THE ANALYST

The Analysis for Residues of Chlorinated Insecticides and Acaricides

A Review*

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WE have attempted to prepare a selective and critical review of the work published up to May, 1965, on the analysis for residues of the most widely used chlorinated insecticides and acaricides, and their principal metabolites. We have made no attempt to present an historical account of previous work, but have attempted to produce a balanced picture of procedures that are relevant at the present time and also to give our own assessment of their relative importance.

A comprehensive treatise edited by Zweig[1] was published during 1963 and 1964 on the analysis of pesticides. The second volume of this work gave detailed accounts of procedures for the analysis of most of the compounds considered in this review. However, few of the methods recommended then can be recommended today as being the best procedures available for chlorinated pesticides.

Residue analysis procedures, particularly for chlorinated pesticides, were revolutionised in 1961 by the application of gas - liquid chromatography (GLC) with either electron-capture or microcoulometric detection systems. The analysis of small amounts of pesticide is possible with such procedures, and the detection of 0·05 ng of some compounds with the electron-capture detector is now routine. However, a few words of caution are necessary. In the absence of a valid control sample, GLC analysis cannot provide positive identification of a particular pesticide when only one retention-time value is obtained. Many naturally occurring products respond to GLC detectors and can be mistaken for pesticides, and often such natural products are not completely removed by the clean-up procedures that are used. This

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behaviour is encountered also with analytical procedures other than GLC. It is a factor, however, that has been ignored by many workers. The problem of the positive identification of a residue in a sample of unknown history is the most important one facing the residue analyst at the present time and it will be discussed in detail in a later section.

We have considered many aspects of the analysis for residues of chlorinated insecticides and acaricides, and the review will be of use to those chemists who wish to analyse samples of unknown history, as well as to those who wish to analyse samples from field trials where adequate controls are available and where the sample history is known.

SAMPLING AND STORAGE

The principles to be adopted when sampling crops and soils for the analysis for residues of chlorinated pesticides are the same as those when sampling for residues of other compounds. Correct sampling, however, is so important that the principles cannot be emphasised too strongly. Experimental studies of sampling procedures for crops have been reported by Huddleston *et al.*,[2] Van Middelem *et al.*[3] and by Poos *et al.*[4]

Lykken *et al.*[5],[6] have reviewed the literature on the sampling of crops and the second of these reviews,[6] in particular, presents a clear account of the correct procedure. Lykken[6] recommends that the gross samples of crops should be 25 to 100 lb or units, and that this sample should be mixed, quartered and sub-divided to obtain representative replicate 2-lb samples for analysis. The size of the gross sample, however, must be related to the size of the plot and to the size of the crop. Studies of the procedures for soil sampling do not seem to have been reported, but the recommendations concerning the representative nature and size of the sample for crops apply also to soils. Soil samples are generally taken as a core and Lichtenstein *et al.*[7] took 30 cores ($\frac{3}{4}$ inch diameter $\times$ 9 inches deep) from 500 square feet of dieldrin-treated soil. The depth to which sampling takes place will depend on the depth of penetration of the pesticide. These chlorinated insecticides and their derivatives are strongly adsorbed on all arable soils and do not leach through the soil to any significant extent. It is usual, therefore, to sample within the cultivated depth unless the total amount or depth variation of the residues is required. The statistical principles of sampling have been summarised by Garber.[8]

The need for the sample to be representative of the plot from which it is taken[6] and the necessity for the participation of the residue chemist in sampling[9] cannot be emphasised too strongly. These needs are so great that it is surprising that more experimental work has not been reported on the comparison of sampling procedures for residue analysis, especially for soils.

STORAGE OF SAMPLES

Crop and soil samples must be stored at a temperature at which the residues and the crop do not decompose further whilst awaiting extraction. This may seem an obvious precaution but experimental evidence for such stability is rarely given in the literature.

Samples are often stored[6] at 1° to 5° C, but this temperature is too high for the extended storage of many crops. Although most of the chlorinated pesticides considered here may be stable for some days when stored at 1° to 5° C, it is recommended that storage for longer than a few days should be at temperatures of −10° C, or below, in closed containers. Whatever the storage temperature, the pesticide residues in the crop and soil must be shown experimentally to be stable under the conditions used. Such evidence is best obtained by analysing field-treated samples after storage for different times at different temperatures. It is advisable to record the weight of samples with a high water content prior to deep-freeze storage, for when they regain room temperature disintegration and moisture loss can be rapid.

The extracts of crops and soils must also be stored, prior to analysis, in conditions under which further decomposition of the pesticide does not occur, and storage at 1° C, or below, and in the absence of light is recommended. If recovery experiments are carried out at the time of the extraction it is possible to obtain evidence for the stability of the pesticide during the storage of the extract.

EXTRACTION PROCEDURES

A universal extraction procedure has not yet been developed and the problems peculiar to the extraction of chlorinated pesticides from soils, crops, water, animal tissues and fatty materials, respectively, will be considered.

Often, insufficient evidence is given for the adequacy of an extraction procedure, particularly for soils. Most workers carry out recovery experiments by introducing known amounts of pesticide at the extraction stage. However, whilst good recoveries are sufficient evidence that the subsequent stages of the analytical procedure (*e.g.*, concentration steps, clean-up) do not lead to losses of pesticide, they do not necessarily mean that the extraction procedure is efficient. It is far more difficult to remove a pesticide from field-treated samples, when the pesticide has penetrated into the sample structure, than to extract a pesticide introduced at the blending stage. The efficiency of an extraction procedure may be established by extracting field-treated samples with a range of solvents for a range of extraction times. When increasing the severity of the extraction conditions (solvent polarity or extraction time) leads to no further increase in the amount of pesticide extracted, one can be reasonably satisfied that the procedure is adequate.

SOILS

Many chlorinated pesticides are strongly bound by dry soils, and the adsorptive capacity of the soil will vary considerably with moisture content, organic-matter content, the polarity of the compound and other factors. For example, hexane will extract aldrin from dry soil, but it is not suitable for the extraction of dieldrin residues.[10] Extraction with acetone will give a good recovery of most chlorinated pesticides but the co-extracted material can interfere with the analysis. Goulden[11] has shown that acetone extraction of soils followed by partition of the pesticide into hexane is a satisfactory procedure when the final analysis is by electron-capture GLC. The use of 10 per cent. acetone in hexane is considered to be adequate for the removal of most chlorinated pesticides from soil without excessive co-extraction of interfering substances.[10] Benzene - IPA,[12] hexane - IPA[13] and pentane - acetone[13] have also been used. Soxhlet extraction of the air-dried soil with acetone has been used for the removal of DDT from soil[14] but air-drying, prior to extraction, cannot be recommended as a general procedure as the pesticide may be volatile.

A procedure that can be applied to the extraction of chlorinated insecticides from a range of soil types has been described.[15] The soil is mixed with anhydrous sodium sulphate to make it friable and is then extracted with 10 per cent. acetone in hexane in an end-over-end tumbler for 1 hour. In order to improve recoveries for a wide range of chlorinated pesticides the acetone content of the extraction solvent can be increased to 20 per cent. without undue interference, especially if the final analysis is to be by GLC.

CROPS

Surface rinsing of crops is simple and results in little interference from co-extractives. It is, however, inadequate for the removal of residues other than those adhering loosely to the crop surface or dissolved in the waxy, surface layer. Since it is rarely used nowadays, we will confine our attention to the extraction of the whole crop.

The extraction of pesticides from crops has been reviewed by Bann,[10] Heinisch,[16] Thornburg[17] and Van Middelem.[18] Useful comparisons of extraction procedures have been described by Bann,[10] Klein and his co-workers[19,20,21] and by Hardin and Sarten.[22] Mills, Onley and Gaither[23] have described a useful acetonitrile extraction procedure applicable to many chlorinated pesticides in a range of crops.

Prior to the extraction, the crop should be subdivided and mixed thoroughly. Grains such as rice, and seeds such as cotton are broken in a mill prior to the extraction.[15]

Bann[10] showed that aldrin and dieldrin were readily extracted from a range of fresh, unprocessed crops such as alfalfa, carrots, corn, dates, figs, beans, turnips and wheat by maceration with hexane followed by tumbling. With many crops, especially frozen or canned foods, low recoveries or emulsion problems were encountered. To overcome these problems the use of a mixture of polar and non-polar solvents or the maceration of the crop with solvent in the presence of sodium sulphate has been recommended. The water-miscible solvent is generally removed by water washing prior to analysis. Klein and his co-workers[20,21] compared the efficiencies of three procedures for the extraction of DDT, aldrin and methoxychlor from spinach, collards and beans. Blending with benzene - IPA was more efficient than either tumbling with benzene - IPA or Soxhlet extraction with benzene or benzene - IPA. Satisfactory recoveries (95 per cent.) were obtained by blending the crop and IPA (in the ratio of 1 g to 1 ml) for 2 minutes followed by the addition of benzene (2 ml) to the mixture and a further 2 minutes maceration. Complete equilibrium existed between insecticide and sample blend, and two pour-offs were necessary to achieve 95 per cent. recovery.

Hardin and Sarten[22] compared the efficiencies of different extraction procedures for the removal of DDT from field-treated collards and their results are summarised in Table I. Blending with IPA followed by the addition of hexane and further blending was the most efficient and rapid procedure, as was found by Klein and his co-workers.[19,20,21]

TABLE I

THE EXTRACTION OF DDT FROM COLLARDS

Extraction procedure	DDT extracted (p.p.m.) (mean values)
Tumbling with hexane (30 minutes)	20·9 ± 1·1*
Blending with hexane (2 minutes) + tumbling (30 minutes)	30·4 ± 2·6
Blending IPA (2 minutes), addition of hexane + tumbling (30 minutes) ..	36·4 ± 1·1
Blending IPA (2 minutes) + addition of hexane + further blending (2 minutes)	35·9 ± 2·9
Grinding sodium sulphate + tumbling hexane (30 minutes)	24·8 ± 1·0

*Standard deviation

Whilst a procedure suitable for the extraction from a wide range of crops of most of the chlorinated pesticides considered here has not been developed, some general indications are possible.

Blending for some minutes with hexane - IPA, benzene - IPA (or with ethanol or acetone instead of IPA) should be suitable procedures. The presence of anhydrous sodium sulphate during the blending should further decrease the emulsification problems.

It does not seem to have been established clearly that prior blending with the water-miscible solvent is necessary, and a simple blending procedure with the mixed-solvent system is an efficient procedure for many pesticides.[10,17]

WATER

The analysis of pesticides in water has been reviewed recently by Hindin, May and Dunstan.[24]

Chlorinated insecticides are of low solubility in water and they may be extracted with water-immiscible solvents, such as hexane or benzene. However, when it is necessary to extract large volumes of water to achieve the necessary sensitivity, batchwise procedures can be time consuming. Rosen and Middleton[25] removed the pesticides from 2000 litres of water with a carbon filter, and desorbed them from the carbon by Soxhlet extraction with chloroform. Recoveries of BHC, chlordane, DDT, aldrin, TDE and endrin were in the range 75 to 86 per cent. at the 2·5 p.p.m. level, but some were lower below the 1 p.p.m. level.

Teasley and Cox[26] preferred a batchwise liquid - liquid extraction process as they considered that several chlorinated pesticides were unstable on activated carbon. Subsequently Kahn and Wayman[27] described a simple apparatus that can be used for the continuous extract of several hundred litres of water with petroleum spirit, and they obtained a 83 to 100 per cent. recovery at the 0·2 to 340 p.p.b. (parts per thousand million) level with a range of compounds.

Previous workers have extracted large volumes of water in order to detect chlorinated pesticides at the p.p.b. level. However, because of the high sensitivity of gas - liquid chromatography, it should now be possible to carry out liquid - liquid extraction by a smaller batchwise process prior to analysis by GLC.

ANIMAL TISSUES AND PRODUCTS

The chlorinated compounds dealt with in this review and many of their metabolites are fat soluble, and some of them tend to concentrate in the lipoid portion of the plant or animal system. Extracts of fatty materials can have such a large amount of co-extracted material that analysis by the usual residue methods is impossible without rigorous clean-up. Extraction merely involves dissolving the product in a suitable solvent before the necessary clean-up procedures are begun. However, attempts have been made to minimise the fat content of the extract, *e.g.*, by the use of polar-extracting solvents, such as acetonitrile[17] or dimethyl sulphoxide,[28,29] or by the use of an aqueous mixture containing an emulsifier.[30]

However, the solubility of fat in these solvents is low and some of the pesticide may remain in the undissolved fat. Extraction with alcoholic alkali is useful for the alkali-stable cyclodiene insecticides,[31,32] and also for DDT and its analogues,[33] although DDT is unstable to alkali.

A general procedure for the preparation and extraction of dairy products has been given by Mills.[34]   Butter was clarified by warming to about 50° C and filtered.   Cheese and milk were blended with sodium (or potassium) oxalate and alcohol, extracted with three volumes of ether and light petroleum, and the combined non-aqueous layers washed with water and evaporated.   The fat obtained from all these products was dissolved in light petroleum.   The procedure used for milk has been improved by Onley[35] with an ether - acetonitrile - dioxane mixture as extracting solvent that gave a cleaner extract more quickly.   A useful general scheme for extracting dairy products has been given by Langlois *et al.*[36] that could also apply to all fatty tissues.   A sample containing not more than 1 g of butter fat was ground with 25 to 30 g of Florisil (containing 5 per cent. of water) to give a free-flowing powder.   This mixture was placed on top of 25 g of clean pre-washed Florisil in a chromatographic column, and the whole column was eluted with 20 per cent. volume of methylene chloride in light petroleum.   Up to 650 ml of eluant removed DDT, DDE, lindane and heptachlor and the more polar heptachlor epoxide, dieldrin and endrin with good recovery and precision.

Other published methods for animal fats and tissues have usually involved grinding with anhydrous sodium sulphate[37,38,39,40] or sand[41] and dissolution in a suitable solvent.

CLEAN-UP PROCEDURES

Column chromatography remains the most widely used method of clean-up and its general use is likely to continue for some time.   Many other procedures are useful, however, for particular problems.

When GLC was first introduced for the analysis of residues of chlorinated pesticides it was hoped that clean-up would no longer be necessary.   This is true for many samples of known history when residues of 0·1 p.p.m., or higher, are present.   However, when it is necessary to detect residues at a level less than 0·05 p.p.m., clean-up is generally necessary.   For analysis by GLC with electron-capture detection or with the newer version of the micro-coulometer, the clean-up may take place on a smaller scale than was necessary with the older colorimetric procedures.   Clean-up is almost always essential with samples of unknown history unless no interference is obtained at the desired level of sensitivity without clean-up.

Gas - liquid chromatography has been used successfully for the clean-up of extracts and this application is covered in other sections.

COLUMN CHROMATOGRAPHY

The convenience and resolving power of column chromatography make it the most commonly used method of clean-up.   Despite much work on the theory of chromatography, selection of particular solid or liquid phases is still largely empirical.   Any separation may be due to the simultaneous action of adsorption, partition and ion-exchange processes, but as one of these factors usually predominates, they will be considered separately.

ADSORPTION CHROMATOGRAPHY—

The adsorptive capacity of a material depends on such factors as its structure, method of preparation, the presence of impurities, the treatment or activation it may have undergone, particle size, moisture content and the eluting solvent.   Variation in the sorptive properties between different batches of many adsorbents tends to be high, and many workers have preferred to work with those with reasonably reproducible properties, such as the synthetic magnesium trisilicate, Florisil.   A wide range of materials has been evaluated, however, and Florisil, alumina, silica gel and carbon are commonly used.   Evaluations of Florisil by Moddes[42] and by Mills and co-workers,[23,34] of silica gel by Moats,[43] and of carbon by Coulson and Barnes,[44] Cassil *et al.*,[45] Baetz[46] and Moats[47] have been reported.   Coulson[48] has advocated the use of aluminium silicate, while cellulose,[49] magnesia[23,31] and clays of the attapulgite type have also been used.[31]

The more powerfully the pesticide is sorbed to the adsorbent, the more polar the solvent needed to desorb and elute it from the column.   Table II gives the adsorbents in order of increasing adsorptivity and the eluting solvents in order of increasing polarity.   In practice, hexane or benzene is the usual solvent with, if necessary, the addition of a small proportion of ether or acetone to remove the more polar pesticides.   The adsorbents may be activated or de-activated as desired by the removal or addition of water.   The mechanism of adsorption by charcoal differs from that of the adsorbents in Table II.   Generally, pesticides containing aromatic groups are more strongly adsorbed on charcoal than are the cyclodiene pesticides.

TABLE II

ADSORPTIVITY OF ADSORBENTS AND POLARITY OF SOLVENTS

Adsorbents	Solvents
Cellulose	Hexane
Kieselguhr (Celite)	Cyclohexane
Magnesia	Benzene
Silica gel	Methylene chloride
Magnesium trisilicate	Ether
Alumina	Ethyl acetate
Clays	Acetone
	Alcohols

PARTITION CHROMATOGRAPHY—

The partition of chlorinated pesticides into polar solvents from less polar solvents is exploited in partition chromatography, particularly for separations from fats and waxes. Abdallah and Landheer[50] have used both acetonitrile and dimethylformamide supported on Celite in the clean-up of lindane and DDT from fat by using pentane or hexane as eluant. Hoskins *et al.*[51] successfully used alumina coated with polyethylene for the clean-up of a variety of crops with 40 to 65 per cent. of aqueous acetonitrile as eluant, and achieved an average pesticide recovery of 88 per cent.   Thornburg[17] has suggested this method as a "universal" type of clean-up.

Coulson *et al.*,[52] Zweig *et al.*[53] and Crosby and Laws[54] have successfully used gas - liquid chromatography as a method of clean-up.   This quite simple technique could have wider application to the stable chlorinated materials.

ION-EXCHANGE CHROMATOGRAPHY—

Ion exchange has proved useful in clean-up[55,56] and metabolism studies[57] of pesticides, but has been used to only a limited extent with chlorinated compounds.   Few members of the group considered here are sufficiently acidic or basic for the technique to be employed, although it has been used for the analysis of DDA in urine.[58]

PAPER CHROMATOGRAPHY

Paper chromatography has been used extensively for the separation of mixtures of pesticides in extracts of plants, animal tissues and dairy products.   It has been used widely for the identification and quantitative analysis of pesticides, but has been little used for the clean-up of extracts prior to analysis by other methods.   For this reason a more detailed discussion of paper chromatography will be considered later in the "Quantitative Analysis" section.   The principles outlined in that section apply also when paper chromatography is used for clean-up.

If paper chromatography is to be used for clean-up, a marker spot should be made visible and the assay sample must be eluted from the relevant section of the paper prior to further analysis.   Any solvent which produces an R_F value of 0·95 or greater for the desired component is satisfactory for the elution.   However, the stationary and mobile phases may also be eluted along with the spot and may interfere in the subsequent analysis; this and the slowness of the method and the low capacity of the paper are the reasons why paper chromatography has been used mainly for the identification of pesticides and not for clean-up.

Recently Heinisch and Neubert[59] have described the application of wedged-shaped paper strips for the clean-up of plant extracts.

THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) may be used for the clean-up of extracts prior to analysis by other methods, for the qualitative analysis of pesticides, and for their direct quantitative analysis.

TLC is more rapid and has a higher capacity than paper chromatography, and is more useful for the clean-up of extracts.   TLC generally gives sharper resolution of chlorinated pesticides than paper chromatography and, unlike the latter, can be carried out successfully without the impregnation of the adsorbent with a stationary phase.   The adsorbents, developing systems and detection systems will be considered in detail in the section on quantitative analysis.

When TLC is used for clean-up prior to analysis by other methods, the pesticide is desorbed from the adsorbent at the R_F value corresponding to the desired pesticide.   The

R_F value is determined by running a marker spot alongside the extract and only the marker spot is made visible.

The correct solvent must be chosen for removal of the separated components from the adsorbent. Little specific information has been given as to a suitable solvent, but of course, any solvent which gives an R_F value of 0·95 or greater for the particular component is suitable. Acetone, ether, alcohol or mixtures of these with hexane should remove most of the pesticides considered here.

Abbott and Thomson[60] have suggested wedge-layers for clean-up by TLC and this system should be useful for chlorinated pesticides. Taylor and Fishwick[61] have used loose-layer chromatography on alumina with hexane for the separation of aldrin, DDT and its metabolites, BHC, heptachlor epoxide, endrin and dieldrin, and this technique is especially useful for the investigation of solvent systems for the separation of pesticides by column chromatography.

LIQUID - LIQUID PARTITION

Jones and Riddick[62] extracted several pesticides including methoxychlor, chlordane, lindane and DDT with hexane from plants, animal tissues and dairy products. The pesticides were partitioned into acetonitrile, and considerably less interference was encountered in the subsequent colorimetric or polarographic analysis as a result.

This liquid - liquid partition procedure has now been used widely, especially for extracts of animal fats and tissues, and its use has been extended to many pesticides and a wide range of solvent pairs are available. Burchfield and Storrs[63] showed that lindane, DDT and aldrin partition from hexane into DMF to a greater extent than into acetonitrile, and that the use of the high-boiling DMF was no drawback as the pesticide may be recovered from this solvent readily by dilution with water followed by partition back into hexane. The use of DMSO - hexane and acetonitrile - hexane was compared by Haenni et al.[64] who showed that BHC, aldrin, dieldrin, endrin and heptachlor partitioned to a greater extent into DMSO than into acetonitrile.

Extraction of crops and soils with acetone and subsequent dilution of the extract with aqueous sodium sulphate solution and partition into hexane was used successfully by Goodwin et al.[65] This sytem was used also for animal tissues[66] but it was found to be unsuitable for animal fats.[40] The animal fats and dairy products were extracted with hexane, and the pesticides (aldrin, dieldrin, pp'-TDE, pp'-DDT, BHC and heptachlor) partitioned into DMF. The DMF was diluted with water and the pesticide was partitioned back into hexane prior to analysis by GLC.

Recoveries from partition processes are generally good, even at the microgram or nanogram level, as long as the mixing of the solvent phases is effective and the partition is repeated a sufficient number of times.[40] The relative volumes of solvents and the number of times that partitioning must be repeated may be determined from the partition coefficients, and

TABLE III

PARTITION COEFFICIENTS[67] OF CHLORINATED PESTICIDES AT 25·5° C

Partition coefficient

Pesticide	Hexane - acetonitrile	Hexane - 90 per cent. aqueous dimethyl sulphoxide	Iso-octane - 85 per cent. aqueous dimethyl formamide	Iso-octane - dimethyl formamide	Iso-octane - dimethyl formamide (with 125 mg butter extractive)
Aldrin	0·73	0·89	0·86	0·38	0·39
γ-Chlordane	0·40	0·45	0·48	0·14	0·16
pp'-DDE	0·56	0·73	0·65	0·16	0·18
oo'-DDT	0·45	0·53	0·42	0·10	0·11
pp'-DDT	0·38	0·40	0·36	0·08	0·09
Dieldrin	0·33	0·45	0·46	0·12	0·13
Endosulfan I	0·39	0·55	0·52	0·16	0·17
Endosulfan II	0·13	0·09	0·14	0·06	0·07
Endrin	0·35	0·52	0·51	0·15	0·16
Heptachlor	0·55	0·77	0·73	0·21	0·23
Heptachlor epoxide	0·29	0·35	0·39	0·10	0·11
Lindane	0·12	0·09	0·14	0·05	0·06
TDE	0·17	0·08	0·15	0·04	0·04
Telodrin	0·48	0·65	0·63	0·17	0·19

several lists of values are available.[40,62,63,67]   It is generally found that co-extractives do not have any great effect on the partition of the pesticide.[67]

Beroza and Bowman[67] have measured the partition coefficients of a range of pesticides (Table III) and have shown that liquid - liquid partition can be useful not only for clean-up, but for more positive identification of the pesticides.   The extract can be analysed before and after a suitable partition procedure.   This will enable the partition coefficient of the component to be determined, and comparison of the results with the values in Table III will allow more positive identification.   This procedure is rapid and is useful during GLC when the particular conditions will not resolve a given pair of insecticides, and may often be quicker than attempting to alter the GLC conditions in order to attain the desired resolution.   More recently these workers[68] have extended their work and have used a counter-current distribution system to clean-up extracts and also to identify pesticides.

PRECIPITATION OF FATS AND WAXES

Precipitation procedures have often been used to remove interfering waxes and fats. Fairing and Warrington[69] cooled acetone solutions of plant and animal tissues to $-15°$ C to precipitate fats and waxes, which were then removed by filtration, and they reported good recoveries of methoxychlor from apple wax.   Williams[70] obtained good recoveries of chlordane when the interfering waxes in tomatoes, cabbages and apples were precipitated by cooling a methanolic solution in an ice-bath.   Precipitation procedures have been used extensively by McKinley, McCully and their co-workers.[38,71,72,73,74,75]   Good recoveries of DDT (*op'* and *pp'*), TDE (Rhothane), methoxychlor and dicofol were obtained[71] when waxes were precipitated at $-70°$ C from acetone extracts of a range of fruits and vegetables.   DDE, DDT and TDE were recovered from a range of animal fats[72] when acetone solutions were cooled and a three-stage cooling procedure, one at $5°$ C and two at $-70°$ C, was necessary to precipitate the fat from large samples.   Recently a simple apparatus has been described[38,74] for the precipitation of fats and waxes which should be useful for the processing of large numbers of samples.   With the apparatus benzene - acetone solutions of plant and crop extracts were cooled to $-70°$ C, and good recoveries were obtained by DDT (*op'* and *pp'*), lindane, heptachlor, aldrin, heptachlor epoxide, endrin and methoxychlor.

Precipitation procedures have also been used in other ways.   Gunther and Blinn[76] cooled benzene extracts of avocados to $0°$ C to crystallise the benzene together with any DDT. The avocado oil was removed from the crystal mush by filtration.   McKinley and Savary[73] deposited an extract of butter fat on a charcoal column and eluted dieldrin without the butter fat with acetone at $-70°$ C.

The precipitation procedure is good for the removal of fats and waxes but not particularly useful for the removal of interference from other sources.   Chromatographic procedures will usually remove several of the interfering classes of compounds and have found more widespread application.

GLC procedures are sometimes less prone to direct interference from fats and waxes than many of the older colorimetric methods.   However, the removal of fats and waxes that do not cause direct interference during GLC is desirable if extended GLC column life with maintained efficiency is required.

CHEMICAL METHODS

Chemical methods of removing or modifying co-extracted material can be applied to a limited range of pesticides but are tending to be superseded for normal use by partition and adsorption methods.   However, the modification of a pesticide by chemical reaction will probably find wider use as an aid to identification.

TREATMENT WITH ALKALI—

Saponification has been widely used as a method of clean-up for the alkali-stable cyclodiene compounds, aldrin,[31] dieldrin[32,77] and endrin,[78] for DDT (with conversion to DDE)[33,79] for lindane,[80,81] heptachlor epoxide[82] and methoxychlor (with conversion to the ethylene compound).[83]   A quicker method of clean-up with alkali has been reported for endrin by Albert,[84] in which a potassium hydroxide - Celite column was used instead of saponification.   One layer of potassium hydroxide - Celite (14 to 17 per cent. water in the mixture) was placed between two layers of magnesium oxide - Celite and the column eluted with light petroleum.

TREATMENT WITH ACID—

Schechter et al.[85] cleaned up chloroform extracts of milk containing DDT and glycerides by hydrolysis with fuming sulphuric acid. Davidow[86] improved the method by percolating the extract through a column containing concentrated and fuming sulphuric acid supported on Celite. This technique has also been used for lindane.[87]

TREATMENT WITH OXIDISING AGENTS—

This technique has occasionally been used for pesticides resistant to oxidation such as DDT, dieldrin and lindane as in the method of Gunther et al.[88] for the rapid determination of DDT in dairy products, and that of O'Donnell et al.[32] for dieldrin in unsaponifiable materials. Alkaline potassium permanganate has been the most favoured reagent, but chromic acid, acid or alkaline peroxide and acid chromic anhydride may also find application.[89]

OTHER MISCELLANEOUS METHODS

STEAM DISTILLATION—

Some of the chlorinated pesticides are steam-volatile, but little attempt has been made to use this property because the volatility is greatly reduced in the presence of crop and particularly tissue extracts. Gunther and Jeppson[90] and Butzler et al.[91] have hydrolysed chlorfenson with alkali and steam distilled the resulting p-chlorophenol, and Gunther and Blinn[92] have described a similar procedure for oxythane. Ott and Gunther[93] have attempted to devise a general method for fat clean-up by using forced volatilisation. The method is rapid and recoveries are good except for TDE and methoxychlor.

HYDROLYSIS WITH ENZYMES—

Clifford[94] showed that clean-up by using enzymes was effective, but the method is lengthy and the enzyme not readily available so that this observation has not been used to any great extent.

QUANTITATIVE ANALYSIS

Methods that have been used for the quantitative analysis of chlorinated pesticides will be discussed in turn, and in each the specificity of the procedure will be considered. In the last sub-section the specificity of residue methods will be considered in detail.

GAS - LIQUID CHROMATOGRAPHY

The rapid application of GLC as a technique and in particular the development of selective methods of detection for halogenated compounds have led to its world-wide use in the residue analysis of chlorinated pesticides. Because of the sensitivity and selectivity that it can offer, GLC has become the preferred method for the whole group of compounds considered here. It was once hoped that the relatively small response from most crop constituents would mean little or no clean-up of extract,[95,96,97] but because of the interest in much lower residue levels, this hope has not been realised. Nevertheless, GLC has many of the characteristics of an ideal residue method.

The high sensitivity of the detectors has meant that only small weights of pesticide, and thus smaller weights of co-extracted materials, are injected and this has led to the use of lower loadings of stationary phases and lower column temperatures, which have given more efficiency, resolution and life to GLC columns.

NON-SELECTIVE DETECTION SYSTEMS—

The first work on the gas chromatography of insecticides in 1958 was carried out by using thermal-conductivity detection.[98] Later workers have also used this detector,[52,53] but the sensitivity is poor and it lacks selectivity. This latter disadvantage also applies to the use of the flame-ionisation detector despite its much greater sensitivity.

SELECTIVE-DETECTION SYSTEMS—

Microcoulometry—

Coulson et al.[96] introduced the microcoulometric titrating system as a GLC-detection device in 1960. The method involves combustion of the vapours eluted from the gas chromatograph and automatic titration of the hydrogen chloride (or sulphur dioxide) produced, and

it has been reviewed by Cassil[99] and by Challacombe and McNulty.[100]   Its usefulness in residue analysis has been assessed by Burke and Johnson[101] and more recently by Burke and Holswade.[102]   They conclude that optimum conditions for the analysis of over a hundred chlorine- or sulphur-containing pesticides are a 6-feet × 4·5-mm i.d. aluminium column, packed with 10 per cent. DC 200 silicone fluid coated on an 80 to 90 mesh Celite support, previously acid and base-washed and silanised.   This column is conditioned and is operated at 210° C with nitrogen as carrier gas at a flow rate of 120 ml per minute.

The maximum sensitivity of the original microcoulometer (Model R-100) is about 0·1 μg chloride, the range to about 1 mg chloride and precision $\pm$3 to 5 per cent.   The detector has the great advantage of internal standardisation, the silver ions being electrically generated. For a limit of detection of 0·01 p.p.m. of pesticide, the extract of 10 g or more of crop must be injected onto the chromatograph, and thus for reasonable GLC column life extensive clean-up of extracts is required.   In addition, the solvent and any materials emerging from the GLC column before the pesticide are usually vented to the atmosphere to prevent incomplete combustion and fouling of the electrodes.   To minimise decomposition of pesticide at the high column temperature that is used, Cassil[99] recommended the use of a quartz insert in the injection block.

The instrument proved immediately popular, particularly in the U.S.A., being put to use not only for analysis of crop residues[103,104,105,106] but also for a great deal of environmental monitoring work.[24,107,108]

A modified design (Model R-200) has a sensitivity about ten times better than the original. An ultimate sensitivity of about 10 ng of chloride is claimed for this later instrument, a great improvement.   The increased sensitivity makes lower column loadings and lower temperatures feasible, which will decrease the tendency for decomposition of some pesticides.

Electron capture—

The quantitative GLC analysis of halogenated compounds with high electron affinity was found to be difficult with some ionisation detectors because of combination of these molecules with electrons.   This disadvantage was exploited by Lovelock and Lipsky[109] for the selective detection of such molecules.   In this detection system the eluted vapour passes into an ionisation cell containing two electrodes.   The cathode is in contact with a source of electrons which is usually a few hundred millicuries of tritium.   The anode collects the electrons accelerated by a d.c. or pulsed potential, and this produces a standing current of 10^{-9} to 10^{-8} amp.   Vapours entering the cell capture electrons to a greater or lesser extent and thus diminish the standing current.   This change of current is measured.   The decrease in current which is normally found is exponentially related to the number of vapour molecules. The working range extends to about 30 per cent. of the standing current, about the first 5 per cent. being rectilinear, the dynamic range being rather less than a thousand.   The sensitivity of the detector varies with the electron affinity of the compound, which itself varies not only with the functional group but also with its molecular environment.[110]   The measurement of the electron affinities of many compounds has revealed an interesting correlation with biological activity.[111,112,113]

The potentialities of the high sensitivity of the detector for the quantitative analysis of traces of polychlorinated insecticides were quickly seen and applied[65,95,97,114] and the purely qualitative aspect has received less attention.   The device has drawbacks, such as the lack of specificity for halogen, the differing sensitivity among even the halogenated materials and the need for frequent calibration.   However, despite these drawbacks, with ordinary care in operation[113,115] the detector has been a great success.   For halogenated pesticides it offers a sensitivity greater than a thousand times that of the microcoulometer, and high selectivity.   The characteristics and performance of the detector have been reviewed by Lovelock,[115] Landowne and Lipsky,[116] Dimick and Hartmann[117] and Clark,[113] and have been shown to be only slightly affected by minor changes in flow rate and detector temperature. For a residue level of 0·01 p.p.m. the extract from only milligram amounts of samples needs to be injected, and although clean-up is necessary at this level, it can be carried out rapidly and conveniently with small volumes of extract, adsorbent and partitioning solvent.

Goodwin *et al.*[65] described a procedure for the analysis to a concentration of 0·1 p.p.m. of a variety of chlorinated insecticides with a commercial detector, and showed that increased sensitivity could be obtained with one made to a design by Lovelock.[110]   Both planar or radial geometry of the radioactive source have since been used, with little apparent difference

in performance. The detector has been used many times for the analysis of chlorinated pesticides in crops, soils and animal tissues and products, and optimum conditions have been reviewed recently by Burke and Giuffrida.[118] These workers recommend as operating conditions a 6-feet $\times$ 4-mm glass column packed with 10 per cent. DC 200 silicone oil (12,500 cS) on 80 to 90 mesh, acid and base-washed and silanised kieselguhr. This column and the detector are maintained at 200° C and 120 ml per minute of nitrogen is passed through. These conditions do not permit the chromatography of endrin without decomposition.[65,119]

Halogen leak detector—

This device was first used as a GLC detector by Cremer et al.[120] for the chlorinated solvents, and was extended to residues of the chlorinated pesticides by Goulden et al.[121] It consists of two concentric platinum cylinders that act as electrodes. The inner anode is sensitised by treatment with alkali and is indirectly heated to about 800° C and maintained at 250 volts d.c. relative to the cathode, which receives a standing current of positive ions. The presence of halogen in a vapour passing through the cell induces an increase in this current, which can be amplified (if necessary) and recorded in the usual way. The system has, at the moment, the disadvantage of poor stability. Improvements in design will, it is hoped, eliminate or at least reduce this defect. However, it has the great merit of excellent selectivity for halogen, and about 10 ng can be detected in a simple circuit without amplification, and less than 1 ng in a more elaborate circuit. With these advantages it may well have a promising future.

Other selective detectors—

Beilstein flame detector—The Beilstein test has been adapted for use as a quantitative GLC detector.[122,123] All or part of the effluent from the GLC column is led through a copper thimble held in the hot zone of a Bunsen burner and an intense green flame ($\lambda_{max.}=473$ mμ) indicates the presence of halogen. The sensitivity by visual observation is in the microgram range for halogen, but this can be increased by the use of instruments. Quantitatively, the time of appearance and disappearance of the coloration can be related to the response from another detector such as a katharometer, coupled prior to the flame or in parallel with it.

Solution-conductivity detector—Sternberg[124] has made use of the properties of combusted vapours to devise a selective detector. The gases emerging from the GLC column, after combustion, contact a flowing film of distilled water. Any soluble, ionised components produce a change in the electrical conductivity. The claims for sensitivity (6×10^{-9} g for DDT, with signal equivalent to twice the noise level), selectivity (lindane is 4000 times more sensitive than hexane), and repeatability are good.[125,126,127]

Although both of these detectors have involved careful development of ingenious ideas, it would seem that the other selective devices previously mentioned have more immediate promise, and these two will probably not be refined for general use.

COLUMN AND INJECTION BLOCK MATERIALS—

In its early days GLC was primarily used by workers in the petroleum industry with much emphasis on the high-temperature separation of the inert hydrocarbons, and on this account the problem of decomposition of more thermally labile compounds has sometimes been overlooked. Exposure to hot metal surfaces or carbonised deposits in the injection block can bring about decomposition of many pesticides such as DDT,[128] dicofol[129] and particularly endrin.[65,119,128] The composition of the column tubing also plays a part in decomposition. Beckmann and Bevenue[128] compared four materials and found increasing recovery of the insecticide injected with columns of copper, stainless steel, aluminium and quartz, respectively. Dimick and Hartmann[117] have shown that Pyrex glass is as effective as quartz. Cassil[99] has overcome decomposition in the injection block by using a quartz liner to the block, and borosilicate glass may be substituted for quartz.[117,130] It is becoming normal practice in analysing thermally unstable compounds such as pesticides to dispense with an injection block and to inject directly on to the GLC column in order to avoid decomposition at this point.

SOLID SUPPORTS—

Although capillary columns have been used for analysis of pesticides[131] their evaluation for residue analysis has so far proved disappointing.[132] In an attempt to use the high efficiencies of capillary columns to secure more positive identification, the drawback of low column capacity proved insuperable. The extreme dilution of pesticides in residue extracts means that the stream-splitting normally used with capillaries is impracticable. Wider-bore capillaries with their higher capacity may improve this picture, but it is doubtful if a resolution superior to that of packed columns can be obtained in a reasonable time.

With packed columns the optimum column bore would appear to be 3 to 4 mm (0·125 to 0·160 inches), and for best column efficiency the particle-size range of the support material should be narrow (a 10 or 20 mesh fraction). Kieselguhr in its various grades has remained the most popular material, being inert, having a high ratio of surface area to volume and with reasonable mechanical strength. Recently, a harder and less adsorptive support specially developed for GLC (Chromosorb G) has been introduced. Glass micro beads[65] have been found to offer no advantage. Low loadings of stationary phases must be used and the packing of the column can be difficult. The lack of porosity of glass beads can lead to high back pressures.

For the compounds under review the most important property of a support material is the absence of any tendency to cause decomposition. Goodwin *et al.*[65] located the main source of decomposition in the support material and showed that nanogram quantities of endrin could be chromatographed successfully by using small amounts of a polar stationary phase (Epikote resin 1001) in admixture with the non-polar phase to act as an active-site suppressor. The proportion of resin used (10 per cent. of the silicone) was insufficient to modify the resolution of the pesticide mixture given by the silicone. The same workers later found that other epoxy additives could be used[132] and that pre-treatment of the support by refluxing with epichlorhydrin gave chromatography without decomposition when silicone alone was used as stationary phase. This approach has also been used by Gunther *et al.*[133]

TABLE IV

RELATIVE RETENTION TIMES OF CHLORINATED PESTICIDES[102]

Pesticide				Relative retention time
Lindane ..	..	..	..	0·52
Heptachlor	..	..	..	0·81
Dicofol ..	..	..	..	*0·99*, 3·82
Aldrin* ..	..	..	..	1·00
op'-TDE olefin ..	..	..	..	1·12
Heptachlor epoxide	..	..	..	1·20
Chlorbenside	..	..	..	1·25
pp'-TDE olefin ..	..	..	..	1·34
γ-Chlordane	..	..	..	1·35
op'-DDE	..	..	..	1·37
Endosulfan	..	..	..	*1·44*, 1·91
Chlorfenson	..	..	..	1·46
β-Chlordane	..	..	..	1·46
pp'-DDA methyl ester ..	..	..	1·48
pp'-DDE..	..	..	..	1·69
Dieldrin ..	..	..	..	1·72
op'-TDE ..	..	..	..	1·72
Endrin ..	..	..	..	*1·90*, 2·09
Chlorobenzilate ..	..	..	..	2·07
pp'-TDE	..	..	..	2·12
op'-DDT ..	..	..	..	2·21
pp'-Methoxychlor olefin	..	..	2·52
pp'-DDT	..	..	..	2·70
pp'-Methoxychlor	..	..	..	3·90
Tetradifon	..	..	..	4·22
Chlordane	..	..	..	0·52, 0·66, 0·74, 0·82
				0·96, 1·14, *1·36*, 1·50, 2·3
Toxaphene	..	..	..	1·29, 1·45, 1·55, 1·87, *2·17*
				2·45, *2·85*, *3·36*, 4·03, 4·52

* Aldrin retention time 5·5 minutes.

Column of 10 per cent. DC 200. Temperature of 210° C.
Carrier-gas flow rate: 120 ml per minute.[21]
Major peaks are in italics.

who used tris-(2-biphenylyl)phosphate (Dow K-1110) as a stabiliser, but Beckman and Bevenue[128] have reported that this is not always successful.

The adsorptivity of the support material has also been reduced by acid and alkali washing, and active hydroxyl groups have been eliminated by conversion to their trimethylsilyl derivatives with hexamethyldisilazane.[102,118,134]

STATIONARY PHASES—

The stationary phase is selected for the separation it offers of compounds actually or potentially present in the mixture to be analysed.   Experience to date has shown that a separation involving the use of silicone compounds based essentially on differences in vapour pressure gives the best resolution of the chlorinated group of pesticides.   Work with the dimethyl siloxane polymers DC 200,[100,102,118] SE 30,[135] SF 96,[136] Dow 11[117,137] and E 301[65,130] and with the almost non-polar methylphenyl siloxane polymer DC 710[138] has been published and tables of retention times have been given by Cassil,[99] Bevenue,[138] Burke and Holswade[102] and Burke and Giuffrida.[118]   The relative retention times of the compounds under review on a non-polar phase are given in Table IV.

From this table it can be seen that on the silicone phase there are pairs of compounds difficult to resolve, notably pp'-DDE and dieldrin, pp'-TDE and op'-DDT, aldrin and dicofol, endosulfan and chlorfenson, heptachlor epoxide and chlorbenside.   Other stationary phases with greater polarity have been chosen for the ability to separate these pairs and also to resolve a pesticide from a peak suspected of being due to a co-extracted natural product. Apiezon hydrocarbon greases,[130,139] the silicone polymers modified with fluorine-containing groups[38,140] or with nitrile groups (GE XE 60[132] and XF 1112[132]), epoxy resins,[65] polyesters[141] and the Nonidet P40[139] have been used to give this extra resolution with chlorinated insecticides, but Ucon fluids,[142,143] the polyglycol amide Versamid 900[143,144] and other esters[143,145,146] have been used for other pesticides.

TEMPERATURE—

Excessively high column temperatures lead to decomposition and loss of resolution. The original work with pesticides[52,98] was carried out at about 250° C, but the advent of more sensitive detectors has meant, among other advantages, that lower operating temperatures can be used and 200° to 210° C has recently been recommended.[102,118] Goodwin et al.[65] have shown that temperatures as low as 160° C give better chromatography with reasonable retention times with a lower percentage of stationary phase (2·5 per cent.) with 0·25 per cent. of epoxy resin added to prevent decomposition on the uncoated support.

To give efficient vaporisation and to prevent condensation the injection block (if used) and the detector must be at a temperature at least as high as that of the column, but it is not necessary for them to be much hotter.

Burke[147] has evaluated temperature programming for the analysis of chlorinated pesticides, and has shown that a complex mixture of compounds with a range of vapour pressures could be conveniently separated, but the procedure did not achieve separations that were not possible with isothermal operation.

Electron-capture detectors containing tritium as the source of radiation cannot be operated at temperatures above 200° C.[148,149]

MEASUREMENT IN GLC ANALYSIS—

The microcoulometer gives an absolute measure of the weight of compound eluted and burnt, but with other detecting systems the amount of pesticide that each peak represents may be found by calibration with an internal standard, but preferably with an external standard.   The peak area or peak height may be measured, although the measurement of the former generally gives more reproducible results[150] except for peaks of short retention time.

IDENTIFICATION—

In the absence of valid untreated control material a single retention time obtained by GLC is not sufficient evidence for the positive identification of a compound.   Many naturally occurring products can respond to the GLC detectors used for residue analysis.   Although the relative response to electron-capture detectors of these naturally occurring co-extractives will generally be much lower than that of the chlorinated pesticides, they are often present in a much higher concentration.

There are several methods available of improving the specificity of GLC. Columns with better resolution may help[132] and retention times on two or more stationary phases of different type offer additional, though not conclusive, evidence.[139] The several-column, single-detector chromatograph produces a characteristic "spectrum" from a single injection and is useful for fairly simple problems,[132,151] but is difficult to interpret for more complex mixtures. Alternatively, information on the identity of a compound may be given by comparison of the responses of the compound to different selective detectors, and this has been successfully demonstrated by Goulden *et al.*[121] with the electron-capture and the halogen-leak detectors.

Conversion into characteristic derivatives prior to GLC has been exploited by several groups of workers for specific pesticides. Klein *et al.*[152] converted DDT and TDE to their ethylene derivatives with alkali, and Beckman and Berkenkotter[153] used reduction with sodium in liquid ammonia.

Conversion on the nanogram scale can be carried out readily and pre-columns filled with suitable reagents have been used by many workers.[154,155,156,157,158] Such methods seem capable of offering conclusive evidence of structure. The use of GLC in combination with other procedures is considered later in this section.

TOTAL HALIDE ANALYSIS

Analysis of pesticide residues by total halide has been thoroughly reviewed in Volume 1 of the treatise edited by Zweig.[1] Wet, dry and oxygen-flask combustion and various types of sodium reduction followed by colorimetric or electrometric end-point determination were discussed. Details of the sodium - diphenyl reduction have been given in Volume 2 of the same text, and Grou and Balif[159] have used sodamide in paraffin oil for dehalogenation. By present standards the limit of detectability of this method is low, (microgram amounts of halide); it is impossible without prior treatment to distinguish between the components of a mixture, and few workers in recent years have concentrated on it.

This latter point also applies to neutron activation, another total-halide technique, reviewed by Schmitt[160] and Guinn and Schmitt.[161] In addition, the sensitivity for chlorine is not particularly high, about $0.1 \mu g$ for the $^{37}Cl (n,\gamma) ^{38}Cl$ reaction, but rather better for bromine, $0.005 \mu g$ for the $^{79}Br (n,\gamma) ^{80}Br$ reaction. This could be a powerful method where many similar samples need to be analysed, but the disadvantages of non-specificity and lack of availability do not encourage its development.

POLAROGRAPHY

Polarography has not been used extensively for the analysis of chlorinated pesticides because the method has not the sensitivity of several of the alternative procedures, and also because extensive clean-up of plant and soil extracts is required before analysis by this method. Furthermore, several of the chlorinated pesticides are not reduced during polarography. The procedure is likely to find more application for the analysis of organo-phosphorus compounds rather than for chlorinated pesticides. The method has been investigated, however, for application to chlorinated pesticides as it is capable of quantitative analysis and also the measurement of the half-wave potentials of the reducible components confers a degree of specificity. Polarography is extremely sensitive for the analysis of reversibly-reducible inorganic components but the sensitivity is poorer for the analysis of irreversibly-reduced organic compounds. For organic compounds pulse-wave polarography is the most sensitive form of this procedure. Gajan,[162] Brazee[163] and Gajan and Link[164] have reviewed the application of polarography to pesticide analysis.

DDT and its metabolites can be determined by polarography[165,166] and tetramethyl ammonium bromide in aqueous acetone has been used as the base solution[167] to determine concentrations of $5 \mu g$ per ml. Analysis of DDT by polarography has also been reported by other workers.[168,169,170] It has been reported[170] that the alkyl chlorine atoms of DDT are reduced more easily than the aromatic chlorine atoms.

Polarography of DDT after nitration has been reported by Davidek and Janicek.[171] The polarography of dieldrin has been described by Swanepoel,[172] and Kosmatyi[173] has used polarography for the analysis of residues of DDT, γ-BHC and heptachlor in crops and soils after chromatography. Kosmatyi and Shlyapak[174] have determined residues of DDT in a range of crops with a limit of detectability of 2.1×10^{-5} M (about $7 \mu g$ per ml). Other references to the polarography of these compounds and to methoxychlor are given by Gajan and Link.[164]

BIOASSAY

The use of bioassay for the determination of pesticide residues, including residues of chlorinated pesticides, has been reviewed within recent years by Needham,[175] Dewey,[176] Sun[177] and Phillips.[178] Most of the work that was reported up to 1962 is considered, and many references are given to the application of bioassay in the determination of residues of chlorinated pesticides.

Bioassay has been used extensively because it is capable of detecting very small amounts of toxicants. Only the simplest apparatus and a ready supply of test organisms are required. A wide range of test organisms has been used, including vinegar flies (*Drosophila melanogaster*),[175,176,177,178] house flies (*Musca domestica*),[175,176,177,178] adult and larval mosquitoes (*Aedes, Anopheles* and *Culex*),[175,176,177,178] alfalfa weevil larvae (*Hypera postica*),[179] brine shrimps (*Artemia salina*),[177] water fleas (*Daphnia magna*),[177] microcrustacea (*Gammarus lacustris*),[180] guppies (*Lebistes reticulatus*),[175,176,177,178] and goldfish (*Carassius auratus*).[177]

Davidow and Sabatino[181] and Dewey and Parker[182] have described a system for the mass rearing of *Daphnia magna* for bioassay, and Gerolt[183] has described methods for the breeding of *Drosophila melanogaster* for bioassay. Satisfactory methods have also been described for house flies and mosquito larvae and are given by Needham.[175] Of these test organisms the vinegar fly, house fly and mosquito larvae have been used most extensively. All three species are easy to rear and are sensitive to toxicants.

Sun[177] has made a useful summary of the susceptibility of several test species to chlorinated insecticides and, although *Drosophila melanogaster* are the most versatile, the choice of test organism will often depend on the pesticide to be assayed and on the possible contaminants.

The three basic techniques generally used are (*i*) direct methods in which the material containing the pesticide is fed directly to the test organism, (*ii*) film methods in which a film of pesticide is deposited on a surface by evaporation of an extract of the sample followed by exposure of the test organism to the film and (*iii*) aqueous methods where the sample extract is transferred to water that contains the test organism, such as fish or larvae.

The direct-feeding method is less sensitive than the other methods but it is quicker and is useful when toxicant levels are high. The results in Table V indicate that whilst the aqueous methods can be used to detect pesticides in very dilute solutions the absolute sensitivity in micrograms is not as good as that of the dry-film method.

In using these procedures it is important that the test conditions should be standardised and the following factors must be considered. Naturally occurring compounds can be toxic or can have a masking effect and clean-up is often necessary to achieve the highest sensitivity. Mosquito larvae are particularly sensitive to co-extractives. The susceptibility of a test organism will vary with the age and sex of the species, for example, male *Drosophila melanogaster* are more susceptible to toxicants than females, and the susceptibility generally decreases as the age increases. Henneberry *et al.*[187] have described a simple method in which a rising air stream is used to separate the male *Drosophila melanogaster* from the females. The presence or absence of food during the bioassay can also affect the susceptibility.[188,189] Apart from these biological factors, physical factors such as the number of test animals, the size of the container, the time of exposure, temperature, humidity and illumination should be standardised.[190]

The mortality obtained with the assay sample is compared with that obtained by using standard amounts of pesticide. Several methods of calculating the results are described by Sun[177]; a plot of percentage of mortality on a probit scale and amount of pesticide on a logarithmic scale is generally rectilinear and can be used within the range of 20 to 80 per cent. mortality.

The susceptibilities of some test organisms by using the three basic procedures are summarised in Table V. The values are not from the same source since a direct comparison of all the basic procedures has not been made. The susceptibilities are generally low in the presence of plant extracts due to masking. The masking effect of a different combination of crops and toxicant has been discussed in detail by Phillips.[178]

When toxicants other than the compound to be assayed are absent, the results of bioassay agree well with the results obtained by chemical or physical methods.[176,177,178] Bioassay is extremely useful in parallel screening tests,[178] as the comparison of the results of bioassay with those of specific methods may indicate the presence of other toxic components that may be metabolites of the parent compound. Bioassay results and the results of other

non-specific methods may be used in combination to provide added confirmation of the presence of a toxicant. However, the use of bioassay has declined during recent years because of its lack of specificity and also because of the difficulties, in comparison with some other procedures, of obtaining reproducibility.

TABLE V

THE SUSCEPTIBILITIES OF TEST SPECIES TO TOXICANTS

	Dry-film method LD_{50} values* (μg per container)	Direct feeding† per cent. mortality for given dosage in p.p.m.	Aqueous solution‡ LC_{50} (p.p.m.)
Aldrin	0·05	0·2 p.p.m. 98%	0·017
Dieldrin	0·05	0·2 p.p.m. 89%	0·0088
BHC	1·0	—	—
γ-BHC	0·10	0·5 p.p.m. 64%	—
Chlordane	0·20	—	—
Endosulphan	0·27	—	—
Endrin	0·30	0·5 p.p.m. 54%	0·017
Heptachlor	0·30	0·1 p.p.m. 55%	—
Toxaphene	10	10 p.p.m. 20%	—
Chlorobenzilate	32	—	—
DDT	15	2·0 p.p.m. 0%	0·035
TDE	18	—	—
Methoxychlor	500	5·0 p.p.m. 0%	—

* Ref. 184. 25 × 200-mm tubes, 24 hours exposure of twenty, 7 to 31-hour old *Drosophila melanogaster* to pesticide in the absence of plant extracts.

† Ref. 185. Twenty grams of macerated potato containing pesticide were exposed to 50 one-day old *Drosophila melanogaster* for 24 hours.

‡ Ref. 186. Twenty-five 4th instar larvae of *Culex p. quinquefasciatus* in 100 ml of water exposed for 24 hours to the pesticide in the absence of plant extracts.

Several attempts have been made to improve the specificity of bioassay. Methods involving a combination of chemical and physical clean-up have been used.[191] Also, the comparison of the response of several different test-organisms to the toxicant can be used to fingerprint the pesticide.[192,193] Other methods of achieving specificity by clean-up, by comparison of the ratio of photomigration, by comparison of rates of mortality, and by the use of several different test-species, have been summarised by Sun,[177] Dewey[176] and Needham.[175]

Although bioassay is useful in parallel screening procedures and for the confirmation of results of other methods, its use cannot be recommended as the sole procedure to be adopted for the measurement of residues of chlorinated pesticides.

COLORIMETRIC AND ULTRAVIOLET-SPECTROMETRIC ANALYSIS

Colorimetry, with ultraviolet or visible radiation, has been until recent years the main source of specific residue methods. Sensitivities of a few micrograms and reasonable specificity have been attained for the whole group of pesticides under review, and a full discussion and details for thirteen of the group are given in volume 2 of the text edited by Zweig.[1] The need for lower levels of measurement and increasing emphasis on the analysis of metabolic products are being met by progress in other methods with much higher intrinsic selectivity and sensitivity, and the usefulness of colorimetry is declining. Comparisons have been carried out between ultraviolet and visible spectrometry and other methods, and agreement of the results has been good.[99,194]

INFRARED ANALYSIS

Infrared spectroscopy is one of the few specific methods available for residue analysis, but there are considerable difficulties involved in its application. The first difficulty, that is gradually being overcome, is the need for a relatively large amount of the pesticide to obtain a spectrum. The second difficulty is the need for extensive clean-up and this difficulty is accentuated by the concentration required before analysis. However, in spite of these difficulties infrared spectroscopy has often been used for the quantitative and qualitative analysis of residues of chlorinated pesticides. Its application in this way has been reviewed

by Blinn and Gunther,[195] Bruce[196] and by Frehse.[197]   These reviews give many references to the analysis of residues of aldrin, chlorbenside, chlordane, DDT, dieldrin, endrin, endosulphan, lindane, methoxychlor and tetradifon in a range of samples including crops, soils, air and water, and these references will not be repeated here.   Blinn and Gunther[195] have also reviewed the use of the different infrared regions and have compared the use of solutions, mulls and potassium bromide pellets.

In much of the earlier residue analysis 60 μg or more of pesticide was required to obtain a recognisable spectrum.   Smaller amounts could be analysed quantitatively by using the strongest absorption band for measurement, but such procedures lose some of the advantage of the specificity.   Blinn et al.[198] carried out measurements with 0·3 ml of carbon disulphide solution in a 5-mm light-path, sodium chloride cavity-cell and, under these conditions, 214 μg of aldrin in solution produced an absorbance (1250 cm^{-1}) of 0·1 unit.

Later, Johns and Braithwaite[199] described a micro-specular attachment which reduced the amount of sample needed to the range 25 to 50 μg.   Kreuger and Volkmann[200] have described a micro attachment for an infrared spectrometer that will increase the instrument's sensitivity 40-fold and will measure the spectrum of 0·1 μg of lindane.   Chen[201] has obtained recognisable infrared spectra with 1 μg of methoxychlor and other pesticides.   Recently Crosby and Laws[54] obtained recognisable spectra for 5-μl solutions containing 5 μg of pesticides by using cavity-cells of 1-mm path length.   This last work was described for organophosphorus compounds, but will be applicable to chlorinated pesticides, although not with quite the same limit of detectability.

Sparagana and Mason[202] have described a dual-beam condensing unit that uses reflecting-type optics and have obtained infrared spectra by using 0·05 μg of a compound.   Although they did not apply the procedure to pesticides the method shows great promise for such applications.

Infrared spectroscopy can often be used, after GLC, to confirm the identity of a component, and Giuffrida[203] has described a useful method of trapping-out components from GLC columns directly on potassium bromide powder.

The sensitivity of infrared spectroscopy has been increased considerably during recent years, and because of its specificity the method will assume increasing importance for residue analysis in the future.

FLUORIMETRY

Fluorimetry is a sensitive method for the analysis of many compounds, and characteristic activation or emission spectra can often be obtained.   MacDougall[204] and Hornstein[205] have reviewed the application of fluorimetry to residue analysis but make no mention of its application to chlorinated pesticides.   Fluorimetry has not been used for the analysis of the chlorinated pesticides considered here, because they are insufficiently fluorescent.   Although they could be converted to fluorescent compounds by suitable chemical methods, such studies are hardly worthwhile in view of the extensive clean-up that is necessary before fluorimetric analysis, and also because several sensitive methods are already available for the analysis.

PAPER CHROMATOGRAPHY

Paper chromatography has been used extensively[37,206,207,208,209,210] for the separation of mixtures of pesticides in extracts of plants, animal tissues and dairy products, and for the qualitative and quantitative analysis of pesticides.   Both aspects will be considered here.   The application of paper chromatography for clean-up has been discussed in a previous section.   The R_F values obtained by paper chromatography are useful for the identification of the components, but a single R_F value is not sufficient for positive identification unless it can be used in conjunction with another parameter such as the GLC retention time.   Paper chromatography is generally used not for clean-up of extracts, but for the identification of pesticides, and the clean-up by other methods is generally necessary to achieve the maximum sensitivity of detection.[37,206]

Early work on the paper chromatography of pesticides has been reviewed by Block et al.[211] and the more recent work has been summarised by Zweig[212] and by McKinley.[213]

The paper chromatography of pesticides was described by Mitchell in a series of papers.[214–224]   In one of these[222] the chromatography of 114 pesticides including almost all of the compounds considered here was described.   Mills[37] showed that chlorinated pesticides in a range of fruits, dairy products and animal tissues, could be readily detected by using

paper chromatography and similar work was reported almost simultaneously by McKinley and Mahon.[206] The basic techniques established by Mitchell, Mills, McKinley and Mahon or minor modifications of them are those used widely with success today.

PAPERS, AND MOBILE AND STATIONARY PHASES—

Whatman No. 1 filter-paper has often been used for paper chromatography, and if phenoxyethanol - silver nitrate is to be the spray reagent the paper is generally washed before the chromatography to remove background interference. Washing procedures have been described with water alone,[223] aqueous silver nitrate followed by water,[206] and aqueous silver nitrate followed by ammonia - water.[209]

Most paper chromatography has been carried out with impregnated papers, and in Table VI are listed a few of the combinations of mobile and immobile phases that have been found to be useful for the separation of chlorinated pesticides, together with the corresponding R_F values. The preferred system will depend on the compounds to be separated, and the use of different systems is recommended to obtain several R_F values if more evidence of identification of a compound is desired.

DETECTION—

Phenoxyethanol - silver nitrate as a spray reagent followed by ultraviolet irradiation has been shown by Mitchell[225] to be capable of detecting as little as 0·01 μg of some chlorinated pesticides. Mitchell[225] has also shown that 4 to 5 minutes of ultraviolet irradiation is the optimum without the production of an excessive background, and he has also compared the limits of detectability with and without water-washing prior to chromatography. Although washing has been an accepted and recommended procedure for some time, these recent results show that the process has little effect on the limits of detectability for many chlorinated pesticides. Indophenol blue in the presence of an aliphatic acid[226] and methyl yellow followed by ultraviolet irradiation[227] have also been used as spray reagents for paper chromatography. Several, but not all, of the spray reagents used for TLC are also applicable to paper chromatography.

When the pesticides are not resolved from one another or from co-extractives they can sometimes be identified by the use of specific spray reagents.

TABLE VI

R_F VALUES OF CHLORINATED PESTICIDES BY PAPER CHROMATOGRAPHY

			R_F values		R_L values (R_F relative to lindane)		
	Mobile phase		2-Methoxy-ethanol[222]	2,2,4-Trimethyl pentane[222]	2,2,4-Trimethyl pentane[206]	40 per cent. aqueous pyridine[206]	70 per cent. aqueous acetone[206]
Component	Immobile phase		Soya-bean oil	2-phenoxy-ethanol	2-phenoxy-ethanol	mineral oil	mineral oil
Aldrin ..	..	..	0·37	0·92	2·63	0·27	0·10
γ-BHC..	..	..	0·65	0·42	1·0	1·0	1·0
Chlorbenside ..	..		0·34	0·65	1·40	0·61	0·32
Chlordane ..	..		0·08 to 0·57	0·8 to 1·0	1·83	streak	0·18
Chlorfenson ..	..		0·74	0·31	0·46	1·00, 1·28	1·00
Chlorobenzilate	..		0·79	0·39	2·26, 0·52	*1·69*, 0·73, 0·30	1·12, 0·85, 0·55
op'-DDT	..	..	—	0·80	1·98	0·55	0·18
pp'-DDT	..	..	0·34	0·67	1·66	0·55	0·18
Dicofol	..	..	0·69	0·30	*0·31*, 2·11	1·09, 0·28, *0·93*, 0·40	0·73, *0·35*, 0·09
Dieldrin	..	..	0·42	0·64	2·64, *1·55*	0·08, 0·33, *0·73*	1·82, *0·33*, 0·14
Endrin	..	..	0·41	0·64	1·48	0·71	0·45
Heptachlor ..	..		0·31	0·94	2·11	0·38	0·15
Heptachlor epoxide ..			0·38	0·77	—	—	—
Methoxychlor..	..		0·68	0·30	0·67	1·09	1·24
Oxythane	..	..	—	—	1·23 and streak	0·42	0·11, *0·50*
Tetradifon	..	..	0·48	0·16	—	—	—
Toxaphene	..	..	0·05 to 0·48	0·36 to 0·92	—	—	—

Figures in italics are the main components.

QUANTITATIVE ANALYSIS—

Quantitative analysis of pesticides can be carried out after paper chromatography by measurement of the size or intensity (or both) of the separated spots and comparison with standards. The principles of such measurements for quantitative analysis by using paper chromatography and thin-layer chromatography are similar and are discussed together under the heading Thin-layer Chromatography.

THIN-LAYER CHROMATOGRAPHY (TLC)

TLC has been used many times for the qualitative and quantitative analysis of chlorinated pesticides and these aspects will be considered here. The application of TLC for the clean-up of extracts has been discussed briefly in a previous section.

Conkin[228] has reviewed the early work on the application of TLC to residue analysis, and other early work on the TLC of chlorinated pesticides has been summarised by Gänshert et al.[229] Walker and Beroza[230] studied the separation of 68 pesticides by TLC, and Kovacs[231] and Morley and Chiba[232] have successfully applied the method to the analysis of residues of chlorinated pesticides in crops. Recently Abbott et al.[233] have studied the separation of chlorinated pesticides by TLC over a wide range of conditions and some of their results are summarised in Table VII.

TABLE VII

R_F VALUES OF CHLORINATED PESTICIDES BY TLC[233]

		$R_F \times 100$ in system														
Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aldrin	88	98	73	58	69	67	70	79	64	62	67	98	82	95	70
γ-BHC	—	58	—	—	37	27	—	47	18	19	46	94	55	78	—
pp'-DDE	87	98	87	74	62	61	68	73	57	56	65	98	78	95	65
op'-DDT	71	90	74	50	58	54	62	71	46	48	59	97	73	89	50
pp'-DDT	72	91	78	52	54	49	60	69	39	40	57	98	69	89	42
Dieldrin	69	58	53	30	48	41	46	63	48	54	65	88	52	37	12
Endosulfan A	—	—	—	—	52	47	63	61	35	31	58	94	64	65	17
Endosulfan B	—	—	—	—	—	—	—	—	—	—	12	86	9	4	2
Endrin	—	—	—	—	52	42	58	65	26	26	49	88	61	51	13
Heptachlor	82	98	69	48	62	61	65	73	53	52	65	88	78	95	58
Heptachlor epoxide	—	—	—	—	—	—	—	—	—	—	39	88	57	49	17
Methoxychlor	—	—	—	28	36	27	30	45	10	13	—	—	—	—	—
pp'-TDE	66	77	58	67	46	33	45	59	26	28	52	92	57	71	25

Key to systems

System No.	Plate	Mobile solvent
1	Silica gel - alumina (1 to 1)	Cyclohexane - liquid paraffin (20%)
2	Silica gel - alumina (1 to 1)	Cyclohexane - silicone oil (8%)
3	Silica gel	Cyclohexane - hexane (1 to 1)
4	Silica gel	Cyclohexane - benzene (1 to 1) - liquid paraffin (10%)
5	Silica gel	Cyclohexane - liquid paraffin (20%) - dioxane (10%)
6	Silica gel	Cyclohexane - liquid paraffin (20%) - dioxane (5%)
7	Silica gel	Cyclohexane - liquid paraffin (10%) - dioxane (3·5%)
8	Silica gel	Cyclohexane - liquid paraffin (5%) - dioxane (2%)
9	Silica gel	Light petroleum (40° to 60°) - liquid paraffin (20%)
10	Silica gel	Light petroleum - liquid paraffin (10%)
11	Silica gel	Light petroleum - liquid paraffin (5%) - dioxane (1%)
12	Kieselguhr	As 11
13	Alumina	As 11
14	Alumina	Hexane
15	Silica gel	Hexane

The measurement of the R_F value may assist in the identification of a chlorinated pesticide although a single R_F value is insufficient evidence. Quantitative analysis may be carried out by measuring the spot area and intensity.

To achieve the greatest sensitivity the layer is generally washed prior to the separation to remove interfering compounds from the absorbents and the extracts are often submitted to clean-up by other methods before the thin-layer chromatography.[231] However, Morley and Chiba[232] were able to measure residues of aldrin, DDE, DDT and heptachlor in wheat, down to 0·1 to 0·2 p.p.m. (0·05 to 0·1 μg) without prior clean-up. After rigorous clean-up

of extracts, however, Kovacs[231] was able to detect residues of many chlorinated pesticides in a range of foodstuffs at the p.p.b. (parts per thousand million) level.

ADSORBENT AND DEVELOPING SYSTEM—

Silica gel, alumina, kieselguhr and some mixtures of these have been used as adsorbents. Kieselguhr is reported[233] to be of limited use for the separation of chlorinated pesticides and silica gel has been the most widely used. Chloroform alone or mixed with polar solvents,[230] hexane[233] or heptane[231] and cyclohexane alone or mixed with polar solvents have been used successfully for development.

The use of a simple system such as hexane or heptane and silica gel or alumina will effect the separation of many chlorinated pesticides.[231,233] It is not possible to recommend one system for general application since no one combination of adsorbent and developing solvent will separate even the small number of compounds considered here. However, by suitable choice from one of the many systems now available[230,231,233] any pair of compounds could be separated. Furthermore, Abbott *et al.*[233] have shown that resolution of chlorinated pesticides, not possible at room temperature with a given system, may be effected at a higher or even lower temperature.

DETECTION—

The most commonly used locating agent is phenoxyethanol - silver nitrate followed by ultraviolet irradiation. Kovacs[231] could detect 0.01 μg of aldrin, DDE, heptachlor and its epoxide, DDT, TDE, endrin, dieldrin, methoxychlor and dicofol, 0.05 μg of BHC and 0.1 μg of toxaphene and chordane. The adsorbent layer was washed with water prior to the chromatography; the limits of detectability may be higher without this washing because of the presence of inorganic chlorides in the adsorbent. Morley and Chiba[232] and Yamamura *et al.*[234] used ammonium hydroxide in place of phenoxyethanol. Abbott *et al.*[233] reported that treatment with ethanolic silver nitrate by ultraviolet irradiation was adequate for the detection of chlorinated pesticides on TLC. Abbott *et al.* obtained interesting results by using combinations of aqueous silver nitrate and a pH indicator (with or without ultraviolet irradiation) and recommended the use of bromophenol blue - silver nitrate,[235] but did not report the limits of detectability.

Other spray reagents that have been used for the detection of chlorinated pesticides on TLC include 0.1 N potassium permanganate,[236] zinc chloride or iodine plus diphenylamine,[237] silver nitrate - formaldehyde - potassium hydroxide - nitric acid - hydrogen peroxide in succession,[238] *p*-dimethylaminoaniline hydrochloride,[239] alcoholic *o*-toluidine or *o*-dianisidine,[240] sulphuric acid,[234] iodine[230] and bromine - fluorescein.[230] With several of these reagents ultraviolet irradiation is necessary. The limits of detectability obtained with these reagents are greater than those claimed by Kovacs, but many of them will detect 5 μg or less.

Although silver nitrate in some form is the most common reagent the other reagents mya serve to increase the certainty of the identity of a particular spot.

QUANTITATIVE ANALYSIS—

The principles for the quantitative measurement of the spots of chlorinated pesticides in thin-layer and paper chromatography are the same as that for other compounds as outlined by Purdy and Truter,[241] and by Truter.[242] Often the spot area alone has been measured and is related to the amount from a calibration graph, preferably obtained by the chromatography of standards on the same plate. The comparison is often made visually however.[37,206,232,243] The area of the spot can be related to the amount of compound present[211] but the relationship is not rectilinear. Evans[210] found that a rectilinear relationship existed between spot area and log (amount) in the range 2.5 to 14 μg for several chlorinated pesticides, and he obtained a precision of ± 10 per cent. for the measurement of 2.5 μg or more of these pesticides with paper chromatography. Purdy and Truter[241] have reported excellent reproducibility (± 6.6 per cent. in the 2 to 30 μg range) with a rectilinear calibration-graph obtained by plotting (area)$^{\frac{1}{2}}$ against log(weight), a relationship first used by Fisher *et al.*[244] Zweig[212] obtained rectilinear plots in the 0.5 to 4.0 μg range for DDT by plotting amount against area and density. It is likely that relationships that consider both area and density will give the most consistent results for a wide range of compounds. Densitometric measurements may be made photometrically by scanning the plate or paper, or by scanning a photograph of them.

TLC is rapid, the equipment is inexpensive and it can be used for clean-up, qualitative and quantitative analysis. These advantages will undoubtedly be exploited even further in future years.

THE POSITIVE IDENTIFICATION OF PESTICIDE RESIDUES

A difficulty becoming more and more prominent in this field of analysis is that of establishing the identity of a residue. Many naturally occurring products in the extract can respond to the analytical procedure and can be mistaken for pesticides when a valid control is not available. Such components are not always removed by the clean-up procedure that is used. For this reason GLC, total halide analysis, polarography, bioassay, colorimetry ultraviolet analysis, fluorimetry, thin-layer or paper chromatography cannot normally be regarded as specific methods, and cannot usually provide positive identification of a given component in the absence of a valid control.

Infrared analysis can be reasonably specific but relatively large amounts of pesticide are required as well as rigorous clean-up of the extracts. These problems are being overcome gradually, and infrared analysis has been used successfully for the positive identification of residues of chlorinated pesticides after clean-up by gas - liquid chromatography, column chromatography or by thin-layer chromatography.

Mass spectrometry can generally provide a positive identification of a component, but little has been reported so far on the application of this technique to residue analysis. The problems are similar to those encountered with infrared analysis, in that rigorous clean-up and relatively large amounts of compound are required. However, Ryhage[245] has obtained recognisable mass spectra with 20 ng of esters of long-chain carboxylic acids that were passed directly from a capillary GLC into a mass spectrometer. Whilst such equipment is expensive it merits application for identification of residues. Such a procedure may perhaps be more convenient than infrared spectroscopy as the effluent from the GLC column is already in the vapour phase, and can be fed directly into the mass spectrometer with perhaps only limited interference from co-extractives and without the need for trapping-out components.

These two procedures can, at best, offer conclusive evidence of identity. The specificity of other methods can be improved, but such improvements cannot make interpretations from them completely unambiguous.

A single retention time obtained by GLC or a single R_F value obtained by thin-layer or paper chromatography is not sufficient for the positive identification of a component. Whilst the measurement of several retention times or R_F values with different operating conditions may show that the component in question is not present, such evidence cannot be used as proof of the presence of the component.

Improvements in specificity can be obtained by the combined use of different clean-up procedures. The use of liquid - liquid partition and column chromatography or chemical treatment prior to GLC improves the specificity, and the additional use of thin-layer chromatography prior to GLC increases the specificity even further.

Increased certainty of identification can better be obtained by analysis using two or more methods. This has been done successfully in the past and extracts have been analysed by colorimetry and bioassay, by total chlorine and bioassay and by GLC. Such combined procedures cannot always be applied, however, especially for the analysis of low residues as few of the other methods are as sensitive as GLC for the analysis of chlorinated pesticides.

If infrared analysis or mass spectrometry cannot be used, reasonable specificity can be achieved if the extract is subjected to partition, column chromatography and thin-layer chromatography in turn, and is analysed by gas - liquid chromatography and by another procedure such as colorimetry or bioassay. Beroza and Bowman[67,68] have used a combination of liquid partition and GLC for the identification of pesticides by using the GLC retention time and the partition coefficient as parameters to identify a component, and this approach shows promise for the positive identification of pesticides.

RECOMMENDED METHODS

In the previous sections, work on the chlorinated pesticides has been reviewed in a general way. In this last section, procedures will be recommended for the extraction, clean-up and analysis which at the present time seem to be the best. Procedures that can be applied to whole groups of compounds will be outlined first. Then each chemical will be considered

individually, pointing out any particular difficulties in the analysis, referring to published work on each chemical to illustrate the use of the recommended methods on a variety of substrates, and giving alternative methods.

GENERAL PROCEDURES

EXTRACTION—

Soils—

Mix a suitable weight of wet soil with anhydrous sodium sulphate and tumble them end-over-end with a solvent mixture. A ratio of 1 g of soil to about 0·5 g of sodium sulphate and 2 ml of a mixture of 20 per cent. acetone in hexane is recommended, tumbling for 1 hour. Filter the mixture and remove the water-soluble portion by water washing.

Crops—

Macerate a convenient weight with anhydrous sodium sulphate and a mixture of water-immiscible and miscible solvents, benzene or hexane and acetone or isopropanol. A ratio of 1 g of crop to about 0·5 g of sodium sulphate and 2 ml of a 1 to 1 volume solvent mixture, with blending for 1 minute is usually sufficient. Filter and remove the water-soluble portion by water washing.

Animal tissues and products—

Grind the tissue with anhydrous sodium sulphate or sand and extract by warming with a suitable solvent such as hexane. Alternatively, the methods of Langlois *et al.*[36] or of Mills[34] already described may be used.

CLEAN-UP—

For the removal of polar interference use column chromatography with adsorbents such as alumina, Florisil or magnesia. Elute the non-polar compounds such as aldrin, DDE, DDT and heptachlor with hexane, and the more polar ones such as dieldrin, endrin and the oxidation products of chlorbenside with hexane containing a small amount of acetone or ether.

For non-polar interference, such as lipid, use liquid - liquid partition with hexane - acetonitrile,[62] hexane - dimethylformamide[40,63] or hexane - dimethylsulphoxide[64] as solvent pairs. To determine low residue levels on materials with a high fat content, further clean-up by using column chromatography is usually necessary.

It should be stressed that while these are the general methods of clean-up, others referred to in a previous section may prove helpful for particular problems.

ANALYSIS—

Gas - liquid chromatography—

The operating conditions given in the papers of Goodwin *et al.*,[65] Burke and Holswade[102] and Burke and Giuffrida[118] should be consulted. The following points will serve as guides in the choice of conditions for particular systems. A non-polar silicone stationary phase has the best separating power; other thermally-stable polar phases may be needed to give particular separations or additional evidence of identification. The following are recommended to minimise the decomposition of pesticides; on-column injection, suppression of support activity by treatment with Epikote resin or hexamethyldisilazane, glass or other non-reactive materials for the column and the use of column temperatures as low as conveniently possible. General screening methods with GLC, designed to reveal the presence of chlorinated pesticides, have been reported by Goodwin *et al.*,[95] Kaufman and Jackson,[246] McCully and McKinley,[38] Minyard and Jackson[135] and Wells.[247]

Thin-layer chromatography—

Where GLC equipment is not available TLC offers an inexpensive and suitable alternative with good separating power and fair sensitivity. Like paper chromatography, it is a useful complementary technique for identification purposes.

The best separations have been achieved with silica gel and a non-polar eluting solvent such as hexane or heptane. Alumina gives poorer separations, but if silver nitrate is used as detecting agent it gives better sensitivity. A 300-μ layer of adsorbent is preferred since variations in layer thickness are less critical than with the normal 250-μ layer.[242]

The best sensitivities have been obtained with Mitchell's silver nitrate - phenoxyethanol spray reagent.[231]

<div align="center">INDIVIDUAL PROCEDURES</div>
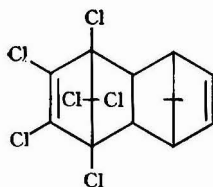
(i) *Aldrin*—

Materials treated directly or indirectly with aldrin must be examined for both aldrin and dieldrin (its oxidation product). Both products are stable to the GLC operating conditions described above. On a silicone stationary phase, aldrin is difficult to resolve from dicofol and *op'*-TDE olefin but these are separable on more polar phases. Residues to a level of 0·01 p.p.m. can be determined readily in crops and tissues.

The GLC method has been used for the determination of aldrin in soil and root crops by Lichtenstein and his co-workers,[248,249] by Stewart *et al.*[250] and by Decker *et al.*[251]; in grapes by Hascoet and Adam[252]; in vegetables and dairy products by Watts and Klein[136]; in milk by Henderson[253]; and in water by Hindin *et al.*[24]

Alternative methods to GLC are the phenylazide colorimetric method and bioassay, both of which are discussed in detail by Porter.[254]

(ii) *γ-BHC (Lindane)*—
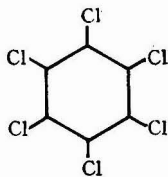
γ-BHC may be measured at the residue level by gas - liquid chromatography[102,118,132,139] and this is the recommended method of analysis. Electron-capture detectors have a large response to this compound and residues of 0·01 p.p.m. can be determined readily and the limit of detectability is even lower. GLC has been applied to the analysis of γ-BHC in a wide range of crops,[11,46,65,255] dairy products[253,256] and tissues.[255]

It is rarely necessary to analyse samples for residues of the other isomers of BHC but the isomers can be conveniently separated by GLC,[102,118,257] TLC[229] or paper chromatography.[258,259]

The Schechter - Hornstein colorimetric procedure or modifications of it have been used by many workers for the analysis of a wide range of samples.[260 to 268] The collaborative studies of the Schechter - Hornstein method are discussed by Klein[269] but the procedure is much less sensitive than GLC. Other methods of analysis that have been used include bioassay,[177] polarography[270] and infrared spectroscopy.[271,272]

(iii) *Chlorbenside (Mitox)*—

Chlorbenside can be analysed with the GLC conditions described previously and is resolved from the other pesticides considered here. GLC does not seem to have been reported for residue analysis of chlorbenside but the previous work[102,118] has shown this to be feasible. GLC of the metabolites of chlorbenside, the sulphoxide and sulphone does not seem to have been reported but should be possible.

Gunther, Blinn and Barnes[273] have measured residues of chlorbenside in pears by infrared spectroscopy. The method is also suitable for analysing for the sulphoxide oxidation product

of chlorbenside.   The sample is extracted with hexane and is subjected to column chromatography which separates the chlorbenside and its sulphoxide.   The separated sulphoxide and chlorbenside are determined by infrared spectroscopy.   The alternative colorimetric procedure of Higgons and Kilbey[274] involves oxidation of the chlorbenside to the sulphone, followed by nitration and spectroscopic measurement at 575 mμ.   Higgons and Kilbey did not separate the chlorbenside and its metabolites before analysis, but there is no reason why the separation procedure by Gunther could not be used for this.   This separation procedure may also be of use before GLC analysis.

(iv) *Chlordane*—
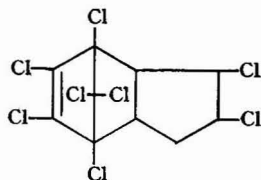
Chlordane is produced by the chlorination of chlordene and contains 60 to 75 per cent. of the β- and γ-chlordanes (the *trans* and *cis* isomers, respectively), and 25 to 40 per cent. of analogous materials containing 6, 7 and 9 chlorine atoms.   The gas chromatogram of technical material has a characteristic appearance, the peaks due to β- and γ-chlordane predominating. While in some respects the gas chromatography of chlordane residues is made easier by the readily identifiable pattern, the feasibility of distinguishing peaks from natural materials and from other pesticides that may be present, such as chlorfenson, chlorbenside and the major isomer of endosulfan, is not an easy matter, and must be overcome by prior clean-up or by the use of more than one stationary phase.

Chlordane has rather specialised uses and little work has been published on residues arising from them, but reference may be made to the paper of Gutenmann and Lisk[255] where a sensitivity of 0·01 p.p.m. of chlordane in soil was achieved.

The alternative method is a colorimetric one, based on its reaction with methanolic potassium hydroxide and diethanolamine, details of which are given by Bowery.[275]

(v) *Chlorfenson (Ovex)*—
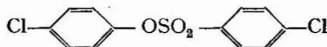
The acaricide chlorfenson can be analysed satisfactorily by gas - liquid chromatography under the conditions previously described, but it is not readily resolved from endosulphan and β-chlordane.   Such resolution might be accomplished by using other, perhaps more polar, stationary phases, but the GLC of chlorfenson has not been studied in detail for residue analysis.   GLC promises to be a sensitive and convenient method for such analysis.

Butzler *et al.*[91] have reported a colorimetric procedure that is sensitive to less than 5 μg of chlorfenson in orange pulp.   In this procedure the chlorfenson is extracted with benzene, and the extract is hydrolysed with alcoholic potassium hydroxide to convert the chlorfenson to *p*-chlorophenol which is separated by steam distillation and nitrosated.   The product is then separated by column chromatography before spectroscopic analysis at 430 mμ.   Other colorimetric procedures have been described.[90,276,277]

The analysis for residues of chlorfenson does not seem to have been reported very frequently in recent years.

(vi) *Chlorobenzilate*—
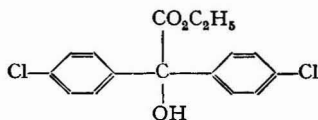
The acaricide chlorobenzilate may be analysed with good sensitivity by gas - liquid chromatography and is stable under the conditions described.   It is readily resolved from the other acaricides considered here.   Its retention time, however, is close to that of *op'*-DDT and *pp'*-TDE when a column temperature of 200° C is used.[118]

The analysis of residues of chlorobenzilate in grapes and cotton seed with a sensitivity of 0·05 p.p.m. has been carried out successfully by microcoulometric GLC[278] with 20 per cent. Dow 11 silicone grease on Chromosorb P. Benzene was used as the extraction solvent and clean-up was by partition into nitromethane followed by column chromatography on Florisil for cotton-seed extracts. Clean-up with Nuchar carbon was used for grapes. Infrared spectroscopy was used to confirm the identity of the residue.

The use of electron-capture has not yet been reported for the determination of chlorobenzilate residues but the work of Burke and Guiffrida[118] shows that this should be possible with high sensitivity.

A suitable alternative method of analysis is the Delley colorimetric procedure as modified by Harris[279] and described in detail by Margot and Stammbach.[280] In this procedure the chlorobenzilate is extracted with benzene, saponified with methanolic alkali to form dichlorobenzilic acid, which is extracted and nitrated and the absorption is measured at 538 mμ. This procedure is sensitive to 2 μg of chlorobenzilate and has been applied to a wide range of samples.[279] Blinn *et al.*[281] have described a similar procedure but without the nitration step, and have applied this to analysis of residues in citrus.

(vii) *DDT, TDE, DDE—*

pp′-DDT
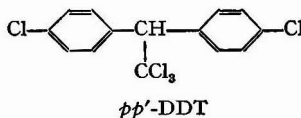
The analysis of residues of the major and minor constituents of technical DDT and the metabolic products is one requiring careful experimentation and interpretation. The technical material contains upwards of 70 per cent. of the *pp′*-isomer, the bulk of the remainder being the *op′*-isomer, but some *pp′*-TDE and a small amount of *op′*-TDE are usually present. The principal metabolic product of DDT is DDA, which can be analysed by GLC after methylation. Other metabolic products are the olefin DDE, but TDE may also be produced and the olefin of TDE. In addition DDT may undergo thermal and catalytic conversion to DDE or TDE. Thus an extract of crop or tissue could conceivably yield DDT and TDE as such, or as dehydrochlorinated products, each as *pp′*- or *op′*-isomers; and to add to the difficulty, derivatives may arise as impurities in the technical material, from metabolism or from decomposition. Exceptional care must therefore be taken when interpreting chromatograms.

On a silicone stationary phase three pairs of the DDT analogues are difficult to resolve and require other stationary phases to show adequate separation. These pairs are *pp′*-TDE olefin and *op′*-DDE; *pp′*-DDE and *op′*-TDE; and *pp′*-TDE and *op′*-DDT. The latter two pairs may also be separated by conversion to their olefins by treatment with alkali, followed by GLC.[141]

GLC has been used for the analysis of residues of DDT and its derivatives in soil,[282] water,[24,283] crops,[136] clothing,[284] dairy products,[152,253,256] tissues,[39,285,286,287,288] and the total diet[289] with a sensitivity sometimes of less than 0·01 p.p.m. Colorimetric methods of analysis have been reviewed by Miskus.[290]

(viii) *Dicofol (Kelthane)—*
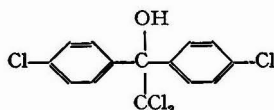
Dicofol is reported[212] to decompose almost quantitatively during GLC and although Burke and Johnson[101] and Burke and Holswade[102] have reported retention results for dicofol they have not shown that the response that they obtained was from dicofol itself. However, Gunther *et al.*[129] have shown that, when the GLC column (silicone on firebrick) was "conditioned" to dicofol the extent of decomposition was small, and that 90 to 95 per cent. of the dicofol was not decomposed during the chromatography, and the remainder was converted to 4,4′-dichlorobenzophenone. Thus, GLC shows considerable promise for the analysis for residues of dicofol, and some results have been given in a study of total-diet samples.[289]

Colorimetric analysis has been carried out successfully for dicofol in a wide range of crops and animal products. The basic procedure is that used by Rosenthal *et al.*[291] involving hydrolysis of dicofol to chloroform which is determined colorimetrically by the Fujiwara reaction. Gordon, Haines and Martin[292] have carried out the hydrolysis with sodium hydroxide (for crops) or with tetraethylammonium hydroxide (for fatty samples) and their procedure has been described in detail by Gordon and Schuckert.[293] This procedure will detect 10 μg of dicofol and it has been applied to the analysis of dicofol in milk,[292] butter-fat and animal body fat,[294] and is applicable to a range of crops and animal products. Similar procedures have been used for a range of samples by other workers,[295,296,297] and Gunther and Blinn[298] have described an alternative procedure involving hydrolysis of dicofol to 4,4'-dichlorobenzophenone which is measured by ultraviolet spectroscopy.

(ix) *Dieldrin*—
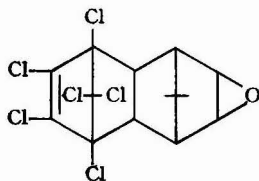
Dieldrin is stable to the operating conditions described previously for GLC analysis. On non-polar stationary phases the retention times of dieldrin and pp'-DDE are almost coincident, and op'-TDE is also close. It is essential therefore to acquire complementary evidence for the identity of dieldrin.[139] It is fairly stable to metabolic change, though several workers have reported metabolites.[299,300,301,302]

Recent examples of the analysis of dieldrin residues by GLC are those carried out by Hardee *et al.*,[303] by Morley and Chiba[304] and Decker *et al.*[251] on soil, by Harvey and Harvey on pasture,[305] by Williams, Mills and McDowell on milk,[256] by Hunter *et al.*[286] and Dale and Quinby[287] on human fat and by Williams[289] on total-diet samples. Residue levels of 0·01 p.p.m. can be attained without excessive clean-up.

The alternative specific method for dieldrin is the phenylazide colorimetric procedure discussed in detail by Porter.[306]

(x) *Endosulfan (Thiodan)*—
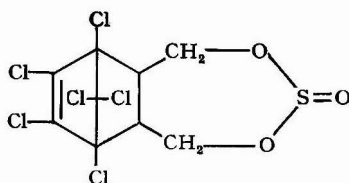
Endosulfan has two stereoisomers, the lower-melting isomer constituting two-thirds of the technical product, the higher-melting isomer being the other major component. These can decompose to endosulfan alcohol, and the so-called endosulfan ether may also be present in technical material. The alcohol may also be produced by metabolism, and the presence of endosulfan sulphate from metabolic oxidation has also been reported,[307,308] so that once again the application of one technical material can give rise to a complex residue analysis.

Zweig *et al.*[53] have used GLC as a means of separation in their work on the infrared determination and identification of endosulfan residues. Details of the GLC microcoulometric procedure are given by Graham *et al.*[309] but few results specifically relating to residues of endosulfan have been reported.[99,105,307,310] The major component of endosulfan may be difficult to resolve under normal GLC operating conditions from β- and γ-chlordane isomers and from chlorfenson, and the other isomer from endrin or chlorobenzilate, but the presence and the ratio of the several peaks from technical endosulfan gives some evidence of identity.

(xi) *Endrin—*
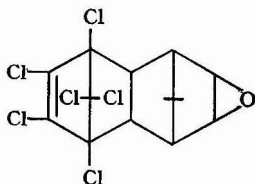
Although endrin is sensitive to temperature,[119] it can be analysed without decomposition by GLC.[65] Endrin has a less toxic product, the so-called "delta keto," an isomeric ketone that occurs in plants but not in animals. Endrin is resolved from other common pesticides on a silicone stationary phase. The pattern of peaks due to decomposition on chromatography at high temperature could be used as an aid to identification. GLC analyses of endrin residues in alfalfa,[255] water,[24] milk,[256] tissue[39] and the total diet[289] have been reported in recent years.

Colorimetric procedures for the analysis of residues of endrin have been reviewed by Terriere.[311]

(xii) *Heptachlor and heptachlor epoxide—*
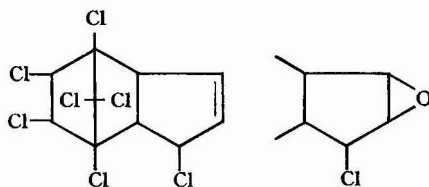
Like aldrin, heptachlor forms an epoxide in biological media, so that both must be determined as residues following heptachlor applications. Both are thermally stable and can be chromatographed on the sub-microgram scale without trouble. Rusk and Fahey[312] have pointed out that γ-chlordane present in technical heptachlor can form a significant proportion of the total residue, and its retention time on non-polar stationary phases is similar to that of heptachlor epoxide. They have given details of a chromatographic separation of heptachlor, its epoxide and γ-chlordane on Florisil. Recent residue work carried out by GLC include that in soil,[248,250] crops,[248,250] milk,[256,313] tissue[66] and the total diet.[289] Colorimetric methods have been reviewed by Bowery.[314]

(xiii) *Methoxychlor—*
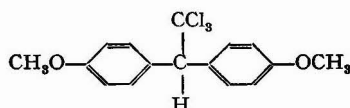
Methoxychlor may be analysed by gas - liquid chromatography under the standard conditions described previously. Because of its long retention time there is little interference from other chlorinated pesticides. The method has not been widely reported for residue analysis but it has been used by Baetz[46] who also described a suitable clean-up procedure, and by Williams.[289]

A colorimetric method of analysis was developed by Fairing and Warrington[69] and is described in detail by Lowen *et al.*[315] The method, which is applicable to crops and animal products, involves partitioning of the pesticide into nitromethane followed by dehydrochlorination, column chromatography and sulphonation, to give a coloured product that is measured spectroscopically. The method is sensitive to 2 μg of methoxychlor. This procedure was used by Cluett *et al.*[316] in combination with total halide analysis to determine methoxychlor and its possible metabolites in milk.

Other methods of analysis have been summarised by Lowen *et al.*[315]

(xiv) *Oxythane (Neotran)*—

Cl—⟨◯⟩—OCH₂O—⟨◯⟩—Cl

Oxythane was not included in the work of Burke and Holswade[102] or of Burke and Johnson[101] but it is very likely that the analysis for this compound can be carried out by GLC. Little work has been published on the analysis for residues of oxythane or on its thin-layer and paper chromatography.

Gunther and Blinn[317] have described a colorimetric procedure involving extraction with benzene followed by hydrolysis with hydrobromic acid to form *p*-chlorophenol. The phenol is separated from interfering compounds by steam distillation, and is reacted with 4-amino-antipyrine, before spectroscopic measurement at 510 mμ.

(xv) *Tetradifon (Tedion)*—

Cl—⟨◯⟩—SO₂—⟨◯⟩—Cl
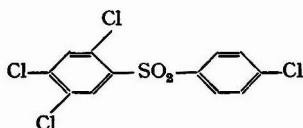
Gas - liquid chromatography is suitable for the analysis of residues of tetradifon, and microcoulometric detectors have been used by Coulson *et al.*,[96] Cassil[99] and by Burke and Mills[105] for the analysis of a range of crops, and less than 5 μg of tetradifon could be detected. The use of electron-capture detection would improve the sensitivity considerably. The GLC of tetradifon has been carried out successfully by other workers who have used both micro-coulometric and electron-capture detection.[102,118]

A colorimetric procedure based on the Fujiwara reaction was developed by Fullmer and Cassil[318] and is described in detail by Cassil and Yaffe[319] who have also considered many of the possible sources of interference in the procedure. Gunther and Blinn[89] have described a total chlorine method, and Gunther *et al.*[320] have measured tetradifon residues by infrared analysis with chromic oxide - acetic acid for the clean-up.

(xvi) *Toxaphene*—

As toxaphene is a multi-component mixture of chlorinated terpenes, it is not possible to recommend a specific method. Residue analysis has generally been carried out by a total-chlorine procedure or the zinc chloride - diphenylamine colorimetric method of Graupner and Dunn,[321] both of which have been described by Dunn.[322] Nikolov and Donev[323] have recently described a modification to the colorimetric procedure that increased the sensitivity 10-fold.

The GLC method with halogen-selective detection is an advance on these in that sensitivity is improved and some semblance of specificity is offered due to the pattern of peaks produced. However, at low residue levels this specificity is unreal as the presence of peaks from natural products or other pesticides would be impossible to distinguish from the Toxaphene components. After rigorous clean-up, determinations of fairly low residue concentrations are possible on short columns which, having poor efficiency, give a single or only a few peaks for the Toxaphene mixture.[324]

An attempt to analyse total diets for toxaphene residues has been reported.[289]

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Spectrophotometric Determination of 0·01 to 0·1 per cent. of Antimony in Lead

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The development of a spectrophotometric method for the determination of small amounts (0·01 to 0·1 per cent.) of antimony in lead is described. The proposed method is based on the extraction of antimony from hydrochloric acid solution with di-isopropyl ether, followed by spectrophotometric determination with the iodide procedure. The presence of other impurity elements usually found in lead causes no significant interference.

CERTAIN physical and chemical properties of lead such as the rate of oxidation,[1] fatigue resistance,[2] and grain size[3] are influenced by the presence of small amounts of antimony. It is therefore important to have a method for determining antimony (0·01 to 0·1 per cent.) in lead that is suitable for routine use in a laboratory.

Many methods have been reported in which lead is separated as lead sulphate and the antimony subsequently determined, either polarographically[4,5,6] or spectrophotometrically.[7] These methods are not however completely satisfactory as we have confirmed in our investigations that an appreciable amount of antimony may be adsorbed on the lead sulphate precipitate.

The object of the present investigation was to devise a more effective separation of antimony from lead, and to determine the antimony spectrophotometrically or polarographically.

Ward and Lakin[8] and others[9,10,11] have reported satisfactory extraction of antimony(v) from hydrochloric acid solution with di-isopropyl ether, while Van Aman and co-workers[12] have drawn attention to the importance of the order in which the reagents are added. They found that, in order to obtain maximum extraction of antimony, the di-isopropyl ether should be added to the concentrated hydrochloric acid solution before diluting the solution with water.

In the preliminary experimental work, the applicability of this solvent extraction procedure to the determination of antimony in lead was investigated by using polarographic and spectrophotometric techniques.

The base electrolytes used in the polarographic studies were sulphuric acid solution,[13] phosphoric acid solution[14] and a mixture of hydrochloric acid and potassium bromide.[15] Results were low, possibly due to loss of antimony during its extraction from the organic phase into the base electrolyte.

A spectrophotometric method[12] with Rhodamine B was examined, the antimony being determined directly in the organic extract. This procedure was found satisfactory when applied to hydrochloric acid solutions containing antimony only, but failed to give reproducible results for antimony in lead.

The second spectrophotometric method examined was that described by McChesney,[16] in which the antimony is converted to the yellow iodo-antimonate ion. The method was applied to the solution obtained by evaporating the organic extract with sulphuric acid. Satisfactory results were obtained.

The separation of antimony by co-precipitation on ferric hydroxide and manganese dioxide was also studied in conjunction with the iodide method of McChesney. Although fairly satisfactory results were obtained, neither procedure was as rapid and convenient as the solvent extraction method.

It was concluded that, of the methods studied, the most satisfactory to be adopted as a routine procedure for determining small amounts of antimony in lead, was extraction with di-isopropyl ether followed by spectrophotometric determination as the iodide.

METHOD

APPARATUS—

A Hilger Uvispek spectrophotometer was used for all the optical-density measurements.

REAGENTS—

All reagents should be of analytical-reagent grade.

Hydrochloric acid, concentrated, sp.gr. 1·18.

Hydrochloric acid - bromine mixture—Shake 25 ml of bromine with 2·5 litres of hydrochloric acid.

Ceric sulphate solution—Dissolve 0·2 g of ceric sulphate in 100 ml of 0·1 N sulphuric acid.

Di-isopropyl ether.

Wash solution—Shake 60 ml of hydrochloric acid with 20 ml of di-isopropyl ether and 28 ml of water. Separate the two layers and retain the aqueous layer.

Sulphuric acid, 50 per cent. v/v, aqueous—Prepare from sulphuric acid, sp.gr. 1·84.

Sulphuric acid, 5 per cent. v/v, aqueous—Prepare from 50 per cent. v/v aqueous sulphuric acid.

Potassium iodide - ascorbic acid reagent—Dissolve 56·0 g of potassium iodide and 10 g of ascorbic acid in 500 ml of water.

Standard antimony solution A—Dissolve 0·6688 g of antimony potassium tartrate, previously dried at 100° C, in 500 ml of water.

$$1 \text{ ml} \equiv 0·0005 \text{ g of Sb.}$$

Standard antimony solution B—Dilute 20 ml of solution A to 100 ml.

$$1 \text{ ml} \equiv 0·0001 \text{ g of Sb } (\equiv 0·01 \text{ per cent. on 1-g lead sample}).$$

PROCEDURE—

Dissolve the sample (1 g for 0·01 to 0·05 per cent. of antimony, or 0·50 g for 0·05 to 0·1 per cent. of antimony) in a mixture of 50 ml of concentrated hydrochloric acid and 10 ml of hydrochloric acid - bromine solution. It is essential that the solution is not boiled at this stage but only warmed. (If the lead sample is rolled out as thin foil of thickness approximately 0·0015 inch, it will dissolve in about 20 minutes.)

When dissolution is complete, cool the solution, add 1 ml of ceric sulphate solution to ensure that all of the antimony is converted to the pentavalent state. Transfer the solution to a separating funnel and make up the volume to 120 ml with concentrated hydrochloric acid. Shake the solution for 1 minute.

Add 40 ml of di-isopropyl ether and shake the solution for a further minute before cooling. Introduce 56 ml of water into the separating funnel and shake the solution for a further period of 1 minute to extract antimony(v) into the ether layer. Cool the solution and separate the two layers. Carry out a second extraction on the aqueous layer by adding 1 ml of ceric sulphate solution, 40 ml of di-isopropyl ether and shaking the solution for 1 minute. Separate the two layers.

To the combined organic extracts add 5 ml of wash solution and, after shaking, separate the organic layer and place it in a 250-ml beaker. Introduce 20 ml of 5 per cent. v/v sulphuric acid and place the beaker on a steam-bath.

When the organic solvent has been removed add to the solution 6·6 ml of 50 per cent. v/v sulphuric acid. Cool the solution, add to it 25 ml of the potassium iodide - ascorbic acid reagent, filter it into a 50-ml graduated flask and dilute to the mark with water. Measure the optical density of the solution after 5 minutes in a 2-cm cell against water at a wavelength of 425 mµ. Deduct the optical density of a reagent blank solution.

CALIBRATION—

Transfer 1·0, 2·0, 4·0, 6·0, 8·0 and 10-ml portions of standard antimony solution B into 50-ml graduated flasks. Add to each flask 8·6 ml of 50 per cent. v/v sulphuric acid, 25 ml of potassium iodide - ascorbic acid reagent and dilute to the mark with water. Mix the solutions thoroughly and measure their optical densities after 5 minutes in a 2-cm cell against water at 425 mµ. Deduct the optical density of a reagent blank solution. Prepare a calibration graph.

RESULTS

RECOVERY OF ANTIMONY IN THE PRESENCE OF LEAD—

The procedure was studied by using 1 g of high-purity lead to which was added a known volume of standard antimony solution consisting of pure antimony dissolved in concentrated hydrochloric acid. The antimony solution was added before the hydrochloric acid - bromine

mixture, as it was found that the presence of antimony increased the rate of dissolution of the lead.

Some typical results are shown in Table I.

TABLE I
RECOVERY OF ANTIMONY IN THE PRESENCE OF LEAD

Antimony added, per cent.	Antimony found, per cent.	Error, per cent.
0·000	0·000	0
0·010	0·010	0
0·020	0·017	− 15
0·030	0·030	0
0·030	0·030	0
0·050	0·050	0
0·050	0·050	0
0·060	0·057	− 5
0·070	0·066	− 6
0·080	0·071	− 11
0·100	0·089	− 11
0·100	0·086	− 14

DETERMINATION OF ANTIMONY IN THE PRESENCE OF OTHER ELEMENTS—

The method was studied in the presence of various elements that normally occur with lead. The results obtained are shown in Table II.

TABLE II
DETERMINATION OF ANTIMONY IN THE PRESENCE OF OTHER ELEMENTS

Impurity added	Form of added impurity	Antimony added, per cent.	Antimony found, per cent.	Error, per cent.
0·050% Cadmium	Cadmium chloride	0·030	0·031	+3
0·040% Bismuth	Lead sample containing 0·040% of bismuth	0·030	0·028	− 7
0·050% Nickel	Nickel chloride	0·030	0·027	− 10
0·050% Zinc	Zinc chloride	0·030	0·027	− 10
0·050% Arsenic	Lead sample containing 0·05% of arsenic	0·030	0·028	− 7
0·050% Tin	Lead sample containing 0·05% of tin	0·030	0·031	+3
0·064% Copper	Lead sample containing 0·064% of copper	0·030	0·028	− 7
0·050% Iron	Ferric chloride	0·030	0·032	+7
0·050% Tellurium	Tellurium dissolved in nitric acid	0·030	0·030	0
0·050% Selenium	Selenium dissolved in nitric acid	0·030	0·032	+7
0·025% Bismuth 0·023% Copper 0·011% Silver	Lead sample containing only bismuth, copper and silver	0·030	0·031	+3

TABLE III
REPRODUCIBILITY OF THE METHOD WITH 0·03 PER CENT. ANTIMONY ADDED TO 1-g LEAD SAMPLES

Antimony found, per cent.	..	0·028	0·027	0·030	0·027	0·030	
		0·032	0·029	0·031	0·033	0·031	0·027
Deviation	.. .. ..	0·002	0·003	0·000	0·003	0·000	
		0·002	0·001	0·001	0·003	0·001	0·003

Mean = 0·030.   Mean deviation = 0·002.

$$\text{Relative mean deviation} = \frac{0·002 \times 100}{0·030} = 6·7 \text{ per cent.}$$

Standard deviation = 0·002.

PRECISION OF METHOD—

The reproducibility of the method was investigated with 1-g samples of high-purity lead, to which the equivalent of 0·030 per cent. of antimony was added as a known volume of standard antimony solution. The results are given in Table III.

DISCUSSION

The method was found satisfactory when using a 1-g lead sample containing the equivalent of 0·01 to 0·05 per cent. of antimony (see Table I). At the higher concentration range, 0·05 to 0·10 per cent., the recoveries of antimony are, however, slightly low. It is therefore recommended that when determining amounts of antimony greater than 0·050 per cent. a smaller sample should be taken so that the concentration of antimony is within the limits giving quantitative recovery. It is not possible to extend the range below 0·01 per cent. of antimony by taking more than 1 g of sample, because of the difficulty in dissolving the larger amount of lead.

According to McChesney,[16] only bismuth, which itself gives a colour with potassium iodide, is likely to interfere in the spectrophotometric determination of antimony by the iodide method. It was found that the di-isopropyl ether extraction separated antimony from bismuth and so eliminated this interference. No serious interference was observed from other elements investigated (see Table II).

We thank Dr. A. I. Vogel, Head of the Chemistry Department of the Woolwich Polytechnic for his interest, and one of us (J.C.H.J.) wishes to thank the Directors of Associated Lead Manufacturers for enabling him to undertake the present research.

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The Use of Lithium-drifted Germanium Diodes for the γ-spectrometric Determination of Radioactive Fission-product Nuclides

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The superiority of a γ-spectrometer incorporating a germanium - lithium diode detector and field-effect transistor head amplifier over the conventional sodium iodide - thallium system, for the resolution of most of the difficult determinations encountered in fission-product radiochemistry, is demonstrated.

DURING the past 6 years, semiconductor solid-state detectors have become generally available for the detection of heavy charged particles. Lately, the discovery of the lithium drift-process, first in silicon[1] and then in germanium,[2] has led to the publication of several papers on their use, initially for conversion electron investigations and most recently for γ-spectrometry.[3,4,5] Because of its higher atomic number germanium has a much better photo-electric response than silicon, and is therefore a much more attractive detector for γ-spectrometry, particularly in the region up to about 1·5 MeV.

In the drift process, lithium is diffused to depths of several millimetres in a piece of p-type germanium to produce a layer of n+-type material, a central layer of intrinsic material and a layer of the original p-type germanium. The whole makes a p-i-n detector. Incident γ-radiation is absorbed by photo-electric or Compton processes, and the ions produced are collected by means of a potential difference applied across the detector. Lithium ions are highly mobile at normal temperatures and so, to achieve stability and a low noise level in the detector, it must be operated at low temperatures. It is usual to mount the detector in a cryostat operated at liquid-nitrogen temperature (77° K).

γ-Spectrometry is one of the most useful techniques available to radiochemists for the analysis of mixed fission products in irradiated nuclear fuel specimens. However, the best sodium iodide detector has a resolution of not less than 50 keV (the full width of the photopeak at half maximum height) at 662 keV (caesium-137). This has been inadequate for the determination of some individual nuclides in fission-product mixtures.

Several difficult problems in fission-product analysis have been overcome by radio-chemical separation, or where this is not possible, by mathematical analysis of the γ-spectrum. There are, in particular, four instances where an improvement in resolution, such as is claimed for lithium-drifted germanium diodes, would enable direct measurement of the activities to be made. These determinations are as follows—

    (*a*)   zirconium-95 in the presence of niobium-95 daughter,

    (*b*)   ruthenium-106 in the presence of ruthenium-103,

    (*c*)   caesium-137 in the presence of caesium-134,

    (*d*)   cerium-144 in the presence of cerium-141.

The three fission-product nuclides most frequently used for the determination of burn-up (the total number of fissions) of irradiated nuclear fuel specimens are zirconium-95, caesium-137 and cerium-144. A preliminary investigation has been carried out to test the feasibility of using the high resolving power of a germanium - lithium detector for the determination of these nuclides in mixed fission products, with a view to extending its application to non-destructive γ-scanning of nuclear fuel. The results of the first experiments are presented in this paper.

EXPERIMENTAL

APPARATUS—

The apparatus used for this experimental work is standard Harwell 2000 series equipment, with the exception of the head amplifier and distribution unit which are experimental and the subject of another paper.[6] The schematic layout is shown in Fig. 1. The input stage of
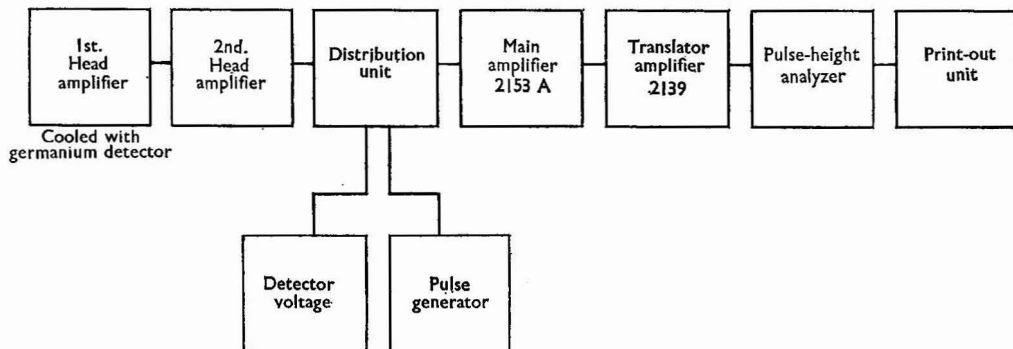

Fig. 1.   Schematic diagram of γ-spectrometer

the amplifier is an n-channel field-effect transistor* which is cooled to about 100° K together with the detector, a lithium-drifted germanium crystal, as shown in Fig. 2. The germanium detector is located on a copper rod which is immersed in liquid nitrogen, and the input stage of the head amplifier is in close proximity with the detector. The source is placed directly on the cold chamber envelope if suitable.

A = Liquid nitrogen
B = Base plate
C = Head amplifier
D = Thermocouple
E = Multiway cable
F = Field-effect transistor
G = Lid
H = Vent
I = Lid core
J = Lid seal
K = Cold chamber
L = Crystal
M = Platform
N = Cap
O = Tag-board assembly holding input
        components
P = Dewar flask
Q = Thermal shield
R = Thermal rod
S = Outer case
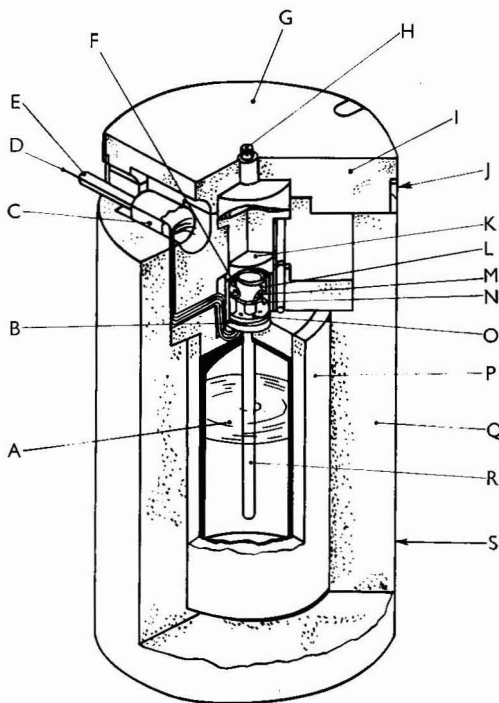
Fig. 2.   Lithium-drifted germanium γ-detector

* Type 2N2346, made by Amelco Semiconductors Ltd., P.O. Box 1030, Mountain View, California, U.S.A.

The advantage of operating the field-effect transistor at low temperature is that the input capacitance of the system can be reduced to a minimum by locating the input stage close to the detector. This reduction in capacitance in conjunction with lower capacity detectors can afford an improvement in resolution. A significant reduction in input noise is achieved by the fact that the thermal noise and gate leakage current noise are reduced to a minimum at this temperature.

The noise performance of the head amplifier with single R.C. differentiation and integration with 10-pF total input capacitance is 160 r.m.s. electronic charges. When used in conjunction with a germanium - lithium detector (2 cm in diameter and 4 to 5-mm depletion depth[7]) with a total input capacitance of 48 pF, the full width of the photopeak at half maximum value for cobalt-57 (122 keV and 136 keV) is less than 3 keV. This performance has been repeated over several months. A limitation in achieving a higher resolution is the over-all drift of the system. It has been shown that a spectrum stabiliser (type 2149) would achieve an improvement for counting periods greater than 15 minutes dependent on the over-all drift of the system.

APPLICATION OF THE APPARATUS TO FISSION-PRODUCT ANALYSIS—

The apparatus described above has been used to resolve the major problem of fission-product analysis, namely the interference of one nuclide with another, which conventional methods, with thallium activated sodium iodide detectors, are incapable of doing.

DETERMINATION OF ZIRCONIUM-95 IN THE PRESENCE OF NIOBIUM-95—

Zirconium-95 is used extensively for the determination of burn-up[8] in short irradiated samples. For the best results it is necessary to separate the zirconium chemically from the main interference, *viz.*, the daughter-product niobium-95. The energies of the γ-rays emitted by these two nuclides differ by only 40 keV and they are not even partially resolved by a sodium iodide detector. Zirconium-95, in fact, emits two γ-rays at 724 and 756 keV, respectively, whereas niobium-95 emits only one γ-ray at 764 keV. The spectra obtained with the conventional method (sodium iodide detector) and the lithium-difted germanium detector are shown in Fig. 3. In the spectrum obtained with the sodium iodide - thallium detector
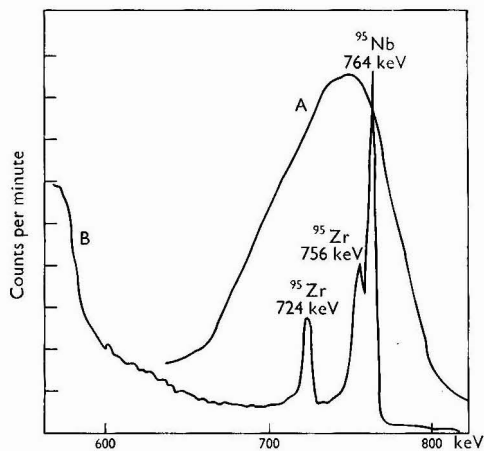

Fig. 3.   γ-Spectrum of niobium-95 + zirconium-95;
curve A, sodium iodide detector; curve B, germanium
detector

only one photopeak is observed which is composed of the three photopeaks expected. These three peaks are clearly seen in the spectrum obtained with the germanium - lithium detector. The two zirconium-95 γ-rays are completely resolved from each other, and the peak for niobium-95 at 764 keV is partially resolved from the peak for zirconium-95 at 756 keV. This spectrum demonstrates that it is thus possible to determine zirconium-95 in the presence of its daughter, niobium-95, without recourse to mathematical methods to calculate the proportion of each nuclide present. It is, in fact, possible to calculate the ratio of niobium-95

to zirconium-95 from this spectrum, provided that the decay scheme is known.   By using the decay scheme shown and the simple arithmetic which follows, this ratio was calculated.

DECAY SCHEME OF ZIRCONIUM-95 AND NIOBIUM-95
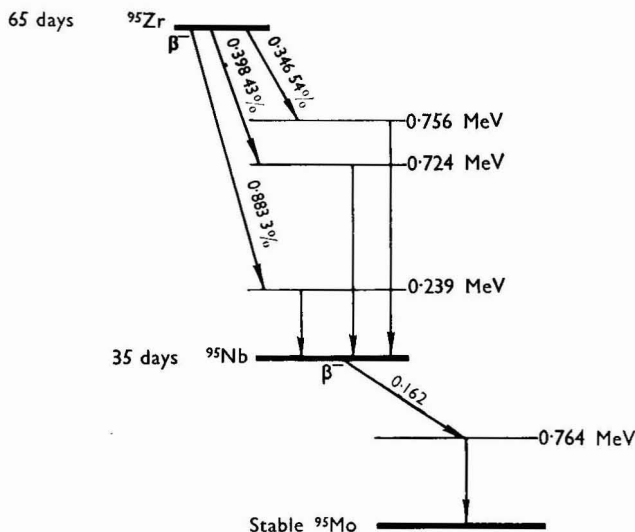
Let—

C$_1$ be the number of counts in the photopeak for zirconium-95 at 724 keV,
A$_1$ be the absolute abundance of the 724-keV γ-ray,
E$_1$ be the efficiency of detection at 724 keV.

Then, if A$_2$ and E$_2$ are the corresponding absolute abundance and efficiency of detection for the 756-keV γ-ray of zirconium-95, the number of counts in this photopeak, C$_2$, is given by—

$$C_2 = \frac{C_1 \times E_2 \times A_2}{E_1 \times A_1}$$

If C$_3$ is the total count in the combined photopeak for zirconium-95 at 756 keV plus niobium-95 at 764 keV, then the true count of the peak for niobium-95 at 764 keV, C$_4$, assuming that the absolute abundance of this γ-ray is 100 per cent., is given by—

$$C_4 = C_3 - C_2$$

If this photopeak is counted with an efficiency of E$_4$, then the ratio of niobium-95 to zirconium-95 is given by—

$$R = \frac{C_4 \times A_2 \times E_2}{C_2 \times E_4}$$

or

$$R = \frac{C_3 \times E_1 \times A_1 - C_1 \times E_2 \times A_2}{C_1 \times E_4}$$

The calculated value was 2·22, which compares well with the theoretical value of 2·18 for an equilibrium mixture, of which Fig. 3 is the spectrum.   The accuracy of this calculation is dependent on the accuracy of the decay scheme and efficiency of the detector for γ-rays of different energies.

MIXTURES OF RUTHENIUM-103 AND RUTHENIUM-106—

Ruthenium isotopes are rarely used as burn-up monitors although both ruthenium-103 and ruthenium-106 are potentially useful.   The chemical properties of ruthenium are such that dissolution of a fuel specimen often results in the loss, by volatilisation, of an unknown amount of this element from the sample solution.   Determination of the absolute amount of ruthenium is therefore of no value.   However, it may still be useful to determine the ratio of ruthenium-103 to ruthenium-106 which will remain undisturbed although the total amount of ruthenium may be decreased.   The fission yield of ruthenium-106 varies by a factor of more than 10 for the two fissile nuclides, uranium-235 and plutonium-239, whereas that of ruthenium-103 differs by about 2.   It should be possible, therefore, to determine the relative proportions to the total number of fissions in specimens containing mixtures of

fissile nuclides, *e.g.*, plutonium dioxide - uranium dioxide and plutonium carbide - uranium carbide fuels from the measured ratio of ruthenium-103 to ruthenium-106. Another case of interest is highly-burned-up low-enriched uranium, where significant quantities of plutonium-239 are produced from uranium-238 by neutron capture, and then undergo fission.
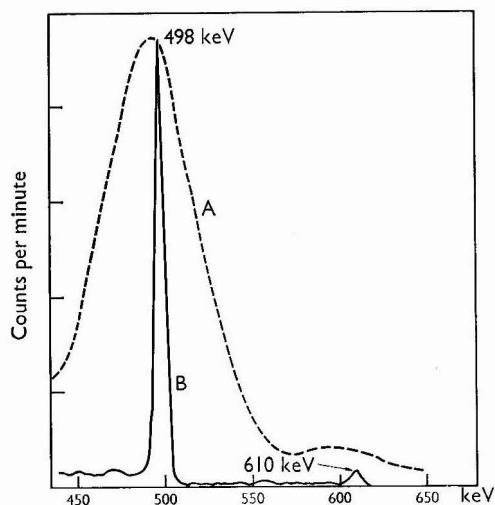

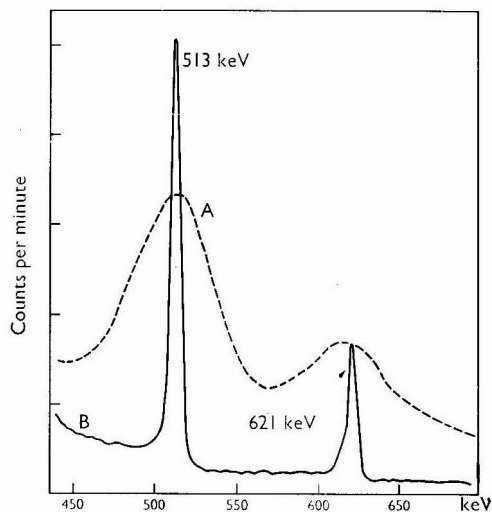Fig. 4.   γ-Spectrum of ruthenium-103; curve A, sodium iodide detector; curve B, germanium detector

Fig. 5.   γ-Spectrum of ruthenium-106 - rhodium-106; curve A, sodium iodide detector; curve B, germanium detector

Portions of the spectra of ruthenium-103 and ruthenium-106 - rhodium-106 are shown in Figs. 4 and 5, respectively. The spectrum of a mixture of ruthenium-103 and ruthenium-106 - rhodium-106 is shown in Fig. 6. From the spectra of the pure nuclides, the ratios of the counts in the peaks for ruthenium-103 at 498 keV and 610 keV, and in the peaks for ruthenium-106 at 513 keV and 621 keV can be calculated. With this knowledge it is possible to determine the amount of each nuclide present in a mixture by mathematical treatment as follows—

If R_3 is the ratio of the counts in the peak at 498 keV to the counts in the peak for ruthenium-103 at 610 keV;

R_6 is the ratio of the counts in the peak at 513 keV to the counts in the peak for ruthenium-106 at 621 keV;

C_5 is the total count in the mixed peak for ruthenium-103 at 498 keV and ruthenium-106 at 513 keV;

C_6 is the total count in the mixed peak for ruthenium-103 at 610 keV and ruthenium-106 at 621 keV,

then it can be derived that the number of counts due to the 498-keV γ-ray of ruthenium-103 in the mixed peak is given by—

$$C_{498}^{103} = \frac{R_3 (C_5 - C_6 \times R_6)}{R_3 - R_6}$$

From the spectrum shown in Fig. 6 the value of C_{498}^{103} was calculated, and was within 3·5 per cent. of the expected value. These values were 21,209 and 20,487, respectively. The ratio of ruthenium-106 to ruthenium-103 was 9·5.

MIXTURES OF CAESIUM-134 AND CAESIUM-137—

Caesium-137, like zirconium-95, is used extensively for the determination of burn-up, and has many advantages. It does, however, suffer from two major disadvantages. One of these, its migration at relatively low temperatures, excludes its use with samples where the temperature of the sample is known to have been greater than 650° C during the irradiation. Where this is not the case, a second disadvantage may still cause severe limitation to its usefulness. Several isotopes of caesium are produced in high yield in fission, including the stable isotope caesium-133. This isotope has an appreciable neutron-capture cross-section which may be greatly enhanced under suitable reactor conditions by a very large resonance

in its capture cross-section spectrum at about 6 eV.   The product of this (n,γ) reaction is caesium-134 (half-life of 2·3 years).   This nuclide has a complex γ-spectrum with γ-rays of high abundance at 605 keV and 796 keV that cause significant interference with the 662-keV γ-ray of caesium-137 when the spectrum of a mixture is obtained by the conventional sodium iodide detector.   The extent of this interference can only be determined with any degree of accuracy by involved mathematical treatment of the spectrum and it is, therefore, of interest to be able to resolve these two nuclides.   Fig. 7 shows the γ-spectrum of a mixture of caesium-134 and caesium-137, in which the ratio of caesium-134 to caesium-137 is 2·75.   The three peaks in question are all well separated from each other, and the determination of caesium-137 from this spectrum should present no difficulty.
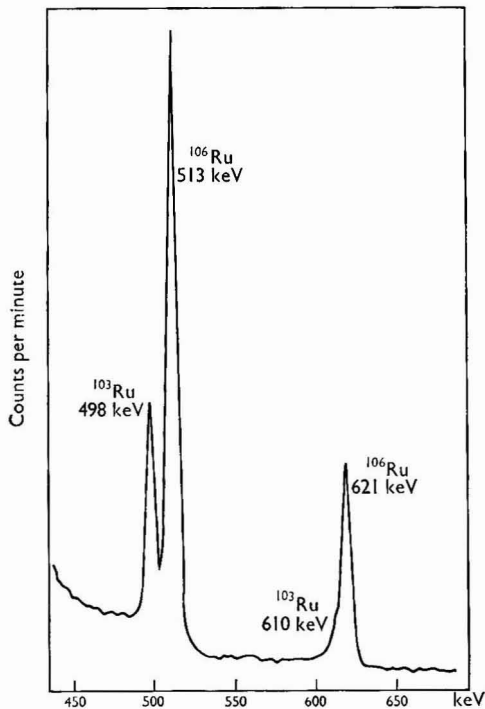

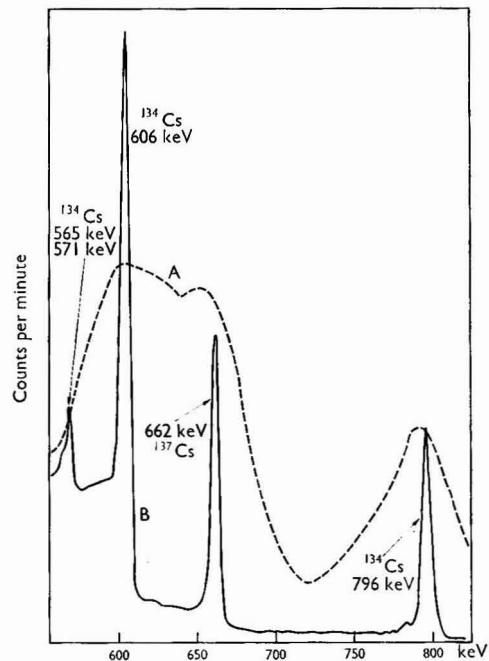Fig. 6. γ-Spectrum of mixed ruthenium-103 and ruthenium-106 - rhodium-106

Fig. 7. γ-Spectrum of mixed caesium-134 and caesium-137; curve A, sodium iodide detector; curve B, germanium detector

MIXTURES OF CERIUM-141 AND CERIUM-144—

The two major γ-active isotopes of cerium produced in fission are cerium-141 (half-life of 32 days), which emits a γ-ray at 145 keV, and cerium-144 (half-life of 285 days) which emits a γ-ray at 134 keV.   The chemical properties of cerium-144, together with its half-life, combine to make it an attractive nuclide to use for the determination of burn-up.   The presence of cerium-141 in specimens that have not been allowed to decay for long periods, however, complicates the determination to a considerable extent.   It is possible to use the 2·18 MeV γ-ray of its daughter praseodymium-144, which quickly reaches equilibrium with its parent cerium-144, but the low abundance of this γ-ray, combined with the low efficiency of γ-ray detectors at this high energy, makes its measurement difficult.

Fig. 8 shows the spectrum of a mixture of cerium-141 and cerium-144 obtained with a germanium - lithium detector.   For comparison, spectra of the same mixture and of pure cerium-144 obtained with sodium iodide - thallium detector are also shown.   The advantage of the germanium - lithium detector over the sodium iodide - thallium detector is clearly demonstrated by the two completely resolved and almost perfectly symmetrical peaks which are obtained by this means.   The resolution at 134 keV is 3·3 keV (the full width of the photopeak at half maximum height).

MIXED FISSION PRODUCTS—

The preceding paragraphs have shown that the apparatus described is capable of resolving many of the problems in fission-product analysis that are difficult to solve by using conventional sodium iodide - thallium detectors. All the sources of activity used were separated chemically, and each contained only one interfering nuclide after separation. It would be of value to be able to resolve the nuclides of interest (zirconium-95, ruthenium-103, ruthenium-106, caesium-137 and cerium-144) from fission-product solutions without previous chemical separation. Three samples, representing different conditions of irradiation and decay, were examined in experiments to determine the feasibility of this approach. The spectra are shown in Figs. 9, 10 and 11. Eight peaks from isotopes of caesium, cerium, ruthenium, niobium and zirconium can be identified between 550 and 800 keV. Solutions were examined in all three cases, and the small amounts of ruthenium present resulted from the loss of this element by volatilisation during dissolution of the specimen. In nearly all instances the photopeaks are well resolved and superimposed on a background that can be estimated with a good degree of certainty. It should therefore be possible to carry out fission-product analysis directly on fission-product solutions without any chemical separation, for a wider range of nuclides and irradiation and decay conditions than has hitherto been possible.
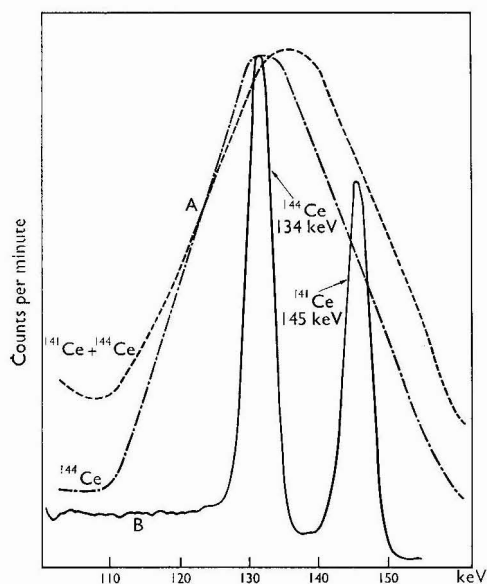

Fig. 8. γ-Spectrum of mixed cerium-141 + cerium-144 - praseodymium-144; curve A, sodium iodide detector; curve B, germanium detector

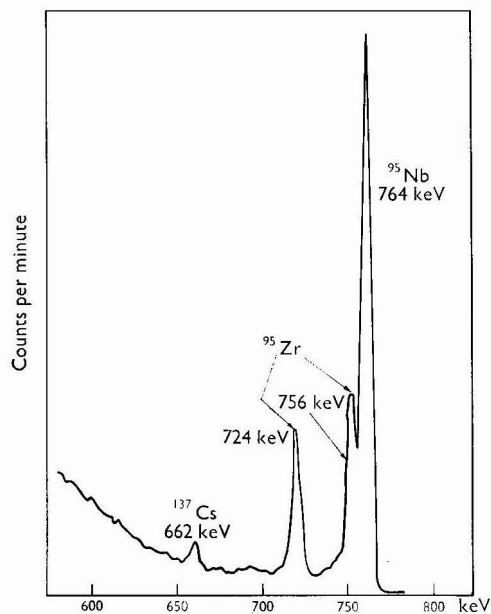Fig. 9. γ-Spectrum of mixed fission products; 72 days' irradiation, 150 days' decay

THE EFFICIENCY OF THE APPARATUS—

The efficiency of the apparatus is defined by the equation—

$$E = \frac{(C_m^n - CB_m^n) \times 100}{A \times D}\text{per cent.}$$

where C_m^n is the total number of counts per second in a photopeak contained in channels m to n,

CB$_m^n$ is the total number of counts per second background in channels m to n,
A is the abundance of the γ-ray in the decay scheme of the emitting nuclide,
D is the disintegration rate of the source.

It has been shown[9] that the efficiency is strongly dependent on γ-energy. The efficiency of the apparatus described was determined at 134 keV for cerium-144 and 662 keV for caesium-137 with standard sources. The values obtained were 0·16 per cent. and 0·0069 per cent., respectively, when the sources were placed as near to the detector as possible, *i.e.*,

directly on the cold-chamber envelope. These values are much lower, and the graph of efficiency versus energy of incident γ-ray is much flatter than reported by other workers.[5] These facts are probably attributable to a low geometry factor, due partly to distance and partly to the fact that the detector was inverted, thereby causing the γ-rays to be incident on the back of the detector. The precision of these measurements is the same as the precision of counting, *i.e.*, ± 1 per cent. on 10^4 events recorded in the photopeak. The degree of accuracy, however, will be less good due to the errors in the absolute calibration of the standard sources (about ± 1 to 2 per cent.) and in the branching ratios in the decay schemes of the nuclides used (about ± 5 per cent.). Most of the sources used had been prepared previously for measurement on a conventional sodium iodide - thallium γ-spectrometer with an efficiency of 10 per cent. at 134 keV, and were in the range 1 to 10 μC in activity. Although counting times with the germanium - lithium detector were usually 1 to 16 hours to accumulate about 10^4 counts in the principal photopeak, the apparatus, even with this low efficiency, is of great value to the radiochemist.
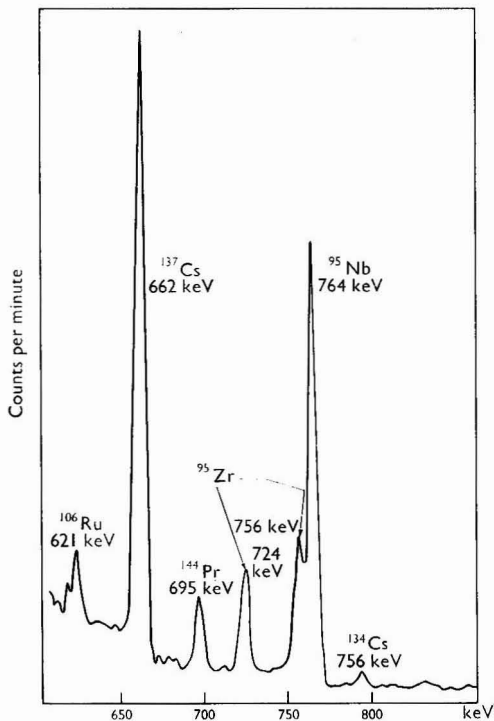

Fig. 10. γ-Spectrum of mixed fission products; 193 days' irradiation, 750 days' decay
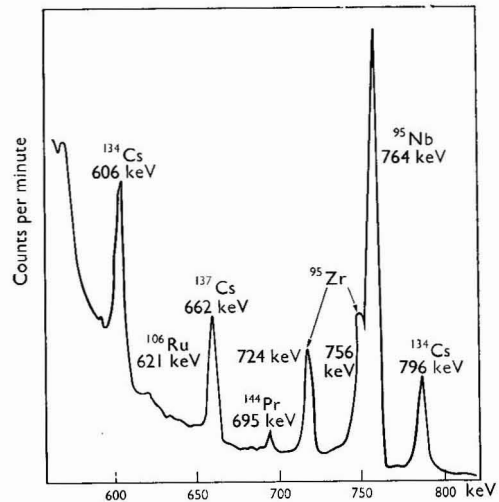
Fig. 11. γ-Spectrum of mixed fission products; 750 days' irradiation, 150 days' decay

CONCLUSIONS

An apparatus incorporating a lithium-drifted germanium detector, and designed primarily for studying low-noise amplifiers, has been used for a preliminary examination of the capabilities of this type of detector for γ-spectrometry.

The results presented here show that, even with poor geometry and low efficiency, the germanium - lithium detector is far superior to the best sodium iodide detector for resolving complex mixtures of γ-ray emitting nuclides such as exist in fission-product mixtures. This high resolving power, coupled with the ability by electronic means to examine small parts of the γ-spectrum in detail, should enable direct quantitative determinations of individual nuclides in intact or dissolved fuel specimens to be made. It should also be possible to scan irradiated fuel specimens, *e.g.*, plates or rods, for individual fission-product nuclides with

little or no interference from other emitters present. This in turn should promote the study of, for example, the migration of fission products in irradiated fuel specimens in greater detail than has hitherto been possible.

The study of the applications of germanium - lithium detectors to fission-product analysis is being continued with a second apparatus with improved geometry and efficiency, whilst the apparatus already described will continue to be in use for investigations of low-noise amplification systems.

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Determination of Catechol in Cigarette Smoke

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A procedure has been devised for the specific determination of catechol in cigarette-smoke condensates. This procedure should also be applicable to the determination of catechol in other materials resulting from pyrolytic or combustion processes.

As the catechol is isolated without recourse to the formation of a derivative, no interference is encountered as a result of the presence of guaiacol or similar compounds. Avoidance of the use of alkaline conditions throughout the procedure has permitted reproducible and high recoveries.

THE presence of catechol in cigarette smoke has been known for many years without any reliable measurements having been reported concerning the amount present. This is principally due to the fact that catechol is rather unstable in alkaline solution, and procedures previously used for its determination in cigarette smoke have utilised an alkaline extraction step to concentrate the acidic materials and remove contaminants.

The simple monohydroxyphenolic materials can be determined reliably by gas - liquid chromatography. Application of gas - liquid chromatography to the quantitative determination of catechol was reported by Janák and Komers.[1] Recoveries of approximately 70 per cent. were achieved. More recently, von Rudloff[2] reported the qualitative separation of catechol from other phenols by using several different gas - liquid chromatographic substrates. Difficulties were noted due to "tailing" or poor elution.

Methylation has been used to furnish derivatives of phenols in cigarette smoke for subsequent separation and determination,[3,4] but this is not a quantitative process for catechol, and furthermore the veratrole measured can be derived from guaiacol, which is also present in the smoke.

Other workers[5] have chromatographed dihydroxybenzene compounds by liquid - liquid partition on silicic acid with cyclohexane as the developing solvent. The amounts of individual phenols were determined by measurement of the optical density at 2800 Å. This procedure gave good resolution and appeared well suited for the routine determination of phenolic constituents in samples of similar composition. Disadvantages were the time required and the need for making optical-density measurements on a large number of fractions.

The successful use of polyamide surfaces for the qualitative separation of phenolic materials, including catechols,[6,7,8] and their suggested utility for quantitative analysis[9] prompted us to adapt this technique for the analysis of catechol in cigarette smoke. The procedure that we have developed was designed to avoid exposure of the catechol to alkali. The smoke condensate is dissolved in aqueous acid and ether. Ether-soluble acidic and neutral components are further fractionated by extraction into aqueous borate at pH 7. Catechol and similar vicinal dihydroxy-compounds are selectively complexed by the borate and made soluble in the aqueous phase. Acidification of the aqueous extract permits recovery of these compounds by extraction with ether. Catechol is then separated from other components of this mixture by chromatography on thin layers of polyamide powder with an acidic developing solvent.

Since catechol is determined in our procedure without the formation of a derivative such as the methyl ether, no interference from guaiacol is experienced. The method has given reproducible results when applied to cigarette smoke and near theoretical recovery of added known amounts of catechol.

EXPERIMENTAL

Catechol was unstable when stored in redistilled analytical-reagent grade ether containing no preservative, but no loss was observed over a 3-week period when trace amounts of sodium diethyldithiocarbamate were added to the ether. Considerable loss of catechol

did occur if it was extracted from ether into an aqueous alkaline solution and was then recovered from the acidified extract by re-extraction into ether. The losses sustained in this treatment were not as great in the presence of other smoke components.

The recovery of catechol was 97 per cent. when subjected to the initial ether extraction used in the present procedure. Similarly, the recovery of catechol in the borate extraction step was 98 per cent. No significant loss was observed in 0·1 M sodium borate at pH 7 for periods of time well in excess of that required for the borate extraction.

Thin-layer chromatography on polyamide powder from another source gave quite different R_F values and less desirable behaviour than did the Woelm polyamide powder. The initial use of the upper layer of a benzene - acetic acid - water (6 + 7 + 3) solvent for the thin-layer chromatography gave unsatisfactory results, apparently due to variations in composition of the solvent. Betts[10] discusses the superior stability of one-phase solvent systems over systems prepared by equilibration of two phases. The solvent mixture that was chosen (100 + 25 + 1) is a single phase and has given reproducible results.

Initially, the recovery of catechol from the thin-layer chromatograms was found to vary from 78 to 96 per cent. of the theoretical value. The extent of loss was usually similar for plates prepared at the same time. Volatilisation of catechol from the polyamide film did not appear to be a source of loss, as the yield was essentially the same whether the spot was eluted immediately, or after one hour, after the drying of the plate. Also, when spots of catechol were applied diagonally across the plate and developed in the usual way, recoveries varied from 92 to 96 per cent., with no indication of a trend related to the extent of travel of the catechol across the plate.

Consistently higher recoveries, 94 to 100 per cent., have been obtained by washing the polyamide powder with ethanol, prior to preparing the slurry. Furthermore, the ethanol-washed polyamide provides layers with less tendency to crack on drying. The recovery of catechol, added to cigarette-smoke condensate and carried through the various steps of the procedure as outlined below, was 95 to 100 per cent.

The catechol spot removed from the thin-layer chromatogram of a typical cigarette-smoke extract was found to have ultraviolet absorption identical to that for an authentic sample of catechol, with little background absorption. It had the same R_F value as authentic catechol when chromatographed on borate-treated silica gel with butanol, saturated with 0·1 M sodium borate at pH 7, and on Whatman No. 1 filter-paper with chloroform (containing 1 per cent. ethanol) which had been equilibrated with methanol - water - formic acid (25 + 24 + 1) as proposed by Reio.[11] The same yellow colour was obtained for both known and unknown by spraying with diazotised *p*-nitroaniline.

The colorimetric procedure used for the quantitative determination of catechol is a modification of the test reported by Mitchell.[12] The test is given, in general, by all di-hydric phenols and it is therefore necessary to achieve an adequate separation of catechol from other compounds of this class prior to applying this test. As catechol is the pre-dominant compound of this class, present in the borate extract of cigarette smoke, the separation achieved by this method is usually adequate. Smoke from cigarettes made entirely with flue-cured tobaccos, contains some interfering substances in concentrations that could warrant extending the development of the chromatogram on longer plates.

It is possible to obtain a measure of the total dihydric phenols in the ether extract from the acidified borate solution. The intensity of the colour is comparable to that obtained with catechol for many of these compounds, such as 3-methylcatechol, 4-methylcatechol and protocatechuic acid. Pyrogallol and caffeic acid give 1·3 times the colour intensity observed for catechol. Protocatechualdehyde, coumarin-diols and naphthalene-diols give an orange colour with absorption maximum at about 450 mμ. These latter compounds, if present, would also contribute to the total colour intensity at 515 to 530 mμ. Phenol, resorcinol, guaiacol and hydroquinone do not give significant colour production at 515 to 530 mμ.

Results were erratic when an aliquot of the ether solution containing dihydric phenols was evaporated to dryness before mixing with the colorimetric reagent. This was remedied by adding a portion of the reagent before evaporating off the ether.

METHOD

APPARATUS—

Smoking apparatus—That described by Keith and Newsome[13] was used.

Smoke-collection flasks—JF–6910 (Scientific Glass Apparatus Co. Inc., Bloomfield, N.J.).

Apparatus for thin-layer chromatography—Desaga-Brinkmann model S–11 (Brinkmann Instruments Inc., Great Neck, N.Y.).

Spectrophotometer—Perkin-Elmer model 350 (Perkin-Elmer Corp., Norwalk, Conn.).

REAGENTS—

α-Cellulose—Solka Floc (Brown Co., Berlin, New Hampshire).

Ether—Mallinckrodt anhydrous analytical-reagent grade is redistilled and stabilised by the addition of 0·05 p.p.m. of sodium diethyldithiocarbamate (Distillation Products Industries).

Woelm polyamide powder—(Alupharm Chemicals, Elmont, Long Island, New York).

Catechol—Re-sublimed (Aldrich Chemical Co., Milwaukee 10 Wis.).

Ferrous salt solution—Dissolve 0·1 g of hydrated ferrous sulphate, $FeSO_4 \cdot 7H_2O$, and 0·5 g of sodium potassium tartrate, $NaKC_4H_4O_6 \cdot 4H_2O$ (Rochelle salt), in 100 ml of distilled water. This solution is stable for approximately 3 days.

Colorimetric reagent—Mix two parts of the ferrous salt solution with five parts of 10 per cent. w/v ammonium acetate and two parts of 0·25 N ammonium hydroxide. This reagent must be freshly prepared for each set of determinations.

Cigarettes—Brands A, B, C and D are non-filter-tipped cigarettes (commercially available in the U.S.A.) which contain blended flue-cured (Bright), air-cured (Burley and Maryland) and sun-cured (Turkish) cigarette tobaccos. Brands E, F, G and H are filter-tipped cigarettes (commercially available in the U.S.A.). These cigarettes also contain blends of the three types of tobaccos listed above. Cigarettes I, J and K were prepared to contain a blend of a single type of tobacco, flue-cured, sun-cured or air-cured, typical of that particular component of a commercial blend. The latter cigarettes were cased with a glycol - sugar mixture.

SMOKING TECHNIQUE—

Cigarettes equilibrated at 60 per cent. relative humidity and 25° C are selected to within ±30 mg of the average weight desired. These are smoked mechanically in a room controlled at 25° C and 60 per cent. relative humidity. Forty-ml puffs of 2 seconds duration are taken at intervals of 60 seconds until an average butt of 30 mm remains. The smoke, produced from the sequential smoking of ten cigarettes, is condensed in a trap containing α-cellulose, which is partially immersed in a solid carbon dioxide - acetone coolant. The weight of smoke condensate is determined by the difference in weight of the cellulose trap, at 25° C, before and after smoke collection. A correction is applied for the amount of moisture introduced from the atmosphere during the puffing.

To obtain the yield of catechol from the filter-tipped cigarettes without filtration, the filter materials are removed from the mouth-piece before smoking and the cigarettes are smoked to a butt length of 30 mm, including the mouthpiece.

EXTRACTION OF THE CATECHOL FRACTION—

The smoke condensate is removed from the cellulose traps by washing with several portions of ether, 0·1 N hydrochloric acid and acetone, totalling 105, 50 and 5 ml, respectively. These extractants are then combined and equilibrated, the layers are allowed to separate, and the aqueous layer is washed five times with equal volumes of ether. The ether extracts are combined and concentrated at 30° to 34° C in a nitrogen stream to a volume of 25 ml.

The ether concentrate is then extracted four times with 25-ml volumes of 0·1 M sodium borate, which has been adjusted to pH 7 with hydrochloric acid. If necessary, the pH of the first borate layer is re-adjusted to pH 7 with cold, dilute sodium hydroxide after contact with the ether concentrate. The borate extracts are cooled and acidified to pH 1 immediately upon collection.

Recovery of the catechol from the acidified borate solution is accomplished by extracting four times with 100-ml volumes of ether. After the ether extract has been concentrated to 50 ml in a nitrogen stream at 30° to 34°C, it is washed twice with 1 ml of water, concentrated further, and dried with sodium sulphate, in the cold, overnight. The sodium sulphate is filtered from the solution, and the filtrates and rinsings are carefully concentrated in a nitrogen stream to exactly 2 ml. Losses of catechol are observed with complete evaporation of the solvent. The extraction of larger samples of cigarette smoke is accomplished, similarly, by scaling up the extracting volumes.

DETERMINATION OF THE TOTAL *o*-DIHYDRIC PHENOLS—

An aliquot of the smoke fraction containing the *o*-dihydric phenols, usually representing one cigarette, is mixed with 5 ml of the colorimetric reagent, the vessel is shaken thoroughly, and the ether evaporated in a nitrogen stream. The coloured solution is diluted to 10 ml with the colorimetric reagent and the visible spectrum is obtained with the colorimetric reagent in the reference cell. The absorption maximum, between 515 and 530 mμ, is calculated from catechol standardisation data, and the amount of *o*-dihydric phenols is calculated as if they were catechol.

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF CATECHOL—

Chromatographic plates (20 × 20 cm) are coated with 0·25 mm of Woelm polyamide powder. Five plates are prepared at one time by spreading a slurry of 5 g of the polyamide powder in 45 ml of methanol - water (3 + 1). A narrow area along each edge is scraped free of polyamide to promote uniformity of the solvent flow.

An aliquot (200 μl) of the catechol extract, equivalent to the smoke condensate from one cigarette, is applied to the lower left corner of each of two plates. A sample of 110 μg of pure catechol is similarly applied to the middle of the lower side of the two plates. After the application solvent has evaporated, development is carried out by ascending chromatography in benzene - acetic acid - water (100 + 25 + 1). The solvent front usually reaches the top of the plate within an hour.

The plates are then removed from the chromatography chamber and the solvents are allowed to evaporate until the plates appear barely dry: extended drying will cause the powder layer to crack. Chromatography is continued by using distilled water to develop the plates in a direction perpendicular to that of the first chromatography. This usually requires about 45 minutes. The plates are partially dried in air, sprayed lightly with the ferrous salt colorimetric reagent, and again partially dried in air. Exposing the damp plate briefly to ammonia vapour will aid in the development of the characteristic purple colour. Both the smoke - catechol spot and the control - catechol spot are marked before the plate has dried completely. After drying thoroughly in air, each spot is removed by suction on to a sintered-glass filter[14] and washed into a 10-ml vessel with the ferrous salt colorimetric reagent.

COLORIMETRIC DETERMINATION OF CATECHOL—

The visible spectrum is measured within 2 hours with the colorimetric reagent in the reference cell. The absorbance is measured at the maximum, which occurs between 515 and 530 mμ. The concentration of catechol in the sample is read off from the curve obtained, by measuring this absorbance for several concentrations of standard catechol. The standardisation curve is re-determined for each colorimetric reagent preparation and, at concentrations between 50 and 300 μg per 10 ml, obeys Beer's law. The value obtained for the smoke - catechol content is corrected for the recovery achieved for control samples of catechol treated in the same manner.

RESULTS AND DISCUSSION

Catechol analyses for smoke from several non-filter-tipped cigarettes are given in Table I. The results are expressed both in terms of μg of catechol per cigarette, and as the catechol percentage of condensate. The latter values tend to compensate for variations in the weights of smoke produced due to differences in length or burning characteristics of the cigarettes. The standard deviation calculated for the results presented in Table I is ±0·024 per cent.

The level of catechol in smoke from cigarettes made with a blend of air-cured (Burley) tobaccos was considerably lower than for smoke from cigarettes made with either flue-cured or sun-cured tobaccos. The reasons for this difference are being investigated.

To evaluate the effect of filtration on the concentration of catechol in cigarette smoke, it is necessary to correct for the moisture content of the smoke. This is required as the filters remove a disproportionate amount of water. When this correction was applied to the analyses performed on smoke from several different brands and types of filter-tipped cigarettes (E, F, G and H, Table II), the results indicated no appreciable effect of the filter on the concentration of catechol in the smoke, *i.e.*, the catechol is removed by these filters to an extent equivalent to the removal of the total dry smoke. This would suggest that negligible

TABLE I

CATECHOL IN SMOKE FROM NON-FILTER-TIPPED CIGARETTES

Type of cigarette	Smoke condensate, mg per cigarette	Catechol content—	
		μg per cigarette	percentage of condensate
Cigarette A, 85-mm blended flue-cured, air-cured and sun-cured tobaccos	48	145	0·30
		161	0·33
	49	127	0·26
		130	0·27
		Average	0·29
Cigarette B, 85-mm blended flue-cured, air-cured and sun-cured tobaccos	37	113	0·31
		131	0·35
	36	118	0·33
		130	0·36
		Average	0·34
Cigarette C, 70-mm blended flue-cured, air-cured and sun-cured tobaccos	33	92	0·28
		93	0·28
	35	102	0·29
		102	0·29
		Average	0·28
Cigarette D, 70-mm blended flue-cured, air-cured and sun-cured tobaccos	35	76	0·22
		95	0·27
	35	97	0·28
		96	0·28
		94	0·27
		100	0·29
		Average	0·27
Cigarette I, 85-mm blend of flue-cured tobacco	53	260	0·49
		180	0·34*
	54	228	0·42
		244	0·45
		Average	0·45
Cigarette J, 85-mm blend of sun-cured tobacco	55	193	0·35
		198	0·36
	52	168	0·33
		186	0·36
		Average	0·35
Cigarette K, 85-mm blend of air-cured tobacco	39	50	0·13
		50	0·13
	39	46	0·12
		43	0·11
		Average	0·12

* Omitted from calculation of average and standard deviation.

amounts of catechol are present in the vapour phase of the smoke, otherwise its selective removal by the cellulose acetate filters, as is noted for the more volatile monohydric phenols, would be anticipated.

TABLE II

CATECHOL IN SMOKE FROM 85-mm FILTER-TIPPED CIGARETTES

			Filter intact			Filter removed		
				Catechol			Catechol	
Brand	mm	Filter-tipped type	Dry smoke,* mg per cigarette	μg	percentage of dry smoke	Dry smoke,* mg per cigarette	μg	percentage of dry smoke
E	17	acetate	26	103	0·39	42	146	0·35
F	20	acetate	20	97	0·49	31	155	0·50
G	20	acetate + charcoal	29	118	0·41	44	176	0·40
H	20	acetate + charcoal	21	92	0·44	26	116	0·45

* The smoke weights are corrected to a dry basis by using moisture values from duplicate smoke collections determined by Karl Fischer titrations.

The colorimetric test for *o*-dihydric phenols, applied to the borate-extractable fraction of cigarette smoke, prior to chromatography on polyamide, and calculated as catechol, indicated a total content for these materials about twice the value of that measured for catechol (see Table III). Studies are in progress to identify these materials.

Smoke samples used for these analyses were prepared under the supervision of D. H. Woods.

TABLE III

TOTAL *o*-DIHYDRIC PHENOLS IN SMOKE FROM NON-FILTER-TIPPED CIGARETTES

	Smoke condensate, mg per cigarette	*o*-Dihydric phenols (as catechol)	
		µg per cigarette	percentage of condensate
Cigarette A, 85-mm blended flue-cured, air-cured and sun-cured tobaccos	49	336	0·69
Cigarette I, 85-mm blend of flue-cured tobacco	54	526	0·98
Cigarette J, 85-mm blend of sun-cured tobacco	53	286	0·54
Cigarette K, 85-mm blend of air-cured tobacco	39	107	0·27

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Determination of Zinc in Trace-element Superphosphate by A.C. Polarography

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Zinc has been effectively determined in "trace-element superphosphate" with an a.c. polarographic technique. This technique is both rapid and accurate and compares favourably with the atomic-absorption method. The zinc is maintained in solution by polarographing in an acid electrolyte of M hydrochloric acid at a pH of less than 1. The method eliminates the time-consuming process of separation from interfering ions.

The presence of the hydrogen reduction wave does not materially interfere with the zinc reduction wave as happens in conventional d.c. polarography.

THE application of conventional d.c. polarography has been used by Heller *et al.*,[1] Walkley[2] and Piper[3] to determine zinc in soil and plant material. They extracted the zinc with dithizone and evaporated the extract to dryness. The residue was dissolved in a basal solution of ammonium chloride and potassium thiocyanate. The zinc was determined polarographically; it has also been determined polarographically in biphthalate,[4] ammoniacal[5] and fluoride[6] electrolytes.

The above investigations were carried out in alkaline, or near-alkaline, solutions because of the interference of the hydrogen wave occurring close to the zinc wave. Breyer, Gutman and Hacobian[7,8] have shown that a.c. polarographic waves 40 mV apart are clearly separable and have, in fact, obtained well defined curves for zinc in 0·1 N hydrochloric acid and 0·5 N hydrochloric acid.

METHOD

REAGENTS—

All the reagents used were analytical-reagent grade chemicals.

Nitric acid, concentrated.

Hydrochloric acid, 10 N.

Sulphuric acid.

Zinc sulphate solution—Prepare a standard solution by dissolving 0·2200 g of zinc sulphate heptahydrate in distilled water. Add to the solution 50 ml of 10 N hydrochloric acid and dilute the solution to exactly 500 ml with distilled water (1 ml of the solution = 0·0001 g of zinc).

SAMPLES—

Sample the normal superphosphate and "trace-element superphosphate" according to A.O.A.C. specifications.[9] Crush the samples to pass through a 40-mesh sieve, dry them at 90° to 100° C for 4 hours and place them in a sealed bottle.

APPARATUS—

Polarograph—A manual a.c. polarograph was used, similar to that described by Breyer, Gutman and Hacobian[10] with a few minor modifications.

The cell consisted of a 100-ml squat beaker into which the test solutions were placed. The dropping-mercury cathode was lowered into the cell to within 15 to 20 mm of the mercury-pool anode. The head of mercury of 100 cm gave a drop time of 1 drop per 4·0 second, with a mass $(m) = 1·44$ mg per second.

Standard polarograms were carried out at the same time as the experimental work and the temperature was maintained constant to within $\pm 0·5°$ C. The peak currents were measured from the interpolated base-line.

Zinc in the trace-element superphosphate samples was determined with an atomic-absorption spectrophotometer similar to that described by Box and Walsh[11] and containing a hollow zinc cathode emitting a resonance line at 213·8 mμ.

PROCEDURE—

Prepare five standard solutions by weighing 2·000 g of the normal superphosphate into a 250-ml beaker. Add to the solid 5 ml of concentrated nitric acid, 5 ml of concentrated hydrochloric acid and evaporate the solution to dryness. Dissolve the residue in 10 ml of 10 N hydrochloric acid and 70 ml of hot distilled water. Boil the solution, then filter it through a Whatman No. 41 filter-paper, and wash the residue with 6 small washings of hot distilled water.

Cool the filtrate, dilute it to exactly 100 ml in a calibrated flask and mix it well. Transfer by pipette a 20-ml aliquot into a 500-ml calibrated flask with 48 ml of 10 N hydrochloric acid, dilute the solution to exactly 500 ml with distilled water and mix it well.

Transfer by pipette a 50-ml aliquot of this solution into each of five 100-ml squat beakers. Introduce 1, 2, 3, 5 and 6 ml of the standard zinc solution to the respective beakers and mix the contents well. Polarograph the solutions between —0·90 and —1·34 volt with a mercury-pool anode and an a.c. potential of 2·87 mV r.m.s.

Transfer a 50-ml aliquot of the above solution to a 100-ml squat beaker and polarograph it as a blank determination on the normal superphosphate and the reagents used.

Place duplicate 2-g samples of the "zinc-trace superphosphate" into 250-ml beakers and evaporate them to dryness with 5 ml of concentrated nitric acid and 5 ml of concentrated hydrochloric acid. Dissolve the residues in 10 ml of concentrated hydrochloric acid and 70 ml of hot distilled water. Boil the solutions and filter them through Whatman No. 41 filter-paper, washing with 6 small washings of hot distilled water.

Cool the filtrates, dilute to exactly 100 ml in a calibrated flask and mix them well. Transfer by pipette a 20-ml aliquot into a 500-ml calibrated flask together with 48 ml of 10 N hydrochloric acid and dilute the solution to exactly 500 ml with distilled water. Transfer a portion of each to separate 100-ml squat beakers and polarograph between —0·90 and —1·34 volt with a mercury-pool anode under the same cell conditions as used for the standard.

Draw a calibration curve from the standard polarogram for the zinc, and determine the zinc in the trace-element superphosphate.

RESULTS

Although the influence of the hydrogen ion reduction on the base-line is evident, it does not interfere with the zinc wave or the determination of its peak height.

Zinc gave fairly well defined peaks in M hydrochloric acid with a half-wave potential at —1·02 volt with a mercury-pool anode. No interference resulted from the presence of phosphate or iron.

The polarograms for some of the additions of standard zinc solution to the normal superphosphate are given in Fig. 1 (*a*), (*b*) and (*c*), and show a corresponding increase in peak height with concentration. The blank determination, represented by Fig. 1 (*d*), consisted of the reagents and the normal superphosphate and gave no wave for zinc.

From the peak heights at different concentrations of zinc, as shown in Table I, the calibration graph was drawn. The calibration graph gave a straight line passing through the origin over the range 0 to $10·7 \times 10^{-3}$ g of zinc per litre.

TABLE I

PEAK HEIGHTS AT DIFFERENT ZINC CONCENTRATIONS

Concentration of zinc, g per litre	Current, μA
$1·96 \times 10^{-3}$	3
$3·85 \times 10^{-3}$	5·5
$5·66 \times 10^{-3}$	8
$9·09 \times 10^{-3}$	13
$10·7 \times 10^{-3}$	15·5

The polarograms of duplicate samples for zinc in trace-element superphosphate carried out under the same cell conditions as the standards gave similar, well defined peaks. With
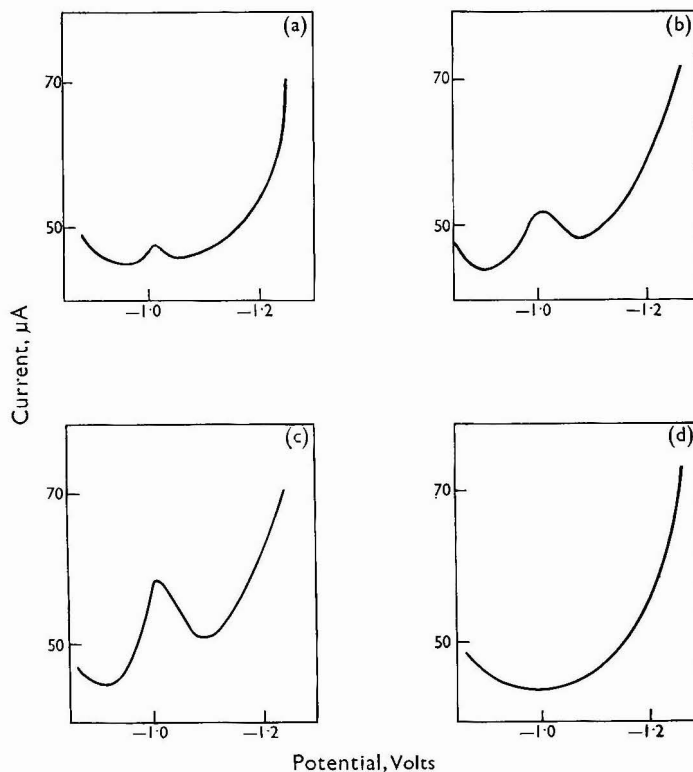
Fig. 1.   Polarogram of zinc standards in "normal" superphosphate at
21·1° C. Base electrolyte M hydrochloric acid, a.c. potential 2·87 mV r.m.s.
(a) $1·96 \times 10^{-3}$ g of zinc per litre; (b) $56·6 \times 10^{-3}$ g of zinc per litre; (c)
$10·7 \times 10^{-3}$ g of zinc per litre; (d) "normal" superphosphate (blank)

high zinc content, increased dilution was necessary. The peak heights were measured and
the percentage of zinc was determined from the calibration curve.

The concentration of zinc in the samples was also determined with an atomic-absorption
spectrophotometer, the comparison of the results being shown in Table II.

TABLE II

COMPARISON OF RESULTS

Sample	Acid digestion, per cent. of zinc	Atomic absorption, per cent. of zinc
Zinc - superphosphate ..	1·25	1·31
	1·25	1·31
	1·21	1·33
	1·25	1·28
	1·23	1·28
	1·24	1·30
	Mean = 1·24	Mean = 1·30
Standard deviation 0·015		Standard deviation 0·017

Zinc may be determined by a.c. polarography in superphosphate containing other trace
elements, such as copper, by using a procedure identical with that described above.

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Simultaneous Determination of Iodine and Bromine in Urine by Neutron-activation Analysis

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Neutron-activation analysis was used for the simultaneous determination of iodine and bromine in urine. The activated iodine and bromine were separated by radiochemical methods. The 0·46 MeV and 0·55 MeV peak areas of ^{128}I and ^{82}Br, respectively, were measured by means of a multi-channel analyser. The amounts of iodine and bromine were found to be of the order of 10^{-7} and 10^{-6} g ml^{-1} of urine, respectively, for normal human beings of different ages.

IODINE is found in urine both in inorganic (80 per cent.) and organic (20 per cent.) chemical forms.[1] Usually, iodine is found in the various biological tissues as an organically bound species, but it cannot be determined until after mineralisation. The order of magnitude of the mineral iodine so obtained, purified by distillation, extraction, ion exchange and so on, is determined by a classical titrimetric or colorimetric method, or from its catalytic action upon the rate of reduction of ceric sulphate by arsenous oxide.[2] The rate of this reaction, slow in the absence of a catalyst, is increased by the presence of micro amounts of iodine. Although the sensitivity of this catalytic reaction is of the order of 0·0002 μg for iodine determination,[3] the results of this method are not good due to the disadvantage of the impurity of the analytical reagents, and to the presence of iodine in the laboratory. Thus neutron-activation analysis, which presents high sensitivity and good precision in comparison with other analytical methods, has been used by several investigators for the quantitative determination of iodine and bromine in a variety of biological materials. Iodine was determined as thyroglobulin iodine,[4] blood-serum and serum organically-bound iodine,[5,6] blood iodine,[7] stable iodine up-taken by the thyroid[8] and as a constituent of different biological materials.[9,10,11,12] Bromine was determined in a series of biological materials.[13,14,15,16,17,18] Although there are many papers on radioactive iodine determination in urine,[19,20,21,22,23,24] milk[9,25] and plasma,[26] not much work has been done on the determination of stable iodine in urine, especially by radioactivation analysis. As a contribution to these studies, iodine and bromine were simultaneously determined in urine by radioactivation-analysis techniques.

On irradiating any material with neutrons, various nuclear reactions take place, those in Table I being of interest for iodine and bromine determination.[27,28]

TABLE I

NUCLEAR REACTIONS

Nuclide	Reaction	Abundance, %	Activation cross-section, barns	Half-life	β energy, max.	%	γ energy	%	Possible interference from
^{127}I	^{127}I (n,γ) ^{128}I	100	5·60	24·99 min.	1·67 2·12	16 76	0·46	17	^{128}Xe (n,p) ^{128}I with a Xe matrix
^{81}Br	^{81}Br (n,γ) ^{82}Br	49·48	1·60	36·87 hr	0·44	100	0·55 0·78 1·04 1·32	75 83 29 28	^{82}Kr (n,p) ^{82}Br with a Kr matrix

EXPERIMENTAL

IRRADIATION—

Transfer an aliquot of about 6 ml of biological liquid, by pipette, into a polythene snap-closure tube of diameter 26 mm and height 50 mm, having along its axis another polythene

snap-closure tube of diameter 15 mm and height 50 mm, that can be stoppered and fused. Add 6 ml of the standard iodine and bromine solution, mentioned below, into the central tube and then stopper and fuse it.
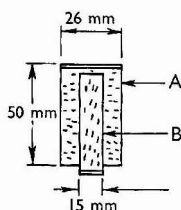
A = External polythene tube containing urine

B = Central polythene tube containing standard solution of potassium iodide and potassium bromide

Fig. 1. Target for irradiation

Irradiate the target for 15 minutes at a flux of 10^{12} n. cm^{-2} second^{-1}. We sent our prepared target, by means of the pneumatic system, to the core of the swimming pool of the "Democritus" nuclear reactor.

REAGENTS—

Use analytical-grade reagents.

Potassium iodide standard solution—Make an aqueous solution containing 10 mg ml^{-1} of iodine.

Potassium bromide standard solution—Make an aqueous solution containing 10 mg ml^{-1} of bromine.

Potassium iodide and potassium bromide standard solution—Prepare an aqueous solution of 10^{-4} mg ml^{-1} of iodine and 10^{-2} mg ml^{-1} of bromine of mixture, taken from the standardised solution of the carrier. Use triply distilled water.

Perhydrol.

Sodium nitrite solution, 2·5 N and N sodium nitrite.

Sulphuric acid, concentrated.

Sodium hydroxide solution, 7·5 per cent. sodium hydroxide.

Carbon tetrachloride.

Nitric acid, concentrated, 6 N and 0·01 N.

Sodium hydrogen sulphite solution, N.

Silver nitrate solution, 0·1 N.

Ethanol.

Potassium permanganate, powder.

*Potassium permanganate solution,* 2 per cent. potassium permanganate.

Hydroxylammonium chloride solution, M.

APPARATUS—

Modified apparatus was used to increase chemical yield. A Pyrex distillation apparatus and a Plexi-glass filter-funnel were used.

ISOLATION OF IODINE AND BROMINE BY DISTILLATON—

After irradiation transfer by pipette into the distillation apparatus, 5 ml of urine containing 1 ml of iodine plus 2 ml of bromine carrier solutions, respectively. Add the following reagents: 2 ml of concentrated sulphuric acid, 2 ml of perhydrol and 1 ml of N sodium nitrite solution. Begin distillation by heating and introducing air. Introduce the distillate and air into 10 ml of 7·5 per cent. sodium hydroxide solution contained in a separating funnel immersed in an ice - water mixture. After a few minutes add the same volumes of perhydrol and sodium nitrite and continue the distillation until white sulphur trioxide vapour is produced. The duration of the distillation procedure was 10 minutes.
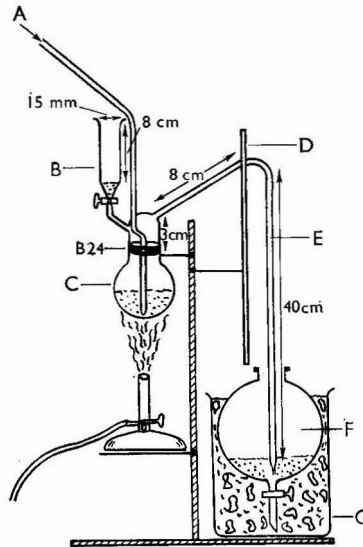
A = Compressed air from cylinder
B = 10-ml funnel containing sodium nitrite, sulphuric acid and a 30% solution of hydrogen peroxide
C = 50-ml round-bottom flask containing urine, potassium iodide and potassium bromide
D = Asbestos shield
E = Air-cooled tube
F = 250-ml flask containing sodium hydroxide
G = Beaker containing ice-water mixture

Fig. 2.   Pyrex distillation apparatus
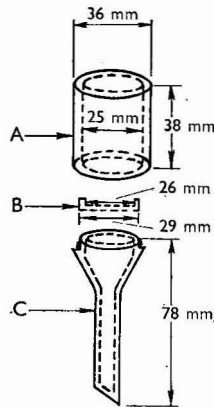
A = Screw-cylinder
B = Filter disc
C = Screw-funnel

Fig. 3.   Plexi-glass filter funnel

ISOLATION OF IODINE BY REDOX AND PRECIPITATION—

Pour 10 ml of carbon tetrachloride into the solution contained in the separating funnel. Gently neutralise and slightly acidify this alkaline solution with 6 N nitric acid.   Add 1 ml of 2·5 per cent. sodium nitrite drop by drop until the organic phase turns pink and separates. Repeat the same oxidation extraction step to the aqueous phase.   Keep the aqueous layer containing bromine so that it can later be subjected to the bromine isolation procedure.

Gather the organic phases containing the iodine and subject them to a reduction step by adding 10 ml of distilled water and a few drops of N sodium hydrogen sulphite. Separate the aqueous and organic phases and repeat the same reduction step with the organic phase, which can then be discarded. Collect the portions of aqueous phase and add 1 ml of 6 N nitric acid. Heat the solution so as to expel the sulphur dioxide and the few globules of carbon tetrachloride present. Add a 2-ml portion of 0·1 N silver nitrate. Filter the silver iodide precipitate, rinse the precipitate with 0·01 N nitric acid solution, distilled water and finally with ethanol. Mount the filter-paper with the precipitate on an aluminium disc for counting. The chemical yield averaged 90 per cent., and the time required was 10 minutes for the redox steps and 5 minutes for the precipitation - filtration procedure.

ISOLATION OF BROMINE BY REDOX AND PRECIPITATION—

Subject the aqueous solution containing bromine to the following treatment. Add a 10-ml portion of carbon tetrachloride, 2 ml of concentrated nitric acid and powdered potassium permanganate, until the pink colour of permanganate is visible. When the organic phase becomes brown, separate it immediately. It was found that, for an instance when separation of the two phases was not achieved due to the excess of permanganate added, one or more mechanical decantations of the mixture from one separating funnel into another gave satisfactory separation of the two phases.

Subject the aqueous phase to two more identical oxidation and extraction processes by using 1 ml of concentrated nitric acid and 2 ml of 2 per cent. permanganate solution. Collect the organic phases and reduce them by using 10 ml of distilled water and 1 ml of M hydroxyl-ammonium chloride. Separate the aqueous and organic layers and repeat the same reduction step, using 0·5 ml of reducing agent, on the organic phase, which can then be discarded.

Gather the aqueous phases and subject them to the redox procedures mentioned above by using N sodium hydrogen sulphite.

Add a 1-ml portion of 6 N nitric acid to the collected aqueous solutions, and then heat so as to expel sulphur dioxide and carbon tetrachloride. Add a 4-ml portion of 0·1 N silver nitrate. Treat the silver bromide precipitate in the same way as the iodide and mount for counting. We found that the chemical yield averaged 70 per cent., and the time required was 15 minutes for the redox steps and 5 minutes for the precipitation - filtration procedure.

The standards were treated in an identical manner to the urine sample.

DETERMINATION OF RADIOACTIVITY—

Count the silver iodide precipitate immediately for 10 minutes on a 3×3-inch sodium iodide (Tl) crystal counter, connected with an Intertechnique 400-channel transistorised analyser, adjusted to count energies from 0 to 2 MeV. Mount a 3-mm thick Plexi-glass block on the crystal to cut off Bremsstrahlung. Print the 0·46-MeV photopeak area of the iodine-128 and compare it with that of the standard.

Count the silver bromide precipitate next day for 10 minutes under the conditions mentioned above. Print the 0·55-MeV photopeak area of the bromine-82 and compare it with that of the standard.

GAMMA SPECTROMETRIC EXAMINATION OF THE ISOLATED RADIO-ISOTOPES—

Gamma-ray spectrometry confirmed the absence of any γ-emitting radionuclides as contaminants in the isolated precipitates of iodine-128 and bromine-82 coming from the analysed urine sample.

DETERMINATION OF THE HALF-LIFE OF THE ISOLATED RADIO-ELEMENTS—

This was accomplished by plotting the decay curves for silver iodide and silver bromide precipitates. The half-lives were obtained from the slope of the straight line calculated by the method of the least squares. A value of 25 minutes with a standard error of $\pm 0·013$ minutes was found for iodine, and a value of 36·88 hours with a standard error of $\pm 0·21$ hours for bromine, compared with 24·99 minutes and 36·87 hours, respectively, reported in the literature.[27],[28]

RESULTS

Iodine and bromine quantitative results in urine were obtained for normal human individuals (male and female) of different ages. One special case is reported of a person

whose first urine sample indicated high iodine content in comparison with the content of other samples, and with those from the same person after a few days from the first sampling. This was due to the fact that the person concerned had sustained food poisoning and was being treated with Mexaform Ciba medicine containing iodochlorhydroxyquinoline. The values found were of the order of 10^{-7} mg ml^{-1} and 10^{-3} mg ml^{-1} for iodine and bromine, respectively. The results are shown in Table II.

TABLE II

CONCENTRATION OF IODINE AND BROMINE IN HUMAN URINE

Name	Age, in years	Sex	Iodine, in p.p.m.	Bromine, in p.p.m.	Remarks
G.A.S.	1	male	0·160	5·38	—
A.C.C.	2	female	0·085	5·82	—
A.G.V.	24	female	0·195	2·78	—
G.A.S.	28	female	0·225	4·31	—
P.C.C.	29	female	0·305	11·9	in pregnancy
A.G.S.	30	male	0·170	9·47	—
A.J.S.	45	male	0·165	8·29	—
S.G.S.	58	female	1·980	9·63	in medical cure
S.G.S.	58	female	0·150	2·41	—
C.A.K.	80	female	0·175	4·28	—

DISCUSSION

By the technique mentioned above iodine and bromine were simultaneously determined by one irradiation. From the values found it is confirmed that urine is enriched in iodine and bromine by a factor of about 2 with respect to blood.[29] The errors in iodine determination by titrimetric, colorimetric and catalytic classical chemical methods, mainly due to the impurity of the analytical reagents, are eliminated. The isolated precipitates of silver iodide and silver bromide showed remarkable radiochemical purity. The results were reproducible within a relative error of less than ±3 per cent., and the sensitivity reached values of 10^{-3} p.p.m. and 10^{-2} p.p.m. for iodine and bromine, respectively, for a neutron flux of 10^{12} n. cm^{-2} second^{-1} and an irradiation time of 15 minutes. This technique could be easily applied for precise investigation of different thyroid diseases due to its high sensitivity and good precision. It could also be applied to the simultaneous determination of these two halogens in almost every biological material.

The authors wish to express their thanks to A. P. Grimanis for helpful discussions. They also appreciate the valuable technical assistance of the N.R.C. "Democritus" nuclear-radio-chemical analysis group personnel.

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SHORT PAPERS

Micro Determination of Inorganic Phosphorus in Plasma

By B. B. BAUMINGER AND G. WALTERS

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SEVERAL methods are available for determining inorganic phosphorus in biological fluids. The macro method described by King and Wootton,[1] which is used in this laboratory, is satisfactory and has produced good results. The possibility was therefore considered of adapting this method to a micro scale, so reducing the amount of plasma required to one-tenth of that used hitherto, as the amount of blood usually available from an infant for a complete analysis is small.

The proposed method has been shown to produce reliable results for phosphorus in plasma of children, and for the last 8 months it has been extended to the routine determination of phosphorus in plasma and dilute urine of adults. Because of the similarity of the two methods, only a short account is given here to show the results of comparative determinations of phosphorus by the proposed micro method and by the macro method.

Table I shows the modification of the amounts of reagents used in the micro method as compared with those used hitherto. The concentration of the blue molybdophosphate was measured at 700 mμ in 4-cm micro cuvettes.

TABLE I

COMPARISON OF AMOUNTS FOR MACRO AND MICRO METHODS

	Marco method, ml	Micro method, ml
Volume of sample	1·0	0·1
Volume of de-mineralised water	—	1·0
Volume of trichloroacetic acid	9·0	3·0
Volume of filtrate	5·0	2·5
Volume of perchloric acid	0·4	0·2
Volume of ammonium molybdate ..	0·4	0·2
Volume of reducing agent	0·2	0·1

METHOD

APPARATUS—

A Unicam SP500 or SP600 spectrophotometer was used for optical-density measurements.

REAGENTS—

Prepare all solutions from analytical-grade reagents and de-mineralised water.

Trichloroacetic acid solution—Make an 11 per cent. w/v aqueous solution. If necessary, filter before use through a Whatman No. 42 filter-paper.

Reducing agent solution—Dissolve 0·2 per cent. of 1-amino-2-naphthol-4-sulphonic acid (purified) in an aqueous solution containing 12 per cent. w/v of sodium metabisulphite and 2·4 per cent. w/v of anhydrous sodium sulphite. Transfer the solution into a brown bottle and store it at 4° C. It is stable for 2 to 3 months.

Stock phosphorus standard solution (100 μg of phosphorus per 0·1 ml)—Dissolve 439·4 mg of potassium dihydrogen phosphate in fresh de-mineralised water and dilute to 100 ml. Add several drops of chloroform as preservative.

Dilute phosphorus standard solution (a) 4 μg and (b) 8 μg of phosphorus per 0·1 ml—Dilute the stock standard solution (a) 1 to 25 and (b) 1 to 12·5.

An accurate automatic delivery of some of the reagents was introduced.

PROCEDURE—

Into glass-stoppered tubes of 5 ml capacity place 1 ml of de-mineralised water, and wash in, by means of a micro pipette, 0·1 ml of unhaemolysed plasma, freshly separated, or 0·1 ml of de-mineralised water for the reagent blank, or 0·1 ml of dilute standard phosphorus solution (*a*) or (*b*). Add to each tube 3 ml of trichloracetic acid, stopper the tubes and mix the solutions well. Allow the tubes to stand at room temperature for 5 to 10 minutes for complete precipitation of the proteins.

Filter off the protein on to a dry 7-cm Whatman No. 42 filter-paper, and collect the filtrate in suitable tubes. Transfer 2·5 ml of the clear filtrate by pipette into small tubes, add 0·2 ml of perchloric acid and mix. Add 0·2 ml of ammonium molybdate and mix; finally, add 0·1 ml of reducing agent and mix well.

After 20 minutes measure the optical densities of the sample, A, the reagent blank, A_B, and the standard, A_{ST}, against water. Calculate the weight of phosphorus in mg per 100 ml of plasma from the formula: $4\ (A - A_B)/(A_{ST} - A_B)$ or $8\ (A - A_B)/(A_{ST} - A_B)$.

RESULTS

COMPARISON OF RESULTS—

In order to establish this micro method, it was compared with King and Wootton's macro method. The results in Table II show that the agreement was generally excellent.

TABLE II

COMPARISON OF MACRO AND PROPOSED MICRO METHODS FOR PHOSPHORUS IN
CONTROL SERUM AND HUMAN PLASMA

	Phosphorus in plasma by—		
Specimen	Macro method, mg per 100 ml	Micro method, mg per 100 ml	Difference
A	6·8	6·7	− 0·1
B	10·0	10·2	+0·2
C	7·7	7·8	+0·1
D	4·8	4·7	−0·1
E	4·2, 4·2, 4·2	4·3, 4·2, 4·2	+0·1, 0, 0
F	4·0	4·0	0
G	1·6, 1·7	1·7, 1·7	+0·1, 0
H	8·1, 8·0	7·9, 8·0	−0·2, 0
I	3·5	3·5	0
J	3·7	3·8	+0·1
K	3·3	3·4	+0·1
L	8·6	8·7	+0·1
M	5·2	5·2	0
N	2·5	2·6	+0·1
O	3·9, 3·8	3·8, 3·8	− 0·1, 0
P	4·2	4·4	+0·2
Q	6·1	6·1	0
R	10·8, 10·8	10·8, 10·7	0, −0·1

PRECISION—

The precision of the micro method was evaluated by determining the standard deviation with a freshly prepared control serum. For each set of tests, 10 sera, three standard phosphorus solutions and two reagent blanks were used. The standard deviation calculated on 20 duplicate determinations was ±0·05 mg of phosphorus per 100 ml.

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An Improved Iodine Determination Flask for Whole-bottle Titrations

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IT has long been recognised that volatilisation losses of iodine may result in significant analytical errors in iodimetry. Matsuyama[1] has pointed out that the volatilisation of iodine may be reduced by complexing with iodide to form the tri-iodide ion. This laboratory has been engaged in a study[2] of the Winkler titration and we have found that significant errors may result from volatilisation, even with tri-iodide complexing, when iodine aliquots are transferred from glass-stoppered bottles before titration with thiosulphate.
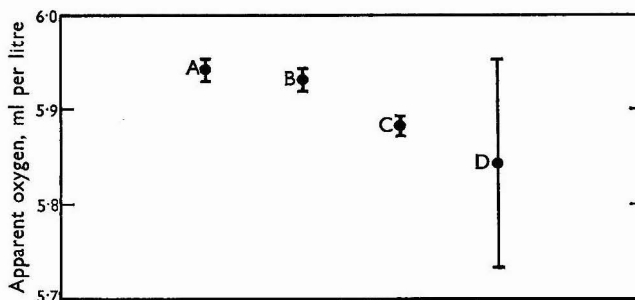

Fig. 1. Iodine volatilisation losses during sample transfers. Apparent oxygen concentration of identically equilibrated samples. Four replicate Winkler titrations performed on each group; means and extremes indicated.

A, whole flask titrated;

B, gently poured into the Erlenmeyer flask;

C, gently poured into Erlenmeyer flask and then back into the flask;

D, 100 ml aliquot was transferred, with a pipette, in an Erlenmeyer flask

Fig. 1 shows the variability of the apparent dissolved oxygen concentration (assumed to be proportional to the iodine concentration in the Winkler titration) of identically equilibrated samples of distilled water. Each point represents the mean of a set of four replicate determinations. It is seen that the iodine concentration found decreases with each transfer step. In the case where aliquots were removed from the sample bottle by using a pipette, it was found that the result was highly variable, possibly a consequence of the variable partial vacuum over the solution during the pipetting step. This conclusion is of some consequence to oceanographic analysts as, in the current Winkler procedure most widely used aboard ship, iodine aliquots are transferred by pipette prior to titration.

Recently Carpenter,[3] in discussing the Winkler titration, has recommended whole-bottle titrations in which volatilisation losses are reduced by elimination of the transfer step. His method requires that concentrated thiosulphate reagent be delivered by micro burette so that only 1 ml of titrant is added and titrations may be performed in the glass-stoppered sample bottle. An alternative method is herein suggested which has been used successfully in this laboratory. It consists in using easily constructed sample bottles (Fig. 2) in which the stoppers displace enough volume such that, when the stoppers are removed, titrations of the entire contents of the flask may be performed without transfer.

CONSTRUCTION

The sample bottle consists, in part, of the ordinary 250-ml Erlenmeyer-shape iodine determination flask available commercially (Corning No. 5400) with a standard taper 22 solid glass
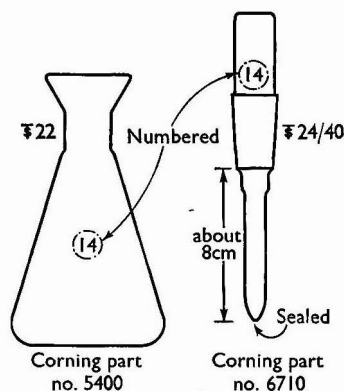
Fig. 2. An improved iodine determination flask for whole-bottle titrations.

stopper. The solid glass stoppers are discarded, and substituted with long nipples blown from standard taper 24/40 full length inner joints with sealed tube (Corning No. 6710). The closed, sealed tube which projects into the flask to within 3 cm from the bottom displaces a volume of about 10 c.c. Bottles and stoppers are engraved with numbers to avoid mismatching when the flasks are calibrated "to contain" by weighing. Repeated fillings and weighings give a precision of better than 0·01 per cent. No attempt was made to make the flask volumes identical. Made as described, the 16 stoppered flasks made in this laboratory contained, on the average, 242 ml with a range of ±4·4 ml. The variation in flask volume removes any unconscious bias on the part of the operator to try to get the same result on replication.

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An Automatic Determination of Thoria in Thoria-Urania Mixtures

By W. A. STUART

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DURING the course of studies into the mixing of solids for nuclear fuel elements, it was necessary to analyse a large number of samples of thoria-urania for their thoria content. The method used should be quick and accurate enough not to contribute significantly to the apparent mixing errors. The chosen method is based on a modification of the manual method of Clinch[1] using thoron [1-(*o*-arsono-phenyl-azo)-2-naphthol-3,6-disulphonic acid sodium salt]. The apparatus used is the Technicon AutoAnalyzer which has been fully described elsewhere.[2,3]

Samples are dissolved in nitric acid with the addition of a small amount of ammonium fluoride to attack the thoria. The solution is made up to a standard volume with distilled water, and an aliquot is placed on the sampler of the AutoAnalyzer. Since fluoride interferes with the colour formation it is necessary to add aluminium to complex it. Also, uranium gives a small, positive interference, which is allowed for by making up standards for the two mixing ratios of urania to thoria used, namely 10 to 1 and 100 to 1.
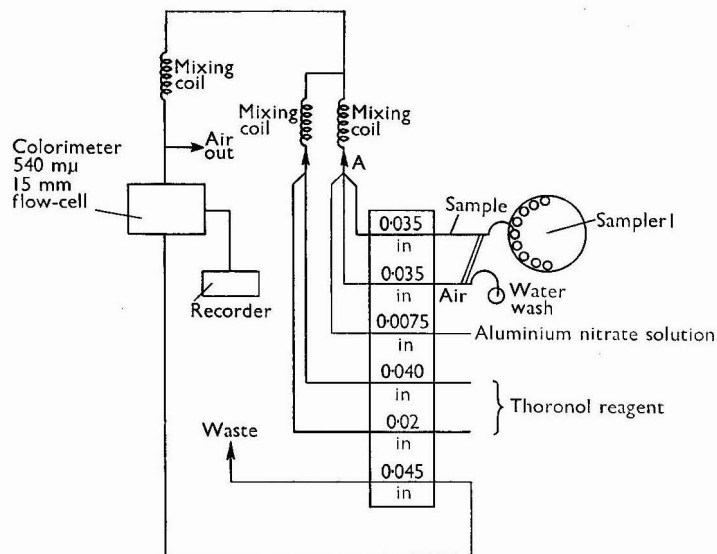
Fig. 1. Flow diagram of the Auto-Analyzer as used in the automatic method for determining thorium. All pump tubing is Tygon, and internal diameters are quoted

EXPERIMENTAL

The flow scheme is shown in Fig. 1. The thoron reagent is highly coloured and, in the absence of a diluting effect due to the sample volume, gives an optical density of about 0·3 with respect to the blank reading obtained with water as diluent. For this reason it is necessary to use air and samples lines of the same size, and to use a "double crook" sample device such that, when the sample line is out of the sample, then the air line is in water. Also, if there is a time when air only enters both lines, an extraneous peak, "an air blip" will be recorded, so the sample and air lines are made the same length from the junction A to their inlet ends (Fig. 1). The sampler rate is 40 per hour, but half the positions are occupied by water to give additional washing between samples.

REAGENTS—

Thoron reagent—Mix 600 ml of 0·1 per cent. w/v aqueous solution of thoron with 200 ml of 10 per cent. w/v aqueous solution of hydroxylammonium chloride, add 80 ml of concentrated hydrochloric acid and make up to 2 litres with distilled water.

Aluminium nitrate solution—Dissolve 100 g of aluminium nitrate, $Al(NO_3)_3.9H_2O$, in distilled water and make up to 1 litre.

Ammonium fluoride solution—Dissolve 0·4 g of ammonium fluoride in distilled water and make up to 0·5 litre.

PROCEDURE—

Dissolve samples weighing up to 100 mg by adding 0·25 ml of ammonium fluoride solution and 2·5 ml of concentrated nitric acid. Heat on a hot-plate until all the thoria has dissolved and no more brown fumes are evolved. After cooling, dilute the solutions in calibrated flasks with distilled water to give a thoria content in the range of 15 to 40 μg ml^{-1}.

Make standard solutions by taking accurately weighed aliquots of thoria and urania in the correct ratio and treating as samples.

Prepare a calibration curve and check it at five points every 15 samples. This frequency of checking is not necessary, but it gave a convenient proof of the reliability of the method at all times. In fact, the same calibration curves were obtained over a period of about 6 months.

DISCUSSION

The reproducibility of the AutoAnalyzer method for one solution was found by feeding 10 aliquots through the system. Table I shows some results.

TABLE I

Method		Ratio of urania to thoria	Mean concentration of thoria, μg ml^{-1}	Coefficient of variation, per cent.
AutoAnalyzer	..	10:1	9·75	0·70
		100:1	16·39	0·52
Manual	.. ..	100:1	660	0·29

These coefficients of variation are acceptable, as the best mixes at present show a coefficient of variation greater than 2 per cent. for ten samples.

The automatic method is capable of producing 20 analyses per hour, and even with a large number of standards is much quicker than the manual method.

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A Rapid Method for Determining the Moisture Content of Gelatin and Animal Glue

By R. T. JONES

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THE British Standard methods[1,2] for determining the moisture contents of gelatins or animal glues require a drying time of 18 ± 1 hours. The reproducibility of the methods, and also the relation to shorter drying times have been examined.[3] It is not possible to determine the absolute moisture content, but the British Standard methods define a set of conditions that gives sufficiently meaningful and reproducible results for most purposes. For many research and commercial applications, however, the time required is too long. This paper describes the investigation of a rapid infrared-heating method of moisture determination.

The temperature and time of drying were varied for several gelatins and glues to determine a suitable set of conditions. The latter were then kept fixed, and moisture determinations for several additional gelatins were carried out to test for reproducibility, and also the effect of gelatin and glue-grist size.

METHOD

APPARATUS—

This consists of an Ediswan 250 watt, 200 to 250-volt infrared lamp, clamped vertically above the centre of a metal pot, 9 inches in diameter, 0·1 inch thick and 5·5 inches high. (These are the dimensions of the vessel that we used but they are not expected to be critical.) The pot has a hole bored in the side at such a height as to enable a thermometer (with its bulb blackened with carbon black), which passes through a rubber bung fitted in the hole, to rest just above the surface of an inverted moisture dish at the centre of the base of the pot. The lamp is connected in series with a Variac transformer (reading to 260 volt) to the mains. This arrangement makes it possible for the temperature above the surface of the inverted dish to be varied by adjusting the Variac without having to alter the position of the lamp. (In the absence of the Variac, calibration of the apparatus can be performed by adjusting the position of the lamp.) In the arrangement used, the lamp remains fixed with its lowest surface at a distance of 8·5 inches above the surface of the inverted moisture dish.

PROCEDURE—

Prepare samples for the moisture determination in exactly the same way as in the British Standard method except for dissolving the gelatin or glue prior to drying. Weigh about 1 g of the sample into a tared stainless-steel dish fitted with an aluminium cover. Introduce 10 ml of distilled water and allow the sample to soak. Switch on the lamp and allow about 1 hour for the system to warm up. To test that reproducible conditions have been achieved, move the thermometer until the bulb rests just above the centre of the inverted dish, and read the temperature after allowing several minutes for equilibrium to be attained. (The temperature normally

varies by $\pm 1°$ C.) Note the Variac reading and then withdraw the thermometer and place the moisture dish containing the sample in position. Adjust the position of the thermometer so that it is just not touching the outside of the dish. Initially, the temperature is lower than indicated in Table I, but when most of the moisture has been removed (after about 45 minutes) the temperature reading remains constant to approximately $\pm 1°$ C. The relationship between the temperature at the centre of the dish and outside the dish is shown in Table I. (Each time the temperature difference is 8° C.)

ESTABLISHMENT AND EXAMINATION OF FIXED CONDITIONS—

Determinations of moisture values for gelatins A and B, and glue C were carried out at different temperatures and for different testing periods to decide upon a set of conditions that would give the best correlation, in a short time, with values obtained by the British Standard method. Results are shown in Table I.

The recommended procedural conditions adopted were—

Temperature outside the dish	=	110° C
Temperature at the middle of the dish	=	118° C
Variac reading	=	232 volts
Time	=	2 hours

With the recommended procedure, moisture determinations for samples A, B and C and also for gelatins D and E (very fine grist size) and for gelatin F (very large grist size) were carried out. The results were compared with those obtained by the British Standard method and are shown in Table II.

RESULTS

TABLE I

EFFECT ON MOISTURE VALUES OF VARYING TEMPERATURE AND TIME

Gelatin or glue	Temperature reading, °C		Variac reading, volts	Time, hours	Moisture values	
	Outside the dish	Middle of the dish			Infrared method	British Standard method‡
A	105	113	215	2	14·1	14·4
				2·5	14·3	
				3	14·4	
				3·5	14·5	
				4	14·5	
	110	118	232	*2	†14·3	
				2·5	14·7	
				3	14·7	
				3·5	14·7	
	115	123	240	1·5	14·6	
				2	14·6	
				2·5	14·9	
B	105	113	215	3	13·07	13·5
				3·5	13·5	
	110	118	232	1·5	13·2	
				*2	13·4	
				3	13·4	
C	105	113	215	2	13·4 ⎫	14·0
				2	13·6 ⎬ 13·6	
				2	13·6 ⎪	
				2	13·9 ⎭	
	110	118	232	*2	14·0 ⎫	
				2	13·9 ⎬ 13·9	
				2	13·8 ⎪	
				2	†13·9 ⎭	
	115	123	240	2	14·2 ⎫	
				2	14·3 ⎬ 14·3	
				2	14·4 ⎪	
				2	14·3 ⎭	

* Recommended procedure.
† Determinations carried out on different occasions.
‡ Mean of five determinations. (The reproducibility of this British Standard method is recorded elsewhere.[3])

TABLE II

MOISTURE VALUES DETERMINED WITH RECOMMENDED PROCEDURE

Gelatin or glue	Moisture values		$\dfrac{\text{Mean infrared value}}{\text{British Standard value}} \times 100$ per cent.
	Infrared method	British Standard method*	
A	14·3 14·4 14·6	14·4	100
B	13·6 †13·7 13·7 13·5	13·5	100·7
C	14·0 13·9 13·8 †13·9	14·0	99·3
D	13·3 13·9 13·8	13·7	100·7
E	†11·6 11·2 11·5 11·8	11·3	101·8
F	†13·3 13·4 13·5 13·5	13·9	96·4

* Mean of five determinations. (The reproducibility of this British Standard method is recorded elsewhere.[3])

† Determinations carried out on different occasions.

DISCUSSION

Similar moisture values obtained for the same gelatin or glue on the same, and on different occasions, show the new infrared method to be reproducible and capable of giving values comparable to those obtained by the British Standard procedure. Agreement with the British Standard method was not quite as good with one sample of very large grist size, a slightly low value being obtained. This discrepancy is probably due to omission, for the sake of speed, of the dissolving-up stage in which a water-bath is used before drying. It is still sufficiently accurate for most purposes where a moisture value is urgently required.

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A Simplified Method for Determining Copper Compounds Present on Leaf Surfaces

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AN accurate, yet relatively simple, technique for determining 0 to 1000 μg of copper on foliage was required for use by unskilled personnel in the field.

The colorimetric method based on the use of bis-cyclohexanone oxalyldihydrazone, described by Martin,[1] is ideal, providing that the final pH of the solution is controlled within the range required for full colour development.[2] The method described was found to be more reliable than the modification used by Martin[3] and, in obviating the dropwise adjustment of pH, it was more satisfactory for unskilled usage. Wetting agent, Lissapol NDB, was added to the acid extracting

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solution and automatic pipettes were used to dispense all reagents to aid determinations in the field. All reagent solutions were prepared in the laboratory with de-ionised water.

METHOD

REAGENTS—

Acid extracting solution, approximately N sulphuric acid—Dilute 14 ml of concentrated sulphuric acid to 500 ml; 0·125 per cent. by volume. Add wetting agent to the solution.

Ammoniacal ammonium citrate—Dissolve 5 g of ammonium citrate and 60 ml of 0·880 ammonia in 500 ml of water. Adjust the strength so that 5 ml of this solution will neutralise 10 ml of the acid extracting solution, with neutral red as indicator.

Borate buffer solution—Dissolve 12·4 g of boric acid in 400 ml of water. Add to this a solution of 1·2 g sodium hydroxide in 60 ml of water.

Bis-cyclohexanone oxalyldihydrazone reagent—Make a 0·1 per cent. solution by dissolving the solid in a 50 + 50 ethanol and water mixture and warming. Store in a dark bottle and renew fortnightly. Dilute the reagent (1 + 1·5) with water for use.

PROCEDURE—

A. *For 0 to 50-μg amounts of copper*—To the leaf sample add 10 ml of acid extracting solution. Allow the solution to stand for 15 minutes, swirling it every 5 minutes. Add to the solution 5 ml of ammoniacal ammonium citrate, 5 ml of borate buffer and 10 ml of bis-cyclohexanone reagent. Again allow the solution to stand for 15 minutes, swirling it every 5 minutes. Measure the optical density at 595 mμ.

B. *For amounts of copper greater than 50 μg*—Add 10 ml of acid extracting solution to the leaf sample, leave for 15 minutes as before. Take 1 ml of this liquid, add to it 10 ml of acid extracting solution and 5, 5 and 10 ml of other reagents as detailed before. Complete the determination as described above. Multiply the copper value by 10 to obtain the total copper in the original sample. Solutions that are just beyond the calibrated range of the instrument may be diluted with 25 ml of copper-free water and 5 ml of borate buffer solution. Multiply the copper value by 2 or 20, as appropriate, to obtain the total copper in the original sample.

Over one hundred determinations of copper, made with standard copper solutions during the development and testing of this procedure, gave completely reproducible colours. Inexperienced operators found no difficulty in using this method; satisfactory readings were obtained with standard copper solutions in the laboratory and field, and determination of copper fungicide on foliage were completed in the field.

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Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

Spectral Characteristics of Eugenol

THE Analytical Methods Committee has received the following Report from its Essential Oils Sub-Committee of an investigation carried out on the Demetrius and Sinsheimer method.[1] The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Essential Oils Sub-Committee responsible for the preparation of this Report was: Dr. G. W. Ferguson (Chairman), Mr. A. J. M. Bailey, Mr. H. E. Brookes, Dr. K. Field, Mr. D. Holness, Mr. H. T. Islip, Dr. T. L. Parkinson, Miss H. M. Perry, Dr. G. B. Pickering, Mr. J. H. Seager, Mr. S. G. E. Stevens and Dr. B. D. Sully.

INTRODUCTION—

The determination of phenols in essential oils has, for many years, been carried out by a method based on the solubility of the phenols in an excess of aqueous alkali.[2] A process of this nature must, however, be somewhat inaccurate, since it makes no allowance for non-phenolic materials that may be soluble in the alkali, nor does it take into account the effect of the possible alkaline hydrolysis of esters producing other alkali-soluble fractions.

Demetrius and Sinsheimer[1] have published a more specific method for the determination of eugenol, based on the difference between the extinction coefficients, at a specified wavelength, of eugenol in alkaline and in acid solution. The method has been studied by the Sub-Committee and the results of collaborative tests are given in this Report.

During the investigation it was found that polythene containers were unsuitable for the storage of solutions for use in spectroscopy owing to the extraction of light absorbing substances from the polythene.

EXPERIMENTAL—

A sample of pure eugenol was examined in seven laboratories by the method of Demetrius and Sinsheimer. The measurements of the wavelength of maximum absorption ($\lambda_{max.}$) and the differences between the extinction coefficients in alkaline and acid solution are shown in Table I. There was general agreement that the wavelength of maximum absorption was 297 mμ (Demetrius and Sinsheimer gave 296 mμ), but the variations in the differential absorption coefficients were unexpected. It was found, however, that these were due to variations between the instruments and could be largely eliminated by calibration with potassium chromate solution[3] as a standard, and adopting the procedure of cell interchange. Members reported that the differences between "matched cells" were negligible.

TABLE I

EXPERIMENTAL VALUES FOR THE WAVELENGTH OF MAXIMUM ABSORPTION AND THE DIFFERENTIAL EXTINCTION COEFFICIENT

Laboratory..		A	B	C	D	E	G	H	Mean
$\lambda_{max.}$ mμ ..	..	297	297·5	297	296·5	297	297	297	297
		297	297·2	297	297	297·5	297	297	
$\Delta\epsilon$ (uncorrected) ..		4065	—	4095	3840	4013	—	3930	
		4025		4080	3970			3996	
								3996	

Re-determination of the differential extinction coefficient after calibration in this way gave the results shown in Table II, derived from a second sample of eugenol shown to be pure by gas-chromatographic and electrometric-titration methods. It was observed that for a single operator using a single spectrophotometer the reproducibility was particularly good (less than 1 per cent. variation), and Table II therefore includes only the average values for each series of determinations.

TABLE I

DETERMINATION OF THE DIFFERENTIAL MOLAR ABSORPTIVITY OF EUGENOL AT 297 mμ

Laboratory ..	..	B	D	E	F	G	H*	Mean
$\Delta\epsilon$ (corrected)	..	3946	3996	4020	3970	3938	3990	3979
							3991	
							3978	
							3988	
							3957	
							3994	

* The six individual figures represent the average values for different instruments.

The mean value for the corrected differential molar absorptivity as determined in six laboratories with a total of eleven different spectrophotometers of the manual and recording types was 3979 with a variation from the mean of ± 1 per cent.

RECOMMENDATION—

Demetrius and Sinsheimer reported that eugenol had a maximum absorption at 296 mμ and a differential molar absorptivity at the maximum of 3886.

In the opinion of the Sub-Committee the physical characteristics of eugenol should be amended to include the following:

"Eugenol, when examined by the Demetrius and Sinsheimer method, shows a maximum absorption at 297 mμ, and at that wavelength has a differential molar absorptivity in 0·1 N acid and 0·1 N alkali of 3979."

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Communications

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Manuscripts must not exceed 300 words; rapidity of publication precludes the use of diagrams, but tables or formulae may be included if the length of text is reduced appropriately. Communications should not be simple claims for priority. This facility for rapid publication is intended for brief descriptions of work that has made some progress and is likely to be valuable to workers faced with similar problems. A fuller original paper may be offered subsequently, if justified by later work.

Manuscripts are *not* subjected to the usual examination by referees. Inclusion of a Communication is at the Editor's discretion; a manuscript not accepted as a Communication may, if the author wishes, be submitted to the Editorial Committee as a possible Short Paper and subjected to the usual scrutiny by referees.

THE DETERMINATION OF FLUORINE BY NEUTRON ACTIVATION

WHEN rapid, repetitive analyses are required, physical methods are usually adopted, as these are readily available for a large number of elements, including most of the metals and many of the non-metals in the periodic table of elements. One notable exception is fluorine. For this we now suggest neutron activation as a means of easily and rapidly examining large numbers of mill products containing fluorite.

TABLE I

NEUTRON-INDUCED REACTIONS OF FLUORINE

Reaction	Product	
	Half-life	$E\gamma$, MeV
^{19}F (n,γ) ^{20}F	10 seconds	1·63
^{19}F (n,2n) ^{18}F*	112 minutes	(0·51)
^{19}F (n,p) ^{19}O	29·4 seconds	0·2 and 1·37
^{19}F (n,α) ^{16}N	7·2 seconds	5 to 7

* Positron emitter.

Four separate neutron-induced reactions (Table I) can be used to determine fluorine. The (n,γ) reaction occurs with neutrons of thermal energies, for which ready access to a nuclear reactor is at present required. This is not practical for industrial use. The (n,2n) reaction produces fluorine-18, a positron emitter which, although it has been used to determine fluorine, may be subject to interference from other positron emitters. The long half-life is also a disadvantage. The (n,p) reaction, giving oxygen-19, is clearly a better choice where a fast-neutron generator is available. An expensive high-resolution multi-channel γ-spectrometer is required to isolate the 0·2 or 1·37 MeV activity.

The (n,α) reaction gives nitrogen-16, which can conveniently be measured with a single-channel spectrometer. Oxygen interferes by giving nitrogen-16 in a (n,p) reaction when a 14-MeV neutron generator is used for the irradiation. However, the threshold value of neutron energy for this latter reaction to occur is about 10 MeV, whilst that for the ^{19}F (n,α) ^{16}N reaction is only about 2 MeV. The interference from oxygen can therefore be avoided by using neutrons of intermediate energy. To demonstrate this we have used an available thorium - beryllium radio-isotope source of 1·5 curies, giving approximately $2·8 \times 10^7$ neutrons (of 3 to 5 MeV) per second. Other isotope sources, such as americium - beryllium, of even higher neutron output, would be preferred.

Samples (75 g) of fluorite ores and concentrates were weighed into specially designed polythene containers, irradiated for 35 seconds and counted for 30 seconds. The analysing system comprised a thallium-activated sodium iodide crystal, photomultiplier and single-channel γ-spectrometer set with a discriminator bias to count only the high-energy activity from nitrogen-16. The fluorine contents were calculated by using a sample of pure crystalline fluorite, ground to pass through

a 70-mesh sieve, as the reference material. The results are shown in Table II in comparison with results obtained with a pyrohydrolysis technique.

TABLE II

SOME FLUORINE RESULTS

	Fluorine found	
Sample material	by neutron activation, %	by pyrohydrolysis, %
Fluorite concentrate 1	44·5	44·7
Fluorite concentrate 2	45·2	44·3
Fluorite head ore	19·5	19·6
Fluorite flotation product 1 ..	15·8	15·7
Fluorite flotation product 2 ..	10·0	10·0
Fluorite tailing	5·2	5·2

Further work on this system will include the examination of products from a commercial fluorite mill and a short field trial in a fluorite-producing area.

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Book Reviews

STATIONARY PHASE IN PAPER AND THIN-LAYER CHROMATOGRAPHY. Proceedings of the Second Symposium held at Liblice, June 10th–12th, 1964. Edited by K. MACEK and I. M. HAIS. Pp. 358. Amsterdam, London and New York: Elsevier Publishing Company. 1965. Price 85s.

This volume, edited by two internationally prominent chromatographic workers, records the proceedings of the Second International Symposium on Chromatography held in Czechoslovakia. This symposium dealt exclusively with Paper Chromatography and Thin-layer Chromatography. The book follows the pattern of the proceedings in being divided into 5 major sections, each of which comprises a review paper that summarises the present state of knowledge of a particular field, and a series of original papers and their related discussions. Approximately half of the 50 or so papers are in German, the rest are in English.

As is usual at such symposia, the introductory papers do not generally do more than collect and collate work that has been previously published. These papers are of such quality, that both the new research worker starting on a problem, or an established research worker needing background information relevant to his own problem, will find an excellent source of information. It would be invidious to separate any one paper or section as being superior to the rest, as each part is a well presented contribution to chromatography. The photographs and line drawings of actual separations are very useful in showing the separation obtained, R_F values alone do not always enable a worker to obtain a fair comparison of his results with those published by the original author.

It is often helpful to be able to relate work done on paper to that done on the newer substrates, and it is possible to obtain much information of this type from a perusal of the book and the excellent indexes provided. The wealth of results obtained with substrates and solvent systems other than cellulose and the more common solvents, is a good indication of the potentialities of thin-layer techniques.

When reviewing the corresponding volume dealing with the First International Symposium it was stated that this volume should surely occupy a place on the bookshelves of all chemists working on chromatographic research and development. This remark applies equally to the present volume, which is a worthy successor, and the editors are again to be congratulated on their work.

L. S. BARK

PROGRESS IN CHEMICAL TOXICOLOGY. Volume 2. Edited by ABRAHAM STOLMAN. Pp. x + 416. New York and London: Academic Press. 1965. Price 100s.

This volume is the second of a series concerned primarily with the analytical aspects of forensic toxicology, but which will also be of interest to those working in allied fields. Nearly one-half of this volume is devoted to recent studies of the absorption, metabolism and excretion of chemicals by the body, and covers the period since the last edition of Professor R. T. Williams' "Detoxication Mechanisms." Although most of the results quoted have been obtained with animals and not with man, this chapter merits the careful attention of the forensic toxicologist, who in the past appears to have limited his attentions to a search for the substance suspected to have been administered. While such a procedure may be appropriate in the analysis of the contents of the gastro-intestinal tract, in the examination of the tissues and excreta it may be more profitable to select a method capable of revealing a major metabolite. It is to be hoped that a later volume in this series will include a scheme for analysing biological specimens for these metabolites, which frequently are conjugated to yield water-soluble derivatives, not readily extractable by the usual procedures.

The analysis of expired air is now a well-known means of determining blood-alcohol concentration, and a chapter on the application of infrared spectrometry to air analysis suggests that this technique may be extended to a wide range of volatile liquids and gases. However, with only a few substances has an attempt been made to establish the relation between the concentration in breath and the likelihood of intoxication, and usually such measurements are limited to a qualitative demonstration of absorption. The section of this chapter dealing with the determination of volatile substances in tissues, makes no reference to those procedures that take advantage of the volatility, as in the determination of blood alcohol by equilibrating the sample with air, which is then analysed by gas chromatography or other means.

The need for rapid diagnostic methods in cases of suspected poisoning is the basis of 2 chapters; one presents a scheme for the determination of basic drugs in biological specimens, though it is limited to those substances that are readily extractable, by ether, from alkaline solution, and which can be steam distilled. Another chapter describes reversed-phase chromatography at elevated temperatures, on paper impregnated with triglycerides, and chromatography and electrophoresis on ion-exchange paper.

A review of sample preparation before spectrographic analysis provides a useful summary of methods for the destruction of organic matter, and for the purification and concentration of the extract prior to trace-metal analysis, but such refined procedures can rarely be necessary in cases of suspected metal poisoning; they seem to be more appropriate for the determination of normal levels in tissues. The value of the final chapter on the application of thin-layer chromatography in toxicology is not in the brief outline of the technique, which could surely be omitted in a volume of this nature, but in the tables listing R_F values of groups of substances of toxicological interest, obtained under defined conditions.   J. C. GAGE

SPECTROSCOPY IN EDUCATION. Volume 2. SPECTROSCOPIC TECHNIQUES IN ORGANIC CHEMISTRY. By A. J. BAKER, B.Sc., Ph.D., and T. CAIRNS, B.Sc. Pp. v + 87 leaves. London: Heyden & Son Ltd. 1965. Price 21s.; $3.50.

This spiral-bound book is Volume 2 of a "Spectroscopy in Education" series, of which the titles of the first four volumes have been announced. The present work deals with infrared spectroscopy (pages 1 to 35), nuclear magnetic resonance spectroscopy (pages 35 to 51), mass spectrometry (pages 52 to 62) and ultraviolet spectroscopy (pages 63 to 85). The stated intention is to provide concise introductions to the basic theory, experimental methods and interpretative procedures for these three branches of molecular spectroscopy proper and for mass spectrometry. The principal application is directed towards the determination of the structure of organic compounds. The book is designed for use in practical classes by organic chemists, to enable them to interpret results obtained on compounds of unknown structure. The interpretative sections are accompanied by examples of their application to actual spectra.

Only for the section on infrared spectroscopy has this ambitious intention been realised to any extent. The 1-page introduction is too brief to be of any value, but the following 12 experiments cover many useful matters of practice, including a simple 3-component analysis and an experiment on hydrogen-bonding. The correlation charts for a few important group frequencies clearly show the changes produced by progressive substitution in a way that emphasises the structural implications.

Apart from the inadequate "Introduction," the chapter on ultraviolet spectroscopy deals with diene, polyene, poly-yne, en-yne, carbonyl, enone and $\alpha\beta$-unsaturated carboxyl systems in some detail, with emphasis on the calculation of $\lambda_{max.}$ for various types of substitution by use of the Woodward and similar rules. The discussion of aromatic and heterocyclic chromophores is too brief to be of much value, and the 6 spectra discussed in detail are mainly concerned with ene-one and poly-yne structures.

In the chapter on nuclear resonance spectroscopy, the short theoretical introduction is not always clear and the tables of τ values are not used directly in the discussion of the 8 rather complex n.m.r. spectra selected for comment. Both the τ and δ conventions for chemical shift are used in this section.

The chapter on mass spectrometry leans heavily on Biemann's summary of fragmentation processes, and the 6 spectra discussed in detail illustrate the occurrence of these processes.

The authors' aims have been achieved with some degree of success in the chapter on infrared spectra and, to a lesser extent, in that on ultraviolet spectra, but the book as a whole, in its present form, is not a satisfactory introductory manual for the student of organic chemistry.

G. H. Beaven

RESEARCH IN MOLECULAR SPECTROSCOPY. Edited by Academician D. V. SKOBEL'TSYN. Pp. viii + 205. New York: Consultants Bureau. 1965.

Authorised translation from the Russian; originally published as Volume 27 of Proceedings of the P.N. Lebedev Physics Institute.

This book is a critical review of the instrumental and the more general experimental aspects of vibrational spectroscopy. There are 4 sections to the work. The first, by G. G. Petrash, is concerned with instrumental errors and their correction in infrared spectroscopy, covering the problems of resolving power and absolute absorption intensities, and the use of computers for error reduction. The second, by A. I. Sokolovksya, reports on experimental methods of studying the effect of temperature on the polarisation, intensity and shape of Raman bands of vapours and liquids. In the third section, A. V. Rahov reviews the effects of molecular rotation on the parameters characterising infrared and Raman bands, and instrumental methods of investigating these effects. The fourth section, by G. V. Mikhailov, gives an account of the effect of pressure on the structure and the width of the rotational and vibrational Raman spectra of gases, and of the apparatus used in studies of the effects. The book is well-documented, and it contains a bibliography of papers on spectroscopy by members of the Lebedev Institute from 1934 to 1962. Research workers in the field of vibrational spectroscopy should find this volume useful, particularly those concerned with instrumentation or with environmental effects on infrared and Raman spectra.

S. F. Mason

GAS CHROMATOGRAPHY OF METAL CHELATES. By Ross W. MOSHIER and ROBERT E. SIEVERS Pp. viii + 163. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1965. Price 35s.

The determination of metals based on the formation of metal chelates and their subsequent examination by gas chromatography is probably the most significant advance in inorganic chemistry over the past decade.

Gas chromatography is usually regarded as being the province of the organic chemist, because it is essentially a process for separating and analysing volatile mixtures, and until comparatively recently the technique had not made any significant incursions into the field of inorganic analysis.

It might have been postulated that some classes of inorganic compounds would be amenable to separation by gas chromatography, *e.g.*, the chlorides of metals such as antimony, niobium, tantalum, tin and titanium are relatively volatile, and a restricted range of certain classes of organo-metallic compounds received early attention in an extension of gas chromatography to inorganic analysis.

In general, however, determinations based on the use of these compounds have serious limitations, because they are usually difficult to prepare, and many are prone to hydrolysis.

To apply gas chromatography more successfully to the analysis of inorganic constituents, it is desirable that a wide range of elements should react readily and quantitatively with a single reagent, and in this respect the metal chelates have outstanding advantages.

Gas-chromatographic analysis, following chelate formation, is of special interest to the analyst with problems in the inorganic field, and the authors have made a detailed appraisal of the use

of the most useful chelating agents for this purpose. Comment on the use of other volatile inorganic compounds is also adequately covered, with the object of providing a complete realistic assessment of the value of gas chromatography in inorganic analysis and the study of co-ordination compounds.

Sensitivity, speed and ease of separating complex mixtures are features of gas chromatography that make it particularly attractive, and its potential in the determination of trace constituents in the inorganic field is impressive.

Conditions for the successful elution, separation and the obtaining of sharp symmetrical gas-chromatographic peaks are described. Many illustrations are given, and factors affecting the volatility of metal chelates, and methods for the preparation of metal acetylacetonates and their fluorinated derivatives are also discussed. Qualitative and quantitative analysis, purification, separation of isomers and the study of kinetics and equilibria of co-ordination compounds receive special attention.

The general editors of this series are to be complimented for selecting this timely topic for inclusion in their "International Series of Monographs," and the authors equally praised for making a readable, comprehensive account of the subject available at a reasonable price. This book is strongly recommended to practical analysts, graduate students and teachers, and, indeed, to all who seek to improve their knowledge of the subject or wish to assess the potentialities of what is unquestionably a useful adjunct to the analyst's working tools.   W. T. ELWELL

LECTURES ON GAS CHROMATOGRAPHY, 1964: AGRICULTURAL AND BIOLOGICAL APPLICATIONS.
    Edited by L. R. MATTICK and H. A. SZYMANSKI. Pp. viii + 256. New York: Plenum
    Press. 1965. Price $12.50.

    *Based on papers presented at the 1964 Cornell University Pesticide Workshop and the 1964
    Canisius College Gas Chromatography Institute.*

The modern practice of publishing, in book form, the papers presented at symposia and conferences has been criticised strongly by the majority of reviewers in these columns. The present book does nothing but strengthen the hand of the critics, despite the attempts of the editors to widen its appeal by selecting "the most significant lectures" from two conferences and supplementing them by several papers "solicited specifically for the purpose of rounding out the presentation." The two conferences were the Sixth Annual Gas Chromatography Institute held at Canisius College, Buffalo, New York, from March 30th to April 3rd, 1964, and the Pesticide Residue Analysis Workshop presented by the New York State Agricultural Experiment Station, Cornell University, Geneva, New York, from April 20th to 23rd, 1964.

The book contains 16 papers. Of these, 6 are presented by representatives of instrument manufacturers and in general, while they would be interesting enough to the analyst who is new to the field, they contain the type of information that is dispensed, free of charge, in the brochures and house magazines of the instrument companies. Two more papers by H. R. Felton, who is the only industrial representative, deal with preparative-scale gas chromatography and chromatogram trouble shooting without attempting to include the special problems of the field covered by the sub-title of the book and therefore giving the impression that they have been shoved in as make-weights. A further 6 papers emanate from the Southwest Research Institute, San Antonio, Texas (with various permutations of 6 authors); 3 of these deal in a general way with instruments, detector systems and so on, and the other 3 with more specific applications, pesticides in water, collection of fruit volatiles and chlorpromazine, its metabolites and related compounds. These latter 3 papers, together with the paper by Lisk and Mattick on the determination of insensitive pesticide residues by the formation of derivatives, are probably the most useful and valuable from the point of view of the practising analyst. The sixteenth paper by Albers and Fahri is an excellent short review of the analysis of blood gases by gas chromatography.

The purpose of publishing a book like this escapes me. Much of the information it contains is already well known or easily accessible and, of the rest, it seems that publication in a recognised journal would have offered a better means of reaching the right audience.   G. E. PENKETH

SEMIMICRO QUALITATIVE ORGANIC ANALYSIS: THE SYSTEMATIC IDENTIFICATION OF ORGANIC
    COMPOUNDS. By NICHOLAS D. CHERONIS, JOHN B. ENTRIKIN and ERNEST M. HODNETT.
    Third Edition. Pp. xii + 1060. New York, London and Sydney: Interscience Publishers,
    a division of John Wiley & Sons Inc. 1965. Price £11.

This is the third edition of a text-book that was first published in 1947; since that time it has established itself as one of the most comprehensive texts available on qualitative organic analysis.

This edition contains considerably more material than earlier ones. The section on laboratory techniques has been expanded and includes descriptions of newer equipment; there is now a detailed account of thin-layer chromatography, and the chapter dealing with the determination of physical constants has been enlarged. Considerably more space has been devoted to theory. In the last edition the physical constants of some 4100 organic compounds and their derivatives were tabulated; in the new edition this number has been extended to 7100.

The authors are rather cautious in their recommendation of the oxygen-flask method for decomposition of compounds before testing for the elements, and they prefer the older methods. They admit that this method does have possibilities, but do not appear to have given it any serious examination. More work has been published in this field since this book went to press, and it is to be hoped that in a future edition the authors will be more enthusiastic about this much simpler technique.

The new edition is a worthy successor to the earlier volumes; there is no similar text known to the reviewer that reaches the same standard in coverage and quality within a single volume.

R. BELCHER

METHODS IN ZONE ELECTROPHORESIS. By JOHN R. SARGENT, B.Sc., Ph.D. Pp. iv + 107. Poole, Dorset: The British Drug Houses Ltd. 1965. Price 8s. 6d.

This slim, paper-bound and moderately priced volume is good value, considering the wide area it is meant to cover. The author has wisely chosen to present the subject matter in the form of a laboratory manual, with a relatively small chapter on the theoretical background and the rest of the book devoted to methodology, equipment and well tried procedures.

The style is reminiscent of that used by I. Smith ("Chromatographic and Electrophoretic Techniques, Volume 2, Zone Electrophoresis," Heinemann, London, 1960).

In the chapters dealing with electrophoresis in which cellulose acetate, starch gel, agar and polyacrylamide are used as stabilising media for the analysis of a variety of proteins in biological fluids, the methods recommended and the practical hints given seem to be based on the author's own experience and sound knowledge in choosing experimental conditions most suitable for specific applications. The references following each chapter are well selected and up-to-date. There are, in addition, excellent, though necessarily brief, chapters on immuno-electrophoresis in agar and on preparative block techniques in which starch, PVC, foam rubber, glass powder and Sephadex are used as media.

By contrast, the treatment given to such important groups of low molecular-weight compounds as the amino-acids and carbohydrates shows certain weaknesses.

Several statements, *e.g.*, that the amino group in zwitterions is dissociated at high pH values (p. 3) and that lysine moves towards the anode and aspartic acid towards the cathode at high pH values (p. 23) are rather incongruous and incorrect. The applicability of paper electrophoresis to carbohydrates is dismissed in a few lines, based mainly on the early paper by Consden and Stanier (p. 27) and their technique, applying 10 v cm^{-1} is inconsistently called a high-voltage technique. There have been great advances in this field since 1952, reported in books and comprehensive reviews.

Chapter 3, dealing with the technique of high-voltage paper electrophoresis, so important for really satisfactory separations of amino-acids and peptides, would have materially benefited from being based on more recent original literature instead of manufacturers' booklets of instructions. The quoted pressure of 5 p.s.i. (p. 32) is excessive and serves no useful purpose.

The book will be welcomed by many, particularly beginners, in the field of proteins.

D. GROSS

DISTILLATION. Edited by E. S. PERRY and A. WEISSBERGER. Second Edition. Pp. xx + 838. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1965. Price 180s.

This comprehensive account of distillation on the laboratory and pilot-plant scale is a completely revised and considerably augmented version of the first edition, published in 1951. During the intervening 15 years, distillation, as a technique for analysing mixtures of volatile substances such as light hydrocarbon fractions from petroleum, has been almost wholly supplanted by gas - liquid chromatography. As a consequence, the 73-page chapter on distillation of liquefied gases and low-boiling liquids in the first edition of this book has been reduced to a single page in the second edition. On the other hand, the second edition contains five chapters on subjects

that were not treated separately in the first edition. The inclusion of two chapters on pilot-scale distillation indicates that the editors have agreed to a widening of the terms of reference of the Weissberger series, which were originally the "comprehensive presentation of the techniques which are used in the organic laboratory."

The book begins with a chapter (239 pages!) on the theory of distillation, including an account of the use of analogue and digital computers in distillation calculations. Chapter II, on vapour-liquid equilibria, contains a useful account of the methods developed at Shell Development Co. for the correlation of activity coefficients in series of related binary mixtures, and the prediction of activity coefficients where no experimental results exist. In other respects this chapter is disappointing: there are numerous minor errors (three distinguished names are mis-spelt), the theoretical section is unsatisfactory, and the section on experimental determination of equilibria is sketchy. The real meat of the book is to be found in Chapters III, IV, V and VI, on ordinary fractional distillation, extractive and azeotropic distillation, distillation under moderate vacuum and distillation under high vacuum, respectively. Together these four chapters provide a detailed account of modern distillation practice on the laboratory scale; it is perhaps noteworthy that, whereas there are several mentions of analytical gas chromatography as an adjunct to distillation, there is no mention of the fact that preparative-scale gas chromatography is increasingly being used, instead of distillation. In Chapter VII, the theoretical and practical aspects of the production of high vacua in connection with distillation are described; the commercially available equipment, to which reference is made, is wholly American. Chapter VIII contains a somewhat laboured description of the use of sublimation for purifying volatile solids, and Chapter IX contains a plea for the more widespread adoption of continuous distillation as a laboratory-scale technique. The book concludes with chapters on pilot-plant distillation and automation in distillation; the editors' objective in including these chapters is stated to be an improvement in communication between the laboratory and the pilot plant, with a feedback to the laboratory of an understanding of the techniques developed for the larger scale.

Workers in those laboratories in which fractional distillation is a major preoccupation will probably feel impelled to buy the book. Those, however, in laboratories in which it is a minor activity, may well think that 180s. is too much to pay when several excellent monographs on laboratory distillation techniques are available at modest cost.   J. D. Cox

MECHANISING LABORATORIES. By E. A. SMITH, B.Sc., M.B.I.M. Pp. x + 205. London: Iliffe Books Ltd. 1965. Price 63s.

In some respects this book is rather stimulating, in others it is disappointing. It is, however, well written and is the kind of book that the executive, in charge of a laboratory, could assimilate in the course of a week-end's study. He will, of course, have to be willing to look into certain questions such as does his laboratory answer enquiries with maximum speed and efficiency, and is he making the maximum and best use of machines rather than human hands?

Perhaps the most important question will be, is he using the best methods of handling and assessing the results that his laboratory provides? Does he, and this might be true of many analytical chemists, shrink at the idea of using computers? If he is a chemist, even an analytical chemist with an open mind on these matters, then it is likely that a reading of this book, which really only scratches the surface of things, may prove to be rewarding.

On the face of it the price of this volume is high, but in particular cases it might prove to be cheap if the right inferences are drawn.   J. HASLAM

Erratum

JANUARY (1966) ISSUE, p. 17. Replace the right hand side of equation 1 by—

$$E_{max.} - [K (E_1 - E_2) + E_2]$$

FEBRUARY (1966) ISSUE, p. 104, 7th line under Table 1. *For* "0·2 per cent. gelatin solutions" *read* "0·1 per cent. gelatin solution".

IBID, p. 108, 6th line under Table VIII. *For* "manganese(II) ions" *read* "magnesium(II) ions".