side-reactions should occur[255]. Obviously, it is not easy to satisfy all of these requirements in every case.

Control of the derivatization by means of thin-layer chromatography was useful in the RGC of $^{14}C$-labelled fatty acid methyl esters and amino acids[259] (Fig. 3). The example of the RGC of $^{14}C$-labelled amino acids demonstrates the problems of derivatization. Side-reactions in the formation of trifluoroacetylated *n*-butyl esters[309a], especially of carrier-free preparations, were caused by impurities (probably aldehydes) at concentrations of *ca.* $10^{-3}$% and led to "radiochemical impurities" of 20–30% (Fig. 4) in spite of a good reagent blank, proposed as a control of contamination[306a]. The extraneous peaks could be suppressed by using larger amounts of car-

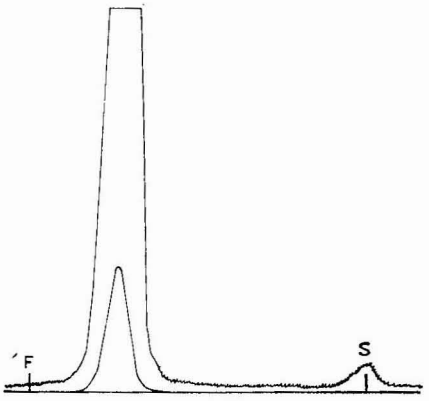



Fig. 3. Thin-layer chromatogram of [$^{14}C$]alanine as bis(chlorodifluoromethyl)-1,3-oxazolidinon-5-one. Solvent, chloroform; plate, Silufol; scanner sensitivities, 300 and 3000 c.p.s. From Matucha[255].

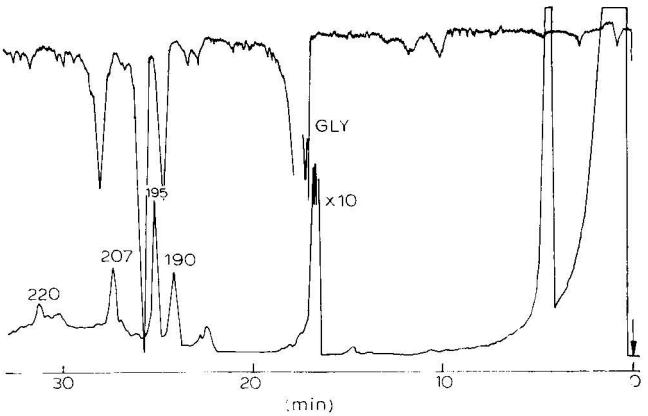



Fig. 4. Radio-gas chromatogram of carrier-free preparation of [$^{14}$C]glycine after butylation and acylation[309a]. The main peak corresponds to N-TFA-butyl ester. Programmed-temperature GC on OV-225 column. From Matucha[255].

rier[255]. Correct results were obtained after the choice of an appropriate derivative (Fig. 5).

Attention should be paid to the choice of solvents and derivatization reagents; their volatility should be markedly higher than that of the derivatives, as the tailing influences the evaluation of the mass record and could make reagent and solvent venting, sparing copper oxide in the combustion unit[251], impossible.

Historically, RGC is closely connected with reaction gas chromatography (loosely defined as a combination of chemical reactions with GC). Kokes *et al.*[223] investigated the hydrocracking of hydrocarbons mixed with [$^{14}$C]ethylene on a pre-column with various catalysts. The chemical reaction can be carried out at any of four positions: ahead of the injection port, in a pre-column reactor, within the chromatographic column or in a post-column reactor. Reaction GC involves pyrolysis, hydrogenation, subtraction, esterification, silylation, saponification and other reac-

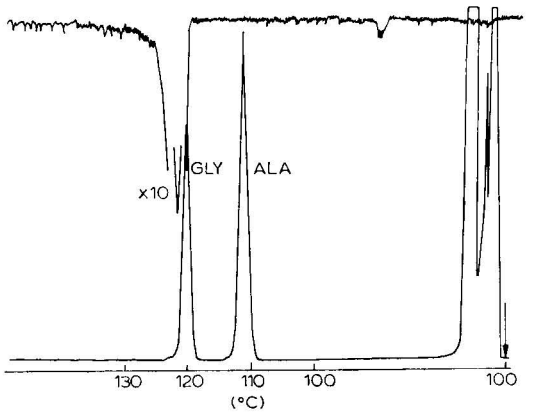



Fig. 5. Radio-gas chromatogram of carrier-free preparation of [$^{14}$C]glycine in the form of bis(chlorodifluoromethyl)-1,3-oxazolidinon-5-one. Non-radioactive alanine added as internal standard. Programmed-temperature GC on OV-225 column. From Matucha[255].

tions. This technique can help in many difficult separations and identifications as well as in establishing structures. For the GC of labelled compounds, interesting applications of the pyrolysis of [$^{3}H$]hexene-1 and [$^{14}C$]hexene-1[342], nucleosides and nucleotides[394,395], degradation of fatty acids[276] and investigation of the degree of uniformity of labelling[191] have been reported and the work of Drawert and co-workers[107,110,391] is of interest.

### *3.2. Reactions in the gas chromatographic system*

One of the potential errors in RGC and GC in general are losses due to retention or decomposition of the solute in the chromatographic system, which can be divided into three parts: the vaporizer, chromatographic column and detector. The breakdown of N-TFA-*n*-butyl esters of amino acids occurred in a heated metal injection port[231a,360a]. Injection-port reactions in the analysis of alkylsilyl derivatives of nucleosides have been described[305], and some trimethylsilyl (TMS) derivatives were decomposed on column[170,354a] or by metal parts of the apparatus[139b,216a,278a,343a]. Therefore, on-column injections and all-glass systems can be recommended. Attention must be paid to the carrier gas, as trace amounts of oxygen and moisture can be sources of errors[228,389a]; the breakdown of TMS-histidine by moisture in a freshly installed septum has been reported[354b].

Interactions with column packings have been reported many times; the stationary phase selected should not be capable of reacting with the compounds being analyzed, and impurities and breakdown products must be also considered. Reactions with solutes, if complete, result in the disappearance of peaks, which may remain unnoticed. If the reaction is slow, a poorly shaped peak can be obtained. Impurities in the sample can also cause reactions of the solute[389a].

Meinertz and Dole[261] estimated the pattern of dispersion of chromatographically pure methyl [$^{14}C$]palmitate; in Apiezon and EGA columns the distribution of activity was not Gaussian and the background remained elevated for several days. The results indicated that a pure substance emerges from a column over a wider interval than is shown from the record of the mass output. Tailing of fatty acid methyl esters was also observed on an SE-30 column[153]. Chemical reactions may occur during passage through a GC system; dehydration of [2-$^{14}C$]-2-methyl-2-undecanol and isomerization of [$^{14}C$]methylenecyclohexane to [$^{14}C$]methylcyclohexene have been reported[147]. Jansen and Baglan[190] followed the recovery of silylated $^{14}C$-labelled glucose, fructose, sucrose, glycerol, cholesterol and stearyl alcohol, which was considerably less than quantitative. The recoveries from Carbowax and SF 96-50 columns of 10–80% were reproducible also after re-chromatography, and the retention of TMS-glucose was significantly higher at the injection end of the column. On the contrary, $^{14}C$-labelled TMS-pyrimidine bases were found to be more stable[308]; the retention on the column was not determined, but the overall recovery of TMS-uracil and TMS-thymine was found to be higher than 96% when a sufficient excess of silylating reagent was used. Errors in the GC analysis of polyunsaturated fatty acids[228] and errors due to trans-esterifications of fatty acid esters on columns with polyester stationary phases[293], etc., are well known. Only chemical inertness of the whole chromatographic system, particularly of the column packing, can lead to sufficiently high recoveries, which should be independent of the sample size.

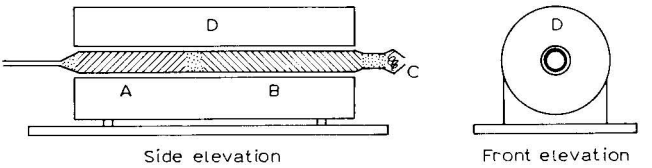



Fig. 6. Combustion tube assembly for conversion of column effluent. A = Copper oxide powder; B = iron fillings; C = spherical joint; D = furnace. Reproduced from *Anal. Chem.*, 35 (1963) 516 (ref. 188).

### *3.3. Conversion of the effluent for counting*

The decrease in sensitivity of a TCD with increasing temperature and the possibility of condensation of substances with high boiling points led to a new approach in GC, namely conversion of the GC effluent into carbon dioxide by combustion[150,250] (used in elemental analysis[295]) or by hydrocracking[431]. Both methods have been used succesfully in RGC; they avoid the difficulties caused by changes in the composition of the gas being counted in radioactivity detection.

The combustion method was applied for the first time by Cacace and co-workers[49,58,60,64] and subsequently by James and Piper[187,188]. They used copper oxide and, in the same tube, converted water of combustion into hydrogen with iron filings or steel-wool. A scheme of the furnace is shown in Fig. 6. Hydrogen is suitable for mass detection by TCD[188] and also for monitoring tritium[47]. The combustion is performed at 650–800°, but the higher temperatures are not convenient because of reduc-

TABLE 3

HYDROCRACKING CATALYSTS USED FOR EFFLUENT CONVERSION

| *Catalyst composition** | *Operating temperature (°C)* | *Compounds converted* | *Ref.* |
|---|---|---|---|
| Raney nickel | 420 | Esters of lower carboxylic and linoleic acids, alcohols, $CO_2$ ($^{14}C$ labelled) | 108, 112, 161 |
| Zn–CoO–NiO–Fe–$V_2O_5$–St (60:40:20:140:10:27) | 620–640 | Hydrocarbons, esters of carboxylic acids, bromo, iodo and nitro derivatives, TMS-carbohydrates ($^{3}H$ and $^{14}C$ labelled) | 347 |
| Zn–CoO–NiO–Fe–$V_2O_5$–Ch (49:16:13:114:8:22) | 660 | Fatty acid methyl esters ($^{3}H$ and $^{14}C$ labelled) | 35 |
| Zn–CoO–G69–$V_2O_5$–St (30:20:20:5:8) | 600 | Benzene, toluene, butanol, methyl benzoate, acetone, dioxan (non-radioactive) | 398 |
| Zn–CoO–G69–$V_2O_5$–NiO–St (25:20:20:5:8:7) | 600 | Benzene, toluene, butanol, methyl benzoate, acetone, dioxan (non-radioactive) | 398 |
| G69–Ch (1:3) | 500–700 | Fatty acid methyl esters, hexadecane, steroids ($^{3}H$ and $^{14}C$ labelled) | 225 |

* Abbreviations: St = sterchamol; Ch = Chromosorb; G69 = zirconium-activated nickel catalyst G69, Girdler Südchemie, Munich, G.F.R.

tion of $CO_2$ and reaction of CuO with quartz; thus 720° was recommended as the optimal temperature[212]. Oxidation of iron caused "memory" effects with tritium (adsorption of $^3H_2O$)[188,212], but the addition of hydrogen gas in the iron part of the conversion tube can eliminate this effect[212]. An auxiliary column for $H_2$–$CO_2$ separation[54,55] or adsorption of water or of $CO_2$ (refs. 188 and 372) for distinguishing between $^3H$ and $^{14}C$ was used. The combustion method was used in most instances for the RGC of $^{14}C$-labelled compounds with flowthrough radioactivity detection[108,112,211,212,225,251,372]; $Co_3O_4$ (refs. 101 and 162) and an FID as the combustion chamber[91,92,135,310] have also been applied.

Hydrocracking of organic compounds is widely used in petrochemical processes. Nickel, iron, cobalt and other elements are generally used for hydrogenation, and oxides of aluminium, silicon, chromium and other compounds for cracking. This approach has often been reported in RGC[35,108,112,225,347,398]; its main advantage is its general applicability to both $^3H$- and $^{14}C$-labelled compounds. Exchange of the catalyst after 60–80 h of operation is recommended[347], so that the application of the catalytic procedure is not so significant an advantage. The temperature for each catalyst and class of compounds to be converted must be tested. A survey of catalysts used in the RGC of labelled compounds is given in Table 3. The hydrocracking reaction (and dead volumes) influences the peak shape (Fig. 7). Hydrocracking is recommended for smaller molecules, combustion for larger ones[398].

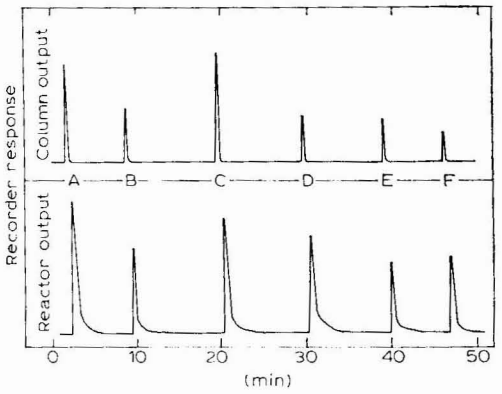



Fig. 7. Influence of the conversion of the effluent on the peak shape. Upper curve, FID response of the effluent without conversion; lower curve, FID response of the main part of the effluent after hydrocracking. A = 1.2 μl of toluene; B = 1.0 μl of butanol; C = 1.0 μl of benzene; D = 1.0 μl of methyl benzoate; E = 1.1 μl of acetone; F = 1.0 μl of dioxan. Reproduced from *Chromatographia*, 2 (1969) 7, by kind permission of R. Tykva[398].

### *3.4. Isotopic effects in the gas chromatography of $^3H$- and $^{14}C$-labelled compounds*

Considerable changes in retention volumes resulting from extensive substitución of deuterium and tritium for hydrogen in organic compounds[418] (reported earlier for radioactive inert gases by Glückauf[144a]) drew attention to isotopic effects in RGC. Broadly defined as any difference in the chemical or physical behaviour between two compounds that differ only in isotopic composition, the isotopic effect in GC has the

character of a secondary effect (chemical bonds are not broken or formed during the GC process). Thus the identification of labelled compounds is rendered more difficult (isotope fractionation must also be distinguished from radiochemical impurities); however, on the other hand, the differences in retention volumes of isotopic species can be utilized for their separation[76,77], *e.g.*, of tritiated methanes[66], olefins[234] or for enrichment of tritium[296,297] and carbon-14[22]. Particular attention has been paid in GC to smaller molecules labelled with stable isotopes, the thermodynamic properties of which could easily be measured[237]. Radioactive substances of high activity can influence the chromatographic process by evolving heat.

Three categories have been considered in the GC of isotopic substances[90]: separation of isotopic molecules, separation of chemical compounds labelled with one or more radionuclides and separation of radioactive materials from other elements, compounds or other matrix materials; the first two categories are similar and related to the GC of labelled compounds. The GC of smaller isotopic molecules has been reviewed by Cram[90], while isotope fractionation during analytical separations of large molecules was discussed by Klein[220].

Two examples of the isotopic effect in the "usual" GC of labelled compounds can demonstrate the phenomenon. Sgoutas[339] observed the fractionation of a mixture of $^{3}H$- and $^{14}C$-labelled fatty acid methyl esters, and found that the $^{3}H$:$^{14}C$ ratio decreased during the appearance of peaks of stearic, oleic and linolenic acid on SE-30 and DEGS columns. Similar results obtained in the GC of steroids labelled with $^{3}H$ and $^{14}C$ can be explained by tritium enrichment in the first fractions of the peaks (an increase in the vapour pressure of $^{3}H$ molecules was predicted), but with [4-$^{14}C$]-testosterone [$^{3}H$]acetate the $^{3}H$:$^{14}C$ ratio also increased in the tail, which remained unexplained[218]. VandenHeuvel *et al.*[402] did not observe fractionation of TMS-[$^{13}C$]-amino acids obtained from algae grown in a $^{13}CO_2$ atmosphere, but a biosynthetic isotope effect[254,287,306] was noted (non-homogeneous distribution of $^{13}C$, which can be explained, according to our experience, by precursor–product interactions as with $^{14}C$-labelled fatty acids[183,256]). Preparations of high specific activity obtained by chemical reactions or biosynthesis, as they are nowadays produced, can have shifted retention volumes, however. More efficient columns for the fractionation of isotopic molecules have been used; TMS-sugars have been separated on packed columns of 40,000 plates[29] and open-tubular column chromatography, *e.g.* of methanes[46,66], has been reported.

## 4. METHODS OF DETECTION OF RADIOACTIVITY

Radioactivity detectors offer the advantage of high sensitivity and selectivity when used in GC; naturally, the use of radioactive compounds is a prerequisite. Exceptionally, a radioactivity detector can be used for detection of some kinds of non-radioactive compounds[245]. If the effluent fractions emerging from GC column are trapped, they can be counted by any appropriate method used for usual radioactivity measurements. Liquid scintillation counting is most commonly employed, but Geiger–Müller (GM) tubes[186] and ionization chambers[415] have also been used. The discontinuous method of analysis has the advantage that collected fractions can be counted with conventional equipment.

For continuous flow-through counting of the effluent, proportional and GM counters, ionization chambers and scintillation methods have been used and the ap-

TABLE 4

SURVEY OF RADIATION DETECTORS USED IN RGC

| *Type of detector* | *Detector volume (ml)* | *Detection efficiency (%)* | | *Background (cpm)* | *Ref.* |
|---|---|---|---|---|---|
| | | $^3H$ | $^{14}C$ | | |
| End-window GM tube | – | 0 | 5–15 | 10–15 | 182 |
| Internal flow proportional counter | 10 | 30 | 88 | 35 | 35 |
| | 10 | 98.4 ± 3.5 | 102.4 ± 3.6 | 50 | 225 |
| | 10 | – | 95 | 3* | 368 |
| | 12 | 64.0 ± 1.5 | 94.5 ± 1.0 | 1* | 349 |
| | 20 | – | 78 ± 2 | 80 | 8 |
| | 20 | | – | 40 | 233 |
| | 22 | 61.5 | 92.5 | 40–50 | 372 |
| | 27 | – | 80 | 60 | 251 |
| Window flow proportional counter | 25.5 | 0 | 20 | 72 | 413 |
| Ionization chamber | 275 | 75** | 33** | *ca.* 700*** | 281 |
| | 275 | – | – | *ca.* 200*** | 350 |
| Plastic scintillator (coiled tube) | 0.27 | – | 58.3 | – | 137 |
| | – | – | 60 | – | 326 |
| Crystalline anthracene | – | 11–23 | 62–86 | 30–50 | 211 |
| Liquid scintillation flow cell (integral) | – | 15–20§ | 40–50§ | 60 | 300 |
| | | 25§§ | 80§§ | 60 | 300 |
| Liquid scintillation flow cell (continuous) | 1.4 | 27 | 85 | – | 336 |

* Gamma shielding and guard counter with the anticoincidence circuit used.
** Signal is also proportional to average radiation energy (see eqn. 1).
*** Background corresponding to the noise current measured.
§ Simultaneous measurement of both isotopes.
§§ Discriminator settings for measurement of individual isotopes.

plication of a semiconductor detector[396] in RGC has also been reported[397]. A survey of the detectors used in GC of $^3H$- and $^{14}C$-labelled compounds is given in Table 4. It is noteworthy that the sensitivity of the mass spectrometer also permits the detection of smaller amounts of radioactively labelled compounds of higher specific activities (of higher isotopic abundances)[146a].

### *4.1. Discontinuous methods*

The approach involving radioassay after fractionation of the effluent from a column is especially convenient for low levels of radioactivity. There are similarities with the isolation of substances from a stream of carrier gas, and preparative GC has also been employed for the preparation of labelled compounds[19,147,277,298,319,401,403]. The outlet systems of preparative instruments[174] and fraction collectors[85,393,426] have been described.

The principle of the methods involves condensation of the vapour of the substance (effluent fraction) from the stream of carrier gas in an exchangeable flow-through collection device. Vapours in the GC effluent tend to form aerosols as they enter a zone of markedly lower temperature. Therefore, the safest method of trapping is the combination of condensation with sorption of aerosols on a sorbent or the use

of a short section of a GC column containing a solid support coated with a non-volatile liquid stationary phase and maintained at room temperature. Sometimes only cooled glass traps have been used[10,11,23,56,89,99,126,145,154,169,288] or PTFE tubing[27,43,288,313]. Traps containing a cooled solvent (ethanol[181], methylene chloride[222] or toluene[283]) or cotton-wool (purified, occasionally wetted)[32,146,186,261,299], cartridges with glass-wool[28,86,156], tubes containing charcoal[95,158,159], fritted filters[328], Millipore filters[153], concave folded glass-fibre paper[69], molecular sieve 5A, glass beads[81,378], Corning porous glass[198,203], uncoated GC supports[99] or coated supports[106,173,200] and cartridges with silicone-coated anthracene, convenient for scintillation measurements[141,171,213,215,411], have been described. Condensation of vapours together with carrier gas ($CO_2$) has also been reported[172]. GC fractions are collected in vessels containing a scintillation "cocktail"[33,117,177,227,263,265,376] or the corresponding $CO_2$, after combustion, is absorbed in ethanolamine[40,122,355], hyamine[390] or sodium hydroxide solution[84,92] for subsequent liquid scintillation counting (Fig. 8). An example of a simple collection device is shown in Fig. 9. Various similar traps have been used elsewhere[120,127,201,202,241,311,320,371].

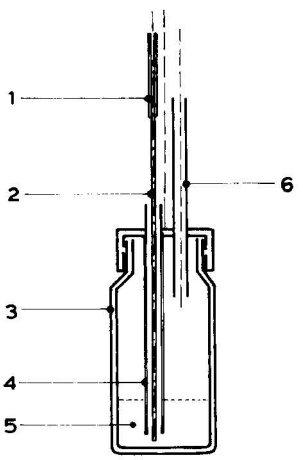
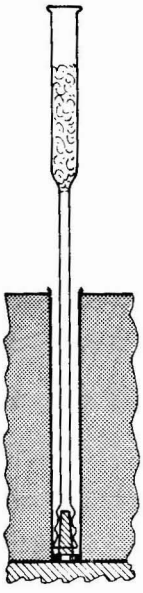



Fig. 8. Vial used for $^{14}CO_2$ trapping in ethanolamine and subsequent scintillation counting. 1 = Silicone rubber tube connecting reactor outlet with the vial; 2 = stainless-steel capillary; 3 = scintillation vial; 4 = glass mixing tube; 5 = trapping solution (3 ml of monoethanolamine in methyl Cellosolve, 2:1). Reproduced from *J. Chromatogr.*, 91 (1974) 507, by kind permission of the authors[163].

Another approach, avoiding aerosol formation, involves collection systems or devices with a temperature gradient which is useful in breaking down aerosols. Axial[226a, 235a] and radial temperature gradients[276a] have been used, while Magnusson[246a] used combined radial and axial gradients in investigating the recovery as a function of the temperature gradient and of the boiling point for substances with b.p. 151–287° at 760 mmHg. Electroprecipitation[226a] was not recommended[246a].

For some purposes, quantitative trapping and isolation of separated labelled compounds are not required (determination of specific activity, isotope dilution method, identifications), but quantitative work requires much effort and is difficult or impossible for some types of compounds or derivatives, such as some TMS derivatives, polyunsaturated long-chain fatty acids and similar labile substances that are sensitive to oxidation or hydrolysis or are otherwise reactive. A very important point in every



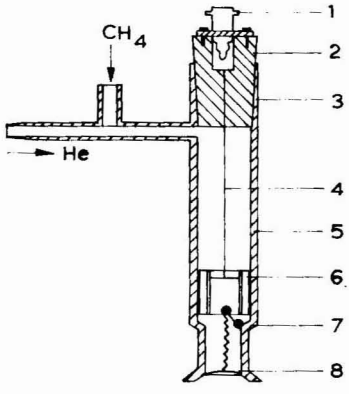



Fig. 9. Unit for collection of gas chromatographic fractions. Reproduced from *J. Lipid Res.*, 3 (1962) 141 (ref. 261).

Fig. 10. Flow-through proportional counter. 1 = Connector; 2 = PTFE plug; 3 = taper joint; 4 = 0.002-in. stainless-steel wire; 5 = brass wall; 6 = Lucite stop (grooved to permit gas flow); 7 = spring; 8 = spherical joint. Reproduced from *Anal. Chem.*, 30 (1958) 903.

discontinuous method is the verification or determination of the recovery of the overall procedure.

The main advantage of the collection method is the possibility of long-term counting, permitting work with low levels of radioactivity, and the possibility of repeating the counting. In some instances the recovery of pure substances for further analysis is possible.

### *4.2. Continuous methods*

#### *4.2.1. Proportional and Geiger–Müller counters*

Counting tubes used in RGC for the continuous monitoring of the effluent can operate in the proportional or GM region and are constructed with or without a window. Windowless counters can be employed with advantage for both radionuclides[271], the counting efficiency for carbon-14 being as high as 100%, while for tritium it is 60% (Table 4); external counting (with window) can be used only for carbon-14. The applications of GM counters are limited by the long dead time of the tubes.

In the first applications of RGC, mostly GM counters were used[223], in connection with a flow cell[25,312] or as a flow-through detector[236,334,335]. Condensation of the effluent in a flow cell cooled by liquid nitrogen combined with a window GM counter was reported[36], and an integral record of the activity was obtained.

Because of their high detection efficiency and low background, proportional counters with internal flow have often been used in the RGC of $^{3}H$- and $^{14}C$-labelled compounds[66,93,94,101,178,225,251,344,359,363,372]. The windowless flow-through proportional counter was proposed by Wolfgang and MacKay[424] and Wolfgang and Rowland[425]; a similar counter had already been used previously for counting carbon-14 during

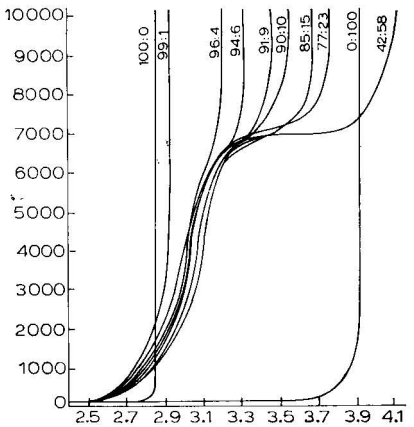



Fig. 11. Counting characteristics of gas-flow proportional counter for various hydrogen to methane ratios obtained with external source of radiation. Counting rate (ordinate) in cpm, voltage (abscissa) in kV. Reproduced from *Angew. Chem.*, 75 (1963) 720 (ref. 108).

distillation[423]. A large number of original designs have been reported[187,188,251,349,422]; they differ mostly in geometry, volume and hydrodynamic properties. An example is shown in Fig. 10. A small-volume proportional counter for open-tubular column chromatography has also been reported[9]. The sensitivity of a flow-through proportional counter changes appreciably during the passage of the effluent through the detector as a result of change in the composition of the gas being counted, *i.e.*, counting characteristics[108,233,238]. This effect is demonstrated in Fig. 11. The influence of temperature was established by Lieser *et al.*[238]; as the temperature of the counter is increased, the length of the plateau decreases and moves to higher voltages (Fig. 12) and even at 200° it is 50 V long. Coincidence losses at higher counting rates[233] can be minimized by operating the counter near the beginning of the plateau in the region of short resolution times. Composition changes in the counter with the passage of a

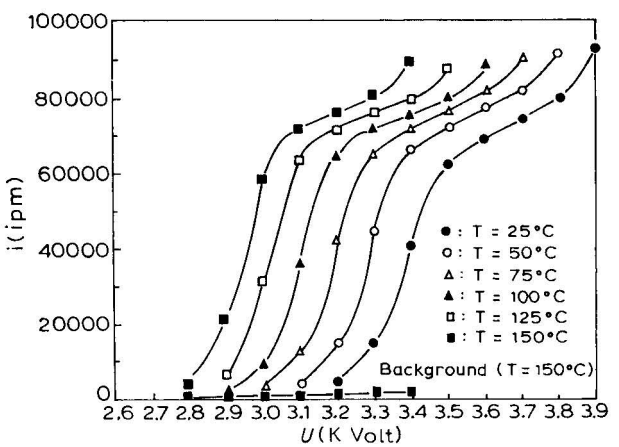



Fig. 12. Influence of temperature on counting characteristics of gas-flow proportional counter. Counting gas, methane (100 ml/min). Reproduced from *Z. Anal. Chem.*, 191 (1962) 108 (ref. 238).

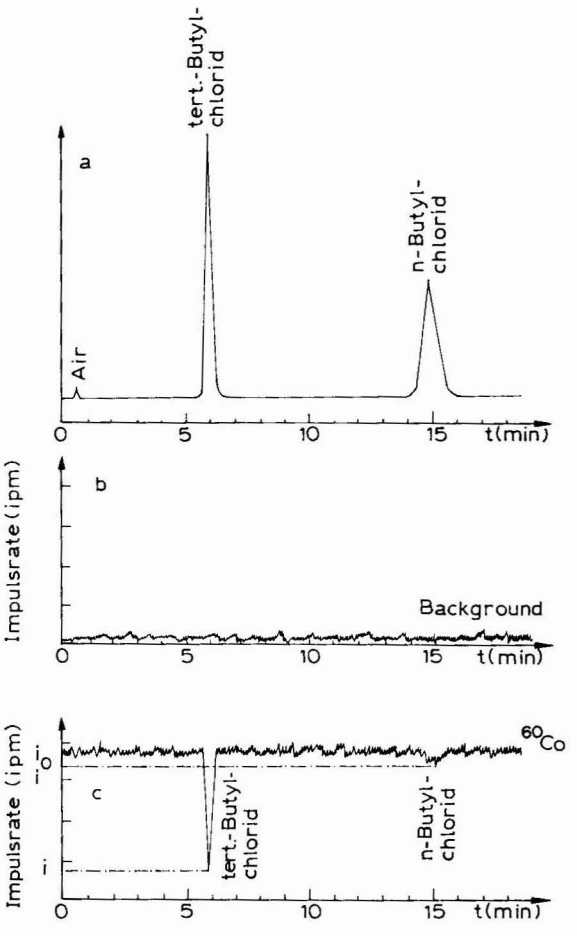



Fig. 13. Examination of influence of effluent on counting properties of gas-flow proportional counter. Injected: 30 $\mu$l of *tert.*-butyl chloride and *n*-butyl chloride (1:1). Carrier and counting gas, methane (60 ml/min). Reproduced from *Z. Anal. Chem.*, 191 (1962) 110 (ref. 238).

large amount of substance in the effluent lead to an increase in the organic to carrier gas ratio, which moves the plateau to a higher voltage, with a consequent drop in the counting efficiency. For this reason, contrary to the case with coincidence losses, operation of the counter at higher voltages makes the drop much less likely. The opposite effect, the shift of the plateau to the left due to changes in gas composition, can cause "elevated" activity or "pseudo-activity" (detection of non-radioactive substances). This effect was reported for aromatic compounds containing a halogen or a nitro group[238,347]. The correct functioning of the counter can be tested by means of an external radioactive source and a non-radioactive substance[238,256] (Fig. 13). Counting characteristics have been established for various gas mixtures, including methane–helium[425], propane–helium[233], methane–argon[9], methane–hydrogen[210] and isobutanol–argon[334,335]. The use of conversion of the effluent and of a suitable carrier gas, *e.g.*, carbon dioxide or carbon dioxide–argon[187,188,368], avoids the above dif-

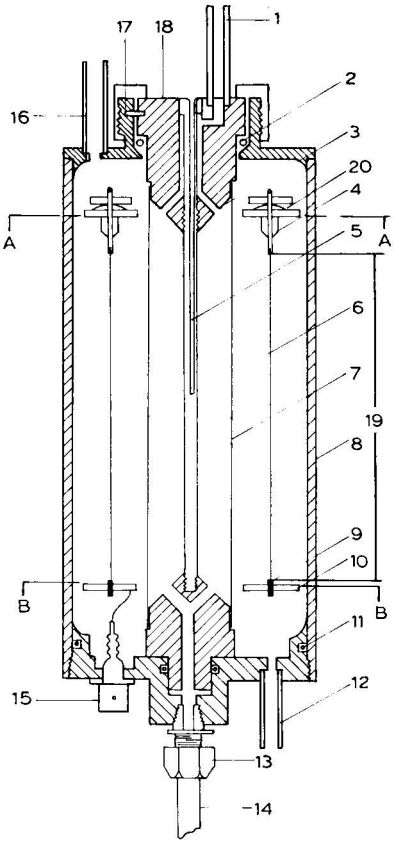



Fig. 14. Cross-section of window flow-proportional counter. 1 = Active gas inlet; 2 = O-ring; 3 = holes connecting gas inlet with active volume of the counter; 4 = spring-loaded PTFE insulators; 5 = hole for insertion of calibration source; 6 = 0.002-in. stainless-steel wire; 7 = 0.970-in. diameter Mylar tube; 8 = 2 $^{15}/_{16}$-in. O.D. brass tube (wall 0.125 in.); 9 = 0.022-in. stainless-steel capillary tube (0.005-in. I.D.); 10 = 2.5-in. I.D. disk of printed-circuit board; 11 = O-ring; 12 and 16 = counting gas inlet (or outlet); 13 = stainless-steel Swagelok connector; 14 = active gas outlet; 15 = connector; 17 = location pin; 18 = counter insert; 19 = active length of the counter; 20 = 0.010-in. U-shaped beryllium–copper tension spring. Reproduced from *Anal. Chem.*, 39 (1967) 276 (ref. 413).

ficulties due to changes in the counting characteristics. Also, a sufficiently high content of quenching gas should be added after the conversion for the same reason.

For $^{14}$C-labelled compounds, window flow-proportional counters[206,363,413,424] (Fig. 14), which have the advantage of stable and reproducible counting characteristics, have also been employed. The detection efficiency is decreased to 20%[413], however, and $^{3}$H cannot be counted at all.

Sensitivity due mainly to the background level, which increases with temperature, has been reported at the $10^{-11}$ Ci level[233,398]. Using equipment[349,368] containing a plastic scintillator, an anti-coincidence guard counter and a gamma shield (Fig. 15), the background has been measured at levels of 1 cpm[349] and 3 cpm[368]. The background is also influenced by the material used for shielding (by its age), and the quality and characteristics of a proportional counter depend on the geometry and performance of the detector; the thin anode wire is also an important component.

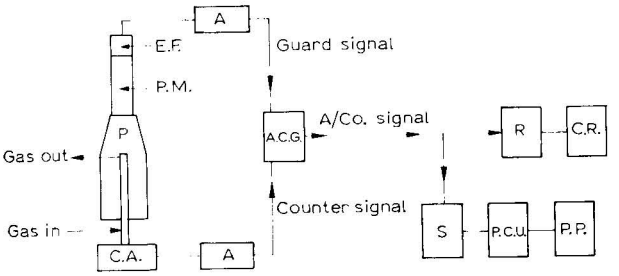



Fig. 15. Sensitive, low-background detector assembly for RGC comprising gas flow-proportional counter, plastic phosphor, anti-coincidence guard and graded gamma shield. P = Plastic scintillator; A = amplifier; R = rate meter; S = scaler; C.A. = charge amplifier; C.R. = chart recorder; E.F. = emitter follower; P.M. = photomultiplier; P.P. = parallel printer; A.C.G. = anticoincidence gate circuit; P.C.U. = printer control unit. Reproduced from *J. Chromatogr.*, 38 (1968) 26 (ref. 349).

*4.2.2. Ionization chambers*

For the detection of low-energy $\beta$-radiation from $^{3}$H and $^{14}$C, ionization chambers are convenient. The first application was reported by Wilzbach and Riesz[418], and such chambers are now in common use[46,58,59,61,119,139,309,317,415]. They can be heated, so that their application is wide[102,114,280,281,350,381–384,418,427]. Various designs have been reported[14,253,280,321,329,349], and two examples are illustrated in Figs. 16 and 17. Gas-phase counting with ionization chambers may give rise to "ghost peaks" when small amounts of unlabelled compounds pass through the detector[212,279,321]. These peaks can be partially or wholly eliminated by maintaining the chamber at a higher temperature[279], by using a relatively large-volume detector in conjunction with a large flow of diluting gas[279,350,419] or by conversion of the effluent[58,60,205,212,415,419].

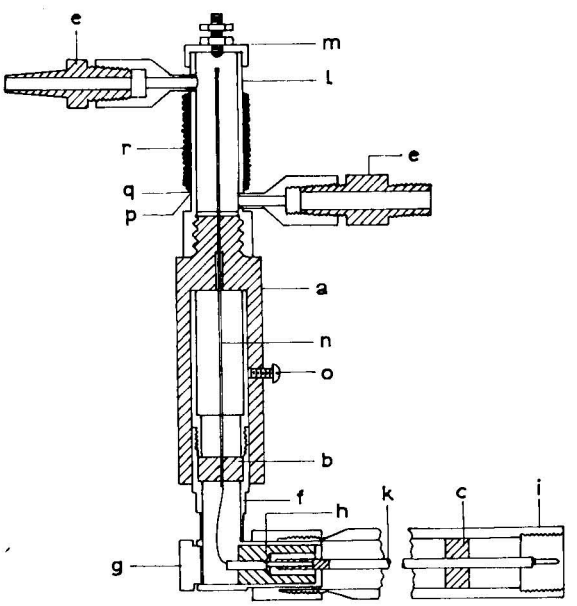
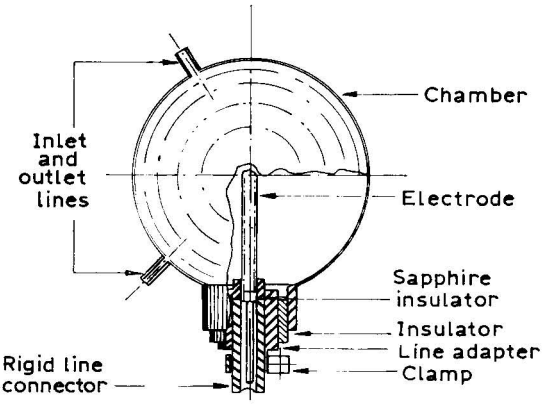



Fig. 16. Low-volume heatable ionization chamber. Reproduced from *J. Amer. Oil Chem. Soc.*, 38 (1961) 635 (ref. 114).



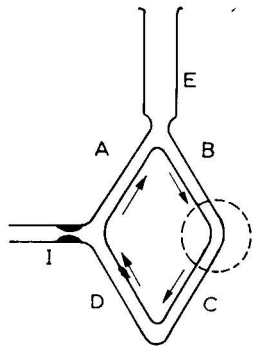




Fig. 17. High-temperature ionization chamber (volume 275 ml). Reproduced from *Anal. Chem.*, 35 (1963) 1576 (ref. 281).

Fig. 18. Liquid scintillator flow cell with integral record of radioactivity. Solution of scintillator circulates in the circuit consisting of tubes A, B, C and D. Carrier gas with radioactive vapour enters through capillary inlet I, the gas bubbles rise in tube A (the liquid circulates in the direction of the arrows) and escape through the chimney E. The dotted circle indicates the position of the photomultiplier. Reproduced from *J. Lipid Res.*, 1 (1959) 30 (ref. 303).

The current ($I$) measured from a radioactive source in an ionization chamber under steady-state conditions is expressed by

$$I = \frac{B\,N\,e\,S}{w} \tag{1}$$

where $I$ is current in amperes, $N$ is the number of disintegrations per second from the sample, $S$ is the average energy per disintegration in electron volts, $w$ is the number of electron volts required to produce an ion pair in the ionizable gas being used, $e$ is the number of coulombs per electron and $B$ is the chamber efficiency. However, the carrier gas containing the active fraction, on entering an ionization chamber, mixes completely with the gas already present so that, immediately after entry, exhaust of the active fraction begins and the signal $E_t$ at any time $t$ after $t = 0$ is given by

$$E_t = E_0 \exp\left(\frac{-f\,t}{v}\right) \tag{2}$$

where $f$ ml/min is the flow-rate and $v$ ml is the volume of the chamber[279]. The chamber response is usually measured with a vibrating reed electrometer.

Although a slightly higher sensitivity can be attained by using proportional counters, the ion chamber technique offers the advantages of being simple, stable, reproducible and usable at higher temperatures; the sensitivity to changes in composition of the measured gas is a disadvantage and conversion of the effluent is to be recommended.

*4.2.3. Scintillation methods*

For counting $^{3}H$ and $^{14}C$, scintillation detection systems are extremely useful

and have been widely employed, especially in connection with the discontinuous method of activity measurement. Continuous scintillation methods involve either liquid scintillation, a flow cell from a plastic scintillator or organic crystalline scintillators. The use of inorganic scintillation glass in the RGC of $^{3}H$- and $^{14}C$-labelled compounds has not been reported.

Scintillation methods have been recommended[205,246,323–325,385–387] for $\beta$- (and $\gamma$-) counting from the viewpoint of the influence of the chemical composition of the column effluent (which must be taken into account in the usual liquid scintillation[345,390]) and counting efficiency. However, a correction for quenching has not been reported in continuous methods using liquid scintillation without conversion of the effluent[243,300–303,336]. The detection efficiency of plastic, organic and inorganic scintillators and glasses[327] is doubtless independent of the gas composition; this fact is, however, not so important when conversion of the effluent is carried out. Presumably because of the low counting efficiency, especially for tritium, chemically inert scintillating glass has not been used in GC.

Short sections of tubing containing a solid support coated with stationary liquid phase have often been used as trapping devices (see Section 4.1). Based on the work of Steinberg[362], who suggested the application of anthracene crystals for counting $^{14}C$ in aqueous solutions, Karmen and Tritch[215] used cartridges packed with anthracene crystals (blue-violet fluorescence grade, coated with 5% of DC 550 silicone oil). In this way, scintillation counting can be accomplished directly, without transfer to scintillation vials. Because of the absorption of radioactive material in the upper layer of the coated scintillator, the counting efficiency is dependent on the position of the cartridge in the photomultiplier compartment. This approach has been reported several times[99,141,171,212,213] and has been combined with combustion methods for continuous counting[170,211,212]. The flow cell was filled with uncoated anthracene, but other crystalline scintillators can be used (*e.g.*, terphenyl[205,212]).

In connection with trapping procedures, conventional liquid scintillation of eluted fractions has been employed many times[153,156,163,261,313,326]. It is advantageous to trap fractions directly in the scintillation solution[177,263,265,376] or in the stream of scintillator, these fractions then being collected[115,376]. When condensation of the radioactive vapour takes place in a flow cell containing scintillation solution (Fig. 18), an integral record of the activity is obtained[243,300–303]. A differential curve is recorded when the effluent is dissolved continuously in a stream of scintillator solution[336]. A

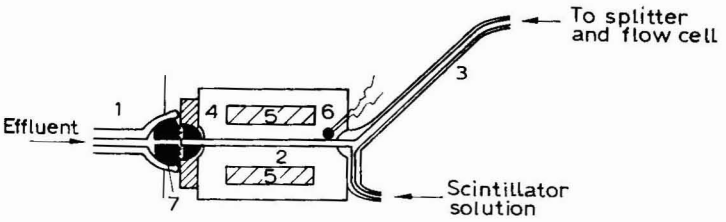
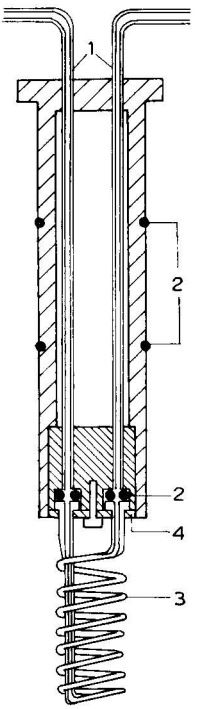
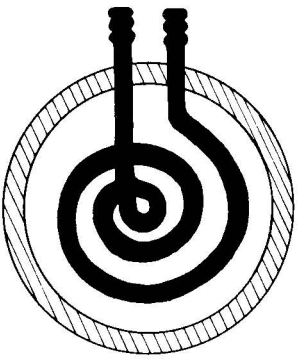



Fig. 19. Detachable mixing tube and effluent-liner extension of the system for continuous detection of radioactivity by liquid scintillation (see Fig. 2). 1 = Glass effluent liner of gas chromatograph; 2 = narrow-bore linear extension of Kovar metal, fused to glass mixing tube and to stainless-steel ball-joint; 3 = glass mixing tube; 4 = aluminium heating block; 5 = heating elements; 6 = thermocouple; 7 = O-ring. Reproduced from *J. Chromatogr.*, 76 (1973) 14 (ref. 336).



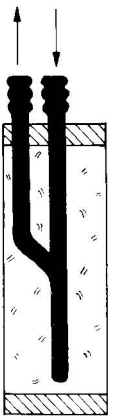


Fig. 20. Liquid scintillation flow cell for continuous measurement of effluent radioactivity. 1 = Stainless-steel pipes; 2 = O-rings; 3 = interchangeable glass cell; 4 = semi-circular collar discs (to press the cell against the O-rings). Reproduced from *J. Chromatogr.*, 76 (1973) 15 (ref. 336).

Fig. 21. Plastic scintillator flow cell. Reproduced from paper 51-47 of Proceedings of Symposium on Organic Compounds Labelled with Radioisotopes, Mariánské Lázne, May 1976, by kind permission of K.-H. Heise[162].

major difficulty of the system constitutes the mixing tube, shown in Fig. 19. Before the solution enters the liquid scintillation flow cell (Fig. 20), the major part of the carrier gas is split off (Fig. 2). The system is also suitable for distinguishing compounds labelled with different isotopes ($^{14}C$ and $^{3}H$), as in the case of liquid scintillation of collected fractions[376] or the use of an integral flow cell[300,302]. Another approach using liquid scintillation involves absorption of the combusted effluent in solutions containing sodium hydroxide[84,91,92,209] ethanolamine[122,163,310] or hyamine[170,390] in a common absorption vial (Fig. 8). This method can be combined with continuous counting[122,163].

Plastic containing scintillating substances were originally employed in liquid chromatography[137,326,366] and are not applicable at higher operating temperatures[326]. Several types of flow cells have been described, such as a plastic scintillator tubing coiled in a spiral cell[137,162,321,324,326] (Fig. 21) and a glass spiral capillary packed with spherical particles of scintillator[321]; the plastic scintillator may also form simple flow cuvette[149,322] or one of its walls[366]. The counting efficiency for gaseous $^{14}CO_2$ was reported to be 60%[137,326].

## 5. DATA OUTPUT AND EVALUATION

### *5.1. Types of record*

In the GC of $^{3}H$- and $^{14}C$-labelled compounds, elution techniques are generally used. Exceptionally, frontal analysis has also been employed[189]. The simultaneous recording of mass and radioactivity is useful but is required or even possible not in every instance (at high specific activities and small sample sizes, some types of analysis follow only the distribution of radioactivity; in the preparative GC of labelled compounds, only a mass detector is commonly employed).

The discontinuous method of detection of radioactivity usually supplies a record of mass; the activity record, if produced, is received in the form of a histogram[116] or has a digital form[361]. Using continuous methods, the record from the rate meter in the form of a differential curve corresponding to the mass trace is usually obtained; sometimes in addition the integral curve is drawn (an example is shown in Fig. 22) or, for methodological reasons, only the integral record is obtained[36,243,300–303] (Fig. 23). An unusual mode of combined record, with a histogram of activity superimposed on the mass trace, has also been reported[334,335]. Development of recording techniques proceeded from the above-mentioned traditional analogue display, *i.e.*, the simultaneous recording of mass and activity curves on the chart of a two-channel recorder or by means of two single-channel recorders, to the digital mode of record[251,373], which is natural in the application of a scaler–timer, electronic integrator or microcomputer-based chromatograph. More sophisticated systems have been used: for the storage of activity data a multichannel analyzer[129] or the data acquisition system of a computer[273,376,412] have been employed, with subsequent data processing.

### *5.2. Factors influencing the data output*

The answer to the most frequent analytical questions, *i.e.*, what substances are present in a sample and how is the activity distributed among the sample components, is based on the record and its evaluation. In RGC usually both mass and radioactivity records are obtained, the latter depending on the RGC system used. There is a time difference between the mass and activity response given by the volume of the activity-detecting part of the system that varies from fractions of a second to a minute. The activity peak is influenced by the dead volumes and also the effluent conversion (Fig. 7) and the "memory effect" (adsorption, condensation, etc.) can contribute to the distortion of the peak shape. This effect can be described by eqn. 2, which gives the perfect mixing of a "sample plug" in the detector volume. The volume of the flow-through activity detector and the flow-rate of the counted gas affect the resolution, accuracy, precision and sensitivity of the method, as expressed by eqns. 3 and 4:

$$V = V_n - V_{n-1} \tag{3}$$

$$c = \varepsilon \cdot \frac{V}{f} \cdot a + b \tag{4}$$

where

$c$ = counts registered during the mean transit through the detector;
$V$ = detector volume;
$V_n$, $V_{n-1}$ = retention volumes of successive resolved components;

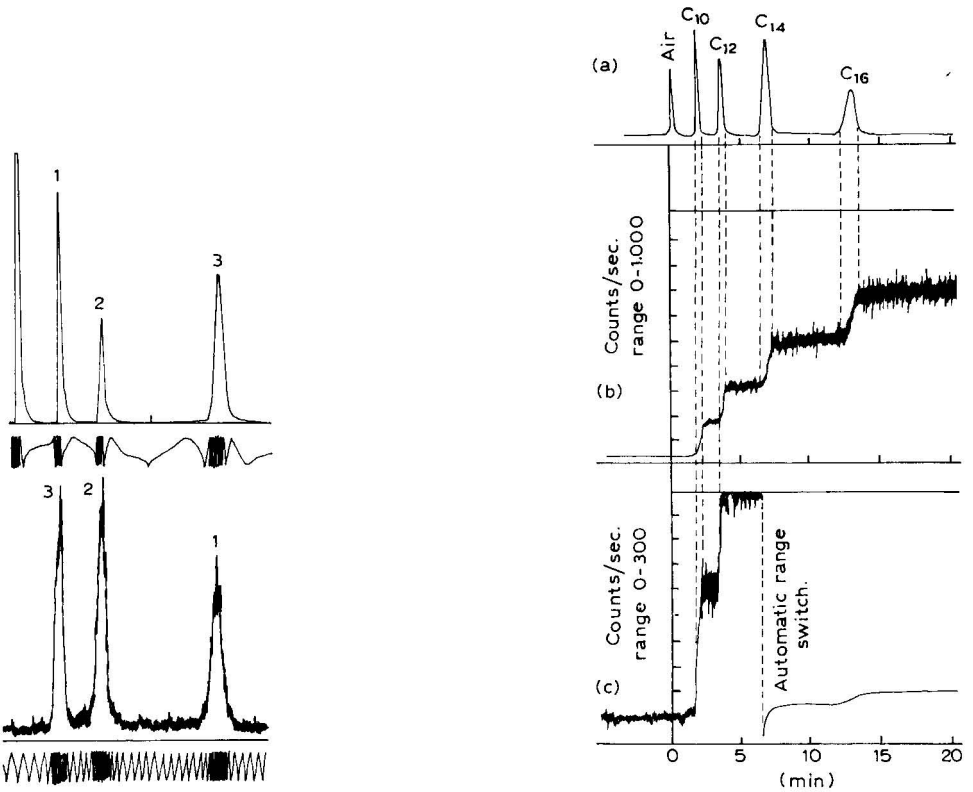



Fig. 22. RGC separation of [$^3$H]dehydroepiandrosterone (peak No. 1, 10 $\mu$g and 8000 dpm), [$^3$H]testosterone (peak No. 2, 8 $\mu$g and 9000 dpm) and [$^3$H]-$\Delta^4$-androstene-3,17-dione (peak 3, 20 $\mu$g and 8000 dpm) on QF-1 column at 240°. Mass detection by FID (one tenth of the effluent); radioactivity detection by gas flow-proportional counter after combustion of the split effluent. Reproduced from *Anal. Biochem.*, 16 (1966) 82 (ref. 372).

Fig. 23. Radio-gas chromatogram of four $^{14}$C-labelled fatty acids separated as methyl esters on a PEGA column at 197°. Sample size: 1.25 mg of esters containing 27 nCi of $^{14}$C. a, Mass record by gas density balance; b and c, simultaneous records of radioactivity detected by integral liquid scintillation flow cell (Fig. 17) at two sensitivities. Reproduced from *J. Lipid Res.*, 1 (1959) 36 (ref. 303).

$\varepsilon$ = counting efficiency;
$f$ = flow-rate of counted gas (if quenching and/or purging gas is used, the detector volume must be correspondingly larger with respect to the resolution desired);
$a$ = activity passing through the detector;
$b$ = background.

The accuracy and precision of the activity determination, especially with low activities, is also limited by the statistical character of the nuclear decay. It seems sometimes to be forgotten that at least $10^4$ disintegrations must be registered if a reproducibility of 1 % is required. From eqn. 4, it follows that the activity measurement depends strongly on the flow-rate of the counted gas (experimentally verified recently[217a]), which can change during peak emergence (with larger sample sizes)

and can be measured by a flow-meter[363]. The variations in the counting efficiency (pseudo-activity, quenching) were discussed in Section 4.2; the possibility of the effect of electronic circuitry has also been mentioned[244]. The background level is the limiting factor of sensitivity; it can be decreased to 1 cpm by use of gamma-ray shielding, guard counter and an anticoincidence circuit[349]. For low-level activity measurements, equipment that has not been in contact with substances of higher activity should be used. The digital output from the scaler is more sensitive than the response from a rate meter[349], the sensitivity of which can be optimized by using an appropriate time constant[349].

When a stream splitter is used, its function must be controlled[8,135,211,213,288,372]. The deviations from linearity of splitting can be explained by changes in the viscosity and density of the split gas causing the "suction effect"[259]. In addition, the changes of the hydrodynamic resistance of the branch leading to the radiation detector influence the split ratio[135]. The explanation of the observed deviation in terms of non-linearity of the FID used as a mass detector[338] must be incidentally taken into account. For nanomolar amounts of fatty acid methyl esters, a too laborious calibration of the splitter was suggested[288]. Calibration of the radiation detector[206–208] or of the entire RGC system[238] has been recommended.

### *5.3. Evaluation of the radio-gas chromatogram*

Radio-gas chromatograms usually contain information on the course of the separation and on the contents and activities of individual components of the sample, from which further data such as the qualitative composition, distribution of radioactivity among the constituents of the mixture and their specific activities can be calculated.

A specific feature of the radioactivity record is the low signal-to-noise ratio. This property influences the evaluation and complicates computation methods (which are also used for the evaluation of related activity records, *e.g.*, in flat-bed and liquid chromatography[343,345], or records of spectroscopic measurements[345]). The measurement of low activity levels is critical owing to the poor counting statistics associated with small peak count rates, the estimation of background and its level. The reproducibility of the results increases with the activity injected[42,163]. Difficulties also arise in the resolution of peaks. Methods of quantitative RGC are the same as those of conventional GC[257]. The standard presentation of GC results involves a strip-chart recorder with interpretation accomplished by manual methods, sometimes supported by a mechanical analogue disc or electronic integrator. GC, however, has now advanced from the simple calculation of analogue or digital results to automatic data processing.

Only a few methods concerning the problems involved in the evaluation of radio-gas chromatograms have appeared. The digital recorder display of radioactive and integrated chromatograms has been discussed[373], and a schematic representation of an RGC, data acquisition and data processing system with a flow diagram of the computer program has been given[273]. The application of a multichannel analyzer in the multiscaler mode has been described[129]: the length of the time during which the response of the counter is recorded in a single channel is set by the variable time controller unit; at the end of a pre-set length of time, the contoller unit advances the

computer response to the next channel, the counts from each channel are stored in the memory of the analyzer at the end of the analysis and the results are printed out (or punched) for quantitative information or an analogue record using $X$–$Y$ plotter can be obtained (the channel number is converted into time). An oscilloscope display in connection with similar systems has also been used[343].

## 6. APPLICATIONS OF RADIO-GAS CHROMATOGRAPHY TO THE ANALYSIS OF $^{3}H$- AND $^{14}C$-LABELLED COMPOUNDS

### *6.1. Applications in the production of labelled compounds*

In addition to the preparative GC of labelled compounds[19,66,121,124,147,260,289,298,403,409], often accomplished with analytical-scale amounts, RGC is also employed for quality control of the substances prepared[71,124,343]. Important applications of RGC in the course of the production process also occur, in the control of synthesis or the analysis of intermediates and reaction mixtures, *e.g.*, the Wilzbach method of labelling [1,2,50,138,309], and in biosynthesis[140,256] (an example is illustrated in Fig. 24). The applications are concentrated on volatile substances or compounds that can be easily derivatized, such as hydrocarbons[16,57,62,66,75,143,162,219,242,269,272,318] (see Fig. 25), alcohols[162,269,358], formaldehyde[358], formic and acetic acids[269,357], fatty acids[32,117,168,249,256,258,259,275,341,364,430] and other compounds[27,405]; non-volatile and polar substances, which can be analyzed by other chromatographic techniques, are assayed only rarely by RGC, *e.g.*, amino acids[20,230,255] (Fig. 26) and pyrimidine bases[290]. Biosynthetic methods of labelling may sometimes produce non-uniformly labelled compounds, and therefore methods for the control of the pattern of labelling with subsequent RGC

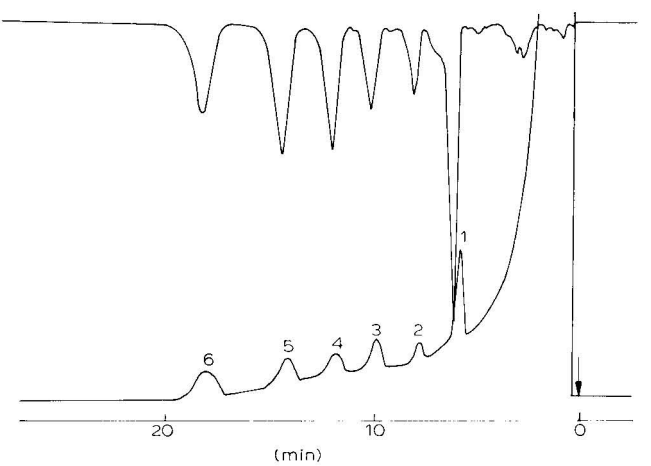



Fig. 24. Radio-gas chromatogram of $^{14}C$-labelled higher fatty acids obtained by cultivation of the alga *Chlorella vulgaris* in an atmosphere of $^{14}CO_2$ separated on a BDS column. Upper curve, activity record obtained with gas flow-proportional counter after combustion of the effluent; lower curve, FID response of the effluent split as follows: 1 = 16:0; 2 = 16:2; 3 = 16:3; 4 = 18:1; 5 = 18:2; 6 = 18:3. Reproduced from *J. Chromatogr.*, 91 (1974) 501 (ref. 256).

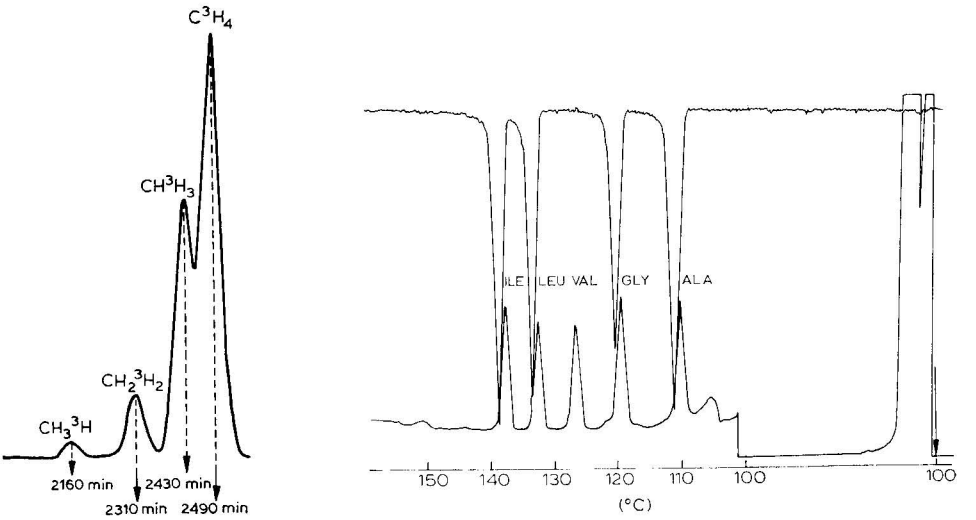



Fig. 25. Separation of tritiated methanes on a 64 m × 0.22 mm I.D. glass capillary column with a layer of active silica. Temperature, 77 °K; flow-rate of helium–nitrogen (3:7), 0.70 ml/min. Detection by 10-ml flow-through proportional counter after addition of methane as quenching gas. Reproduced from *J. Labelled Compd.*, 11 (1975) 319 (ref. 66).

Fig. 26. Determination of the specific radioactivity of carrier-free amino acids labelled with carbon-14 using non-radioactive valine as internal standard. Derivatives used, bis(chlorodifluoromethyl)-1,3-oxazolidinon-5-ones[175]; column, 1% OV-225 on Chromosorb G-HP, 100–120 mesh, 2 m × 2 mm I.D.; temperature programme, 10 min at 100° then heated to 160° at 3°/min; carrier gas, argon (40 ml/min). Upper trace, activity record of gas flow-proportional counter; lower trace, FID response. Splitting ratio, 1:1. From Matucha[255].

analysis have been developed[73,184,256,346]. A high specific activity of the preparations requires an appropriate sensitivity of the mass detector, which also gives information on the chemical purity of the sample being analyzed. The application of RGC to the analysis of molecules that contain labile $^3$H that is exchangeable with a convenient carrier gas has been reported[407].

### 6.2. *Applications in "hot-atom" chemistry*

The development of RGC is closely connected with investigations of the Szilard–Chalmers effect[374]. Evans and Willard[124a] utilized GC in the analysis of a mixture of products obtained by the irradiation of *n*-propyl bromide in a reactor and detected more than 20 compounds containing radioactive bromine, formed by interaction of "hot" atoms with the medium. The recoil of $^3$H obtained by the reactions $^6$Li(n,$\alpha$)$^3$H and $^3$He(n,p)$^3$H has been extensively studied; $^{14}$C, produced by the reaction $^{14}$N(n,p)$^{14}$C, forms compounds with lower specific activities.

The $^3$H recoil reactions studied by means of RGC include reactions with alkanes[79,97,123,129,304,314,354,370,379,423], alkenes[24,194,231,235,247,314], arenes[52,53,332], alkyl halogenides[232,267,286,291,353,375], methylsilanes[96–98], amino acids[348] and methyl isocyanide

[380,412]. More general problems have also been investigated[74,138,148,155,164,315,412]. The possibilities of utilizing $^{14}C$ recoil reactions as a labelling method, with GC as a separation technique, have been discussed[406,420,421]. Several reactions of $^{14}C$ with heterocyclic nitrogen-containing compounds[18,134], solid benzene[239], magnesium nitride[132] and gaseous anhydrous ammonia[428] have been reported.

### 6.3. *Applications in organic chemistry and chemical processing*

RGC has been used successfully for investigations of various organic reactions, *e.g.*, the analysis of benzoic acid nitration products[11], chlorination of benzene[56], products of methylene reactions in photolytic systems[78], kinetics of hydrogenolysis of mesitylenesulphonic acid and sulphuration of mesitylene[404], oxidation of propylene[195,415], hydrogenation of acetophenone[179], propene[307], the interconversion of cyclohexane and benzene[180] and of propenes[131]. Technological processes have also been studied, *e.g.*, mechanism of the Fischer–Tropsch synthesis[37,333], catalytic cracking[21,165] and other catalytic reactions[100,195,196,268,312] and the sequence length distribution of ethylene–propylene copolymers[125]. A radio-gas chromatogram of the aromatic products from the hydrocracking of a light catalytic cycle oil[8] (Fig. 27) illustrates an application in chemical processing.

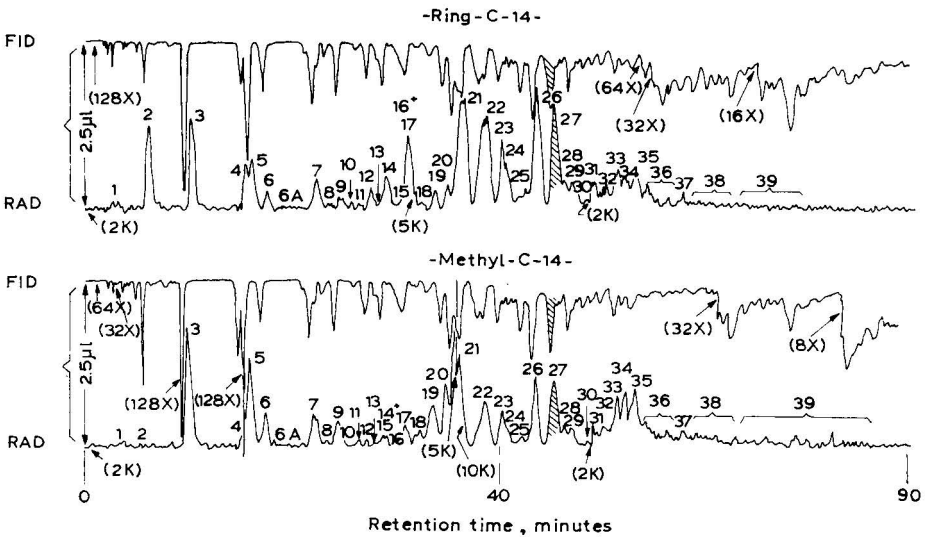



Fig. 27. Example of application of RGC to the determination of petroleum processing products. Comparison of aromatic products from ring- and methyl-labelled feeds of a light catalytic cycle oil containing 2-methylnaphthalene (labelled in the ring or in the methyl group with carbon-14). The upper curve demonstrates the FID response of one tenth of the column effluent. Column, 20 ft. × 1/8 in. O.D. stainless-steel packed with 10% OV-101 on Chromosorb W, 80–100 mesh; carrier gas, helium (30 ml/min); temperature programme, 4 min at 80°, then heated to 300° at 2°/min. Lower curve: corresponding radioactivity record obtained by gas flow-proportional counting without effluent conversion at a helium flow-rate of 60 ml/min (additional helium) with propane (5 ml/min) as quenching gas. Peak No. 27 includes 2-methylnaphthalene. Reproduced from *J. Chromatogr. Sci.*, 12 (1974) 190 (ref. 8).

### *6.4. Applications in biochemistry and clinical biochemistry*

Compounds of biochemical interest can be analyzed by RGC without derivatization only in exceptional circumstances, *e.g.*, fermentation gases[94,282]. For investigations of biosynthesis, intermediates and metabolic pathways, appropriate derivatives must be prepared. Applications of RGC in lipid analysis are well known, *e.g.*, fatty acid methyl esters[7,13,31,34,38,44,99,118,122,140,141,146,151,156,157,161,166,171,183,186,193,199,200,216,217,224–226,256,258–261,265,273,278,284,285,294,313,331,340,365,367,377,378,399,408,416], diglycerides[17,45], triglycerides[42,82] and cholesteryl esters[163]. Many applications in steroid analysis have been described[4,23,26,33,39,41,86,126,145,218,225,229,239a,264,283,299,316,330,359,372]. RGC has also been employed in analyses of amino acids[20,43,67,128,233,248,255,256,336,352,410], pyrimidines [290,308], aroma substances[110,111,391,392], alcohols[224], pyrazine compounds[222], alkaloids[252], carbohydrates[190] and other substances[15,68,69,109,127,136,144,400]. The determination of 5-hydroxyindole-3-acetic acid in cerebrospinal fluid[30], investigations of lipid synthesis in the rat[103], the identification of hormone metabolites in tissue[330], the determination of homovanillic acid in cerebrospinal fluid[351] and of short-chain carboxylic acids in biological material[360], the effect of hormones on fat synthesis in mammary explants of pseudo-pregnant rabbits[369] and investigations of the fat metabolism of new-born domestic mammals[377] have been reported.

## 7. CONCLUSIONS

More than 20 years have passed since the first report on the GC of labelled compounds. During this period, RGC and labelled compounds have found valuable applications in various fields of chemical research. Investigations of many complicated problems became possible and new aspects of known processes were revealed. Moreover, the tracer technique is a powerful tool and has already contributed (and may contribute still further) to the methodological development of chromatography itself.

RGC methods have specific features, *e.g.*, requirements on derivatization, stability of the solute, inertness of the chromatographic system and problems with the handling of samples, the fractionation of isotopic species activity detection, recording and data processing. Some aspects of conventional GC that are applicable to the GC of labelled compounds have therefore been mentioned in this review. However, the main purpose was to point out the methodological problems and to summarize the current state of the art. Many problems can be solved by RGC in several different ways, but any recommendation of a universal RGC system or of a method convenient for any type of problem is questionable, and for each new application appropriate efforts must be made to work out a suitable method.

RGC plays an important role in the analysis of labelled compounds produced or transformed during various processes and its advantages, *viz.*, sensitivity, separation efficiency, possibility of simultaneous detection of mass and activity and speed of analysis, can be successfully exploited in many fields.

## 8. ACKNOWLEDGEMENT

The authors are indebted to Professor A. I. M. Keulemans for many helpful discussions and remarks.

## 9. SUMMARY

GC methods of analysis of $^{3}H$- and $^{14}C$-labelled compounds that have been published in the period from 1955 to mid-1976, together with important applications, are reviewed. In addition to its obvious use for identification purposes, RGC is mostly used for the determination of the distribution of radioactivity among the components of mixture under analysis and for the determination of their contents and specific activities. It can be also combined with chemical reactions (reaction gas chromatography). Applications that require derivatization have some limitations connected with the derivatization reaction and the stability of the derivatives. The separation of isotopic molecules by GC is also possible. For the measurement of radioactivity, a discontinuous method involving the collection of fractions and subsequent counting is often used, while for continuous monitoring of effluent activity flow-through radiation detectors (Geiger–Müller and proportional counters, ionization chambers and scintillation methods) are used, mainly after conversion of the effluent. The possibilities of various recording techniques with respect to quantitative evaluation are surveyed and applications of RGC in the production of labelled compounds, "hot-atom" chemistry, organic chemistry, chemical processing, biochemistry and clinical chemistry are discussed.

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CHREV. 97

# POLYCHLORINATED NAPHTHALENES

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CONTENTS



## I. INTRODUCTION

Laurent[1], in 1833, observed that wax-like materials result from the reaction of chlorine with naphthalene in the presence of certain catalysts. Almost 50 years later, these chlorination products were further studied by Fischer[2] and, shortly after 1900,

Aylsworth[3–5] patented the use of chlorinated naphthalenes for impregnating wood, paper, textiles and other materials. Chlorinated naphthalene waxes first became of importance during World War I as protective coating materials, particularly in Germany. In Germany, several firms took up the production of polychlorinated naphthalenes (PCNs), including Chemische Fabrik Griesheim Elektron, which used its so-called Perna waxes to impregnate paper inlays in gas-masks.

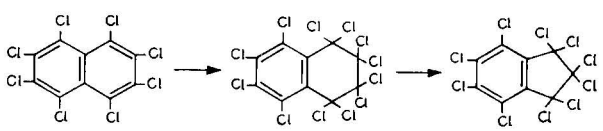
To-day, well known series of PCNs include the Halowaxes, Nibren waxes and Clonacires (see Section II.1), for which a variety of uses has been suggested related to their electrical, flame-retardant and fungus-resistant properties, stability and compatibility with other materials. The physical and chemical properties of PCNs and polychlorinated biphenyls (PCBs) are rather similar, and PCNs are manufactured for uses analogous to those of PCBs. As is now well known, PCBs are widespread and persistent industrial pollutants of the environment, and so are PCNs. Unfortunately, little information is available on the world-wide production of PCNs. According to one source[6], in 1956 the output of PCNs in the U.S.A. was about 3500 short tons, while the present total world market for PCNs has been estimated at "... probably less than 800 tons"[7]. The production of PCNs has also been claimed[8] to be at least 10% of that of PCBs, which has been estimated[9] at 33,000 and 13,000 metric tons for domestic sales of Monsanto's Aroclors only, in 1970 and 1972, respectively*.

Prior to this review, Fishbein[10] and Sherma[11] discussed the analysis of PCNs, and Kimbrough[12] wrote an extensive survey of toxicological aspects of PCNs and similar compounds. Much technical information can be derived from an early paper by Hardie[6] and from manufacturer's bulletins[13,14]. Recently, Kover[15] published a comprehensive environmental hazard assessment report on PCNs.

## II. SYNTHESIS

### *1. PCN mixtures*

Commercially available mixtures of PCNs are generally produced[6] by chlorinating naphthalene with chlorine gas in the presence of *ca.* 0.5 wt.-% of iron(III) or antimony(V) chloride. Chlorination is begun at 80° and the temperature is slowly increased as the reaction proceeds. During the process, the mixture is agitated continuously. When the desired pour-point has been reached, the chlorinated product is neutralized by stirring it in the molten state with aqueous alkali solution, washed with water and finally dried under vacuum. It should be noted that as chlorination proceeds, there is an increasing tendency towards the formation of products of simultaneous substitution and addition of chlorine. Thus, if chlorination is continued beyond the octachloronaphthalene stage, at temperatures above 200° in the presence of iron(III) chloride, four additional chlorine atoms can be readily introduced; simultaneously, liberation of carbon tetrachloride and transformation of the naphthalene ring system occur[16]:



(1)

Octachloronaphthalene Perchloroindane

* The large decrease in output after 1970 was due to voluntary restriction by Monsanto of sales of PCBs essentially to uses in closed systems.

Manufacturers of PCNs produce series of materials appropriate to the various uses to which the substances are to be put. In this section, the commercially available products are exemplified by reference to four such series.

*(a) Halowaxes*

Koppers Co. (Pittsburgh, Pa., U.S.A.) markets[13] a large series of light-coloured PCN mixtures under the trade-name Halowax. These products range from a liquid with a melting point of −33° to a mixture containing *ca.* 90% of octachloronaphthalene and melting at 185°. Information regarding the composition of the Halowaxes is presented in Table 1; their main physical and chemical properties are summarized in Table 2.

TABLE 1

APPROXIMATE COMPOSITIONS (WT.-%) OF HALOWAXES

| *Halowax* | *Type of PCN* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Mono-* | *Di-* | *Tri-* | *Tetra-* | *Penta-* | *Hexa-* | *Hepta-* | *Octa-* |
| 1031 | 95 | 5 | | | | | | |
| 1000 | 60 | 40 | | | | | | |
| 1001 | | 10 | 40 | 40 | 10 | | | |
| 1099 | | 10 | 40 | 40 | 10 | | | |
| 1013 | | | 10 | 50 | 40 | | | |
| 1014 | | | | 20 | 40 | 40 | | |
| 1051 | | | | | | | 10 | 90 |

Halowaxes 2141 and 2148, not mentioned in these tables, are special-purpose blends for use in the electrical industry.

*(b) Nibren waxes*

These materials, produced[14] by Bayer (Leverkusen, G.F.R.) and formerly by I.G. Farbenindustrie, are crystalline solids with a chlorine content of *ca.* 50–60%; they are marketed as powders or flakes. Important physical characteristics of the vacuum-distilled Nibren D88, D116N and D130 waxes are recorded in Table 3.

Several further types of Nibren waxes have been reported[14,17], such as Nibren RN88 and RN130, and Nibren D130CM and D130CM/10. The RN-type products are dark-coloured non-vacuum-distilled analogues of Nibren D88 and D130. The other two products are modified PCN mixtures whose crystal structures oppose the penetration of water vapour very effectively.

*(c) Seekay waxes*

These waxes have been produced in Great Britain by ICI (Runcorn, Great Britain) and one of its forerunners since 1919. They are described[6] as wax-like solids with a light odour, and range in colour from pale yellow to black. Approximate melting points are from 67–73 to 120–125°. Two grades, produced in order to conform

TABLE 2

PHYSICAL AND CHEMICAL PROPERTIES OF HALOWAXES

| *Property* | *Halowax* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *1031* | *1000* | *1001* | *1099* | *1099B* | *1013* | *1014* | *1051* |
| Physical form | liquid | liquid | flakes | flakes | flakes | flakes | flakes | powder |
| Cl content (approx. wt.-%C) | 22 | 26 | 50 | 52 | 52 | 56 | 62 | 70 |
| Specific gravity (at 25°C) | 1.20 | 1.22 | 1.58 | 1.59 | 1.65 | 1.67 | 1.78 | 2.00 |
| Initial boiling point at 760 mm Hg (°C) | 250 | 250 | 308 | 315 | 322 | 328 | 344 | 310** |
| Approx. melting point (°C) | −25 | −33 | 93 | 102 | 115 | 120 | 137 | 185 |
| Flash point (°C, C.O.C.) | 135 | 130 | 200 | 210 | 210 | 230 | 250 | none to 430 |
| Fire point (°C, C.O.C.)* | 165 | 170 | ntb | ntb | ntb | ntb | ntb | ntb |
| Max. acidity (mg KOH/g) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.1 |
| Approx. viscosity (Saybolt univ. sec.) | 35 (25°) | 34 (25°) | 30 (130°) | 31 (130°) | 31 (130°) | 33 (130°) | 35 (150°) | — |
| | | | *Temperature (°C)* | | | | | |
| | | | *100* | *100* | *115* | *130* | *150* | |
| Dielectric constant at 60 Hz | — | — | 4.1 | 4.1 | 4.0 | 3.8 | 3.7 | — |
| Dielectric constant at 1000 Hz | — | — | 4.1 | 4.1 | 4.0 | 3.8 | 3.7 | — |
| Power factor at 60 Hz | — | — | 0.37 | 0.37 | — | 0.45 | 0.99 | — |
| Power factor at 1000 Hz | — | — | 0.005 | 0.005 | 0.01 | 0.04 | 0.44 | — |
| Resistivity (MΩ·cm) | — | — | $1 \cdot 10^5$ | $1 \cdot 10^5$ | $1 \cdot 10^5$ | $1 \cdot 10^5$ | $1 \cdot 10^5$ | — |

* ntb = none to boiling.

** At 30 mm Hg.

TABLE 3

CHEMICAL AND PHYSICAL PROPERTIES OF NIBREN WAXES

| *Property* | *Nibren* | | |
|---|---|---|---|
| | *D88* | *D116N* | *D130* |
| Approx. melting point (°C) | 90 | 113 | 135 |
| Specific gravity (at 20 °C) | 1.57 | 1.66 | 1.77 |
| Shrinkage from 150° to 20 °C (%) | 11 | 10 | 10 |
| Viscosity (°*E*) at 150 °C | 0.96 | 0.98 | 1.1 |
| Acid number | 0.01 | 0.02 | 0.03 |
| $Cl^-$ content (%) | $3 \cdot 10^{-4}$ | $3 \cdot 10^{-4}$ | $5 \cdot 10^{-4}$ |
| Dielectric constant at 800 Hz, 20 °C | 5.0 | 4.7 | 4.5 |
| Power factor (tg $\delta$) at 800 Hz, 20 °C | $1 \cdot 10^{-3}$ | $1 \cdot 10^{-3}$ | $1 \cdot 10^{-3}$ |
| Resistivity ($\Omega \cdot$cm) at 100 V, 1 min | $1 \cdot 10^{14}$ | $1 \cdot 10^{14}$ | $1 \cdot 10^{15}$ |
| Disruptive strength ($kV \cdot cm^{-1}$) | 150–200 | 150–200 | 200 |

to special electrical specifications, are known. Data[18] on the chlorine contents of the Seekay waxes, production of which was ceased about a decade ago, are summarized in Table 4.

*(d) Clonacire waxes*

Small amounts of three Clonacire waxes (90, 115 and 130) are produced by Prodelec (Paris, France). They differ in their degree of chlorination; the numbers correspond to the melting points.

Further information on the properties and uses of the PCN mixtures is presented below, while their spectral characteristics and behaviour in chromatography are discussed in the pertinent sections on analysis.

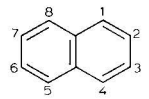
*2. Individual PCNs*

Theoretically, 75 different PCNs exist, as demonstrated in Fig. 1. However, on mechanistic and statistical grounds it is unlikely that all of them are formed in technical chlorination processes. More important, physically, the difficulty of isolating isomers from the mixtures produced by chlorination of naphthalene is such that indirect routes must be employed for all individual PCNs except 1-monochloro- and octachloronaphthalene. So far, the synthesis of 55 individual PCNs has been reported.

TABLE 4

CHLORINE CONTENTS OF SEEKAY WAXES

| *Grade* | *Seekay wax* | *Cl content (%, w/w)* |
|---|---|---|
| R (refined or white wax) | 68 | 46.5 ± 1 |
| | 93 | 50 ± 1 |
| | 123 | 56.5 ± 1 |
| | 700 | 43 ± 1 |
| RC (electrical grades) | 93 | 50 ± 1 |
| | 123 | 56.5 ± 1 |




| No. of Cl atoms in one ring | No. of Cl atoms in other ring | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| 0 | N | 2 | 4 | 2 | 1 |
| 1 | | 6 | 12 | 8 | 2 |
| 2 | | | 13 | 12 | 4 |
| 3 | | | | 6 | 2 |
| 4 | | | | | 1 |

Fig. 1. Naphthalene (N) and its chloro derivatives.

There is one main route according to which most PCNs are obtained in the laboratory. In order to synthesize, for example, a tetrasubstituted PCN, (one of) the corresponding trichloronaphthalenesulphonyl chloride(s) is heated for several hours with phosphorus(V) chloride at *ca.* 200°. The required sulphonyl chloride is often obtained by treating a suitable lower (tri-) substituted PCN with chlorosulphonic acid. Alternative routes include the use of a sulphonic acid instead of a sulphonyl chloride, and hydrolysis of a sulphonyl chloride with hydrochloric acid, in which event no additional chlorine atom is introduced into the naphthalene nucleus. As an illustration, a reaction scheme for the synthesis of several tetra- and one pentachloronaphthalene, taken from ref. 19 and simplified, is shown below.

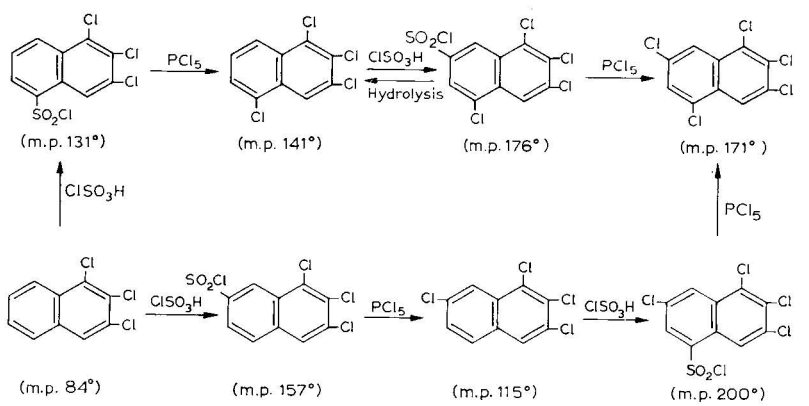



Table 5 records relevant characteristics of all PCNs that have been reported as pure products in the literature. Consultation of the papers by Wynne and co-workers[19,22,25,26,28–30,34] will usually suffice for those workers who are interested in PCNs synthesized from (poly)chloronaphthalenesulphonyl chlorides or -sulphonic acids. Therefore, further references have been omitted from Table 5. For all other compounds, details are listed in Table 6. Some further comments are given in the following sections.

TABLE 5

PHYSICAL PROPERTIES AND METHODS OF SYNTHESIS OF INDIVIDUAL PCNs

Methods of synthesis (see text): 1, $SO_2Cl_2$ + $PCl_5$; 2, sulphonic acid + Cl-containing reagent; 3, hydrolysis of sulphonyl chloride; 4, see Table 6.

| *PCN* | *Melting point (°C)* * | *Boiling point (°C)* | *Density* ($d_4^t$) | $n_D$ | *Dipole moment (D)* * | *Route* | |
|---|---|---|---|---|---|---|---|
| | | | | | | *No.* | *Ref.* |
| 1 | − 4 to − 2.3 | $259.3^{760}$ | $1.1938^{20}$ | $1.6326^{20}$ | 1.50–1.59 | 2 | 20 |
| 2 | 58–60 | 256 | $1.1377^{70.7}$ | $1.6079^{70.7}$ | 1.57–1.72 | 2 | 21 |
| 1,2 | 34–37 | 295–298 | $1.3147^{48.5}$ | $1.6338^{48.5}$ | 2.47 | 1 | 22 |
| 1,3 | 61.5–62 | $291^{775}$ | | | 1.78 | 1 | 22 |
| 1,4 | 68–72 | $286–287^{740}$ | $1.2997^{75.9}$ | $1.6228^{75.9}$ | 0–0.48 | 1 | 23 |
| 1,5 | 106.5–107 | subl. | | | 0 | 1 | 24 |
| 1,6 | 48.5–49 | subl. | | | 1.44 | 1 | 25 |
| 1,7 | 61.5–64 | 285–286 | $1.2611^{99.5}$ | $1.6092^{99.5}$ | 2.55 | 1 | 26 |
| 1,8 | 88–89.5 | decomp. | $1.2924^{99.8}$ | $1.6236^{99.8}$ | 2.82 | 3 | 27 |
| 2,3 | 119.5–120.5 | | | | 2.55 | 3 | 28 |
| 2,6 | 135–141 | 285 | | | 0–0.60 | 1 | 23 |
| 2,7 | 114–116 | | | | 1.53 | 1 | 29 |
| 1,2,3 | 81–84 | | | | | 4 | 19 |
| 1,2,4 | 92 | | | | | 3 | 28 |
| 1,2,5 | 74–79 | | | | | 1 | 19 |
| 1,2,6 | 90–92.5 | | | | | 1 | 29 |
| 1,2,7 | 88 | | | | | 1 | 29 |
| 1,2,8 | 83 | | | | | 2 | 27 |
| 1,3,5 | 102–103 | | | | | 1 | 28 |
| 1,3,6 | 80.5–81 | | | | | 1 | 19 |
| 1,3,7 | 112.5–113 | | | | | 1 | 30 |
| 1,3,8 | 89.5 | | | | | 1 | 30 |
| 1,4,5 | 130–131 | | | | | 1 | 19 |
| 1,4,6 | 65–68 | | | | | 1 | 28 |
| 1,6,7 | 109–109.5 | | | | | 1 | 28 |
| 2,3,6 | 90–91 | | | | | 1 | 19 |
| 1,2,3,4 | 196–198 | | | | | 4 | 19 |
| 1,2,3,5 | 141 | | | | | 1 | 19 |
| 1,2,3,7 | 115 | | | | | 1 | 19 |
| 1,2,4,6** | 111 | | | | | 1 | 19, 31 |
| 1,2,4,7** | 140–144 | | | | | 1 | 19, 32 |
| 1,2,5,6** | 164 | | | | | 1 | 19, 33 |
| 1,2,5,7** | 114 | | | | | 1 | 19, 31 |
| 1,2,6,8 | 125–127 | | | | | 4 | 34 |
| 1,3,5,7 | 178–181 | | | | | 1 | 19 |
| 1,3,5,8 | 131 | | | | | 1 | 19 |
| 1,3,6,7 | 119–120 | | | | | 1 | 19 |
| 1,4,5,8 | 183 | | | | | 4 | 35–37 |
| 1,4,6,7 | 139 | | | | | 1 | 19 |
| 2,3,6,7 | 135 | | | | | 4 | 38 |
| 1,2,8,*x* | 135 | | | | | 1 | 19 |
| 1,4,5,*x* (*x*=3?) | 144 | | | | | 1 | 19 |
| 2,3,6,*x* | 218 | | | | | 1 | 19 |
| *x*,*x*,*x*,*x* | 176 | | | | | 4 | 38 |
| 1,2,3,4,5 | 168.5 | | | | | 4 | 39, 40 |

*(Continued on p. 210)*

TABLE 5 (*continued*)

| *PCN* | *Melting point (°C)* * | *Boiling point (°C)* | *Density* ($d_4^t$) | $n_D$ | *Dipole moment (D)* * | *Route* | |
|---|---|---|---|---|---|---|---|
| | | | | | | *No.* | *Ref.* |
| 1,2,3,4,6 | 147 | | | | | 1 | 19 |
| 1,2,3,5,7 | 171 | | | | | 1 | 19 |
| 1,2,3,5,*x* | 177 | | | | | 4 | 37, 41 |
| 1,2,4,6,*x* ($x = 8$?) | 135 | | | | | 1 | 19 |
| 1,3,5,8,*x* | 155 | | | | | 1 | 19 |
| 1,3,5,8,*x* | — *** | | | | | 4 | 42 |
| 1,4,6,7,*x* ($x = 2$?) | 131 | | | | | 1 | 19 |
| 1,2,3,4,5,6 | — | | | | | 4 | 43 |
| 1,2,4,5,6,8 | 175–177 | | | | | 4 | 38 |
| 1,2,3,4,5,6,7 | — *** | | | | | 4 | 44 |
| 1,2,3,4,5,6,8 | 194 | | | | | 4 | 44, 45 |
| 1,2,3,4,-5,6,7,8 | 197.5–203 | | | | | 4 | |

* Range of values found in the literature.
** See text for structure assignment.
*** Still impure.

*(a) Mono-PCNs*

Apart from the PCN mixtures, 1-monochloronaphthalene is the only chlorinated naphthalene that has so far proved to be of industrial utility. It is produced industrially by passing chlorine into molten naphthalene and fractionating the product. It can also be obtained in good yield[17,47,48] by chlorinating molten naphthalene in the presence of iodine, iron(III) chloride or antimony(V) chloride.

1- and 2-monochloronaphthalene can be prepared on a smaller scale from naphthalene-1-sulphonic acid on heating with copper(II) chloride[20] and from 2-monochloronaphthalene-1-sulphonic acid on treatment with dilute sulphuric acid[21].

*(b) Di- and tri-PCNs*

For some disubstituted PCNs, preparation from the sulphonyl chloride is not the preferred method. 1,3-Dichloronaphthalene, for instance, is obtained[49] in better yield by heating a solution of diazotised 2,4-dichloro-1-naphthylamine in dilute sulphuric acid with ethanol. With trisubstituted PCNs, both nitro and amino compounds have been used[19,27,28] as starting products instead of a sulphonyl chloride or sulphonic acid. For further details, the reader should consult compilations such as refs. 50 and 51.

*(c) Tetra- and penta-PCNs*

Turner and Wynne[19] were not able to elucidate the exact structures of several tetrasubstituted PCNs. According to Cencelj[31], 1,4,7,*x*-tetrachloronaphthalene (probably $x = 8$; ref. 19) is identical (melting point, IR spectrum) with the 1,2,4,6-tetrachloronaphthalene he synthesized himself. On the basis of melting point data, Cencelj also concluded that $x = 7$ in 1,2,5,*x*-tetrachloronaphthalene (m.p. 114°). Piggott and Slinger[33] have demonstrated that 1,2,5,*x*-tetrachloronaphthalene (m.p. 164°) is iden-

TABLE 6

DETAILS OF THE SYNTHESIS OF PCNs NOT PREPARED ACCORDING TO REACTION SCHEMES 1–3 IN TABLE 5

| *PCN* | *Starting product* | *Method* | *Reference* |
|---|---|---|---|
| | *Tetralin* | | |
| 1,2,3 | 1,1,2,3,4-Pentachlorotetralin | Boiling with $C_2H_5ONa$ | 19 |
| 1,2,3,4 | 1,1,2,3,4,4-Hexachlorotetralin | Boiling with $C_2H_5ONa$ | 19 |
| | *Nitro derivative* | | |
| 1,2,6,8 | 1,2,6,8-Tetranitronaphthalene | $PCl_5$ and some $POCl_3$ at 180° | 34 |
| 1,4,5,8 | 4,8-Dichloro-1,5-dinitronaphthalene | $PCl_5$ | 35–37 |
| 1,2,3,5,*x* | 1,2,3,5-Tetrachloro-*x*-nitro-naphthalene | $PCl_5$ | 37, 41 |
| 1,3,5,8,*x* | 1,3,5,8-Tetranitronaphthalene | Conc. HCl at 240° | 42 |
| 1,2,3,4,5,6 | 1,2,3,4,5,6-Hexachloro-7-nitro-naphthalene | (1) Zn–acetic acid; (2) $NaNO_2$; (3) $H_3PO_2$ | 43 |
| | *Quinone* | | |
| 1,2,3,4,5 | 2,3-Dichloro-1,4-naphthoquinone | $PCl_5$ and some $POCl_3$ at 180–200° or $PCl_5$ only at 200–250° | 39, 40 |
| | *Miscellaneous* | | |
| *x*,*x*,*x*,*x* | Dichloronaphthalene | (1) Chlorination; (2) KOH | 38 |
| 2,3,6,7 | 2,3,6,7-Tetrachloronaphthalene-tetracarboxylic acid | Basic copper carbonate in quinoline at 240° | 38 |
| 1,2,4,5,6,8 | 1,3,5,7-Tetrachloro-4,8-bistosyl-aminonaphthalene | (1) $H_2SO_4$; (2) $NaNO_2$; (3) HCl + $CuCl_2$ | 38 |
| 1H-hepta | Octachloronaphthalene | $LiAlH_4$ | 44, 45 |
| 2H-hepta | Halowax 1051 | Fractional crystallization | 44, 45 |
| Octa | See text and ref. 46 for details | | |

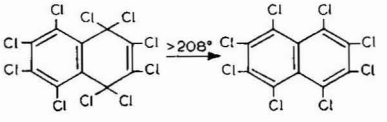
tical with 1,2,5,6-tetrachloronaphthalene. Hardy *et al.*[32] have confirmed that the 1,2,7,*x*-tetrachloronaphthalene synthesized by Turner and Wynne indeed has $x = 4$, as already suggested by Wynne[52]. Lastly, the *x*,*x*,*x*,*x*-tetrasubstituted PCN mentioned in Table 6 has been isolated[38] from the oily by-product obtained in the synthesis of 1,2,3,4,5,8-hexachlorotetralin by chlorination of crude dichloronaphthalene, followed by treatment with alcoholic potassium hydroxide. The unknown structures of the various pentachloronaphthalenes recorded in Tables 5 and 6 have not yet been elucidated.

*(d) Hepta- and octa-PCNs*

Recently, Clark and co-workers[44,45] described the preparation of a pure heptachloronaphthalene (probably 1H-heptachloronaphthalene) by reduction of octachloronaphthalene with aluminium lithium hydride. The other, still impure, heptachloro isomer has been obtained by fractional crystallization of Halowax 1051 from toluene and carbon tetrachloride. The assignment of structures to the heptachloronaphthalenes is discussed in Section III.

Octachloronaphthalene is prepared by exhaustive chlorination of naphthalene with, for example, chlorine and phosphorus(V) chloride[53], or chlorine, iron and iodine at 100–150°[16,54]. Nearly quantitative yields are realized[55] in vapour-phase chlorination using *e.g.* charcoal or rhodium(III) chloride–alumina at 300°. A short survey of the literature on perchlorination was given by Suschitzky[56], while a discussion on the use of perchlorination for PCN analysis is presented in Section III.2.

Lastly, it should be noted that decachloro-1,4-dihydronaphthalene is obtained in good yield from Halowax 1014[46] or a 1-phenylmethylnaphthalene derivative[57] and a mixture of disulphur dichloride, sulphuryl chloride and aluminium trichloride. When decachloro-1,4-dihydronaphthalene is heated above its melting point (208°), octachloronaphthalene is formed:




(2)

It has been suggested[46] that such a thermal reaction may account for the fact that both compounds give an identical mass spectrum (corresponding to that of octachloronaphthalene) and display identical retention times in gas–liquid chromatography (GLC).

*(e) Deuterated compounds*

The synthesis of partly deuterated naphthalenes and octadeuteronaphthalene[58], their main physical properties[58] and IR[59] and Raman[58] spectra have been reported.

*(f) Melting points*

The melting point data of the disubstituted PCNs have been analyzed by De Laszlo[60]. The $\alpha,\beta$-dichloronaphthalenes, which exhibit no degree of symmetry, have lower melting points than have the $\beta,\beta$- and $\alpha,\alpha$-dichloronaphthalenes, all of which possess a plane of symmetry. The di-$\beta$-substituted isomers melt at higher temperatures than do the di-$\alpha$-substituted isomers, and 2,6-dichloronaphthalene, which has its substituents in positions analogous to those in *para*-substituted benzenes, has the highest melting point. The same sequence is observed for the corresponding dibromonaphthalenes.

*3. General properties and uses*

Most information is available on the Halowaxes and Nibren waxes and these particular types will therefore serve for further discussion[6,13,14,17] of PCNs in general.

*(a) Properties*

As is evident from the data in Section II.1, except for Halowaxes 1031 and 1000, the commercially available PCN mixtures can be described as having chlorine contents from 40 to 70 wt.-%, melting points in the range 80–185°, specific gravities (at ambient temperature) from 1.5 to 2.0, dielectric constants of $4.5 \pm 1$, and direct-current resistivities of about $10^{14}\,\Omega\cdot$cm at room temperature and $10^{11}\,\Omega\cdot$cm at their melting points.

The chlorinated naphthalenes are excellent dielectrics and possess a high degree of chemical stability, indicated by their resistance to concentrated bases and acids, except concentrated nitric acid. PCN mixtures remain stable even at temperatures up to their boiling range, and are stable to oxidising agents. At 120–125°, they are unaffected by copper and mild steel in a dry atmosphere, and at 40–50° in the presence of moisture. In the presence of moisture at 120–125°, they tarnish copper, owing to the liberation of small amounts of hydrogen chloride. Other desirable characteristics include inherent flame resistance and resistance to fungus growth. The solid products melt to liquids of extremely low viscosity.

Chlorinated naphthalenes are generally compatible with petroleum waxes, chlorinated paraffins, polyisobutylenes, low-molecular-weight styrene, phenolic resin solutions and several plasticizers. They have limited compatibility with ethylcellulose, polyethylene and vinyl resins, and are not compatible with nitrocellulose or cellulose acetate.

PCNs have good solubility in chlorinated and aromatic solvents and in petroleum naphthas. They have limited solubility in ketones, ethers, acetates and mineral oils, and are insoluble in alcohols and water.

The toxicity of the PCNs, which is discussed in Section IV.1, calls for special precautions in their use. Poisoning by PCNs may take the form of acne or of toxic jaundice. Systematic poisoning is a consequence of inhalation of the fumes from the molten substances, rather than from handling the cold solids. Damage from inhaling the fumes can be severe and occasionally fatal. Before being employed on work with PCNs, one should undergo a medical examination in order to ascertain whether one has suffered or is suffering from any disease that affects the liver, as it is known that such persons are predisposed to further liver damage by PCN poisoning.

The principal precautionary measures to be taken against PCN poisoning are the provision of forced ventilation, washing facilities and protective clothing, which should be dry-cleaned frequently. The use of barrier creams, to be applied to the hands before working with chlorinated naphthalenes, has also been recommended. After handling PCNs, one should wash with soap and apply lanolin to the skin. For general precautions to be taken when using PCNs industrially, the reader is referred to a paper by Greenburg[61].

1-Monochloronaphthalene is the only pure PCN that is liquid at ordinary temperatures. It is miscible with most of the common organic solvents and, at moderate temperatures, is unaffected by water and alkali and has no corrosive action on the common materials of construction. So far, 1-monochloronaphthalene has not been reported to have the harmful effects associated with the higher chlorinated naphthalenes. However, it is recommended that contact with the skin should be avoided and adequate ventilation should be provided.

*(b) Uses*

PCN mixtures are used chiefly in the electrical industry, *e.g.*, as separators in storage batteries, as capacitor impregnants and as high-temperature and flame-resistant seals for condensers and coils. They are also employed as binders for electrical-grade ceramics and sintered metals. PCNs, as the molten wax or in the form of emulsions, are used in cable-covering compositions, and to impregnate wood, paper and textiles, to which they impart water-proofness, flame resistance and

fungicidal and insecticidal properties. Some grades of PCN mixtures, and 1-monochloronaphthalene, have been applied as insecticides.

When compounded with materials such as resins, rubber, plastics, talc, kaolin and PCBs, the PCNs form a wide range of mouldable masses of appropriate hardness, plasticity, etc. Further, PCNs are used to dissolve sludge and varnish formed by petroleum oils and as ingredients in motor tune-up compounds and photoelastic immersion fluids. They are employed as plasticizers, as additives in automobile and industrial gear oils and cutting oils, and in protective coatings, lacquers and underwater paints. Halowax 1051 is used in organic fillers when flame retardancy is required, and Halowax 1031 has been suggested as a raw material for dyes. The PCN waxes are easily coloured, *e.g.*, with Ceres dyes.

## III. ANALYSIS

### *1. Chromatography*

Armour and Burke[62] were among the first workers to study the behaviour of PCNs in view of their possible interference in the analysis of organochlorine pesticides and/or PCBs. They demonstrated that under the GLC conditions used for the analysis of organochlorine pesticide residues[63], Halowax 1099 and 1014 exhibit peaks throughout the retention-time region of the pesticides (retention time relative to aldrin, 0.5–6); the highly chlorinated Halowax 1051 is eluted beyond the retention times of the common pesticides (retention time relative to aldrin, 11). A study of Florisil column-chromatographic clean-up[63] showed that Halowax 1014, 1099 and part of Halowax 1051 are eluted by 200 ml of 6% diethyl ether in light petroleum, which also elutes pesticides such as DDT and its analogues. Recovery studies with Halowax 1014 (50 $\mu$g added to 100-g samples of fish, milk and spinach) using the FDA method[63] revealed recoveries of 67, 68 and 90%, respectively. According to the authors, the low recoveries from fish and milk resulted from unfavourable partitioning between light petroleum and acetonitrile.

As an alternative, Armour and Burke carried out chromatography on a silica gel (+3% added water)–Celite (4:1, w/w) column, using the procedure reported in ref. 64. All Halowaxes tested were completely eluted by the 250 ml of light petroleum used as eluent, in which fraction PCBs were also recovered. No Halowax was found in the subsequent 200-ml acetonitrile–*n*-hexane–dichloromethane (1:19:80, v/v) mixture, which contained most of the common organochlorine pesticides (Fig. 2). The recovery of Halowax 1014 added to a trout sample (50 $\mu$g to 10 g of fish) was over 90%. The procedure, which has also been recommended by Goerlitz and Law[65], suffers from the disadvantage that large volumes of solvents (*ca.* 0.5 l per sample) are required.

Separation of PCNs plus PCBs from organochlorine pesticides also occurs in the alumina–silica gel column-chromatographic procedure developed by Holden and Marsden[66] and modified by Zitko[67]. A summary is given in Table 7. Both PCNs and PCBs are eluted in fractions I and II; *p,p'*-DDE is also partly eluted in the *n*-hexane fractions, but separation from most other common pesticides is successful. Zitko emphasized that in order to achieve reproducible chromatographic conditions, the activity of the adsorbents must be carefully controlled, the activation procedures described in detail, and the quality of the solvents used for elution specified.

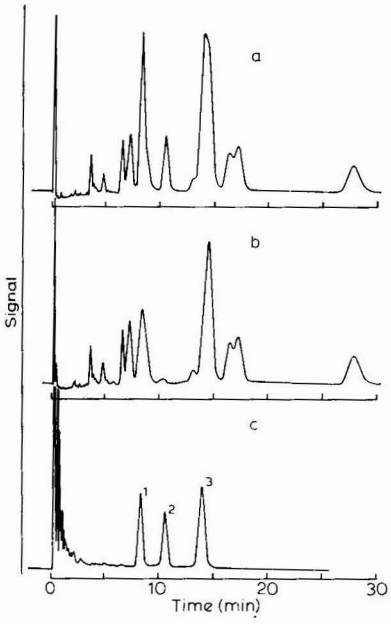



Fig. 2. GLC of brown-trout Florisil column eluate fortified with 2.5 ppm of Halowax 1014, 0.3 ppm of *p,p'*-DDT and 0.2 ppm of *p,p'*-TDE and containing 0.19 ppm of *p,p'*-DDE residue. (a) Before separation. (b) Light petroleum eluate from silica gel column. (c) Polar eluate from silica gel column containing (1) *p,p'*-DDE, (2) *p,p'*-TDE and (3) *p,p'*-DDT. A 10-mg sample was injected for each curve. For GLC conditions, see ref. 62.

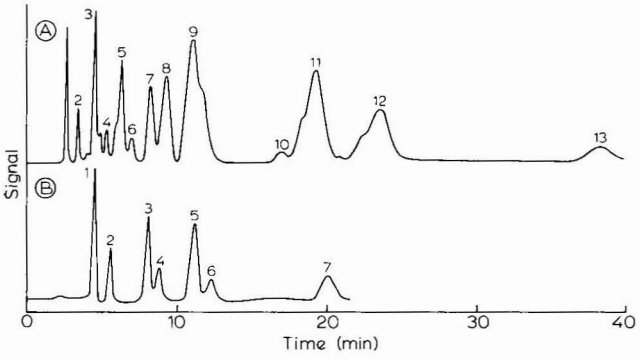
As an alternative method for the discrimination between PCNs and other types of organochlorine compounds, Gulan *et al.*[68] trapped the components of a mixture as they were eluted from the gas chromatograph (2% SE-30–2% QF-1 on 70–80-mesh Anakrom ABS at 180°), added a small amount of *n*-hexane, exposed the solution to the light from a 100-W, medium-pressure UV lamp and re-chromatographed the irradiated products on a 4% SE-30–4% QF-1 on 70–80-mesh Anakrom ABS column, the temperature being programmed from 170° to 190°. As an illustra-

TABLE 7

PROCEDURE FOR SEPARATION OF PCNs AND PCBs FROM ORGANOCHLORINE PESTICIDES[66,67]

| *Step* | *Procedure* |
|---|---|
| 1 | Sample (5 g) ground with anhydrous $Na_2SO_4$ |
| 2 | Ground sample extracted with pesticide-grade *n*-hexane, Soxhlet, 1 h, final volume of extract 100 ml |
| 3 | Chromatography on alumina (Fisher No. A-540), aliquot of extract (1–50 ml) in 1.5 ml of *n*-hexane, alumina activated at 800° (4 h), 5% water added. Column 45 × 0.7 cm, 2 g of alumina, 20 ml of effluent collected |
| 4 | Chromatography on Silicar silica gel. Effluent from alumina in 1.5 ml of *n*-hexane, Silicar activated overnight at 130°, 3% water added, column 45 × 0.7 cm, 2 g of Silicar |
| 5 | Effluent: *n*-hexane, 10 ml: fraction I; 20 ml: fraction II; 10% diethyl ether in *n*-hexane, 10 ml: fraction III |

tion, data on the so-called optimal irradiation time (*i.e.*, the minimal time that yields about equal areas for the main degradation peak and for the parent peak) of the 13 peaks obtained on GLC of Halowax 1014, and the fingerprint degradation patterns observed after re-chromatography, are shown in Fig. 3. The insecticides heptachlor, aldrin, heptachlor epoxide, *o,p'*- and *p,p'*-DDE, dieldrin and *o,p'*- and *p,p'*-DDT, which interfere in GLC, can easily be distinguished from the Halowax peaks 3, 4,



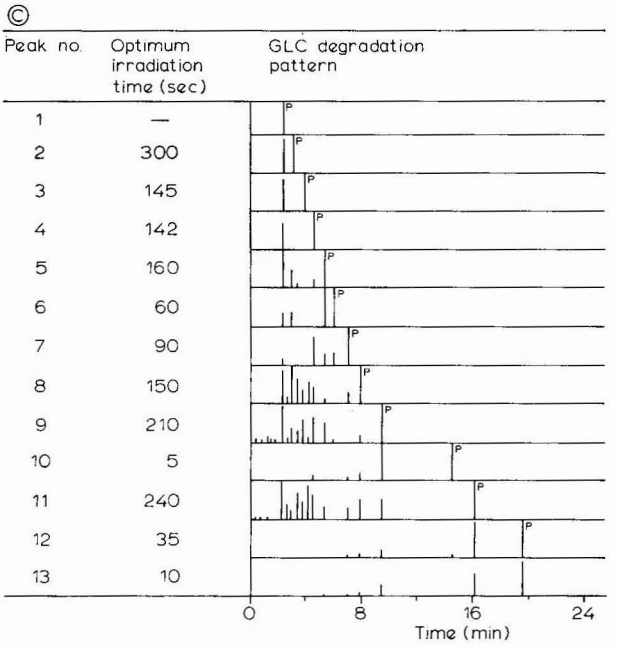




Fig. 3. GLC (ref. 68) of (A) Halowax 1014 and (B) a mixture of insecticides: (1) heptachlor, (2) aldrin, (3) heptachlor epoxide, (4) *o,p'*-DDE, (5) *p,p'*-DDE, (6) dieldrin and *o,p'*-DDD, (7) *p,p'*-DDT. (C) Irradiation procedure and degradation patterns of peaks 1–13 of Halowax 1014 presented as peak area *versus* $t_{ret.}$. P = parent peak. Trapping GLC on 2% SE-30–2% QF-1 on 70–80-mesh Anakrom ABS. Temperatures: injection and detector ($^3$H), 200°; oven, 180°. Gas flow-rate, Ar–$CH_4$ (19:1), 60 ml/min. Re-chromatography on 4% SE-30–4% QF-1 on 70–80-mesh Anakrom ABS. Temperatures: injection, 240°; detector, 210°; programmed from 170° to 190° at 2°/min. Gas flow-rate, $N_2$, 30 ml/min.

7–9 and 11, as their degradation products are largely resolved from those of the PCN degradation products. The authors claim that the same holds true for the PCB mixture Aroclor 1254 for all but one pair of corresponding GLC peaks.

Goerlitz and Law[65], who also studied the extent to which PCNs may interfere in the analysis of pesticides, presented chromatograms of Halowax 1013 and 1014, analyzed by electron-capture GLC on 3% OV-101 (on Gas-Chrom Q) and 3% OV-101–5% OV-210 (on Gas-Chrom Q) columns at 180° and 175°, respectively. Identification of the chlorine content of the peaks was achieved with the aid of a computer-controlled GLC–mass spectrometry (MS) system. Fig. 4 shows representative chromatograms and the chlorine-number assignments of each peak. According to the authors, the pattern of compounds and isomers of a particular PCN preparation, as it appears on the chromatogram, is not as characteristic or distinctive as that of PCB formulations. In other words, one cannot readily assess the occurrence of PCNs in previously analyzed samples simply by reviewing the chromatographic records.

The combined use of thin-layer chromatography (TLC) and GLC for the analysis of PCNs has been proposed by Stalling and Huckins[69]. The components of Halowax 1099, 1013 and 1014 were resolved by reversed-phase TLC on Kieselguhr-coated glass plates impregnated with paraffin oil. Three successive developments were carried out in a saturated atmosphere, using methanol–acetonitrile–acetone–water

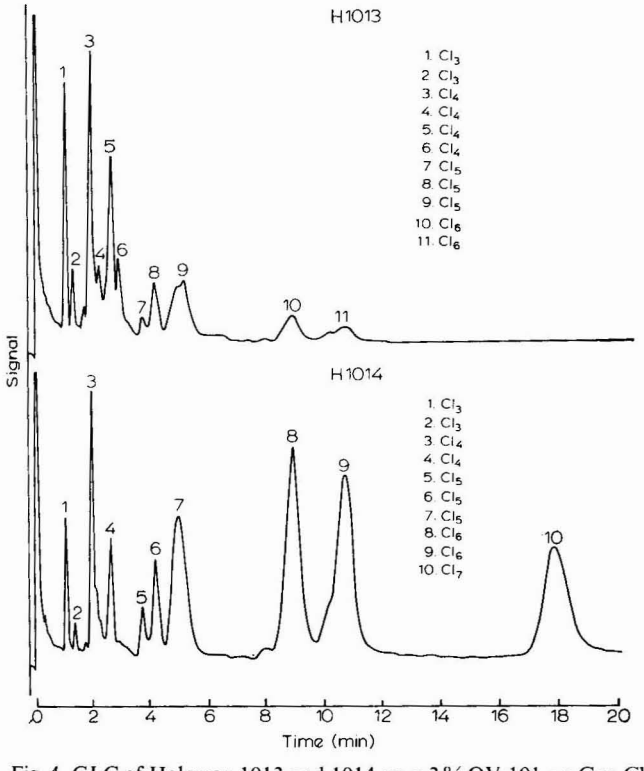



Fig. 4. GLC of Halowax 1013 and 1014 on a 3% OV-101 on Gas-Chrom Q column[65]. Temperatures: injection, 205°; column, 180°; detector ($^3H$), 200°. Gas flow-rate, $N_2$, 37 ml/min; He used instead of $N_2$ in GLC–MS.

(8:8:3:1, v/v) saturated with paraffin oil as the mobile phase. Identification was effected by spraying with a solution of silver nitrate (1.7 g) in ethanol (200 ml) to which ammonia solution (sp.gr. 0.880) (1 ml) is added just before application. The plate was then held over a steam-bath for a few seconds and finally placed under a UV lamp for several minutes. All chlorinated components appeared as dark spots on a light background. Approximately 8–15 $\mu$g of the Halowaxes are required in order to detect the major components. In order to relate the TLC data to results obtained by GLC, they eluted the pertinent zones on the chromatogram with light petroleum–diethyl ether (19:1, v/v) and analyzed the resulting solutions on a 0.3% OV-7 on glass beads column at 160 or 190°, using a nickel-63 electron-capture detector. Temperature-programmed GLC–MS was used to characterize Halowax standards in terms of the chlorine contents of the individual peaks. From the results, which are summarized in Table 8, it is apparent that spots with lower $R_F$ values generally correspond to GLC peaks with longer retention times, *i.e.*, to Halowax components with more chlorine atoms. Stalling and Huckins[69] also presented gas chromatograms of Halowax 1031, 1000 and 1051, but did not comment on them.

According to Brinkman *et al.*[70], reversed-phase TLC of PCNs on Kieselguhr

TABLE 8

CORRELATION OF REVERSED-PHASE TLC SPOTS AND GLC PEAKS OF HALOWAX 1099, 1013 AND 1014[69]*

| *Halowax* | $R_F$ *in TLC*** | *Retention time relative to aldrin or p,p'-DDE in GLC**** | *GLC–MS* | |
|---|---|---|---|---|
| | | | *No. of Cl atoms* | *Mol. wt.* |
| 1099 | 0.52 | 1.97 | 5 | 298 |
| | 0.67 | 0.83, 1.03 | 4 | 264 |
| | | 2.48, 2.82 | 5 | 298 |
| | 0.77 | 1.18 | 4 | 264 |
| | 0.81 | 0.44, 1.28 | 3, 4 | 230, 264 |
| | 0.85 | 1.53, 0.62 | 4, 3 | 264, 230 |
| | 0.89 | 0.23 | 2 | 196 |
| 1013 | 0.46 | 1.67, 4.64 | 5 | 298 |
| | 0.50 | 4.98, 5.70 | 6 | 332 |
| | 0.58 | 0.70, 1.97 | 4, 5 | 264, 298 |
| | 0.66 | 0.82, 1.02 | 4 | 264 |
| | | 2.48, 2.82 | 5, 6 | 298, 332 |
| | 0.76 | 1.27 | 4 | 264 |
| | 0.81 | 1.52, 0.43 | 4, 3 | 264, 230 |
| | 0.85 | 0.60 | 3 | 230 |
| 1014 | 0.35 | 1.60, 1.89, 4.21 | 6, 7 | 332, 366 |
| | 0.44 | 0.72, 0.84 | 5 | 298 |
| | | 1.98, 2.28 | 6 | 332 |
| | 0.56 | 0.30, 2.53 | 3, 6 | 230, 332 |
| | 0.61 | 0.38, 1.05, 1.19 | 4, 5 | 264, 293 |
| | 0.81 | 0.23, 0.58 | 4, 3 | 264, 230 |

* For TLC and GLC conditions, see text and subsequent notes.

** $R_F$ for *p,p'*-DDE, 0.92.

*** Column temperature, 160° (Halowax 1099 and 1013) or 190° (Halowax 1014); retention times relative to aldrin (Halowax 1099 and 1013) or *p,p'*-DDE (Halowax 1014).

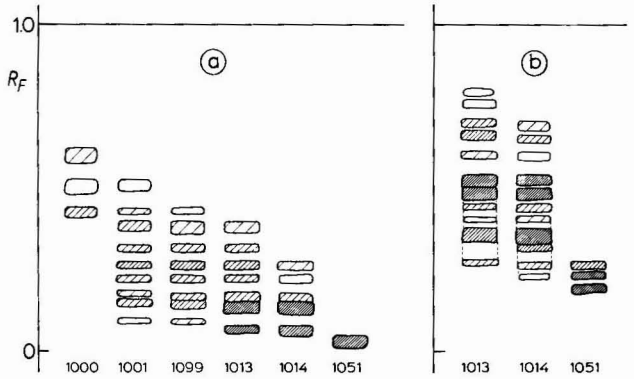



Fig. 5. TLC of Halowax 1000–1051 on (a) Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–water (8:9:3); (b) Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–acetone–water (20:20:9:1)[70]. Detection: spraying with a solution of 0.3% tolidine in 80% ethanol containing 0.3% glacial acetic acid and subsequent UV irradiation.

impregnated with paraffin oil is superior to TLC with the system silica gel–dry *n*-hexane (*cf.* ref. 176). With the former technique (Fig. 5), about twice as many spots are observed. Acetonitrile–methanol–water (8:9:3, v/v) is recommended for the separation of the low-chlorinated Halowaxes; improved resolution of the more highly chlorinated mixtures is obtained with acetonitrile–methanol–acetone–water (20:20:9:1, v/v) as the mobile phase. The data compare favourably with those of Stalling and Huckins[69], who reported the presence of 5–7 spots in the reversed-phase TLC of various Halowaxes (Table 8). The excellent separation obtained with Halowax 1051 is discussed below.

Challen and Kučera[71] studied the detection of PCNs and other commercial wood preservatives using TLC and GLC. With chloroform extracts of wood (46 species), the several preservatives were separated best on silica gel with *n*-hexane–ethyl acetate (17:3, v/v) as the mobile phase. Spraying with a 0.1% solution of diiodofluorescein in alcohol, and subsequent exposure to bromine vapour, allowed the detection of 50–100 $\mu$g of PCNs. In GLC, the large number of peaks observed with samples containing PCNs allows their detection; however, it renders impossible their identification in mixtures with other preservatives.

An extensive study on the identity of the PCNs present in Halowax 1031, 1000, 1001 and 1099 was reported by Beland and Geer[8]. Chromatograms were run on two different columns, *viz.*, a 10% Carbowax 20M on 60–80-mesh Chromosorb W and a mixed 5% Bentone 34–10% OV-101 on 100–120-mesh Supelcoport column, using an oven temperature of 180° (Fig. 6). From the data collected in Table 9, it can be seen that both monochloronaphthalenes were found to be present, together with all of the disubstituted isomers (except 2,6-dichloronaphthalene) and several of the possible tri- and tetrasubstituted naphthalenes. In addition, at least three components appear to be present that do not seem to be PCNs. Four PCNs identified in Halowax 1001 have also been shown[72] to be present in Nibren D88, which has a comparable chlorine content. A sample of Nibren D88 was separated into 22 fractions on a column of activated alumina, using light petroleum as solvent and light petroleum–benzene (99:1, v/v) as the mobile phase. Detection by means of IR absorption measurement indicated

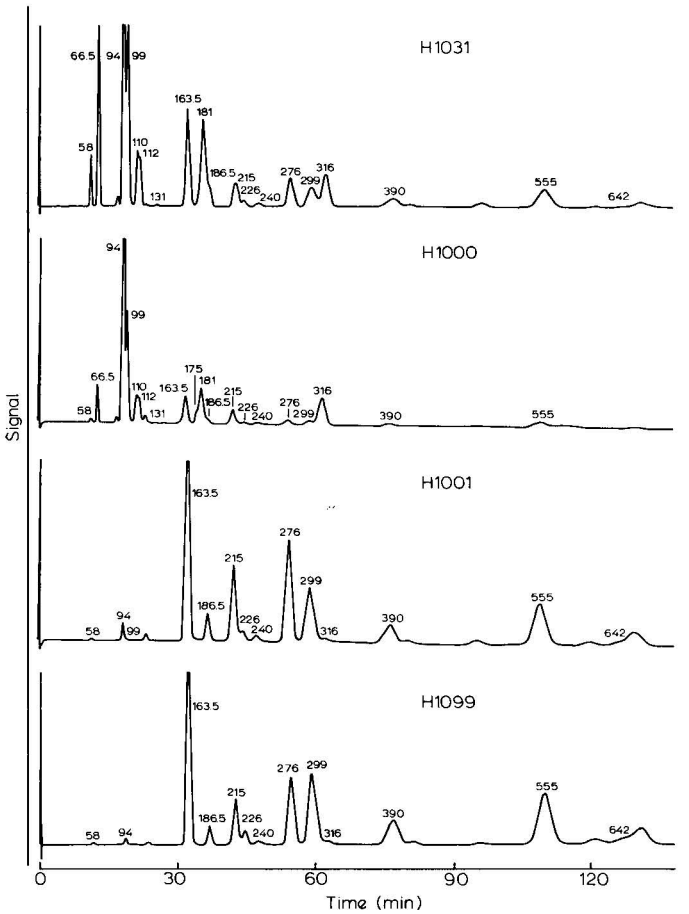



Fig. 6. GLC (ref. 8) of Halowax 1031 (3 $\mu$g), 1000 (0.3 $\mu$g), 1001 (30 ng) and 1099 (30 ng) on a Bentone 34–OV-101 packed column. Temperatures: injection, 190°; oven, 180°; detector ($^3$H), 190°. Gas flow-rate, $N_2$, 30 ml/min.

that the first three fractions contained 1,3,5,7-tetrachloronaphthalene, and that 1,4,6-trichloro-, 1,4,5-trichloro- and 1,4,5,8-tetrachloronaphthalene were the main components of fractions 4–9, 10–15 and 16–22, respectively.

Beland and Geer[8] pointed out that electron-capture gas chromatograms give misleading values of the amounts of each individual PCN present. They observed that an increase in the detector response occurs largely in the mono- to trichloro range, although the magnitude of the effect is not yet known. For instance, Halowax 1031 contains a minimum of 96% of monochloronaphthalenes, yet the chromatogram in Fig. 6 shows a clear indication of tetrasubstituted isomers being present, although at very low concentrations.

High-performance liquid chromatography (HPLC) in the system silica gel–dry *n*-hexane has been used[70] to characterize the behaviour of three series of commercially available PCN mixtures, *viz.*, Halowax 1031–1051, Nibren D88–D130 and Clonacire 90–130. Chromatograms of the Halowax series are presented in Fig. 7; they were re-

TABLE 9

RETENTION TIMES AND IDENTIFICATION OF COMPONENTS OF HALOWAX 1031, 1000, 1001 AND 1099

For GLC details, see text and Fig. 6.

| *Retention time (min) on* | | *PCN* | *Halowax* | | | |
|---|---|---|---|---|---|---|
| *Bentone* | *Carbowax* | | *1031* | *1000* | *1001* | *1099* |
| 58.0 | 32.0 | 2 | + | + | + | + |
| 66.5 | 32.0 | 1 | + | + | | |
| 94.0 | 62.0 | 1,4 | + | + | + | + |
| 99.0 | 62.0 | 1,5 | + | + | + | |
| 110.0 | 57.0 | 1,3 | + | + | | |
| 112.0 | 69.0 | 1,6 | + | + | | |
| 131.0 | 77.0 | 2,7 | + | + | | |
| 163.5 | 84.0 | 2,3 | + | + | + | + |
| 163.5 | 108.5 | 1,4,6 | + | + | + | + |
| 175.0 | 77.0 | 1,2 | | + | | |
| 181.0 | 69.0 | 1,7 | + | + | | |
| 186.5 | 94.5 | 1,3,5 | + | + | + | + |
| 215.0 | 108.5 | 1,2,4 | + | + | + | + |
| 226.0 | 127.0 | 1,3,5,7 | + | + | + | + |
| 240.0 | 154.5 | 1,2,6 | + | + | + | + |
| 276.0 | 199.0 | 1,4,5 | + | + | + | + |
| 299.0 | 177.0 | 1,2,4,6 | + | + | + | + |
| 316.0 | 127.0 | 1,8 | + | + | + | + |
| 390.0 | 254.0 | 1,3,5,8 | + | + | + | + |
| 555.0 | 526.0 | 1,4,5,8 | + | + | + | + |
| 642.0 | 272.0 | 1,2,3,4 | + | + | + | + |

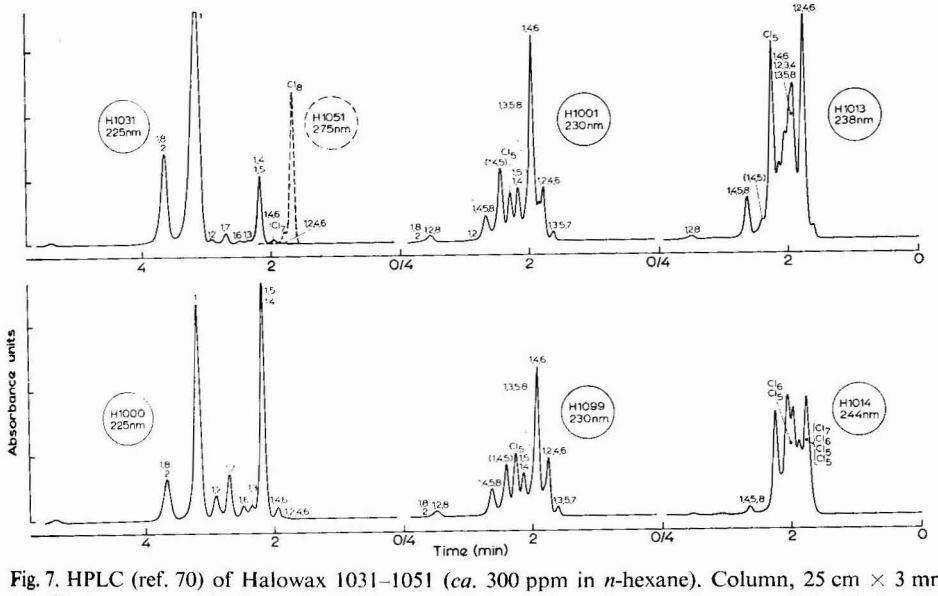



Fig. 7. HPLC (ref. 70) of Halowax 1031–1051 (*ca.* 300 ppm in *n*-hexane). Column, 25 cm × 3 mm I.D. filled with 5-$\mu$m LiChrosorb SI 60; mobile phase, dry *n*-hexane; flow-rate, 1.4 ml/min; UV detection at the wavelengths indicated in the figures; full scale, 1.28 (Halowax 1031, 1000, 1001 and 1099), 0.64 (Halowax 1013 and 1014), 2.56 (Halowax 1051) absorbance units; temperature, 27 ± 1°. Tentative assignments are indicated by parentheses.

TABLE 10

HPLC RETENTION TIMES, UV AND IR SPECTRAL DATA AND REDUCTION POTENTIALS OF INDIVIDUAL PCNs AND NAPHTHALENE

Data on HPLC ($t_{ret.}$) and UV spectra taken from ref. 70; for UV spectra of 1,4-di-, 1,5-di- and 1,4,5,8-tetrachloronaphthalene, also see ref. 35, and for those of all mono- and disubstituted PCNs, ref. 60; data on reduction potentials taken from ref. 75; for IR spectra, references have been quoted only.

| *Substituted PCN* | $t_{ret.}$ *(min)* | $\lambda_{max.}$ *(nm)* * | |
|---|---|---|---|
| | | *$B_b$ band* | *$L_a$ band* |
| Naphthalene | 5.40 | 218s, 221 | 257, 265, 274, 285 |
| 1 | 3.15 | 219s, 223 | 264, 273, 284, 295 |
| 2 | 3.60 | 221s, 225 | 257, 267, 277, 288 |
| 1,2 | 2.90 | 215s, 218s, 224s, 230 | 267, 275, 285, 297 |
| 1,3 | 2.30 | 214s, 225s, 229 | 267, 276, 286, 297 |
| 1,4 | 2.15 | 213s, 224 | 272, 281, 292, 309 |
| 1,5 | 2.15 | 217s, 223s, 227 | 272, 281, 292, 309 |
| 1,6 | 2.45 | | |
| 1,7 | 2.65 | | |
| 1,8 | 3.65 | 217s, 220s, 226s, 230 | 274, 285, 295, 307 |
| 2,3 | 3.30 | 216s, 220s, 225s, 230 | 263, 272, 283, 294 |
| 2,6 | 2.80 | 215s, 219s, 226s, 230 | 259, 268, 277, 288 |
| 2,7 | 2.80 | 216s, 221s, 228s, 231 | 262, 270, 280, 290 |
| 1,2,3 | 2.55 | 217s, 230s, 234 | 268, 278, 289, 300 |
| 1,2,4 | | | |
| 1,2,5 | 2.00 | 216s, 221s, 228s, 233 | 272, 282, 293, 304 |
| 1,2,6 | 2.35 | 217s, 230s, 234 | 264, 274, 284, 296 |
| 1,2,7 | 2.60 | 220s, 223s, 229s, 234 | 268, 278, 289, 302 |
| 1,2,8 | 3.50 | 218s, 231s, 235 | 275, 287, 298, 310 |
| 1,3,5 | | | |
| 1,3,6 | 2.00 | 219s, 230s, 234 | 268, 279, 290, 302 |
| 1,3,7 | 2.15 | 217s, 222s, 229s, 234 | 260, 274, 284, 296 |
| 1,3,8 | 2.70 | 217s, 222s, 230s, 235 | 275, 287, 298, 311 |
| 1,4,5 | | | |
| 1,4,6 | 1.95 | 217s, 222s, 227s, 233 | 275, 285, 296, 309 |
| 1,6,7 | | | |
| 2,3,6 | 2.75 | 220s, 230s, 235 | 263, 273*, 284, 295 |
| 1,2,3,4 | 1.95 | 227s, 232, 238 | 274, 285, 296, 309 |
| 1,2,3,5 | 1.90 | 240 | 276, 287, 298, 311 |
| 1,2,3,7 | 2.35 | 227s, 237, 240 | 270, 280, 290, 303 |
| 1,2,4,6 | 1.75 | 221s, 235s, 237 | 275, {286 {291** {298 {303' 310 |
| 1,3,5,7 | 1.60 | 220s, 227s, 233, 239 | 279, {287 {291** {300 {303' 311 |
| 1,3,5,8 | 2.00 | 221s, 238 | 285, 297, 308, 321 |
| 1,3,6,7 | 2.05 | 223s, 233s, 239 | {268 {273' {280 {284' {291 {296' 303 |
| 1,4,5,8 | 2.60 | 223s, 234s, 238 | 294, 307, 321, 336 |
| 1,4,6,7 | 1.90 | 223s, 228s, 241s, 245 | 280, 290, 302, 314 |
| 1,2,3,5,7 | 1.65 | 220s, 226s, 238s, 244 | {272 {279' {284 {290' {296 {301' {309 {314' |
| 1,2,3,6,7,8 | | | |
| 1,2,3,4,5,6,7,8 | 1.65 | 240s, 260s, 268s, 275 | 310, 322, 332, 345 |

* In the $B_b$ band, the principal maximum is invariably found at the high-wavelength side; in the $L_a$ band, the third, and occasionally the second, $\lambda_{max.}$ recorded has the highest intensity.

| *Reduction potentials, $E_{2a}$ vs. S.C.E. (V)* | | | | | | | | | *Ref. on IR* |
|---|---|---|---|---|---|---|---|---|---|
| −2.326 | −2.197 | | | | | | | | |
| −2.326 | −1.940 | | | | | | | | 76 |
| −2.326 | −1.975 | | | | | | | | 76 |
| −2.326 | −1.957 | −1.726 | | | | | | | 72 |
| −2.326 | −1.965 | −1.752 | | | | | | | 72 |
| −2.326 | −1.944 | −1.751 | | | | | | | 72, 77 |
| −2.326 | −1.933 | −1.765 | | | | | | | 72, 77 |
| −2.326 | −1.948 | −1.802 | | | | | | | 72 |
| −2.326 | −1.944 | −1.793 | | | | | | | 72 |
| −2.326 | −1.938 | −1.704 | | | | | | | 72 |
| −2.326 | −1.960 | −1.769 | | | | | | | 72 |
| −2.326 | −1.957 | −1.844 | | | | | | | 72, 77 |
| −2.326 | −1.959 | −1.838 | | | | | | | 72 |
| −2.326 | −1.975 | −1.761 | −1.554 | | | | | | 72 |
| −2.326 | −1.939 | −1.752 | −1.565 | | | | | | 72 |
| −2.326 | −1.942 | −1.783 | −1.581 | | | | | | 72 |
| −2.326 | −1.955 | −1.827 | −1.620 | | | | | | 72 |
| −2.326 | −1.955 | −1.898 | −1.613 | | | | | | 72 |
| −2.326 | −1.941 | −1.785 | −1.512 | | | | | | 72 |
| −2.326 | −1.938 | −1.781 | −1.578 | | | | | | 72 |
| −2.326 | −1.958 | −1.824 | −1.634 | | | | | | 72 |
| −2.326 | −1.974 | −1.853 | −1.635 | | | | | | 72 |
| −2.326 | −1.960 | −1.806 | −1.541 | | | | | | 72 |
| −2.326 | −1.932 | −1.758 | −1.540 | | | | | | 72 |
| −2.326 | −1.941 | −1.776 | −1.618 | | | | | | 72 |
| −2.326 | −1.938 | −1.778 | −1.599 | | | | | | 72 |
| −2.326 | −1.956 | −1.820 | −1.657 | | | | | | 72 |
| −2.326 | −1.946 | −1.751 | −1.563 | −1.393 | | | | | 72 |
| −2.326 | −1.948 | −1.784 | −1.591 | −1.411 | | | | | 72 |
| −2.326 | −1.959 | −1.832 | −1.627 | −1.445 | | | | | 72 |
| −2.326 | −1.954 | −1.783 | −1.609 | −1.445 | | | | | 72 |
| −2.326 | −1.960 | −1.836 | −1.615 | −1.444 | | | | | 72 |
| −2.326 | −1.946 | −1.783 | −1.616 | −1.373 | | | | | 72 |
| −2.326 | −1.955 | −1.782 | −1.607 | −1.490 | | | | | 72 |
| −2.326 | −1.929 | −1.755 | −1.535 | −1.345 | | | | | 72, 77 |
| −2.326 | −1.957 | −1.729 | −1.576 | −1.431 | | | | | 72 |
| −2.326 | −1.985 | −1.833 | −1.639 | −1.506 | −1.340 | | | | — |
| | | | | | | | | | 77 |
| −2.326 | −2.028 | −1.840 | −1.706 | −1.600 | −1.411 | −1.298 | −1.081 | −0.940 | 59, 72, 77 |

corded at or near the wavelength of maximum absorption. Nibren D88 and Clonacire 90 display chromatograms, as well as melting points and UV spectra (*cf.*, Sections II.1 and III.3), that are virtually identical with those of Halowax 1001. The more highly chlorinated Nibren D116N and Clonacire 115 strongly resemble Halowax 1013; Nibren D130 displays the same behaviour as Halowax 1014. For Clonacire 130, a composition intermediate between those of Halowax 1013 and 1014 was suggested.

Comparison of the chromatograms in Fig. 7 with data on PCBs in ref. 73 reveals that the major peaks of low-chlorinated PCB mixtures are eluted separately from those of all Halowaxes. However, more highly chlorinated PCB mixtures, as well as polychlorinated terphenyls[74] and various common chlorinated pesticides, are eluted in the retention-time region characteristic for PCNs. However, it was demonstrated that detection at two different wavelengths, lying in the 195–215 and 275–320 nm regions, will help to discriminate between, and even determine quantitatively, PCNs and PCBs. The results will be best for PCNs, because PCBs and polychlorinated terphenyls show negligible absorption above 275 nm. Admittedly, detection of the Halowaxes in the 275–320-nm region instead of at their wavelength of maximum absorption causes an approximately 10-fold decrease in sensitivity. In practice, another limitation to the UV approach is the background generated by UV-absorbing compounds eluted from the column material and/or extracted from the sample to be analyzed[67].

Data[70] on the retention times of 33 individual PCNs in the HPLC system silica gel–dry *n*-hexane are presented in Table 10. The retention behaviour appears to be determined by two main effects: (1) increasing introduction of chlorine atoms into the naphthalene nucleus decreases the retention, non-adjacent $\alpha$-substitution having a greater effect than non-adjacent $\beta$-substitution in this respect; (2) substitution in the adjacent 1,8- and, although less so, in the 2,3-positions promotes retention. Illustrative examples are the relatively high retention time of 1,4,5,8-tetrachloronaphthalene compared with those of the 1,4- and 1,5-dichloronaphthalenes, and the very short retention time of 1,3,5,7-tetrachloronaphthalene. With the last PCN, the complete absence of substitution in adjacent positions causes it to move ahead of all PCNs studied, including octachloronaphthalene.

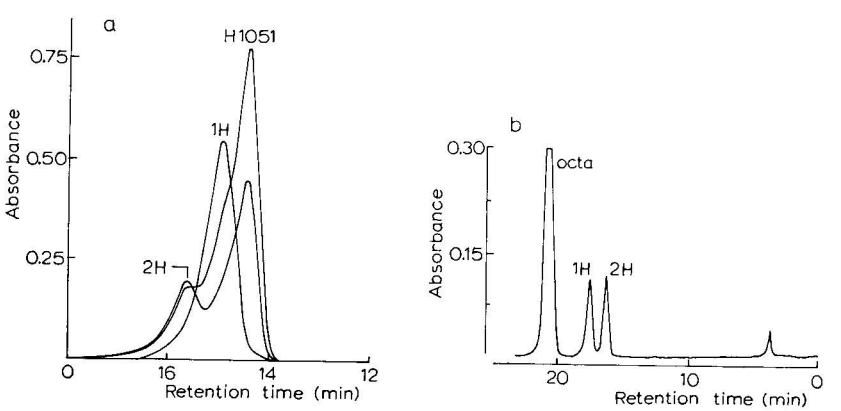



Fig. 8. HPLC (ref. 78) of Halowax 1051 in the systems (a) silica gel–dry *n*-hexane at a flow-rate of 0.2 ml/min (temperature, 27 ± 1°); (b) LiChrosorb RP-8/methanol–water (8.5:1.5) at a flow-rate of 0.9 ml/min (temperature, 20 ± 1°). UV detection at (a) 256 and (b) 230 nm.

TABLE 11

CHROMATOGRAPHIC AND SPECTRAL DATA FOR HEPTA- AND OCTACHLORONAPHTHALENES

| *Substituted PCN* | *$R_F$ in reversed-phase TLC** | *$t_{ret.rel.}$ in GLC** | *$t_{ret.}$ in HPLC (min)** | *$t_{ret.}$ in reversed-phase HPLC (min)** | *$\lambda_{max.}$ (nm)** | *$^1$H-NMR signal ($\tau$)*** |
|---|---|---|---|---|---|---|
| 1H-Hepta | 0.23 | 0.63 | 1.70 | 18.5 | 256 | 1.53 |
| 2H-Hepta | 0.27 | 0.63 | 1.80 | 16 | — | 2.15 |
| Octa | 0.19 | 1.00 | 1.65 | 21 | 275 | — |

* Data from ref. 78.
** Data from ref. 72.

In several recent papers[44,70,78], special attention has been devoted to the analysis of Halowax 1051, which is a mixture of octachloronaphthalene (90%) and heptachloronaphthalenes (10%). GLC provides[44,69] a sharp separation of the octa- from the hepta-substituted naphthalene(s), but as yet does not allow the separation of the 1H- from the 2H-isomer. Surprisingly, three separate zones show up in reversed-phase TLC (Fig. 5). HPLC in the system silica gel–dry *n*-hexane also reveals the presence of at least three constituents (Fig. 8a), while an excellent separation occurs in reversed-phase HPLC on a LiChrosorb RP-8 column, using methanol–water (9–8:1–2, v/v) mixtures as the mobile phase (Fig. 8b). Comparison of the NMR and UV spectra and the HPLC retention times of the hepta-substituted isomers with those of related PCNs strongly suggests that the assignments made in Fig. 8 and Table 11 are correct. As a consequence, the isomer produced by reduction of octachloronaphthalene with aluminium lithium hydride (*cf.*, Section II.2) is believed to be the 1H-heptachloronaphthalene.

### *2. Differentiation of PCNs from PCBs*

In the previous section, it has been shown that an efficient separation of PCNs and/or PCBs from a large number of organochlorine pesticides can be achieved if the chromatographic conditions are closely controlled. However, no method has yet been devised for the differential elution of PCNs and PCBs, although HPLC[70], multi-wavelength detection[67,70], UV irradiation[68] and GLC–MS[65] may help to discriminate between these two closely related types of compounds. Several further attempts to differentiate between PCNs and PCBs are discussed below.

Holmes and Wallen[79] treated a mixture of PCNs and PCBs with an excess of chromium trioxide. After reaction for 20 min at *ca.* 100°, examination of an *n*-hexane extract by electron-capture GLC revealed the presence of peaks due to PCB isomers only; the PCNs were apparently oxidized completely. However, one should bear in mind that, according to another study[80], treatment of PCBs with chromium trioxide–sulphuric acid also leads to considerable decomposition of several low-chlorinated PCB isomers.

In order to simplify the analysis of a heterogeneous mixture of PCNs, PCBs and other similar compounds, conversion into a single derivative has often been suggested, *e.g.*, by exhaustive chlorination to give the fully chlorinated product (per-

chlorination), or by removal of all chlorine atoms to obtain the parent hydrocarbon (dechlorination). The former technique is usually preferred, both on account of the high sensitivity of the electron-capture detector (ECD) towards octachloronaphthalene and decachlorobiphenyl, and of the relatively high volatility of naphthalene and biphenyl, which may incur significant losses during treatment and further handling of the samples.

Hutzinger *et al.*[46] carried out perchlorination by heating the PCN mixture, under reflux, with sulphuryl chloride–antimony(V) chloride (9:1, v/v) for 1 h. Octachloronaphthalene was obtained in good yield; however, no accurate data on the percentage conversion have been published. Treatment with a mixture of disulphur dichloride, sulphuryl chloride and aluminium chloride, and subsequent heating at a temperature exceeding 208° in order to convert the initially formed decachloro-1,4-dihydronaphthalene into octachloronaphthalene, may also be recommended. For obvious reasons, perchlorination is usually combined with electron-capture GLC as a method of analysis. Unfortunately, under the conditions normally employed[46,81], octachloronaphthalene and decachlorobiphenyl (resulting from perchlorination of PCBs) have approximately the same retention times. This effect causes serious interference in the simultaneous determination of PCNs and PCBs in their mixtures. However, according to our experience[82], if GLC is carried out on a 4% OV-101 on 80–100-mesh Chromosorb W (HP) column at 260°, decachlorobiphenyl shows a stronger retention than octachloronaphthalene. The relative retention is *ca.* 1.15 and well resolved peaks are obtained for mixtures of octachloronaphthalene and decachlorobiphenyl containing 10–90% (w/w) of either compound. An excellent separation of both fully chlorinated products has also been obtained by HPLC, using the reversed-phase system previously used for the separation of 1H- and 2H-heptachloronaphthalene (*cf.*, Fig. 8); octachloronaphthalene precedes decachlorobiphenyl, and the relative retention and resolution are *ca.* 1.1 and 2.0, respectively.

Perchlorination with chlorine gas in the presence of iodine as a catalyst has been recommended[81] for discriminating chlorodibenzo-*p*-dioxins from PCNs, PCBs and chlorodibenzofurans. In GLC on a 3% XE-60 on 100–120-mesh Chromosorb W (HP) column at 210°, octachlorodibenzo-*p*-dioxin has a longer retention time than have the fully chlorinated naphthalene, biphenyl and dibenzofuran. Perchlorination with disulphur dichloride, sulphuryl chloride and aluminium chloride, followed by chromatography on an alumina column and final analysis by GLC, has been used[83] to verify the absence of chlorodibenzofurans from samples of Halowax 1014 and technical naphthalene.

With dechlorination, Zimmerli[84] has shown that PCB mixtures are quantitatively converted into biphenyl on a partly deactivated palladium catalyst. Under the same conditions, PCNs are converted into naphthalene plus some tetralin. As Zimmerli did not quote quantitative data on the behaviour of PCNs, no meaningful conclusions can be drawn concerning the merit of so-called carbon-skeleton chromatography.

Hutzinger *et al.*[46] stressed that treatment of PCNs with the very powerful perchlorination reagents antimony(V) chloride–iodine and pure antimony(V) chloride leads to extensive degradation of chlorinated naphthalenes, whereas they are the preferred reagents for the perchlorination of PCBs. In our laboratory, this conclusion has been confirmed for Halowax 1000, 1099, 1014 and 1051. Therefore, the powerful

TABLE 12

UV ABSORPTION DATA FOR PCN MIXTURES[70]

| *Type* | $\lambda_{max.}$ | | *Type* | $\lambda_{max.}$ | |
|---|---|---|---|---|---|
| | *$B_b$ band** | *$L_a$ band* | | *$B_b$ band* | *$L_a$ band* |
| *Halowax* | | | *Clonacire* | | |
| 1031 | 224 | 274, 284 | 90 | 233 | 295, 304 |
| 1000 | 224 | 284, 292 | 115 | 238 | 297, 305 |
| 1001 | 233 | 297, 304 | 130 | 238 | 306 |
| 1099 | 233 | 298, 305 | *Nibren* | | |
| 1013 | 238 | 306 | D88 | 233 | 296, 304 |
| 1014 | 244 | 310 | D116N | 238 | 306 |
| 1051 | 275 | 332 | D130 | 244 | 313 |

* log $\varepsilon_{max.}$ = 4.7–4.8 except for Halowax 1031, which has log $\varepsilon$ = 5.0.

perchlorination of PCN–PCB mixtures appears to be a promising alternative to the chromium trioxide oxidation technique discussed above.

### 3. *UV and IR spectrometry*

Apart from an early paper on the UV absorption of dichloronaphthalenes[60], only one systematic study on the UV spectra of PCNs has been published[70]. Data for commercially available mixtures are recorded in Table 12, while results for individual PCNs are included in Table 10. Several spectra are shown in Fig. 9.

In the UV spectra of aromatic hydrocarbons, the $B_b$ band is the most intense

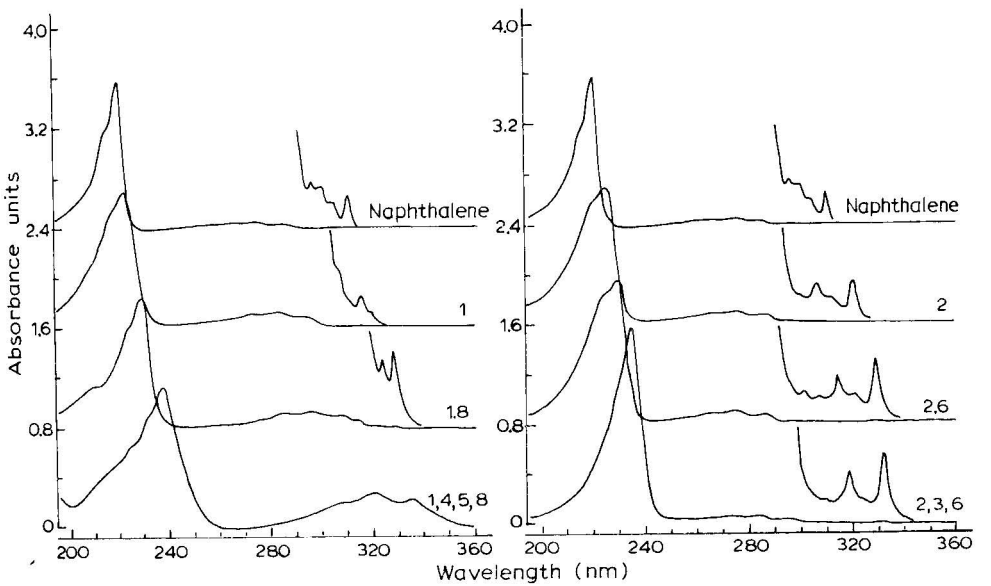



Fig. 9. UV absorption spectra[70] of naphthalene (10 ppm) and 1-mono-, 1,8-di-, 1,4,5,8-tetra-, 2-mono-, 2,6-di- and 2,3,6-trichloronaphthalene (*ca.* 25 ppm in *n*-hexane). Cell pathlength, 2 mm. $L_b$ band, ×60.

(log $\varepsilon$ = 4–6). With the PCNs, introduction of chlorine atoms into the naphthalene nucleus induces a bathochromic shift of this band; the magnitude of this shift is chiefly determined by the number, not the positions of the substituents[70]: mono-, 223–225 nm; di-, 224–231 nm; tri-, 233–235 nm; tetra-, 238–245 nm. $\alpha$-Substitution causes batho- and hyperchromic shifts predominantly in the transverse-polarised $L_a$ band. This effect is clearly demonstrated by comparing the spectra of 1,4-, 1,5- and 1,8-dichloronaphthalene with those of 2,3-, 2,6- and 2,7-dichloronaphthalene. The effect of $\alpha$-substitution is even more pronounced with the fully $\alpha$-substituted 1,4,5,8-tetrachloronaphthalene. With $\beta$-substituted PCNs, the position of the $L_a$ band hardly changes and, instead, an intensification of the relatively weak $L_b$ band (high-wavelength maxima, 320–330 nm) occurs. These conclusions are in good agreement with the data on disubstituted PCNs published by De Laszlo[60] nearly 50 years ago. Mosby[85] commented briefly on the considerable broadening of the $B_b$ band and the strong bathochromic shift observed in the spectrum of octachloronaphthalene compared with those of less highly substituted PCNs. These features are probably derived to a large extent from the non-planar nature of the fully chlorinated naphthalene.

The IR spectra of PCNs have been studied by Cencelj and Hadži[72], who reported spectra between 1650 and 660 $cm^{-1}$ of the complete range of dichloro- and trichloronaphthalenes and of nine tetrachloronaphthalenes; a selection of these spectra is presented in Fig. 10. Parallel runs with each substance were made in the solid state (Nujol mull) and in solution (carbon tetrachloride and cyclohexane). In most instances there was little difference between the positions of the bands in the solid and the solution state. However, notable exceptions occurred, *e.g.*, with 1,5-, 1,7- and 2,3-dichloronaphthalene, and occasionally there were even differences as regards the number of bands observed. The authors limited the discussion to the 690–900-$cm^{-1}$ region, where a number of strong bands appeared in the spectra of substituted naphthalenes that are known to arise from the out-of-plane deformation vibrations of the hydrogen atoms attached to the rings. Following the method of Thompson[86], Cencelj

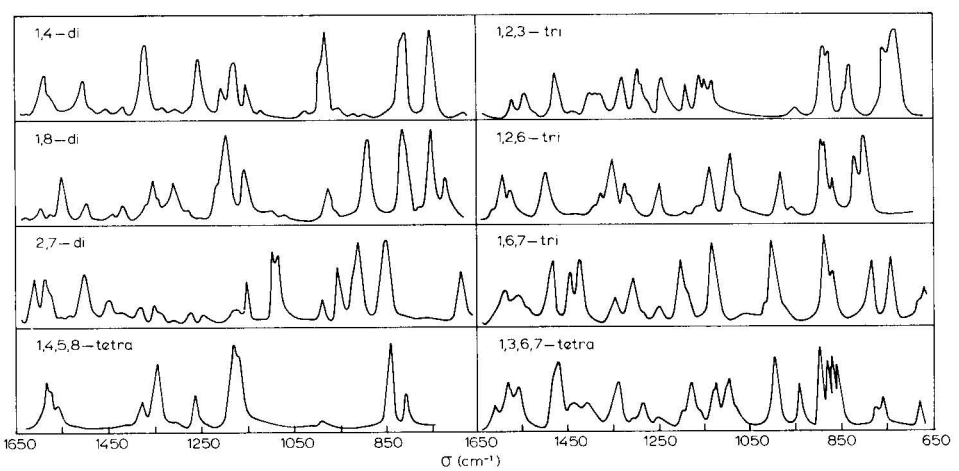



Fig. 10. IR spectra of eight PCNs (Nujol mull) in the 1650–660 $cm^{-1}$ region[72]. The band at 735–770 $cm^{-1}$ is characteristic of four adjacent hydrogen atoms, and a band at 860–900 $cm^{-1}$ is expected to appear for one isolated hydrogen atom (also see ref. 59).

and Hadži[72] quoted several examples that indicate that the pattern of the absorption bands in the above region seems to be characteristic of the types of substitution, and independent of the nature of the substituents. However, there is a serious limitation to the applicability of the correlation rules for the determination of the type of substitution: the presence of a band is not sufficient proof of the presence of the corresponding group of hydrogen atoms. However, the absence of a band required by a particular type of substitution seems to exclude it (*cf.*, Fig. 10 and ref. 87).

Kalmykova *et al.*[77] also noted that changes in the shape of the IR spectra are due to changes in the shape of the molecule and the nature of the spin–orbit interactions. To quote an example, from analysis of IR spectra (1650–150 $cm^{-1}$) recorded at room temperature, and electronic spectra measured at 4 °K, they concluded that the coplanar nature of 1,4,5,8-tetrachloronaphthalene is significantly disrupted. Remarkably, 1,2,3,6,7,8-hexachloronaphthalene turned out to be planar, while the fully substituted octachloronaphthalene was non-coplanar, although its deformation vibrations were not as intense as those of 1,4,5,8-tetrachloronaphthalene.

The IR spectrum of octachloronaphthalene in carbon disulphide and in potassium bromide (3000–650 $cm^{-1}$) was studied by Luther *et al.*[59]. They reported frequency assignments made on the basis of a comparison with octadeuteronaphthalene and the system benzene–hexachlorobenzene.

A list of IR spectra of PCNs recorded in the literature, and the pertinent references, are included in Table 10.

### *4. Mass spectrometry*

The mass and ion kinetic energy (IKE) spectra of the two isomeric mono- and several dichloronaphthalenes have been recorded by Safe and Hutzinger[88]. Pertinent data are given in Table 13. The primary ion mass spectra of 1- and 2-monochloronaphthalene are similar, and the fragmentation pattern shows loss of both Cl· and $C_2H_2$ from the molecular ion. The IKE spectra of both compounds are also identical

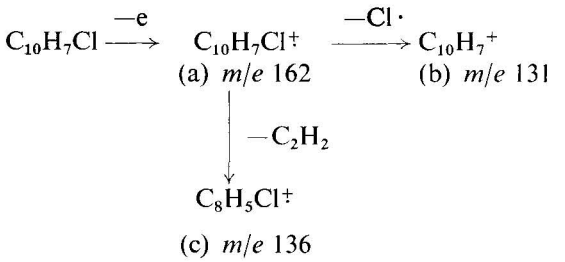
TABLE 13

PRIMARY ION MASS AND IKE SPECTRA INTENSITIES OF MONO- AND DICHLORONAPHTHALENES*

| *PCN* | *Primary ion mass* | | | | *IKE* | | | |
|---|---|---|---|---|---|---|---|---|
| | *M* | *M − Cl* | *M − $C_2H_2$* | *M − $Cl_2$* | *0.867E* | *0.821E* | *0.782E* | *(0.867E)/(0.821E)* |
| 1 | 100 | 20 | <1 | | | | | |
| 2 | 100 | 18 | <1 | | | | | |
| 1,2 | 100 | 11 | <1 | 18 | 10.5 | 100 | 54 | 0.105 |
| 1,3 | 100 | 12 | <1 | 20 | 10.5 | 100 | 54 | 0.105 |
| 2,3 | 100 | 14 | <1 | 25 | 11.0 | 100 | 55 | 0.110 |
| 1,4 | 100 | 11 | <1 | 19 | 10.5 | 100 | 54 | 0.105 |
| 1,5 | 100 | 12 | <1 | 21 | 10.0 | 100 | 55 | 0.100 |
| 1,7 | 100 | 14 | <1 | 18 | 10.5 | 100 | 56 | 0.105 |
| 1,8 | 100 | 14 | <1 | 19 | 10.0 | 100 | 54 | 0.100 |

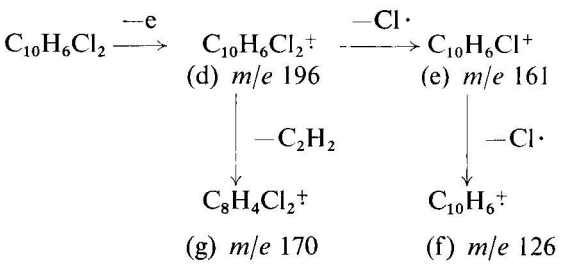
* Recorded[88] at 70 eV, 80 kV with a DuPont CEC-21-110B instrument.

with daughter ion peaks at 0.840 and 0.785 E (ratio, 0.13), corresponding to the reactions a → b and a → c:




These results are evidence of chlorine randomization, at least in the substituted ring of the naphthalene nucleus.

For the disubstituted PCNs, the relative intensities of both the primary ion and IKE spectra are similar for all of the isomers studied. Moreover, the ratios obtained for the two daughter ion peaks resulting from unimolecular decomposition of the molecular ion (0.867 *E*/0.821 *E*) are also virtually indistinguishable; the fragmentation pattern is as follows:




The results suggest that chlorine randomization occurs over all of the carbon atoms in the naphthalene nucleus. This suggestion contrasts with the conclusion of Safe *et al.*[89] concerning IKE spectra of PCB isomers: with substituted biphenyls, the relative peak abundances are different for each isomer and therefore are thought to be of some use in structure analysis.

Mass spectra have been recorded for tri- to heptachloronaphthalenes[65], octachloronaphthalene[46] and Halowax 1031 and 1014[90]. Two examples are shown in Fig. 11a and 11b. It is worthwhile stressing that, contrary to general ideas, the odd-electron ions are always more intense than neighbouring even-electron ions. This phenomenon, together with the highly characteristic $^{35}Cl/^{37}Cl$ isotope distribution, considerably facilitates the recognition of (poly)chloroaromatic compounds. A conveniently readable bar chart of the isotopic abundance ratios for 1–15 chlorine atoms was presented by Hutzinger *et al.*[46]. Calculations[91] on the theoretical probability of the occurrence of ions of different masses in the molecular ion cluster for from mono- to octachloronaphthalenes are summarized in Table 14. The identification of a pentachloronaphthalene in a pesticide–Halowax 1014 mixture by means of mass spectrometry has been reported by Bonelli[92].

A detailed study has been made[44] of the mass spectra of octa- and heptachloro-

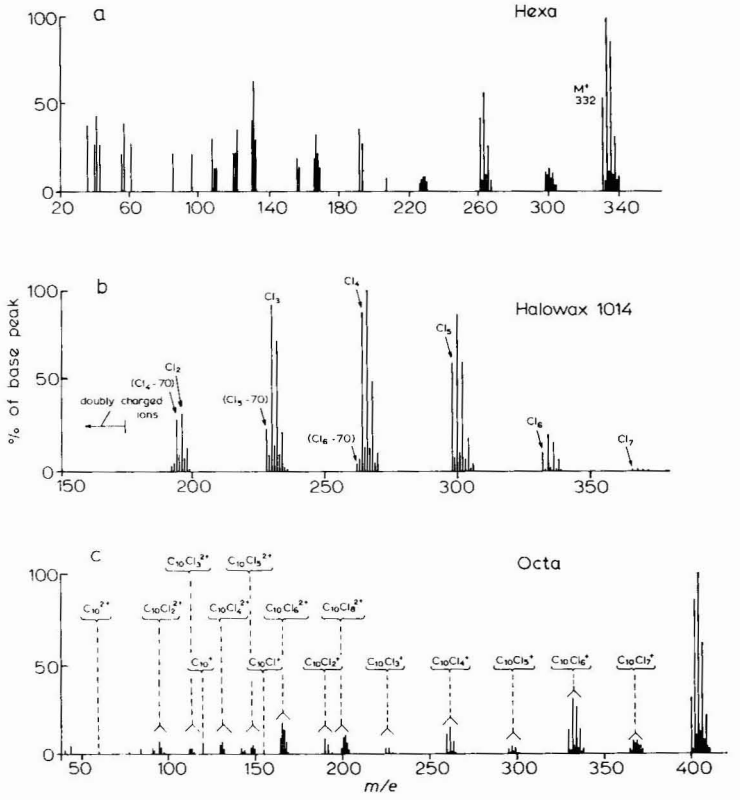



Fig. 11. Mass spectra of (a) a hexachloronaphthalene[65], (b) Halowax 1014[90] and (c) octachloronaphthalene[44]. The last spectrum was recorded at 8 kV, 70 eV on an AEI MS 902S spectrometer.

naphthalenes. With the former compound, the molecular ion can be seen (Fig. 11c) to be the base peak and the only important mode of fragmentation is by successive loss of chlorine atoms. To a large extent, the atoms are lost singly, but some simultaneous loss of two chlorine atoms may also occur. Moreover, all eight chlorine atoms may be shed successively without loss of a carbon atom, as indicated by the peaks for the series of $C_{10}Cl_8^+$–$C_{10}^+$ ions. In addition to the series of singly charged ions, a corresponding series of doubly charged ions occurs. The general abundance of odd-electron ions such as $M^+$ and $[M - Cl_2]^+$ has already been noted above. Interpretation of the spectra of the heptachloronaphthalenes, which are indistinguishable except for minor differences in the relative intensities of some of the peaks, is in accord with that of the mass spectrum of octachloronaphthalene. Again, the major mode of fragmentation is by successive loss of (all seven) chlorine atoms, the odd-electron ions are most abundant and doubly charged versions of all of the ions are apparent. Loss of chlorine is strongly preferred to loss of the hydrogen atom, which does not occur to an appreciable extent until at least four chlorine atoms have been shed.

Lastly, it is interesting to note the presence of a group of low-intensity peaks due to $C_{10}HCl_7^+$ ions in the mass spectrum recorded in Fig. 11c. Clark *et al.*[44] produced evidence that suggests that these ions are due mainly to slow incorporation of

TABLE 14

THEORETICAL PROBABILITY OF THE OCCURRENCE OF IONS IN THE MOLECULAR CLUSTER OF PCNs[90]

Values expressed relative to the most intense ion in the cluster; parent ion always lowest *m/e* in the group.

| *m/e* | *Theoretical probability* | *m/e* | *Theoretical probability* | *m/e* | *Theoretical probability* | *m/e* | *Theoretical probability* |
|---|---|---|---|---|---|---|---|
| *Mono-PCN* | | *Tetra-PCN* | | *Hexa-PCN* | | | |
| 162 | 100.00 | 264 | 76.35 | 332 | 50.98 | 375 | 1.95 |
| 163 | 11.28 | 265 | 8.61 | 333 | 5.75 | 376 | 3.48 |
| 164 | 33.17 | 266 | 100.00 | 334 | 100.00 | 377 | 0.38 |
| 165 | 3.68 | 267 | 11.23 | 335 | 11.24 | 378 | 0.39 |
| | | 268 | 49.25 | 336 | 81.83 | | |
| *Di-PCN* | | 269 | 5.49 | 337 | 9.16 | *Octa-PCN* | |
| 196 | 100.00 | 270 | 10.86 | 338 | 35.79 | 400 | 33.44 |
| 197 | 11.28 | 271 | 1.19 | 339 | 3.98 | 401 | 3.77 |
| 198 | 65.77 | 272 | 0.92 | 340 | 8.84 | 402 | 87.40 |
| 199 | 7.35 | | | 341 | 0.97 | 403 | 9.83 |
| 200 | 11.00 | *Penta-PCN* | | 342 | 1.17 | 404 | 100.00 |
| 201 | 1.20 | 298 | 61.14 | | | 405 | 11.22 |
| | | 299 | 6.89 | *Hepta-PCN* | | 406 | 65.44 |
| *Tri-PCN* | | 300 | 100.00 | 366 | 43.71 | 407 | 7.31 |
| 230 | 100.00 | 301 | 11.24 | 367 | 4.93 | 408 | 26.81 |
| 231 | 11.27 | 302 | 65.54 | 368 | 100.00 | 409 | 2.98 |
| 232 | 98.37 | 303 | 7.33 | 369 | 11.25 | 410 | 7.05 |
| 233 | 11.03 | 304 | 21.55 | 370 | 98.12 | 411 | 0.78 |
| 234 | 32.44 | 305 | 2.39 | 371 | 11.00 | 412 | 1.16 |
| 235 | 3.59 | 306 | 3.57 | 372 | 53.56 | 413 | 0.13 |
| 236 | 3.65 | 307 | 0.39 | 373 | 5.98 | | |
| 237 | 0.39 | 308 | 0.24 | 374 | 17.58 | | |

hydrogen atoms by thermal reaction on the surface of the inlet system and source of the mass spectrometer. This indicates that the spectra of labile polychloroaromatic compounds must be interpreted with care, especially if the compounds are present in low concentrations and are introduced other than on a direct insertion probe. As another limitation of mass spectrometry for the analysis of polychloro compounds, Clark *et al.* mentioned the fact that these compounds are often very difficult to remove from the source of the spectrometer and affect subsequent spectra for many hours.

### 5. *Electrochemistry*

Farwell *et al.*[75,93] studied the use of voltammetry for the identification of PCNs and other polychlorinated compounds. Polyhaloaromatics are known to be irreversibly reduced at a mercury electrode, usually in a stepwise manner, the voltammetric reduction potentials of the various C–Cl bonds depending upon the structural positions in the parent molecule. In order to improve significantly the quantitative resolution of the individual peaks, Farwell *et al.* used interrupted-sweep voltammetry instead of normal voltammetry. They described[75] an inexpensive interrupted-sweep instrument that permits the resolution of reduction peaks separated by less than 60 mV, while it has a potential resolution of 43 mV for overlapping reduction peaks. Illustrative

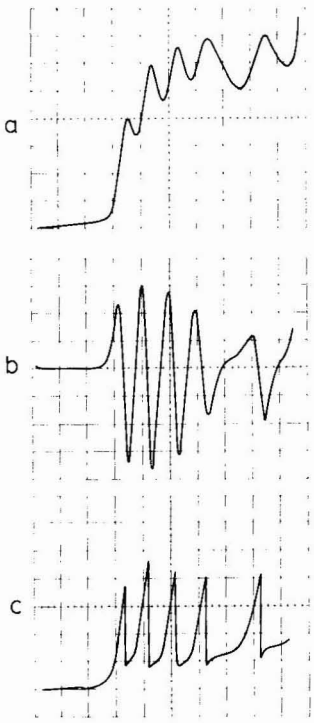



Fig. 12. Voltammograms of 1,2,3,4-tetrachloronaphthalene[75]. Voltage range: −0.700 to −2.600 V *vs.* S.C.E. (a) Normal scan; (b), second-derivative scan; (c), interrupted-sweep fingerprint.

voltammograms of 1,2,3,4-tetrachloronaphthalene are shown in Fig. 12. The most cathodic peak corresponds to the reduction of naphthalene and the remaining peaks represent the stepwise removal of the chlorine atoms.

Characteristic reduction-potential data for 37 PCNs are given in Table 10. The first zero-crossing of the second derivative of the cell current has been chosen as the interrupt potential $E_{2d}$ on the reduction wave, as it is readily determined experimentally and is sufficiently anodic relative to the peak potential to provide good resolution between waves. Fairly large day-to-day variations in the reproducibility of the $E_{2d}$ data have occasionally been observed. However, each reduction peak varies by the same magnitude; *i.e.*, the $\Delta E_{2d}$ values are constant. Further, all of the voltammograms contain the reduction peak for naphthalene ($E_{2d} = -2.326$ V *vs.* S.C.E.), which can be used as an internal standard.

Using the equipment described, the fingerprint technique requires at least 9 $\mu$g of relatively pure compound in order to obtain positive identification. That is, the components of a complex mixture must be separated by a chromatographic technique before they can be identified.

### 6. *Photochemistry*

In recent years, increasing attention has been paid to the photochemistry of chlorinated compounds such as organochlorine pesticides, PCBs and, to a lesser extent,

PCNs. In view of the high chemical stability of these compounds, environmental breakdown initiated by the photochemically active part of the solar spectrum is of particular interest*. Photodegradation of the pollutants may lead to their removal from the environment, *e.g.*, through conversion into insoluble polymeric material, or to the formation of much less or, alternatively, more highly toxic products. It is known that halogenoaromatic compounds chiefly undergo reductive dechlorination, dimerization and substitution by the solvent; however, chlorination and, possibly, isomerization may also occur. In this section, the limited amount of information available on the photodegradation of PCNs is discussed in more detail.

In the photolysis of 1-monochloronaphthalene in a mixture of *n*-hexane and ethanol, Szychlinski[95] observed $Cl^-$ formation, which increased when the proportion of ethanol in the mixture was increased. Robinson and Vernon[96] reported that in ethanol, 1-monochloronaphthalene (concentration *ca.* 0.1 %) is reduced to naphthalene noticeably faster than it gives 1-phenylnaphthalene under similar conditions but in benzene. This difference is probably due to predominant absorption and inefficient sensitization by benzene at the low concentrations of substrate employed, whereas in ethanol the halogen-containing compound itself absorbs the incident light. The photolysis of 1-monochloronaphthalene ($8.5 \cdot 10^{-2}$ m*M*) in degassed solutions of 50% aqueous methanol containing potassium hydroxide has been carried out[97] using light with a wavelength of 280 ± 10 nm. Naphthalene was formed, its quantum yield increasing with increasing concentration of the base; however, the reaction also took place in neutral medium. Under the conditions employed, no formation of naphthol (quantum yield $<10^{-4}$) was observed. Replacement of a halogen by a hydrogen atom probably takes place by electron transfer from the nucleophile ($OH^-$) to an excited PCN molecule, and subsequent dissociation of the resulting chloroaromatic radical anion into a chlorine atom and an aromatic radical, which splits off a hydrogen atom from one of the components of the medium (however, also see below).

Ruzo *et al.*[98] carried out irradiations of 1- and 2-mono- and 1,2-dichloronaphthalene at 300 nm using methanol, cyclohexane and acetonitrile–water (4:1, v/v) as solvents. The results are summarized in Table 15. In a subsequent paper[99], the results of the photodegradation of a large series of mono- to tetrachloronaphthalenes were presented. In cyclohexane, naphthalene and binaphthyl were the major products; in methanol, however, methoxylated and more highly chlorinated naphthalenes and binaphthyls were also formed. However, no evidence was found for the isomerization of 1- to 2-monochloronaphthalene, previously claimed by Mamedov and Nasibov[100] to occur next to dechlorination. Ruzo and co-workers reported a wide range of dechlorination: dimerization ratios that indicated marked substituent effects, which are probably both electronic and steric in nature. Dechlorination is favoured with PCNs that have adjacent (*vicinal* and *peri*) chlorine atoms, while unhindered PCNs give mostly dimers; moreover, the former type of PCNs show large relative reaction rates.

The major organic products found suggest that free radical intermediates are involved; sensitization experiments in the presence of benzophenone and experiments with atmospheric oxygen indicated the occurrence of an intermediate triplet excited

* For a general discussion of the choice of conditions of irradiation, laboratory models for natural conditions, general theoretical aspects of photochemistry, etc., the reader should consult, *e.g.*, ref. 94 and ref. 9, Ch. 6.

TABLE 15

DISTRIBUTION OF PHOTOPRODUCTS AND QUANTUM YIELDS FOR REACTION OF SIMPLE HALONAPHTHALENES[98]

| *PCN* | *Solvent* | *Φ** | *Dehalogenation*** | *Binaphthyls* | *Substitution* | *Chlorination* |
|---|---|---|---|---|---|---|
| 1 | $CH_3OH$*** | 0.005 | 74 | 25 | | <1 |
| 1 | $CH_3OH$–$O_2$ | 0.002 | 76 | 23 | | <1 |
| 1 | $C_6H_{12}$ | | 88 | 12 | | |
| 1 | $CH_3CN$–$H_2O$ | | 1 | 94 | 5 | <1 |
| 2 | $CH_3OH$*** | 0.007 | 58 | 38 | 4 | |
| 2 | $CH_3OH$–benzophenone | 0.007 | 2 | 97 | 1 | |
| 2 | $C_6H_{12}$ | | 72 | 28 | | |
| 2 | $CH_3CN$–$H_2O$ | | 2 | 94 | 4 | |
| 1,2 | $CH_3OH$ | 0.012 | 32 | 66 | 2 | |
| 1,2 | $CH_3OH$–benzophenone | 0.014 | 28 | 68 | 4 | |

* Degassed solutions, 20–60-h irradiations.

** Yields were estimated as percentage of total product formation by comparison with standard concentrations of naphthalene and binaphthyl.

*** The material balance on naphthyl residues was ~95% in the early stages of the reaction (6 h).

state as the principal precursor of both free radical and methoxylated products. Attempts[98,101] to determine the triplet lifetimes of the PCNs by quenching experiments led to abnormal behaviour, in that enhanced quantum yields for reaction (PCN disappearance and naphthalene formation) were found with potential triplet quenchers such as 1,3-cyclohexadiene, biacetyl and *trans*-stilbene.

On the basis of the evidence, Ruzo and co-workers concluded that the major products in their studies clearly resulted from C–Cl bond fission, which, however, is more likely to involve electron transfer:

$$\mathrm{ArCl} \xrightarrow{h\nu} \mathrm{ArCl}^* \xrightarrow{\mathrm{X}} \mathrm{Ar}\cdot + \mathrm{Cl}^- \qquad (3)$$

rather than direct homolytic fission:

$$\mathrm{ArCl} \xrightarrow{h\nu} \mathrm{ArCl}^* \longrightarrow \mathrm{Ar}\cdot + \mathrm{Cl}\cdot \qquad (4)$$

This postulate is supported by the fact that the quantum yield for the disappearance of 1-monochloronaphthalene increases 8-fold in the presence of triethylamine, a known electron donor. Subsequently, the aryl radical either abstracts a hydrogen atom from a solvent molecule or attacks a molecule of starting material to form a dechlorinated product and a binaphthyl, respectively. Recently, it has been suggested[94,98] that in dimerization and substitution reactions, an aryl radical cation rather than an aryl radical reacts with a substrate or solvent molecule.

In aqueous acetonitrile[98], which can be considered to be a good model system for environmental photochemistry, the main photoproducts from monochloronaphthalene are chlorobinaphthyl, 1-naphthol and a hydroxylated dimer. In the presence of oxygen, the dimers are largely suppressed and 1-naphthol is the major product. A typical reaction scheme[94] for the photolysis of a polychloronaphthalene in an aqueous system is shown in Fig. 13.

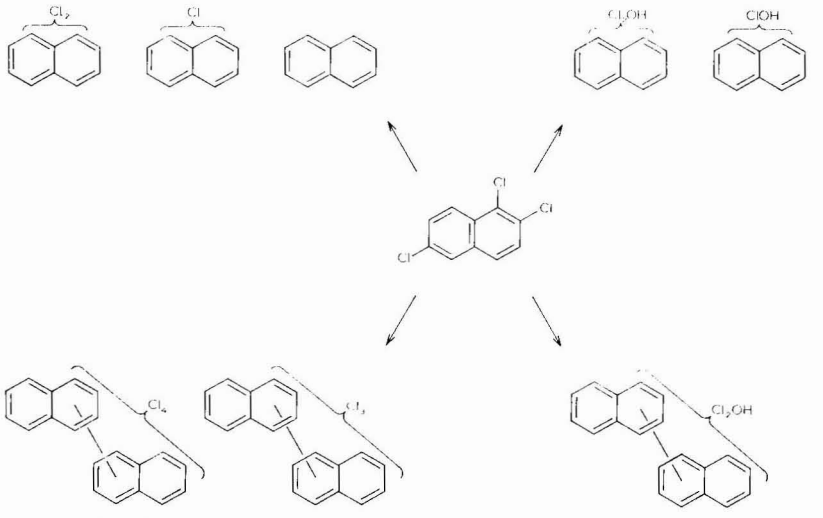


Fig. 13. Photolysis (at 300 nm) of 1,2,6-trichloronaphthalene in an aqueous medium[94]. Dechlorination, substitution and dimerization reactions are seen to occur.

Lastly, irradiation with sunlight[99] of Halowax 1014 and 2-mono- and 1,5-dichloronaphthalene as solid films on quartz produces only insoluble polymeric material. In view of the facile generation of radicals in solution photochemistry, this result is not surprising.

## IV. TOXICITY AND METABOLISM

### *1. Toxicity*

PCNs (and many other types of chloroaromatic compounds) have been implicated in various diseases, such as chloracne, X-disease in cattle and chicken oedema. Therefore, in studies on the toxicity of such compounds, *e.g.*, chlorinated naphthalenes, one should always be aware of the role possibly played by small amounts of highly toxic contaminants, such as chlorinated dibenzodioxins and dibenzofurans, for example.

#### *(a) Chloracne*[12,102]

The symptoms of this disease, which derives its name from the fact that early workers blamed nascent chlorine for its occurrence, are the formation of comedones with or without cysts and pustules[12]. The follicular orifices are filled with sebaceous and keratinous material, and melanosis and a secondary inflammatory reaction may exist. In some cases aetheroma, pigmentation and photodermatitis have been observed[103]. Chloracne is one of the most frequent forms of occupational dermatitis, and many cases due to PCNs have been reported (*e.g.*, refs. 103–126). The use of chlorinated naphthalenes as substitutes for natural waxes and rubber in Germany during World War I led to the first large outbreaks of chloracne. Massive outbreaks also occurred in the late 1930s and 1940s, chiefly in the manufacture and use of electrical cables whose covering was a fabric impregnated with penta- and hexachloronaphthalenes.

There is little doubt[102] that most cases in industry were due to external contact with PCNs alone, especially when only one side of the body was involved. However, it has been proved conclusively that chloracne can also result from systemic intoxication of the human subject with no external contact. Fumes of PCNs are by far the most potent, solutions less so, and contact with the solid substance is of little importance except under special conditions, such as when friction also occurs[127]. Even more important is the degree of chlorination of the PCNs. Contrary to the ideas of early workers that as the degree of chlorination increases so do the systemic toxicity and acneigenic properties, studies by Shelley and Kligman[128], Hambrick[123] and Crow[102] (and the large outbreaks of acne around 1940) have shown unequivocally that whereas mono-, di-, tri-, tetra-, hepta- and octachloronaphthalenes are entirely non-acneigenic, the penta- and hexachloro derivatives (Halowax 1014) produce very severe chloracne. In refs. 123 and 128, and also in a paper by Plewig[125], details can be found concerning the experimental production of acne in male adults, the PCNs being acneigenic in man in every area of the body tested.

*(b) Liver damage*

The disease occurs independently from chloracne[115], and usually manifests itself after an exposure of 4–6 months, but may occur in as short a time as 7 weeks. Occasionally loss of appetite, nausea and oedema of the face and hands are the first symptoms. Abdominal pain and vomiting follow, and then jaundice develops[12,129]. Flinn and Jarvik[130] reported nine cases with a fatal outcome following exposure to PCNs; at autopsy, yellow atrophy of the liver was found. Further reports of liver damage in human subjects are given in refs. 112–114 and 131–136. In rats, guinea-pigs and other test animals, acute yellow atrophy and other liver changes have been observed[122,130–132,137–141] in feeding and inhalation experiments. For example, upon feeding hexachloronaphthalene to rats, Schoettle *et al.*[141] observed mild to moderate fatty degeneration of the liver with centrilobular vacuolation of the hepatic cells. Oral administration of pentachloronaphthalene to guinea-pigs caused fat degeneration of the liver, loss of weight, conjunctivitis and death[122]. Lastly, degenerative lesions of the liver and kidneys occurred[142] in young swine fed with hexachloronaphthalenes, while necrosis and cirrhosis of the liver have been observed in sheep after ingestion of feed containing highly chlorinated naphthalenes[143]. From the above results, one can conclude that PCNs with high chlorine contents produce liver damage in several species. In man, effects such as leucopenia, lymphopenia, reduced amounts of haemoglobin and glucose in the blood and hyperacidic gastritis have also been observed[124].

*(c) X-disease*

In the U.S.A. in 1941, the term X-disease was applied to a cattle disease of unknown etiology. After further outbreaks in 1942–1946, Olafson described[144] the disease in 1947 and, a few years later, produced evidence[145,146] to show that it is caused by highly chlorinated naphthalenes[147].

The symptoms of poisoning include complete weakness, draggy loop, excessive lacrimation, night-blindness, diarrhoea, polyuria, marked salivation and discharge from the nostrils[12,148]. Cattle show a rapid decline in vitamin A plasma levels[148,149]. A chronic cough, poor appetite and numerous red maculae in the buccal mucosa develop

and hyperkeratosis of the skin follows. Degeneration of cells in the pancreas and liver and gall-bladder and renal-cortex damage have also been observed[12,150]. In a study on the ability of various PCNs to cause X-disease, Bell[151] reported that di- and trichloro derivatives do not produce the disease in calves, tetrachloronaphthalenes have an effect and the highly (penta- to octa-)chlorinated naphthalenes cause severe disease. However, octachloronaphthalene is less toxic than hexa- and heptachloro derivatives. Similar results have been obtained in various other studies[147,152–157]. Vlachos and co-workers[158,159] reported a rapid decrease in the semen quality of a bull following feeding of highly chlorinated naphthalenes. The first change observed was a decrease in mobility of the sperms and a marked increase in the proximal protoplastic droplets; spermatogenesis then stopped. Cats, dogs, rats and chickens, impregnated or fed with PCNs with a high chlorine content, suffer[141,160,161] typical symptoms of X-disease, which appears after varying periods of time. No symptoms have been found after administration of PCNs with relatively low chlorine content.

Chronic periodic inhalation of chloronaphthalene vapour by rats causes a decrease in the urea level of the urine and in the blood-sugar level[140]. Other changes include an increase in the cholesterol and a decrease in the ascorbic acid concentrations in the blood. Octachloronaphthalene, when fed to rats, greatly accelerates the loss of vitamin A from the liver[162]. There is no effect on vitamin A or E in the blood, nor does vitamin E in the liver change. According to Hill and Siegmund[163], vitamin E inhibits the loss of vitamin A from the liver.

Pigs do not show the characteristic symptoms of X-disease[142], although hexachloronaphthalene produces hyperplasia of the vaginal epithelium with keratin formation and a depression of the vitamin A plasma level. According to another report[164], pigs have a much greater tolerance than cattle for PCNs. A similar conclusion was drawn by Brock *et al.*[143] when comparing sheep and cattle.

*(d) Chicken oedema*[12]

In 1957, a disease occurred in a large number of chickens and it was discovered that the residue of certain distilled animal fats produces the condition when they are added to the chicken diet. The disease was called chicken oedema because it manifests itself with hydropericardium and ascites in chickens. Ducks and turkeys experience a reduction in growth.

Toxic fat is not the only product capable of producing the chicken oedema syndrome. A mixture of penta- and hexachloronaphthalenes (Halowax 1014), when fed to chickens, results in chicken oedema[165]. On the other hand, Halowax 1051 has no adverse effects[166]. In a study[167,168] on the toxicity of some European PCB mixtures (Phenoclor DP6 and Clophen A60) and one from the U.S.A. (Aroclor 1260), the two European products were found to have greater toxicity and to produce centrilobular liver necrosis. Moreover, chicken oedema is a common finding, rare with Aroclor 1260. All three mixtures produced porphyria. Fractionation of the PCB mixtures over a Florisil column, followed by GLC and mass-spectrometric and microcoulometric analyses, revealed the presence of tetra- and pentachlorodibenzofurans and hexa- and heptachloronaphthalenes in the European samples but not in the Aroclor sample. In view of the known high toxicity of polychlorodibenzofurans, these compounds must be assumed largely to determine the (high) toxicity of the Phenoclor and Clophen preparations.

The interference of Halowaxes and other chloroaromatics in the TLC/GLC detection of chicken oedema factor has been studied by Huang *et al.*[169].

*(e) Miscellaneous*

Craigie and Hutzinger[170] compared the effects of several types of chloroaromatic compounds on marine phytoplankton. Halowax 1099, at the 100 ppm level, kills *Olisthodiscus* and strongly depresses the growth of *Thalassiosira fluviatilis*. However, it is not toxic to *Dunaliella tertiolecta*.

*2. Metabolism*

Cleary *et al.*[171] fed a mixture of PCNs (mainly penta- and hexachloronaphthalenes) to rats and dogs and found no significant storage of material in the lungs, skin or kidneys, nor was any significant amount excreted in the urine. Apparently, the animals are able to remove the chlorinated compounds promptly. In the dog, an increase in the urinary ethereal sulphate fraction, but no significant change in the neutral sulphur excretion, was noted after PCN feeding. Drinker[138] reported a high percentage of chloride in the urine of a dog after administration of hexachloronaphthalene.

An extensive study on the metabolism of PCNs was made by Cornish and Block[172], who administered naphthalene, 1-monochloronaphthalene and di-, tetra-, penta-, hepta- and octachloronaphthalenes to rabbits. Analysis of the urine samples for a large number of excretory products during 4 days indicated that naphthalene and mono- and dichloronaphthalene are metabolized readily. Tetrachloronaphthalene is metabolized to a lesser extent in the 4-day period, while the more highly chlorinated products do not yield urinary metabolites that could be detected by the procedure used by Cornish and Block. Possibly these compounds are metabolized by pathways that yield excretory products not included in the study, or they may be deposited in tissues and metabolized or excreted unchanged over longer periods of time. Cornish and Block suggested that a high degree of chlorination interferes with the formation of a 1,2-dihydro-1,2-diol type of compound, which has been proposed[173] as an intermediate in naphthalene metabolism.

Ruzo and co-workers[43,174,175] also studied the metabolism of PCNs. When frogs and pigs were given Halowax 1031, whose main constituents are monochloronaphthalenes (Table 1), or one of several mono-, di- and tetrachloronaphthalenes, the

TABLE 16

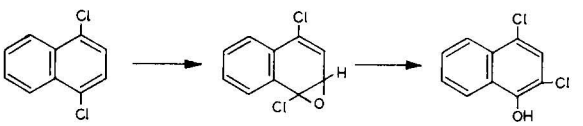
SURVEY OF PCN METABOLITES

| *PCN* | *Metabolite* | *Animal* | *Reference* |
|---|---|---|---|
| 1 | 4-Chloro-1-naphthol | Frog, pig | 174, 175 |
| 2 | 3-Chloro-2-naphthol | Pig | 175 |
| 1,2 | 3,4-Dichloro-1-naphthol | Pig | 43 |
| 1,4 | 2,4-Dichloro-1-naphthol | Pig, frog | 43, 174 |
| 1,2,3,4 | 5,6,7,8-Tetrachloro-1- and -2-naphthol | Pig, frog | 43, 174 |
| 1,2,3,4,5,6 | * | Pig | 43 |

* No isolatable metabolite transformation products obtained.

major metabolites were invariably phenolic products; dechlorination has not been observed with any of the PCNs investigated.

The structure of several major metabolites has been assigned on the basis of TLC, GLC and mass-spectrometric data; the results are summarized in Table 16. The mechanism of the metabolism of 1,4-dichloronaphthalene has been explained[43,174] in terms of the formation of an initial epoxidation of the naphthalene nucleus to give an arene oxide, its decomposition being accompanied by a 1,2-migration of a halogen atom:




(5)

In contrast with the lower chlorinated naphthalenes, 1,2,3,4,5,6-hexachloronaphthalene does not yield any urinary metabolites.

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