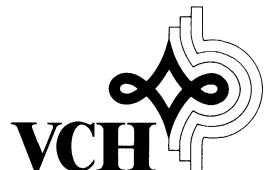


**DFG**

Manual of Pesticide  
Residue Analysis  
Volume I



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# Manual of Pesticide Residue Analysis Volume I

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# Preface

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With the advancement and growing importance of pesticide residue analysis in the late sixties, the Working Group on Pesticide Residue Analysis of the “Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel” (Pesticides Commission), Deutsche Forschungsgemeinschaft (DFG), began to edit a loose-leaf Manual containing a compilation of suitable analytical methods. During the past decades, the Working Group has continued to augment and update the Manual by issuing a series of instalments each containing new and revised methods.

The principal criterion on which the Working Group always has based its selection of methods for inclusion in the Manual is reliability of a particularly high standard. Therefore, in contrast to many other publications, all the methods contained in this Manual have been validated, without exception, by at least one other specially qualified laboratory. Experience has shown that the dialogue between the laboratory of the author of a method and the validating laboratory is extremely useful, having led to modification of many an originally suggested procedure. The ultimate outcome has been the creation of an optimal version for every method included in the Manual.

The Editorial Committee also has established a uniformity of structure for all the methods, and has ensured that they are presented in such detailed and readily understandable form that analysts using them cannot possibly ever be left in doubt about how to proceed in any of the steps. Each of the single methods specifies the substrates to which it is suited and on which it has been validated. These include food crops, stored commodities, processed food of vegetable and animal origin, feedstuffs, forage and fodder crops, soil and water. The multiresidue methods obviously could not be validated on all pesticide – commodity combinations. Notwithstanding, they too have all been tested for their reliability on particularly typical pesticide – commodity combinations or have been validated by collaborative studies and experiences of other laboratories or working groups.

The methods are divided into two groups according to their definition. The compound-specific methods (single methods) are suitable primarily for investigating aspects directly relating to crop protection, e. g. for the construction of degradation curves of the specific compound; however, they also are most appropriate for analyses undertaken in food inspection and monitoring programmes in those instances where the respective compound cannot be determined by a multiresidue method. The multiple analytical methods (coded by the letter S), on the other hand, permit the identification and determination of large groups of compounds in one and the same procedure. They are intended mainly for use in food inspection and monitoring programmes. Multiresidue methods of a special subgroup included in the Manual are those designed for the analysis of water (they are coded by the letter W, Vol. 2). In addition, the Manual presents special techniques for the processing of extracts, for the separation of co-extractives and for the separation of groups of compounds.

The German edition of the Manual, again augmented and updated in 1985 by the issue of the 8th Instalment, contains a total of 163 single analytical methods for 133 specific compounds, 28 multiresidue analytical methods (of which 4 are for the analysis of water) and 6 cleanup methods. With them, some 277 pesticides, metabolites and chemically related compounds and

280 substrates can be analyzed. The importance of the Manual is reflected more clearly in the very large number of about 3500 pesticide – commodity combinations which can be analyzed with the described methods.

As all the methods presented have been validated in at least one independent laboratory, the Manual has met with acceptance far beyond the frontiers of the Federal Republic of Germany, especially in English-speaking countries. For this reason, both the multiresidue analytical methods and very many of the compound-specific methods contained in the Manual have been included in the List of Recommended Methods for Residue Analysis compiled and issued by the Codex Committee on Pesticide Residues (CCPR) of the FAO/WHO Codex Alimentarius Commission. Many residue analysts in other parts of the world are, however, not well versed in German. Therefore, to overcome this language barrier and to render the methods accessible to a far wider international circle of analysts, the Working Group has begun to translate the most important sections of the Manual into English. This mission is sponsored by the Deutsche Forschungsgemeinschaft.

It has been decided not to translate the entire contents of the German Manual because since initiation of its compilation residue analytical methodology has undergone continual change and improvement. The methods contained in the German edition are listed in the section on types and uses of methods. Some of them are now obsolete and have been superseded by more modern ones. Others are still recommended for use only under special conditions or else the specific compounds are no longer contained in the currently marketed formulations.

Therefore, a choice has been made of up-to-date methods which are of most value to the analyst. The first volume of the English edition contains 23 compound-specific analytical methods selected from the 6th and 7th Instalments (issued in 1982 and 1984, respectively) of the German edition, 17 multiresidue analytical methods and all cleanup methods (1984 status). It also contains all pertinent general sections, e.g. on the collection and preparation of samples, on the limits of detection and determination, and on micro methods and equipment for sample processing.

In the German edition, the compound-specific analytical methods are arranged according to the numbers under which the compounds are registered with the Biologische Bundesanstalt für Land- und Forstwirtschaft (Federal Biological Research Centre for Agriculture and Forestry) in Braunschweig. For presentation in the English edition, it was considered more convenient to arrange them in the alphabetical order of the compound names. However, the above-mentioned registration numbers are given additionally to establish the connection with the German edition and with the numbers in the CCPR List of Recommended Methods. The multiresidue analytical methods are designated by exactly the same codes used in the German edition. However, they begin with the designation S 6 because methods S 1 to S 5 contained in the German Manual no longer satisfy present-day requirements.

Needless to say, the Manual contains the requisite indexes for quick access to the particular method deemed suitable for determining a specific compound in an analytical material. The single methods each contain the chemical name and the structural formula of the respective compound. The multiresidue analytical methods, however, do not contain chemical names and structural formulae because this would have proved most confusing. Therefore, the multiple methods are preceded by a table in which these data are presented jointly for all compounds.

The first volume of the English edition will soon be followed by a second one. It will contain more single methods for the determination of recently developed compounds. These methods will be adopted from the latest instalments of the German edition (the 8th Instalment issued in

1985 and the 9th scheduled for publication in 1987); some of them, however, will originate also from earlier instalments. The next volume will also contain the new multiresidue analytical methods (coded S) published in German since the first volume went to press, and the multiple methods for analysis of water (coded W). It is also planned to include cumulative indexes for Volumes 1 and 2 in the forthcoming issue. As soon as further methods become available in sufficient number, they will be presented in a 3rd English volume.

The Working Group on Pesticide Residue Analysis hopes that by publishing its selection of validated methods in English, it will have rendered a major contribution to pesticide residue analytical technology. The Working Group requests all users of the Manual to communicate their experiences with the presented methods to the Deutsche Forschungsgemeinschaft, Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel, Arbeitsgruppe "Analytik", Kennedyallee 40, D-5300 Bonn 2, Federal Republic of Germany.



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# **Part 1**

## **Introduction and Instructions**



# **Explanations**

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## **General**

The common names approved by ISO are used wherever possible to designate the compounds mentioned in this Manual. Some of these names are registered trade names. Should this not have been indicated in certain cases, it does not imply that the respective names may be used by parties other than the owners.

Besides the compound name given in the heading, each of the individual pesticide residue analytical methods contained in Part 3 is identified by a BBA Number. These are the numbers under which the compounds are registered with the Biologische Bundesanstalt für Land- und Forstwirtschaft (Federal Biological Research Centre for Agriculture and Forestry) in Braunschweig. In the German version of this Manual, the individual pesticide residue analytical methods (single methods) are arranged according to these numbers; in the English edition, however, they are arranged in alphabetical order. The multiple pesticide residue analytical methods contained in Part 4 are arranged in chronological order. The multiple methods for the determination of residues in substrates of vegetable and animal origin and in soil are marked thus S 1, S 2, etc. The multiple methods for the determination of residues in water (see Volume 2) are marked thus W 1, W 2, etc. In those cases where several methods have been included in the Manual for the determination of one compound or one compound class, such methods are marked by additional code letters (A, B, C, etc.). The heading of each method lists the crops, foods, feeds or other substrates like soil and water on which the method has been tested. Whether the respective method may be employed also for the analysis of materials other than those listed must be decided from case to case.

## **1. Introduction**

The chemical names given for the parent compounds, metabolites and related compounds are based on either IUPAC or C.A. nomenclature, and thus correspond in most cases also to the names used in the Federal German Regulation on Maximum Pesticide Residue Limits.

The physical data given for each compound comprise those parameters considered most likely to be useful to the analyst.

Data on compound solubility in the solvents required for the analysis have been standardized in accordance with the solubility gradations of the German Pharmacopoeia. Numerical data sometimes are given additionally in parentheses, except for compounds very sparingly soluble in water because the data given in the literature for such compounds usually vary considerably.

For the multiresidue methods, physical data are of minor significance because, inter alia, the solvents or solvent mixtures used represent a compromise for the compounds or any derivatives detectable by the method. Therefore, the table of compounds preceding the multiresidue methods gives only the common names, chemical names and structural formulae of all the parent compounds, metabolites and other residue-forming compounds detected by the presented multiresidue methods.

## 2. Outline of method

This section presents a brief description of the major steps of the analytical procedure.

## 3. Apparatus

It has emerged that it is expedient to list all requisite glassware and apparatus to ensure that every single item needed will have been obtained prior to initiation of an analysis. In the development of the methods, the different authors often used various models or makes of one and the same kind of apparatus, for example household mixers. The choice was dictated usually by what apparatus was available in the laboratory at the time or by favourable purchase prices rather than by special requirements of the respective method. The types used generally were required to have no influence on reproducibility of the results obtained. Therefore, the names of manufacturers often given in parentheses should not be construed as mandatory but simply as a suggestion. On the other hand, an exact specification of the apparatus and accessories used for measurements, in particular gas-chromatographic determinations, is intended to express that satisfactory results were obtained under the given conditions with the very instrumentation specified. As the quality of a gas-chromatographic determination may depend also upon design features of an apparatus, e.g. sample introduction system, transfer from column to detector, detector geometry, etc., users of this Manual are urgently recommended, should they prefer to use systems and/or operating conditions other than those given, firstly to test them thoroughly for their suitability.

The analytical material often must be comminuted in the presence of flammable solvents. When commercial type mixers are used, there is the likelihood of solvents or their vapours infiltrating into the motor (risk of explosion). Therefore, residue laboratories are urgently recommended to use only explosion-proof apparatus for comminuting samples.

Every residue laboratory should be equipped with apparatus suitably designed for gentle concentration of solutions. Particularly the removal of the very last millilitre often results in losses of compound. Large surfaces and strong local heating must therefore be avoided. Rotary vacuum evaporators are fully proven tools for the evaporation of the bulk of a solvent. Generally, a Kuderna-Danish concentrator can also be used successfully. For the removal of the last traces of a solvent, it can be converted into a useful micro-evaporator. The use of streams of dry, purified air or nitrogen for removing solvent remnants may result in evaporative losses of pesticide. To avoid losses during evaporation, additional measures often may be necessary, e.g. by adding paraffin oil as a keeper.

For photometric measurements, a filter instrument is often sufficient. Some determinations, however, require the use of a spectrophotometer wherever it is necessary to carry out measurements at a certain wavelength or at several wavelengths or even to record the whole spectrum.

The basic equipment of a residue laboratory must include a deep freezer. Freezing is the best way of avoiding compound losses during storage of the laboratory sample or the subsamples taken from it for analysis. The containers in which the samples are frozen should be made of polyethylene or a like material that does not adsorb any of the compound. Such plastic containers should be tested for their suitability before they are used; for more information, see p. 17 ff, p. 21 f and p. 23 ff.

## 4. Reagents

The reagents are listed in the following order: solvents, solvent mixtures; compound standard solutions; acids, bases, salt solutions, liquid organic substances that are not solvents, inorganic liquid substances; solid substances like salts; other solid materials like Celite, Florisil, glass wool; gases; all substances with specification of required purity. The reagents are listed in alphabetical order within each group. Mention of the names of manufacturers in parentheses does not imply that it is mandatory to use their products; they are given simply as a guide to sources of purchase. Wherever it is necessary to use a definite reagent, this is indicated e. g. by giving the Catalogue Number next to the name of the suppliers.

Wherever specific directions are given for purifying or checking supplied batches, they should always be strictly observed.

The term 'water' always means aqua dest. or fully deionized water. Wherever double-distilled water is required, this is explicitly stated. The term 'solutions' without mention of the solvent always means aqueous solutions.

A number of reagents recommended for use in the Manual are subject to special use restrictions for reasons of health protection. The Chapter "Important Notes on the Use of Reagents" (see p. 14) should therefore be strictly observed.

## 5. Sampling and sample preparation

As the guidelines on sampling and sample preparation are identical for numerous methods, added to which they take up considerable space, reference is made in this section only to the specially compiled chapters, viz. Preparation of Samples, Collection and Preparation of Soil Samples, and Collection and Preparation of Water Samples in so far as the respective analytical materials were included in the procedure. Wherever necessary, special additional instructions are given in this section.

## 6. Procedure

In this section, the analytical procedure is described in such full detail as to ensure reproducibility of the results also when it is used for the first time. Wherever possible, the steps comprising this section are subdivided into *Extraction* of the compound from the analytical material, *Cleanup* of the extract e. g. by column chromatography, and *Measurement* or *Determination* of compound concentration by spectrophotometric, gas liquid chromatographic or high-performance liquid chromatographic methods. Sample weight and volumes of extracts and solutions are marked by code letters in so far as they are included in the calculation of the residues.

A general guideline is for three analytical samples to be included in the analysis wherever possible. In the case of large series, two samples may be sufficient although a third one should then always be kept in reserve for the clarification of any discrepancies.

Within each analytical series, several samples of comparable material should be analyzed that have not been treated either with the compound being analyzed or with a related compound that might interfere with the analysis (control sample). A complete residue analysis carried out with

such material gives the sample blank value; this includes the possibly present reagent blank value. Unexpectedly high sample blank values often may be due to contaminated reagents, adsorbents, etc. In such cases, the reagent blank value should be checked by performing a complete analysis without plant or other analytical material.

## 7. Evaluation

### 7.1. Calibration values and methods of evaluation

*Evaluation of photometric and polarographic measurements.* Such measurements usually are evaluated by means of calibration curves obtained from measurements of compound standard solutions. The curves themselves usually are not reproduced in the methods. They are characterized by individual values given for the respective compounds or their reaction products.

*Evaluation of gas and high-performance liquid chromatograms.* In methods for the analysis of individual pesticide residues, the chromatograms obtained usually are not reproduced. Multi-residue methods, on the other hand, often contain reproductions of chromatograms to illustrate separation efficiency.

The quantitative interpretation of a gas or high-performance liquid chromatogram is based on either peak height or peak area. The procedure for calculation is the same in either case. Therefore, an example will now be given only for quantitation by the peak area method.

The area of the peak ( $F_A$ ) of the compound from the cleaned-up extract solution to be analyzed is measured and compared with the area of the peak ( $F_{St}$ ) measured for the compound from a standard solution, and from this comparison the amount of compound in the sample solution is calculated. The two peak areas  $F_A$  and  $F_{St}$  should be of comparable size. It is recommended to repeat the determinations several times, and to use the mean values of the areas in the calculation. Appropriately equipped laboratories will use electronic quantitation aids like integrators or computers.

*Quantitation by external standardization.* A standard solution of the compound to be quantitated, that is of known concentration, is injected into the gas or high-performance liquid chromatograph before or after injection of the sample solution. In multiresidue methods, the standard solution may contain several compounds. With this method of evaluation, errors may arise particularly from the injection of unequal volumes by hand, from conducting determinations in the non-linear range of detector response and from effects of sample co-extractives on instrumental sensitivity. Therefore, equal volumes of the sample solution and the standard solution should be injected. To ensure that  $F_A$  and  $F_{St}$  will be of comparable size, standard solutions of appropriate concentration must be chosen. Injection of the standard solution should be repeated after each injection, or at least after every third injection of sample solution, also when conducting determinations in the same concentration range. With some detectors, checks must be made from time to time to ensure that the determinations are being performed within the linear or approximately linear range of response. The simplest procedure is to run an analysis of a solution diluted to double volume; the area of the resultant peak must be half that obtained with the undiluted solution.

*Quantitation by internal standard method.* In most methods, evaluation is described by the use of an external standard. The reliability of the results can be improved, however, in many

cases by performing and evaluating the analysis with the aid of an internal standard. By the internal standard method, a compound is used that can be chromatographed under the same conditions as the pesticidal compound required to be determined but which has a different retention time. The standard should be detected with the same sensitivity as the sample. Prior to the analysis, the area ratio ( $f$ ) of the peaks of equal amounts of sample and standard must be determined for the concentration range of interest. Then a few microlitres of the standard solution are added to the sample solution prepared for injection, and the mixture is injected into the gas or high-performance liquid chromatograph. The amount of standard solution added should be such that  $F_A$  and  $F_{St}$  again show comparable sizes. Quantitation is performed by comparing the peak areas of sample ( $F_A$ ) and standard ( $F_{St}$ ), taking account of  $f$ .

An advantage of using an internal standard for quantitation is that inaccuracies during injection and alterations of detector response have hardly any influence on the analytical result. Nonetheless, care must be taken also when using the internal standard method that the quantitation is performed within the linear response range of the detector.

## **7.2. Recovery, limit of detection, limit of determination, lowest determined concentration**

Every processing (cleanup) procedure incurs losses of compound. For the appraisal of analytical results, the rate of recovery i. e. the portion of the compound residue recovered after processing in the final determinative step, is of special importance. Recoveries may be subject to considerable variations induced by dissimilar handling during processing/cleanup and by remnants of interfering substances in the final determinative step. A factor of similar importance to the appraisal of analytical results is also the range of variation of the recovery. Therefore, several recovery experiments always should be run, from which the recovery mean and its standard deviation are calculated. The recovery and its range of variation often are greatly dependent upon the amount of pesticide (mg/kg) in the analyzed material. They must be determined separately in every range of interest (e.g. 0.05, 0.1, 0.5, 1.0 mg/kg).

With the aid of the recovery mean, the analytical results can be converted to a non-loss i.e. theoretical recovery. The recovery factor used for this calculation is

$$F = \frac{100}{\% \text{ recovery}}$$

The recovery and its range of variation must be determined by every analyst in his/her own laboratory. The values given in the different methods were obtained during testing of the respective method, and constitute only guide values. For the determination of recovery, the comminuted analytical material is fortified with the pesticide in a suitable solvent (e.g. in that used for the following extraction), and the solution is allowed to absorb into the sample. The fortified control sample is then extracted and analyzed in accordance with the specified method. Any departures from this procedure that are necessary in special cases are stated in the respective method e.g. under Section 8, Important points.

Besides the recovery, the following data are given in the methods contained in the Manual: *Either* the limit of detection and the limit of determination which particularly underline the efficiency of an analytical method,

or the lowest determined concentration at which the sought compound could still be measured quantitatively (routine limit of determination, see p. 43).

Factors having decisive influence on the magnitude of these values are the sensitivity of the detection reaction or the gas chromatographic or high-performance liquid chromatographic system, the recovery, the level of the sample and reagent blank values, and the range of variation of these quantities. The procedure for the calculation of the limit of detection and the limit of determination is given on p. 37 ff.

### **7.3. Calculation of residues and reporting of results**

In most of the analytical methods contained in this Manual, formulae are given for calculating the level of residue in the analyzed sample. Wherever it is required to analyze foods for compliance with a regulation on maximum residue limits, other methods of calculation may be specified. This applies, for example, to cases where joint maximum residue limits have been established for several compounds or for the total residue of a compound and its metabolites or where it is required to relate the residues to certain portions, e. g. the fat content of a food.

An analytical result can only be appraised objectively when full details are given on the nature and preparation of the sample and on the procedure employed for the analysis. The analysis report should therefore contain all appropriate data such as detailed also in the Chapter "Use of Forms in the Reporting of Analytical Results". Where several analyses are performed on one and the same sample, the individual values measured should also be given and not just a mean.

Wherever the repeatability of an analysis has been determined in the examining laboratory or where the reproducibility of an analytical method has been tested in several laboratories, the corresponding repeatability standard deviations or reference standard deviations, together with the relevant degrees of freedom, should be reported.

## **8. Important points**

This section includes details on peculiarities of the analysis; difficulties encountered with certain analytical materials; other pesticides that interfere with the analytical procedure; similar compounds that can be determined by the method; methodical modifications for special cases; use of the method for analyzing foods for compliance with regulations on maximum residue limits; and other important information.

## **9. References**

The bibliography listed in this section relates to analytical principles and utilized methods. The titles of periodicals are abbreviated in accordance with Chemical Abstracts Service Source Index (CASSI) 1975 (with supplements).

## **10. Authors**

This section gives the names of the authors of the respective methods and their laboratories.

# Notes on Types and Uses of Methods

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The residue analytical methods presented in the Manual are divided into two main categories. One category embraces specific methods for determining residues of individual compounds (single or compound-specific methods). The other category comprises multiple methods for analysis of plant material, soil, etc., including special procedures for determination of residues in water (multiresidue methods).

The *single methods* are intended principally for serial analyses of substrates for residues of a specific compound of known structure, e.g. for constructing degradation curves of the compound under defined conditions. Since the whole procedure is specially tailored to the respective compound, the limits of detection and determination of the method usually are very low. Prior to its inclusion in the Manual, each single method is tested for efficiency in at least one independent laboratory that had no part in its development. The test is always performed on all the analytical materials listed in the heading of the method. In this test the method is evaluated on fortified samples for recovery rates, blank values and limits of detection and determination, and its efficiency is validated.

The *multiresidue methods* are intended mainly for the simultaneous analysis of residues (also of unknown nature) of several compounds in one and the same procedure. Therefore, they are suitable especially for use in food inspection and monitoring programmes. As the number of pesticide-commodity combinations can be extraordinarily large, obviously these methods cannot be tested as comprehensively as the single methods. Therefore, the multiresidue methods are validated only on a select number of pesticide-commodity combinations. This is done either in an independent laboratory just as for the single methods or, however, in interlaboratory collaborative studies as undertaken e.g. by the Working Group on Pesticides, Food and Forensic Chemistry Division of the Gesellschaft Deutscher Chemiker (German Chemical Society). The results of collaborative studies are outlined in the respective multiple methods included in the Manual.

The first German edition of the Manual was published in the year 1969, the second edition appearing in 1972, some fifteen years ago. Since then, pesticide residue analytical methodology has constantly undergone tremendous improvement and changes, as evidenced in each of the subsequent editions of the Manual. For example, the earlier spectrophotometric techniques, especially those involving measurements in the visible wavelength region, have been very largely superseded by gas chromatography with selective detectors. In the meantime, many multiresidue methods have also been developed which permit simultaneous and reliable determination of residues of chemically related compounds in numerous analytical materials.

The majority of the methods, especially those published in the most recent German editions of the Manual, take account of the current state of residue analytical technology, so that their application can be recommended unreservedly.

Some of the earlier compound-specific methods, although not reflecting the latest state of the art, nonetheless continue to be of value for analyses undertaken for special purposes. Likewise for analyses performed under special circumstances, for example where certain instruments are not available or where the technical requisites for their operation are wanting. These methods

also contain information on how compounds behave during processing so that they are useful for making any necessary procedural modifications. To assist users of the Manual in choosing a suitable method, those single methods which, according to the current status of residue analysis, deserve special recommendation are listed below. They are followed by a list of older compound-specific methods.

Compound-specific methods (single methods) considered worthy of special recommendation according to the present status of residue analytical technology

Compound	Principle of determination*)	Published	German version Method No.
Acephate (+ methamidophos)	GC-PND	1982	358-365
Acrylonitrile	Pho-Vis	1972	120
Aldicarb	GC-FPD	1984	250
Arsenic	Pho-Vis	1969	230
Azinphos-ethyl	GC-ND	1976	62-A
Azinphos-methyl	GC-ND	1976	63-A
Binapacryl	GC-ECD	1974	8-A
Binapacryl (+ dinoseb + dinoseb acetate)	GC-ECD	1979	43-430-8
Bromacil	GC-ND	1976	222
Bromophos	GC-PD	1974	210-A
Bromophos-ethyl	GC-PD	1974	263
Captafol	GC-ECD	1982	266
Captafol	HPLC-PhLD	1984	266-A
Captan	HPLC-PhLD	1984	12-A
Carbendazim	GC-ECD	1985	378
Chlordecone (+ kelevan)	GC-ECD	1976	247
Chlorfenvinphos	GC-PD	1972	239
Chlorthiophos	GC-PND	1984	465
Copper	Pho-Vis	1969	147
Copper oxychloride	AAS	1985	147-A
Cymoxanil	GC-PND	1985	513
2,4-D (+ dichlorprop)	GC-ECD	1985	27-A-38-A
Dalapon	GC-ECD	1982	28
Dialifos	GC-PD	1979	281
Di-allate (+ tri-allate)	GC-FPD	1976	135-34
Diazinon	GC-PD-ECD	1969	35-B
Dichlobenil	GC-ECD	1982	225
Dichlorprop (+ 2,4-D)	GC-ECD	1985	27-A-38-A
Dichlorvos	GC-PD	1972	200
Diclofop-methyl	GC-ECD	1984	424
Dicofol	GC-ECD	1976	69
Dicofol	HPLC-UV	1979	69-A
Dimethoate (+ omethoate)	GC-PD	1979	236-42
Dinoseb (+ dinoseb acetate + binapacryl)	GC-ECD	1979	43-430-8
Dinoseb acetate (+ dinoseb + binapacryl)	GC-ECD	1979	43-430-8
Diquat	Pho-Vis	1969	37
Ditalimfos	GC-PND	1979	372
Endosulfan	GC-ECD	1974	50
Ethiofencarb	GC-FPD	1976	393
Ethoxyquin	GC-ND	1979	500

Compound	Principle of determination *)	Published	German version Method No.
Ethylene thiourea	GC-FPD	1982	389
Fenitrothion	GC-PD	1972	58
Folpet	HPLC-PhLD	1984	91-A
Fonofos	GC-PND	1985	288
Heptenophos	GC-FPD	1982	427
Indolylbutyric acid, -acetic acid, -propionic acid	TLC	1976	144-145-423
Iprodione	GC-ECD	1979	419
Kelevan (+ chlordecone)	GC-ECD	1976	247
Lenacil	GC-ND	1979	237
Medinoterb acetate	GC-ECD	1976	364
Mercaptodimethur	GC-FPD	1974	79-A
Metalaxyl	GC-PND	1984	517
Methamidophos	GC-PD	1976	365
Methamidophos (+ acephate)	GC-PND	1982	358-365
Methidathion	GC-PD	1972	232
Methomyl	GC-FPD	1982	299
Metribuzin	GC-PND	1985	337
Mevinphos	GC-PD	1974	93
1-Naphthylacetic acid	GC-ECD	1982	434
Nicotine	Pho-UV	1969	85
Nitrofen	GC-ECD	1982	340
Omethoate (+ dimethoate)	GC-PD	1979	236-42
Oxamyl	GC-FPD	1985	441
Paraquat	Pho-Vis	1976	134
Paraquat	Pho-Vis	1982	134-A
Parathion	GC-PD	1972	87-B
Parathion-methyl	GC-PD	1972	88-B
Phenmedipham	GC-ECD	1974	233-B
Phosphine	Pho-Vis	1972	13
Phoxim	GC-PD	1974	307
Pirimicarb	GC-ND	1984	309
Pirimiphos-methyl	GC-PND-FPD	1982	476
Propiconazole	GC-PND	1985	624
Propyzamide	GC-ECD	1979	350
Pyrazophos	GC-FPD	1982	328
Sulfotep	GC-PD	1972	104
Sulphur	HPLC-UV	1985	184-B
2,4,5-T	GC-ECD	1972	105
TCA	GC-ECD	1974	107
TCA	GC-CD	1976	107-A
Tetrachlorvinphos	GC-ECD	1982	317
Thiabendazole	TLC-Flu	1979	256-A
Thiabendazole	Pho-UV	1979	256-B
Tri-allate (+ di-allate)	GC-FPD	1976	135-34
Triazophos	GC-FPD	1982	401
Trichlorfon	GC-PD-ECD	1972	112
Trichloronat	GC-PD-ECD	1972	249
Triforine	GC-ECD	1979	338
Vinclozolin	GC-ECD	1982	412

## Older compound-specific methods

Compound	Principle of determination *)	Published	German version Method No.
Atraton	TLC	1972	6a-A
Atraton	Pho-UV	1972	6a-B
Atrazine	TLC	1969	6-A
Atrazine	Pho-UV	1969	6-B
Azinphos-ethyl	Pho-Vis	1976	62
Azinphos-methyl	Pho-Vis	1969	63
Barban	Pho-Vis	1969	7
Binapacryl	Spepho	1969	8
Bromophos	Pho-Vis	1969	210
Buturon	Pho-Vis	1972	213
Captan	Pho-Vis	1969	12
Carbaryl	Pho-Vis	1969	100
Chinomethionat (quinomethionate)	GC-ECD	1969	189
Chlorbufam	Pho-Vis	1969	9
Chloridazon	Pho-Vis	1969	89
Chlorpropham	Pho-Vis	1972	21
Cycluron	GC-ECD	1969	86
2,4-D	GC-ECD	1972	27
DDT	Pho-Vis	1969	30
Desmetryn	TLC	1972	244-A
Desmetryn	Pho-UV	1972	244-B
Diazinon	Pho-Vis	1969	35-A
Dichlofluanid	Pho-Vis	1969	203
Dichlorprop	GC-ECD	1972	38
Dieldrin	GC-ECD	1969	39
Dimethoate	Pho-Vis	1969	42
Dinocap	Pho-Vis	1969	68
Dithianon	TLC	1974	45-A
Dithianon	Pho-Vis	1974	45-B
Ethylene oxide	Titr	1972	126
Fenaminosulf	Pho-Vis	1976	291
Fentin: Fentin acetate, chloride, hydroxide	Pol	1979	55-B-188-349
Fentin acetate	Col	1969	55-A
Folpet	Pho-Vis	1969	91
Fuberidazole	Pho-UV	1972	214
Hydrogen cyanide	Pho-Vis	1969	11
Linuron	Pho-Vis	1969	71
Malathion	Pho-Vis	1969	72
Maleic hydrazide	Pho-Vis	1976	297
Mercaptodimethur	Pho-IR	1969	79
Metaldehyde	Pho-Vis	1969	151
Methabenzthiazuron	Pho-Vis	1974	245-A
Methabenzthiazuron	GC-ND	1974	245-B
Methoprottryne	TLC	1972	219-A
Methoprottryne	Pho-UV	1972	219-B
Methoxychlor	Pho-Vis	1969	80
Methyl bromide	Pho-Vis	1969	149
Metobromuron	Pho-Vis	1972	217-A

Compound	Principle of determination *)	German version	
		Published	Method No.
Monolinuron	Pho-Vis	1969	82
Naled	GC-PD	1974	36
Parathion	Pho-Vis	1969	87-A
Parathion-methyl	Pho-Vis	1969	88-A
Phenkaption	TLC	1969	92-A
Phenkaption	GC-PD-ECD	1969	92-B
Piperonyl butoxide	Pho-Vis	1969	163
Prometryn	TLC	1972	96-A
Prometryn	Pho-UV	1972	96-B
Propazine	TLC	1969	97-A
Propazine	Pho-UV	1969	97-B
Propham	Col	1969	66
Propineb	Pol	1969	117-B
Propoxur	Pho-Vis	1972	216
Pyrazon	Pho-Vis	1969	89
Quintozone	Pho-Vis	1969	99
Rotenone	Pho-Vis	1972	193
Selenium	Pho-Vis	1972	257
Simazine	TLC	1969	101-A
Simazine	Pho-UV	1969	101-B
Sulphur	Pho-Vis	1972	184-A
Tecnazene	GC-ECD	1969	108
Terbutryn	TLC	1972	246-A
Terbutryn	Pho-UV	1972	246-B
Tetradifon	GC-ECD	1969	109
Tetasul	GC-ECD	1969	110
Trichlorotrinitrobenzene	Pho-Vis	1969	157

\*) Key to abbreviations:

Col = Colorimetry

GC = Gas chromatography (CD = coulometric detector; ECD = electron capture detector; FPD = flame photometric detector; ND = nitrogen detector; PD = phosphorus detector; PND = phosphorus/nitrogen detector)

HPLC = High-performance liquid chromatography (UV = UV detector; PhLD = photoconductivity detector)

Pho = Photometry (IR = in IR region; Spepho = with recording of a spectrum; UV = in UV region; Vis = in visible region)

Pol = Polarography

Titr = Titrimetry

TLC = Thin-layer chromatography (Flu = with fluorometric evaluation)

# Important Notes on the Use of Reagents

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Use of some of the reagents prescribed in this Manual is subject to special restrictions for reasons relating to health and safety. The maximum allowable concentrations (MACs)\* established in the Federal Republic of Germany for these reagents when present as gases, vapours or suspended particulates in the workplace atmosphere are given in a List of "Maximale Arbeitsplatzkonzentrationen" (MAC Values for Chemical Substances in Working Environments) published newly each year by the DFG Senate Commission for the Evaluation of Hazardous Industrial Substances in Workplaces. This List also designates those substances from which there is a risk of exposure giving rise to allergic reactions (e.g. after sensitization of skin or air passages) or to poisoning as the result of dermal absorption.

In elucidation of the reasons governing the establishment of MACs, the DFG Senate Commission for the Evaluation of Hazardous Industrial Substances has edited a manual entitled "Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten" (Toxicological and Occupational Hygiene Documentation and Validation of MACs) (published by VCH Verlagsgesellschaft, Weinheim).

With the continual emergence of new scientific findings, both the individual data on certain substances and the range of substances in question may be subject to substantial alterations. Therefore, users of this Manual are urgently advised in cases of doubt always to consult the latest official Lists of MAC Values\* such as published also by the American Conference of the Governmental Industrial Hygienists (ACGIH), the British Health and Safety Commission and Executive, and the European Community.

Two of the solvents frequently mentioned in this Manual are benzene and chloroform.

Use of *benzene* should be completely avoided. The Working Group on Residue Analysis, DFG Senate Commission for Pesticides, urgently advises analysts to examine whether benzene can be substituted by another potentially suitable solvent, e.g. toluene, in methods providing for the use of benzene.

*Chloroform* is one of those substances suspected of having a notable carcinogenic potential, and which urgently require further investigation. In those instances where the use of such substances is of technical necessity, special protective measures and monitoring procedures must be enforced. If at all possible, however, chloroform should be substituted by dichloromethane (methylene chloride) in those methods that provide for the use of chloroform.

In this context, users of the Manual are referred to the Preface in which they are requested to communicate their experiences with the methods to the editorial committee because the use of solvents other than those prescribed may reduce the efficiency of the methods.

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\*<sup>1</sup>) also referred to as *Occupational Exposure Limits* and *Threshold Limit Values*

# Abbreviations

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a	ar (1 a = 100 m <sup>2</sup> )
A	ampère
A (λ)	spectral decadic absorption dimension (formerly: extinction)
bar	bar (1 atm = 1.01325 bar)
cm	centimetre(s)
conc.	concentrated, in conjunction with reagents
corr.	corrected, in conjunction with physical units of measure
cryst.	crystallized
d	day(s)
D	density
dist.	distilled, in conjunction with solvents
g	gram(s)
h	hour(s)
ha	hectare (1 ha = 10 000 m <sup>2</sup> )
kg	kilogram(s)
kHz	kilohertz
l	litre(s)
μA	microampère
μg	microgram(s)
μg/kg	microgram(s) per kilogram (formerly: ppb)
μl	microlitre(s)
μm	micrometre(s)
μs	microsecond(s)
m	metre(s)
mbar	millibar (1 Torr = 1.33322 mbar)
mg	milligram(s)
mg/kg	milligram(s) per kilogram (formerly: ppm)
min	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mol/l	mol per litre
mV	millivolt(s)
n-	normal, for isomerism
ng	nanogram(s)
ng/kg	nanogram(s) per kilogram (formerly: ppt)
nm	nanometre
NS	standard joint
p. a.	pro analysi
pg	picogram(s)
r. p. m.	revolutions/rotations per minute

16        Abbreviations

s	second(s)
s <sup>-1</sup>	reciprocal second ( $1 \text{ mCi} = 37 \cdot 10^6 \text{ s}^{-1}$ )
S	standard deviation
V	volt(s)
v/v	volume per volume
W	watt(s)
w/v	weight per volume
°C	degrees Centigrade/Celsius
°C/min	degrees Centigrade per minute
%	percent (dimensionless)
dia.	diameter
i. d.	inside diameter
o. d.	outside diameter
≈	approximately equal, approximated
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
≡	equivalent to, corresponds to

# **Preparation of Samples**

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(German version published 1982)

Meaningful residue data can only be obtained provided special care is taken in collecting samples and the collected samples are truly representative. The procedure employed for sampling must be fully accommodated both to the commodities involved and to the levels of pesticide residues to be determined. Sampling should be done only by trained personnel.

The valid field sample collected should be mixed and reduced, by random selection of the required number of portions, to the gross laboratory sample; unripe, rotten or otherwise anomalous portions should be discarded.

Whenever fruit and vegetable samples are collected for official analysis to determine whether they are in compliance with established maximum residue limits, they must be taken as specified in official regulations.

## **1. Size of laboratory sample**

The size of the laboratory sample submitted to the residue analysis laboratory should be as follows:

For homogeneous products	approx. 0.5 to 1 kg
For products consisting of small units each weighing, on average,	
less than 25 g	approx. 1 kg (at least 50 units)
from 25 to 100 g	1 to 3 kg (at least 30 units)
from 100 to 250 g	2 to 5 kg (at least 15 units)
more than 250 g	at least 10 units

These values are given as a guide. In some instances (e. g. for cereals, tea, melons or the like), laboratory samples of sizes differing from those given above may be required.

## **2. Preparation of laboratory sample**

Immediately after delivery to the residue analysis laboratory, the laboratory sample should be further processed as required and reduced to an analytical size sample. If, for some exceptional reason, this cannot be done on the same day, the laboratory sample should be stored immediately in a refrigerator.

Firstly, the laboratory sample should be assessed for appearance, aroma and, if applicable, flavour (taste); particular note should be taken of any variations from the normal physical state or appearance. At the same time, any contaminations or visible deposits possibly arising from application of crop protection measures should be noted.

The next step is to remove any impurities like stones or perhaps even pests as well as any visibly blemished, spoilt or rotten portions. The material should not be washed. However, root vegetables, potatoes and the like should be rinsed lightly to remove adhering soil. Then the

laboratory sample should be weighed and, if applicable, the number of units it contains should be counted.

For numerous crop commodities and for most foods of animal origin, the laboratory sample can be reduced directly to the analytical sample according to Section 3. Examples of such commodities are:

- Legume vegetables
- Potatoes
- Citrus fruit
- Coffee and cocoa beans
- Spices incl. mustard and culinary herbs
- Tea and tea-like commodities
- Cereal grains incl. rice and maize
- Feeds
- Oilseeds
- Fats of vegetable and animal origin
- Muscle (meat) and organs of slaughtered animals incl. poultry
- Milk and dairy products

The analytical results obtained later usually are required to be related to the whole of the sampled commodity. In analyses of foods, however, it is expedient to remove from the laboratory sample, prior to further preparation, certain portions not intended or unsuitable for consumption. Examples of such portions are those given in the right-hand column for the following commodities:

Root vegetables (e.g. carrots, radish, beet)	Tops
Onions, leeks	Roots
Cabbage (e.g. head cabbage)	Inedible and wilted outer leaves
Cauliflower, rhubarb	Leaves
Other leafy vegetables and stem vegetables (e.g. endives, lettuce, spinach)	Inedible and wilted leaves
Fruiting vegetables (e.g. cucumbers, melons, peppers, tomatoes)	Stems
Pome fruit (e.g. apples, pears)	Stems
Stone fruit (e.g. apricots, cherries, peaches, plums)	Stems and stones
Grapes	Stems and stalks
Small (soft/bush/cane) fruit (e.g. strawberries, raspberries)	Stems and where present sepals (caps, hulls)
Pineapples	Crowns (leaves at top of fruit)
Dates, olives	Stems and stones
Nuts (e.g. peanuts, almonds)	Shells
Eggs	Shells

In *cases of doubt*, it should be left to the *analyst* to decide which portions can be removed from the laboratory sample, taking into consideration customary procedures (see also Codex Committee). After inedible portions have been removed and discarded, the laboratory sample

should be re-weighed. The resultant loss of weight should be taken into account in the calculation of the residues.

The maximum limits for the residues of some crop protection products are established explicitly for certain harvested commodities less peel (e.g. for bananas, citrus fruit, potatoes). In these cases, the peel must be removed and discarded; the residues are related to the weight of the laboratory sample less peel.

### **3. Reduction of laboratory sample to analytical size sample**

The gross laboratory sample should be composited in such manner that representative analytical samples can be obtained from it.

Products that are of a homogeneous nature or finely comminuted should be intensely mixed.

With products that contain solid and liquid portions (e.g. canned fruit and vegetables), the solid portions should be homogenized and intensely mixed with the liquid portion.

Products consisting of small units each having an average weight of more than 25 g should be quartered; two opposite quarters should be homogenized. Frozen products should, if possible, be processed in a frozen state.

Animal tissues (meat, fish) should be comminuted in portions in a meat grinder (mincer), intensely mixed, and passed again through the meat grinder.

For compositing products that are comprised of large units (large blocks, wedges or wheels of e.g. cheese or solid fats) where residues of crop protection products are not expected to be present on the surface, aliquots of the units should be taken if it is not practical to homogenize the entire laboratory sample. The aliquot portions that can be obtained for example as slices by making diagonal cuts, are then comminuted and mixed.

From the thus composited laboratory sample, several portions should be selected, viz. the analytical samples required for the determination and a further analytical sample that is retained as reserve. For the analysis of foods, at least two analytical samples should be taken for the determination. If the analysis cannot be performed immediately after compositing and preparation, the analytical samples must be placed in a deep-freezer at once.

All retained analytical samples must be stored in such manner as to prevent decomposition of product and residue. At the same time, steps must be taken to ensure preservation of the integrity of samples (for example, by storing check samples in sealed containers).

### **4. Special cases**

Where it is required to determine the residue levels of pesticides that tend to undergo rapid degradation upon intensive comminution of the gross laboratory sample (e.g. dithiocarbamates), the procedure employed for reducing the laboratory sample to the analytical size sample must be modified accordingly. Details on this are given in the respective analytical methods.

Where it is established with certainty that the residues of a pesticide are present only on the surface of the commodity, extraction of the surface will suffice for the analysis. In such cases, the gross laboratory sample need not be finely comminuted but is reduced to the analytical size sample simply by randomly selecting a few portions.

## 5. References

- Pesticide Analytical Manual. U.S. Department of Health, Education and Welfare. Food and Drug Administration, Washington D.C., Volume I, Section 142.22b.
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- Bundesgesundheitsamt (ed.), Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Beuth Verlag Berlin und Köln, Band I/3, Abschnitt L 29.00 1 (EG), see also Bundesgesundheitsbl. 23, 397 (1980).
- Codex Committee on Pesticide Residues, Guide to Codex recommendations concerning pesticide residues. Part 6: Portion of commodities to which Codex maximum residue limits apply and which is analysed, Codex Alimentarius Commission, FAO/WHO, CAC/PR 6-1984.

## 6. Author

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# **Collection and Preparation of Soil Samples**

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(German version published 1979)

## **1. Sampling**

Soil samples are taken with a corer e. g. with an auger as specified in DIN 19671 Sheet 1 Form A, or with a similar perhaps shorter tool or with a spade.

Cores usually are collected down to depths at which the soil is tilled; in grassland down to 10 cm, in forest land and non-cropland down to 20 cm. In vineyards, orchards, hop gardens and asparagus fields, it may be necessary to collect cores from two soil layers (0–25 cm and 25–50 cm). Where it is required to study the vertical movement of a pesticide in soil, cores are collected e. g. from depths of 0–2.5, 2.5–5, 5–10 and 10–20 cm; such subdivision of soil sampling depths is easy to achieve by making corresponding marks on the sampling tool.

The sampling sites should be spread evenly across the field or the experimental plot e. g. along a diagonal or an S-shaped line. Samples should not be taken from marginal strips, headlands and places where pesticides have been transferred from containers to tanks or where sprays have been mixed. In large fields, the distance between sampling sites and the field periphery or the above-mentioned places should be at least 5 m; in experimental plots, this distance should be appropriately shorter.

Where special treatment methods have been employed (planting hole treatment, sub-surface injections or the like), the sampling procedure must be modified accordingly.

## **2. Sample size**

The number of sampling sites will depend upon the size of the field and the uniformity of the terrain. The following figures are given as a guide:

In large fields (size of about 1 ha), 30 cores should be taken per ha. If the field is not homogeneous with respect to terrain and soil texture, up to twice as many cores should be collected per unit area. In particularly difficult cases, the field should be subdivided into sections, and separate samples should be taken from each section.

In small fields (size of about 1 a) and experimental plots, a larger number of cores must be taken per unit area so that the field sample will have a minimum size of 1–2 kg.

The same principles are applied for the collection of untreated samples, should these be required. However, considerably fewer cores can be taken.

The collected cores are combined to give the field sample.

## **3. Packing and transport**

The field samples should be transported in tightly closed, rinsable glass or metal containers. If there is no alternative but to pack the field samples in plastic bags or in paper bags lined with aluminium foil, this type of packing should be used only for transportation of the samples to

the laboratory. Upon arrival there, the field samples must be transferred immediately to other suitable containers.

#### 4. Sample preparation

In the laboratory, the field sample should be mixed thoroughly and reduced to the laboratory sample. While the soil is being dried, there is a risk of compound loss. Therefore, if it cannot be analyzed in a moist state, the soil should be air-dried only to a degree that will allow it to be sieved to remove stones, roots and the like. The analytical samples are taken from the sieved soil.

If the analytical samples cannot be analyzed immediately after drying and sieving, they should be stored at about  $-20^{\circ}\text{C}$  in glass bottles fitted with screw caps (without plastic seals). Containers and bags made of plastic material may be used only if it is certain that their use will not result in any losses of compound or any interferences with the analysis.

As the analytical result usually relates to dried soil, the loss of moisture upon drying at  $105^{\circ}\text{C}$  should be determined in a separate test (see DIN 19863 Sheet 4 No. 3.1), and the analytical result should be corrected accordingly.

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# Collection and Preparation of Water Samples

(German version published 1979)

## 1. Containers for collection and transport of water samples

The concentration of pesticides in groundwaters and surface waters usually is close to the limit of determination of the most sensitive analytical methods. Therefore, it is essential to avoid losses of compound or contamination of water samples with interfering substances that would otherwise occur through the use of containers made of unsuitable material for collecting and transporting samples. In view of this likelihood, choice of the right material evidently is of considerable importance.

Water samples are collected with scoops, bottles or other like containers. For taking samples from depths of down to 10 m, a suitable sampler is that described by Meyer. It consists of a 5-l amber glass bottle fitted with a ground stopper and an outer polyethylene container with a weighted bottom; a lever is provided for opening the bottle after it has been lowered into the water (see Figure 1).

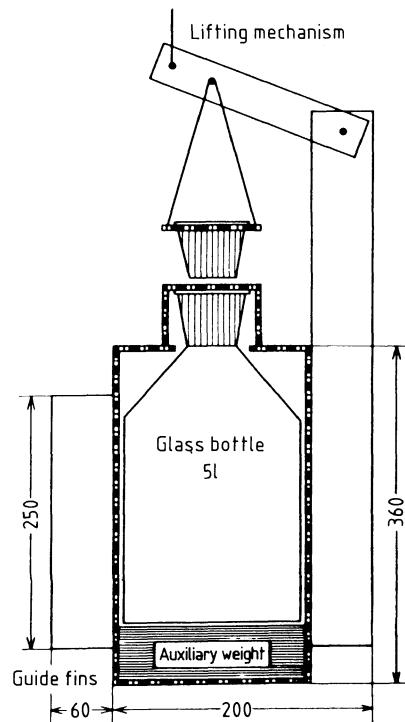


Figure 1. Water sampling device after Meyer (modified). Dimensions in mm.

Different types of scoops for collecting water samples from large depths are described in the literature. A proven implement for organic trace analysis is a stainless steel sampler described by Stadler; it is fitted with an inlet valve, and can be opened at the required depth by means of a falling weight.

For taking samples from the surface layer of a body of water, a special implement has been designed, constructed and tested also by Stadler.

Glass containers fitted with ground stoppers are best suited to the collection and transport of water samples. Containers made of stainless steel are also useful. The use of aluminium containers may result in losses of compound in some cases. Hoses, containers, etc. made of plastic may not be used because plastic materials absorb substantial amounts of pesticide residues; these materials also may release traces of organic compounds (plasticizers, etc.) into the water resulting in considerable interference with the subsequent gas-chromatographic analysis especially when electron capture detectors are used. Losses of residues may be caused also by the use of stoppers or seals made of plastics when the water is in contact with them for a prolonged period. For rinsing samplers, use may be made only of distilled water or of the water that is to be analyzed; use of e.g. deionized water usually results in interferences with the analysis (appearance of extraneous peaks).

## 2. Sampling

The sampling strategy is orientated to the objective of the study and to hydrologic conditions. For the analysis of water samples collected to characterize the quality of the water at a given geographical location (the sampling site) at a given point in time, it will suffice to take grab (single; instantaneous) samples. However, if it is required to monitor e.g. the contamination of a body of water over a given period of time, this can be done only by continuous sampling viz. collection of samples at regular intervals proportionate either to time or to flow rate.

The collection of *grab (single) samples* from conduits and springs usually poses no problems given provision for thorough mixing by turbulent flow (especially for water samples that also contain particulates). However, grab samples collected from a surface water do not always furnish meaningful data on the state or quality of the entire body of water, also when they are taken from different areas (e. g. bank area, inflow and outflow area, middle etc. of a lake). In this case, further studies on stratification and flow conditions as well as the evaluation of profiles are required. Groundwater samples are collected from wells or boreholes. Where several aquifers are present, it will be found possible in favourable cases to collect separate water samples from each aquifer by introducing packers in the borehole. Groundwater samples collected by using booster pumps usually are representative of the groundwater reservoir.

For *continuous sampling*, automatic samplers equipped with continuously operating (peristaltic pumps) or intermittently operating (scoops; water splitters; overflow or immersion vessels) dosing devices are needed. Continuous sampling of water from waste discharge and small outfalls must be done proportionate to flow rate because of sudden fluctuations in flow rate and water quality. Continuous collection of samples proportionate to time (at intervals shorter than those in which a marked change of flow rate is possible) should be confined to large rivers. When intermittently operating samplers are used, which are the most commonly marketed types, the intervals between sampling should always be so close that the collected

samples will be representative also of sudden fluctuations. For the monitoring (e.g. observance of guide or threshold values) or characterization (e.g. determination of the contamination of a water; recording of incidental or periodic fluctuations etc.) of a body of water, a sampling programme must be designed that is matched to the objective of the proposed study.

### 3. Sampling programmes

A sampling programme can be designed only on the basis of detailed preliminary studies. Important considerations in the design of the sampling programme include the frequency of sampling, the number and size of the required samples (factors that should all be based on statistical calculations), location of sampling sites as related to hydrologic conditions, and an estimation of the influence of water volume, seasonal-related use of pesticides and similar factors on the contamination of the water. As the level of noxious organic substances in surface waters is particularly subject to extremely high fluctuations, an evaluation of the individual analytical results by statistical methods is essential. In view of the temporal and spatial inhomogeneity of a body of water, single values cannot provide conclusive evidence of its actual contamination.

Hardly any simple contingencies in the sense of a direct relationship of cause and effect occur in the water-pesticide system due to its complex structure. To describe the contamination of a water, it is therefore necessary to measure not only the concentrations of pesticides but also other parameters. These include e.g. pH, electrolytic conductivity, oxygen content, total organic carbon, chemical oxygen demand (COD), biological oxygen demand (BOD), number of colonies, turbidity (concentration of suspended matter), water and air temperature, amount of rainfall, insolation, discharge volume and the geological and pedological characteristics of the sampling site. Therefore, separate samples also should be collected for the customary chemical and bacteriological analyses of the water.

The contamination of a water is determined by means of a statistical model incorporating a principal component analysis and a multidimensional discriminant analysis. Examples of sampling programmes including all requisite statistical calculations are given in the proposed standards included in the list of references.

### 4. Sample size

Samples of 5 to 10 l per sampling site usually are of sufficient size. These are reduced, with thorough mixing, at the sampling site to the size of the analytical samples by filling them into 1 to 2-l bottles. In special cases, however, it may be necessary also to collect larger samples.

### 5. Sampling protocol

The sample containers must be labelled immediately after being filled. At the same time, the following information, of importance to the analyst, must be recorded in the form of a protocol:

Designation of the body of water (in the case of groundwaters, geography, depth and diameter of the borehole); exact location (incl. e.g. which side of bank, distance from bank, sampling depth), date and time of sampling; water volume, water and air temperature, weather conditions and data on other natural or anthropogenic factors that will assist in the interpretation of analytical results (e.g. silvicultural and agricultural uses of land and other activities in the vicinity of the sampling sites, waste discharge); in the case of groundwaters, also groundwater level and hydrogeological data on the aquifer; method of sampling, in the case of continuous sampling details of sampling programme; name of person responsible for collecting samples.

## 6. Sample preparation

Samples generally should be prepared or at least extracted immediately after collection or after arrival in the laboratory. The entire contents of a sample container must always be prepared, and the empty container must be thoroughly cleaned by rinsing and shaking it out with fresh extraction solvent. The washing should be combined with the sample extract. The amount of sample is determined firstly by weighing the bottle while it is still filled with the sample and again weighing it after it has been emptied, cleaned and dried. It is not recommended to take aliquots from the sample container for the analysis because experience has shown that false results are obtained when the level of residue determined in an aliquot is re-calculated in relation to the entire sample or to the unit.

If it is not possible for water samples to be prepared or extracted immediately and they are to be examined for residues of compounds of different groups, they must be transported and stored in metal containers under refrigeration at  $-25^{\circ}\text{C}$  to preclude the risk of hydrolysis e.g. of organophosphorus compounds or other changes. Samples that are to be analyzed only for residues of persistent organochlorine compounds can be stored for a short period also in a non-frozen state since these compounds display considerable stability. Experiments disclosed no appreciable differences between 14-day storage at room temperature and that at  $+5^{\circ}\text{C}$ ; during this period, no transformation or degradation products were formed.

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ISOTC 147/6/1 Normvorschlag DP 5667 Water Sampling Programmes.

ISOTC 147/6/2 Normvorschlag DP 5668 Water Sampling Techniques.

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## Use of the Term “Water”

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Besides crops, foods, feeds and soil, water often must also be analyzed for residues of crop protection products and related compounds. Therefore, numerous methods published in the Manual include the analysis of water. Difficulties posed by the determination of residues in water generally tend to increase with increasing levels of pollution. A method developed for the analysis of waters showing little pollution may fail where heavily polluted waters are involved. Therefore, it is essential always to state in a method the constitution of the water on which it actually has been tried. As from the 8th German Edition (1985) this information is given where applicable in Section 7.2 of a method; however, as in previous editions, only the word “Water” appears in the heading of the respective method.

On the grounds of this information, it will be possible to assign, from the analytical viewpoint, the different waters to either of two basic categories differing in their degree of purity:

- Drinking water, equivalent to groundwater or other natural waters fit for human consumption;
- Surface water, e.g. river water, pond water, rainwater and other waters that do not satisfy the standards of purity established for drinking water.

It can be assumed that a method tried on a certain water will be applicable also to waters of comparable purity. As a rule, a method developed for the analysis of drinking water will be suitable also for groundwater, and a method devised for pond water will be useful also for river water. On the other hand, it cannot be predicted whether and, if so, to what extent a method tried on drinking water will be useful also for the analysis of surface water.

Effluents from industry or from households are not taken into consideration in the methods published in this Manual.

# **Micro Methods and Equipment for Sample Processing**

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(German version published 1982)

## **1. Introduction**

In the conventional procedures employed for processing samples for residue analysis, it is customary to use portions weighing 50 to 100 g. For the extraction step and especially for the ones that follow it, large volumes of solvents and correspondingly big amounts of other chemicals and reagents are therefore required so that consequently correspondingly large units of apparatus and equipment as well as relatively large areas of working space are needed. These high inputs are completely disproportionate to the amount of substance required for the quantitative gas-chromatographic determination, which as a rule is equivalent to no more than about 1/1000 of that extracted from the analytical sample.

By using apparatus of greatly reduced size and simple design, a number of the routine operations performed daily in a residue analysis laboratory can be drastically rationalized. Consistent use of this approach makes for a big saving of working space, reduced requirements of materials, in particular dangerous solvents, less physical energy input and increased operational economy. Provided the workplace is suitably designed and organized, analysts will be able to take full advantage of every one of the benefits afforded by this system.

## **2. Description of the system**

All the processing operations performed with the total extract by the conventional procedure are carried out with a very small aliquot of the sample extract in the micro apparatus. These operations include partitions between two immiscible phases, phase separations, chromatographic cleanup steps, reactions, dilutions and evaporation.

## **3. Steps of the procedure and required apparatus**

The basic unit of equipment is the commercially standardized centrifuge tube (10-ml capacity) with a fused-on KS 18 spherical socket joint illustrated in Figure 1, referred to hereinafter as the standard vessel.

Apart from the other units of equipment described in the following sections, additionally required items include 2 to 10-ml beakers, conical beakers (Figure 2), micro spatulas, micro rods, microlitre syringes, stands (made of stainless steel welding wire or glass) and racks for the standard vessels and conical beakers. Work is made much easier by using a glass work top (approx. 40 cm × 50 cm) and a good table lamp.

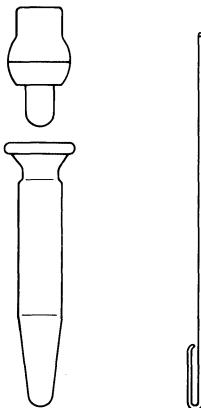


Figure 1. Standard vessel with KS 18 ground socket joint and wire stirrer.

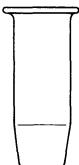


Figure 2. Conical beaker.

### 3.1. Homogenization and extraction

Starting with a sample of conventional size, the analytical material is extracted by the procedure described in Section 6.1 in the different methods. The extract is made up to the next suitable volume, and well shaken. An aliquot equivalent to a weighed portion of 1 to 2 g is then transferred volumetrically to a standard vessel and, if necessary, concentrated as described below in 3.5.

### 3.2. Transfer of liquids

Liquids can be added and withdrawn conveniently with all-glass syringes (0.5 to 10-ml capacity). A most suitable apparatus for directly transferring a liquid phase e.g. from one standard vessel to another is also the siphon illustrated in Figure 3. By applying a gentle vacuum regulated at one orifice with the tip of the index finger, the liquid is drawn in and simultaneously transferred to the second standard vessel connected to the siphon by a spherical joint clip.

This apparatus and the syringes are used also for siphoning off the upper or lower phase of immiscible liquids. To remove the lower phase, the syringe needle or the suction tube is inserted through the upper phase.

Separation of a liquid from solid constituents is effected by centrifugation, by decantation or by using a fritted micro filter tube (Figure 4) connected by Teflon tubing to a siphon with an appropriately shortened suction tube. Instead of the micro filter tube, use can be made also of a short glass tube, with constricted neck, in which a filter paper roll is inserted (Figure 5).

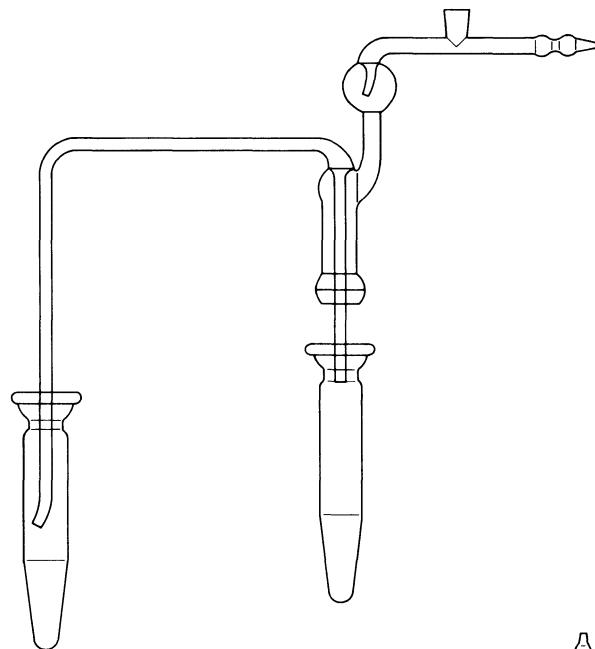


Figure 3. Liquid siphon (made from a capillary tube).

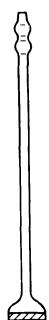


Figure 4. Fritted micro filter tube.

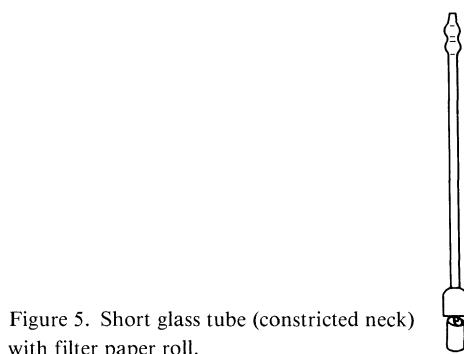


Figure 5. Short glass tube (constricted neck) with filter paper roll.

### 3.3. Partition between two immiscible liquids

The standard vessel is used to partition a substance between two liquid phases (e.g. hexane/acetonitrile). Partitioning is effected by whisking the phases with a wire stirrer consisting simply of a length of bent stainless steel welding wire. If clear separation of the phases is not achieved instantly, the vessel should be centrifuged for 1–2 min at approx. 2000 r.p.m.

### 3.4. Heating of solutions

The solutions are heated by placing the glass vessels in the drilled holes of an aluminium block mounted on a hotplate provided with energy regulator control (e.g. 100 W).

### 3.5. Concentration of solutions

Solutions are concentrated, with heating (3.4), mainly by directing a stream of purified nitrogen, blown through capillary tubes, onto the liquid surface. For this purpose, several tubes are assembled in a rack and spaced so that they are in complete alignment with the bores in the heated aluminium block.

Concentration operations must be monitored very carefully. In many cases, the solvent must not be allowed to evaporate completely because this may result in uncontrollable losses if solutions are left to stand for prolonged periods. All evaporation work is best carried out in a small fume hood (e.g. made of Plexiglas) fitted with an exhaust pipe, especially in those cases where hazardous substances have to be handled.

### 3.6. Chromatographic separations

A description of the apparatus required for chromatographic separations need not be given here. Micro columns for chromatographic cleanup on Florisil and silica gel are often described in the literature, e.g. constructed on the basis of Pasteur pipettes. For separations by gel permeation chromatography, it is recommended to use glass columns having a length of 25 cm and an internal diameter of 7–8 mm.

Before use, the retention volume must be determined for the respective compound with a solution of known compound content.

### 3.7. Adjustment of small liquid volumes

A certain problem is posed by the use of micro graduated cylinders (0.1, 0.5, 1, 2, 5 ml) (Figures 6 and 7). They are commercially obtainable (e.g. from Ehle) but they have to be re-calibrated which is best done gravimetrically with water at 20°C.

The 0.1-ml micro graduated cylinders are not required very often. Therefore, analysts are best advised to make their own from heavy wall capillary tubing (2 mm bore) cut into lengths of

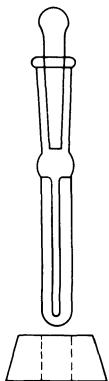


Figure 6. Micro graduated cylinder,  
0.1 ml, and cork with bore.

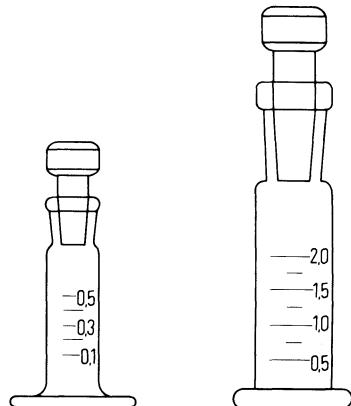


Figure 7. Micro graduated cylinders, 0.5 and 2.5 ml.

30 mm. One end is sealed, and a D 50 ground socket joint is fused onto the other end. The base is made from a piece of cork in which a hole of appropriate size is bored (Figure 6). This prevents transfer of heat when the cylinder is handled. The volume mark is determined gravimetrically with water and carefully scratched on or etched on with glass-marking ink.

For adjustment to e.g. 0.5 ml, the residue remaining after evaporation is dissolved in a few drops of solvent, the resultant solution is transferred to the micro graduated cylinder using a microlitre syringe (100 µl), and then filled up to the mark with solvent.

For adjustment to volumes of less than 100 µl, the largely concentrated solution is transferred to a 100-µl micro graduated cylinder using a microlitre syringe; the solution is continuously evaporated with nitrogen in the cylinder thus creating space for the next transferred portions and the washing. The concentrated solution is mixed thoroughly with a thin wire or glass stirrer, and adjusted to the mark either by blowing over a stream of nitrogen or by topping up.

It must be noted that with readily volatile solvents the adjusted volumes do not remain constant because the ratio of volume to surface area is unfavourable and the ground joints are never absolutely tight. Therefore, after the volume has been adjusted exactly, the solution must be gas-chromatographed as quickly as possible.

### 3.8. Gas-chromatographic determination

For the gas-chromatographic determination, an aliquot of the cleaned-up analytical solution transferred to a micro graduated cylinder of appropriate volume, is injected into the gas chromatograph. The determination is performed under the same conditions described in the methods for the individual compounds.

## 4. Organization of workplace

The advantages of working with miniature apparatus can be fully exploited especially in large series of analyses, particularly when the workplace is appropriately designed and organized.

Most of the work is done sitting down. Therefore, all items of equipment should be within easy reach to avoid any interruptions otherwise caused by having to stand up and leave the workplace. A suitable working surface is e.g. an L-shaped bench top set at a height of about 80 cm. Two roll-fronted cupboards fitted with drawers are placed under the bench to hold all requisite centrifuge tubes and bottles, beakers and other miniature apparatus and materials. By appropriately assembling the cupboards side by side as a separate unit and covering them with a single bench top, the layout of the overall working surface can be extended and converted to a U-shape. The actual working surface can consist e.g. of a 40 cm × 50 cm glass plate underlaid with a sheet of white paper. The test tube rack and the stand are mounted on it, and the hotplate and the nitrogen stream delivery tube rack are placed either on the right or on the left of them depending upon which is more convenient to the analyst.

The nitrogen is supplied through a length of tubing from a gas cylinder equipped with a pressure regulator and a fine adjustment valve. A second length of tubing is connected to a vacuum source (a water jet pump will be sufficient).

The small centrifuge can be placed on the second half of the L-shaped bench. Behind it, there is enough space for the sample bottles and the containers for holding used glassware.

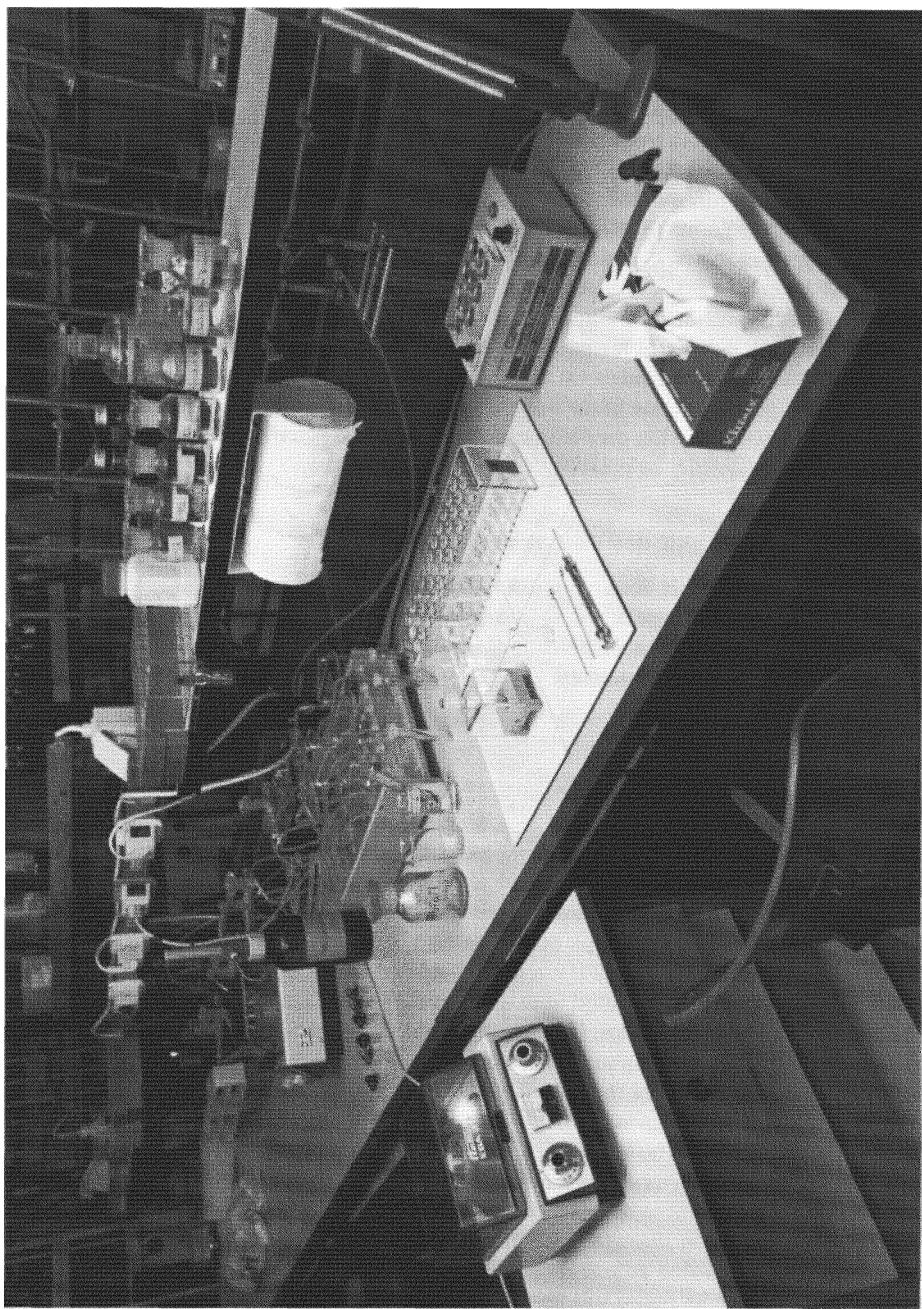


Figure 8. View of workplace.

The most frequently required chemicals are also placed within easy reach on a shelf preferably behind the actual work top. For most solvents, no more than a 250-ml stock of each need be kept at the workplace.

To ensure that all operations will proceed smoothly, it is recommended to use a separate, appropriately labelled syringe for each solvent. To avoid contamination of the stock of solvents, sub-portions should be filled into 50-ml glass bottles for use as required during the procedure. Centrifuge bottles, centrifuge tubes and beakers should be placed in a detergent solution immediately after use; they need not be finally cleaned until a sufficiently large number has accumulated. The other items of glassware and syringes, however, should each be rinsed immediately after use, while analytical work is still in progress. For cleaning these items, a sufficiently large stock of acetone and distilled water filled in 250-ml glass bottles as well as a plentiful supply of cellulose fibre wiping cloths, paper tissues and towels should be kept handy. Some items of equipment like the liquid siphon need no extra cleaning; they are cleaned automatically by the after-washing done during the analytical procedure. Rinsings and solvent remains should be deposited in a 500-ml glass bottle also kept at the workplace. A view of such a workplace is presented in Figure 8.

## 5. Appraisal of the micro procedure

By this micro method, 12 samples can be run simultaneously through all steps, apart from the extraction, by a single person seated at the bench and who has the operations completely within his view all the time. All items of apparatus are within easy reach and are clearly arranged. These features, combined with the miniature size of the apparatus and the neat layout of the workplace, make for a considerable improvement of work quality and are conducive to a smooth and precise flow of operations.

In comparison with conventional residue analysis, handling of the miniature apparatus is much less tiring even though the number of samples that have to be processed is two to three times greater.

The consumption of glassware is minimal, and the items are very easy to clean. The centrifuge bottles and tubes are small, very sturdy and relatively low-priced. A large stock of them can be purchased without incurrence of considerable financial outlay, and they can all be kept within easy reach at the workplace e.g. in the bench drawer, thus making for a saving of space.

Although the customary amount of solvent generally is still required for the extraction step, the amounts needed for processing the extracts are minimal, e.g. as little as 40 ml of dichloromethane for analyzing 12 samples for pyrazophos residues.

A stock of 250 ml of each solvent at the workplace usually will last for several days. This greatly reduces exposure of the analyst to solvent vapours. Generally, the use of micro apparatus considerably improves work safety.

With regard to accuracy and reproducibility of the analytical results, the micro procedure fully matches conventional methodology. In about 200 recovery experiments in which untreated control samples were fortified with pyrazophos and triazophos at levels of 0.01 to 1 mg/kg, the average recovery was  $85\% \pm 10\%$  ( $S = 0.95$ ) at a routine limit of determination of about 0.01 mg/kg.

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# Limits of Detection and Determination

(German version first published 1979, revised 1982 and 1987)

The limit of detection (LDC) is an important criterion of the efficiency of an analytical method. We have convened another characteristic, namely the limit of determination (LDM) the definition of which is matched to the specific conditions obtaining in residue analysis. Accordingly, a compromise had to be drawn between practicability and the application of exact statistical procedures to problems of residue analysis.

## 1. Definition of limit of detection

The limit of detection is characterized by the smallest value of the concentration of a compound in the analytical sample, for which the particular analytical method produces signal values which differ with 95% probability from those given at "nil" concentration in the analytical sample.

A compound is considered to have been detected when the found value of its concentration in the matrix is greater than the limit of detection. If the found value is above the limit of detection but below the limit of determination, the analytical result cannot be expressed as an exact numerical value; at the best, the order of magnitude of the concentration can be estimated.

## 2. Definition of limit of determination

The limit of determination is defined in residue analysis, by convention, as the smallest value of the concentration of a compound in the analytical sample, that satisfies the three following requirements:

- (i) It is greater than or equal to the limit of detection ( $LDM \geq LDC$ ); see Section 3.
- (ii) The sensitivity (recovery) for the limit of determination is equal to or greater than 0.70 ( $S \geq 0.70$ ); see Section 4.
- (iii) The coefficient of variation V at the limit of determination is equal to or smaller than 0.2 (equivalent to 20%):

$$V = \frac{\sigma}{q} \leq 0.2; \quad \text{see Section 5.} \quad (I)$$

Using the same method, differing limits of determination may be obtained for different analytical materials. In this case, closely lying limits of determination should best be grouped in classes, e. g. 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 mg/kg (see *W. D. Weinmann and H. G. Nolting, Nachrichtenbl. Dtsch. Pflanzenschutzdienstes Braunschweig* 33, 137–141, 1981).

The concentration of a compound in a substrate can be determined with sufficient reliability at the level of a maximum residue limit only by means of a method whose limit of determination

is substantially lower than the maximum residue limit, being equivalent at the most to one-third, if possible however to one-fifth of the permissible limit, or even less. For example, the limit of determination should be 0.01 mg/kg at an MRL of 0.05 mg/kg.

The values given for the limit of determination in this Manual can however provide the user of a method with no more than an indication of the performance rendered by the particular method in the laboratory of the author. The user must himself determine the values for his own laboratory and his own particular analytical problems. To do this, he requires analytical material that contains no residues of the sought compound (control sample).

The definition of the limit of determination indicates that to calculate it, the following parameters must be measured:

- limit of detection;
- sensitivity;
- coefficient of variation.

### 3. Determination of limit of detection

Residue-free analytical material originating from control tests, shown by control analyses to be free from residue or possibly obtained by removal of contaminated external portions, is analyzed for residues by the particular analytical method.

Theoretically, no signal values ought to be obtained with these samples. In practice, however, signals of "apparent residues" may occur, so-called blank signals. They may be due to several causes. For example,

- non-removed co-extractives,
- solvent and reagent impurities or
- instrument noise

may simulate the presence of residues.

If the blank signals vary only little, a mean blank signal  $\bar{B}$  can be calculated together with its standard deviation  $\hat{\sigma}_B$ , by measuring  $n$  (at least 3) blank signal values  $B_1 \dots B_i \dots B_n$ . The mean blank signal  $\bar{B}$ , measured e.g. in cm peak height, is computed from the formula

$$\bar{B} = \frac{1}{n} \sum_{i=1}^n B_i \quad (2)$$

and an estimate of the standard deviation of the blank signals is given by

$$\hat{\sigma}_B = \sqrt{\frac{\sum_{i=1}^n (B_i - \bar{B})^2}{n - 1}} \quad (3)$$

The degree of freedom of this estimate is  $f = n - 1$ .

If, however, the blank signals vary considerably, e.g. due to inadequate separation of co-extractives from the analytical material, or if they tend to become progressively smaller or greater e.g. due to constant decline or increase of a contamination, it will not be possible to correct the

value obtained for the analytical sample. In this case, the method or its implementation must be improved by the respective user, if necessary by incorporating additional cleanup steps.

There are several ways of estimating the limit of detection:

a) Rough estimate from blank values

When blank signals are measured several times, a rough estimate of the limit of detection can be obtained from the formula

$$LDC = \frac{2 \cdot t_{f=2; 95\% \text{ one-sided}} \cdot \hat{\sigma}_B}{S} [\text{mg/kg}] \quad (4)$$

(using the one-sided value for  $t_f$ ) where  $S$  is the sensitivity of the analytical method, determined as described in Section 4. Note that the dimension of  $\hat{\sigma}$  is e.g. cm peak height, and that the dimension of  $S$  is accordingly  $\frac{\text{cm}}{\text{mg/kg}}$ !

LDC can also be estimated by simply calculating the standard deviation of the mean blank signal from only three blank measurements. Then, when  $S$  is not known but is greater than 0.7,

$$LDC \approx \frac{2 \cdot 3 \cdot \hat{\sigma}_B}{0.7} \approx 9 \cdot \hat{\sigma}_B [\text{mg/kg}] \quad (5)$$

(with  $t_{f=2; 95\% \text{ one-sided}} \approx 3$ ) where the value 0.7 represents a rough estimate of the sensitivity (slope of calibration curve) of the method.

When it is certain that the recovery in a given case is practically complete ( $S \approx 1.0$ ), the limit of detection decreases to  $LDC \approx 6 \cdot \hat{\sigma}_B$ .

b) Improved estimate from recovery experiments

Recovery experiments are conducted as described in Section 4 for determining sensitivity. The standard deviation  $\hat{\sigma}_A$  is then estimated for the experiments with the lowest fortification level, and the standard deviation  $\hat{\sigma}_{\text{com}}$  is computed from  $\hat{\sigma}_B$  and  $\hat{\sigma}_A$  by applying the formula

$$\hat{\sigma}_{\text{com}} = \sqrt{\frac{(m - 1) \cdot \hat{\sigma}_A^2 + (n - 1) \cdot \hat{\sigma}_B^2}{m + n - 2}} [\text{mg/kg}] \quad (6)$$

where  $m$  is the number of analytical values and  $n$  is the number of blank values. The degree of freedom  $f$  of this estimate is  $m + n - 2$ .

An estimate of the limit of detection is then obtained by applying the formula (4), substituting  $\hat{\sigma}_{\text{com}}$  for  $\hat{\sigma}_B$ ,

$$LDC = \frac{2 \cdot t_{f=2; 95\%} \cdot \hat{\sigma}_{\text{com}}}{S} [\text{mg/kg}] \quad (7)$$

## 4. Determination of sensitivity

Recovery experiments are run in which the compound to be determined is added at several graded concentration levels to residue-free analytical material, and the slope of the calibration curve is measured. It is important to ensure that a linear plot of concentration vs. signal can be obtained over the entire measured range.

The calibration curve signifies the relationship between concentration and signal value. Its slope represents the sensitivity of the analytical method. Therefore, the sensitivity signifies the change in signal value per change of concentration. The dimension of sensitivity is thus equal to the dimension of the signal (e.g. cm peak height) divided by concentration (e.g. mg/kg).

The compound is added at fortification levels  $q$  beginning at the assumed limit of determination and terminating in the probable working range. Suitable fortification levels have proved to be, for example,  $q = 0.003, 0.01, 0.03, 0.1, 0.3 \text{ mg/kg}$  or  $q = 0.005, 0.01, 0.05, 0.1, 0.5 \text{ mg/kg}$ . At least four experiments ( $m \geq 4$ ) are conducted at each graded concentration; therefore,  $m$  signal values  $y$  are obtained for each concentration level.

By applying the usual formulae or, more simply, by using an appropriately programmed pocket or desk-top computer, a linear regression analysis is conducted with the single values obtained, and the parameters of the regression line are computed from the equation

$$\hat{y} = a_0 + \hat{S} \cdot q$$

where  $q$  is the given concentration (e.g. in mg/kg) and  $\hat{y}$  is the signal (e.g. in cm) pertaining to  $q$ . The slope of the calibration curve  $\hat{S}$  is an estimate of the sensitivity  $S$ .

In gas-chromatographic determinations, the usual practice is to convert the measured signals (e.g. cm peak height), either vs. the co-run standard or by means of a calibration curve, to the corresponding concentrations (e.g. mg/kg). Therefore, the signal values are expressed as concentrations in mg/kg;  $\hat{S}$  then becomes nondimensional.

## 5. Determination of the coefficient of variation

From the results of the recovery experiments conducted to determine sensitivity as described in Section 4, the coefficient of variation  $V$  is computed for each graded concentration  $q$  from the equation

$$V = \frac{\hat{\sigma}_q}{q}.$$

To determine  $V$  for the lowest concentration level, the weighted standard deviation from the signal values and the blank signals  $\hat{\sigma}_{\text{com}}$ , computed according to (6), is used.

## 6. Stipulation of the limit of determination

The limit of determination is the lowest concentration level  $q$  of the recovery experiments, for which the conditions (i) and (ii) are satisfied (see Section 2) and for which the coefficient of variation  $V$  is  $\leq 0.2$ .

## 7. Examples

### 7.1. Rough estimate of the limit of detection from the blank values

Blank values  $B_i$ : 0.012, 0.015, 0.017, 0.026 mg/kg.

Mean blank  $\bar{B}$ , computed from (2): 0.017 mg/kg.

Standard deviation  $\hat{\sigma}_B$  of blanks, computed from (3):

0.006 mg/kg with  $f = 3$  degrees of freedom.

When the lower estimate of sensitivity  $S$  is assumed to be 0.70, then equation (4) gives

$$LDC = \frac{2 \cdot 2.353 \cdot 0.006}{0.70} = 0.04 \text{ mg/kg} .$$

### 7.2. Improved estimate of the limit of detection from the results of recovery experiments

Blank values  $B_i$ : 0.007, 0.010, 0.012, 0.021 mg/kg.

Mean blank  $\bar{B}$ , computed from (2): 0.012 mg/kg.

Standard deviation  $\hat{\sigma}_B$  of blanks, computed from (3):

0.006 mg/kg with  $f = 3$  degrees of freedom.

Lowest concentration level used in recovery experiments:  $q = 0.10 \text{ mg/kg}$ .

Analytical values  $A_i$ : 0.090, 0.091, 0.096, 0.098 mg/kg.

Mean analytical value  $\bar{A}$ : 0.094 mg/kg.

Standard deviation  $\hat{\sigma}_A$  of analytical values:

0.004 mg/kg with  $f = 3$  degrees of freedom.

Standard deviation  $\hat{\sigma}_{\text{com}}$ , computed from (6):

0.005 mg/kg with  $f = 6$  degrees of freedom.

Rough estimate of sensitivity  $S$  from  $\bar{A}$  and  $q$ :

$$\hat{S} = \frac{0.094 \text{ mg/kg}}{0.10 \text{ mg/kg}} = 0.94 .$$

The estimated limit of detection given by applying (7) is:

$$LDC = \frac{2 \cdot 1.943 \cdot 0.005}{0.94} = 0.02 \text{ mg/kg} .$$

### 7.3. Estimate of limit of detection and limit of determination from results of recovery experiments

#### 7.3.1. Table of measured values and relevant statistical quantities

Amounts added mg/kg q	Measured values mg/kg y	Means mg/kg $\bar{A}$	Standard deviation mg/kg $\hat{\sigma}_A$	Coefficient of variation V
		$\bar{B}$	$\hat{\sigma}_B$	
0 (blank values)	0.0009			
	0.0014		0.0031	0.0022
	0.0048			
	0.0051			
0.01	0.011			
	0.011	0.0120		0.0014
	0.012			
	0.014			
0.05	0.041			
	0.044	0.0435		0.0017
	0.044			
	0.045			
0.10	0.071			
	0.077	0.081		0.0088
	0.085			
	0.091			
0.50	0.46			
	0.47	0.482		0.022
	0.49			
	0.51			

#### 7.3.2. Estimate of sensitivity

From the results of the blank samples and the recovery experiments (given values q, measured values y), the parameters of the regression line

$$\hat{y} = a_0 + \hat{S} \cdot q$$

are computed to be  $-0.00312$  for  $\hat{a}_0$  and  $0.966$  for  $\hat{S}$ .

The estimated value of sensitivity is therefore  $\hat{S} = 0.97$ .

#### 7.3.3. Estimate of the limit of detection

The standard deviation  $\hat{\sigma}_{\text{com}}$  given by equation (6), on the basis of the standard deviation of the blanks ( $\hat{\sigma}_B$ ) and the fortification levels at  $q = 0.01$  mg/kg ( $\hat{\sigma}_A$ ), is  $0.0018$  mg/kg with  $f = 6$  degrees of freedom.

Hence, the estimate of the limit of detection given by equation (7) is

$$\text{LDC} = \frac{2 \cdot 1.943 \cdot 0.0018}{0.97} = 0.0072 \text{ mg/kg} .$$

#### 7.3.4. Estimate of the limit of determination

At the lowest fortification level of 0.01 mg/kg, the coefficient of variation from  $\hat{\sigma}_{\text{com}}$  is 0.18, in other words smaller than 0.2, so that condition (iii) is satisfied (see Section 2). At this concentration, the recovery is greater than 70% ( $S \geq 0.7$ , corresponding to condition (ii)). Further,  $q = 0.01$  is greater than  $\text{LDC} = 0.0072$ .

The limit of determination of the method is thus  $\text{LDM} = 0.01 \text{ mg/kg}$ .

#### 7.3.5. Remarks

In this example, it is observed that at the point where the computation is transitional  $q \rightarrow 0$ , the regression line has a negative intercept on the ordinate ( $a_0 = -0.0031$ ) although the actual measured values give a positive mean blank  $\bar{B} = 0.0031$ . This clearly demonstrates that a calculation of the mean blank by extrapolation of the regression line from recovery experiments is not permissible. If no control samples are available, the mean blank can be estimated only by analyzing samples in which the concentration of the compound sought is below the limit of detection of the method.

## 8. Important points

In numerous methods reported in this Manual, only a **routine limit of determination** is given. It is not a value derived by statistical methods or on the basis of the relationships described above. Rather, it denotes the lowest residue concentration successfully used by the author and/or the analytical laboratory in the development of the method, or which was at least expected to produce satisfactory results. The routine limit of determination, therefore, provides the users of the Manual with an indication of the concentration range in which it is most expedient to test the method for its performance in their own laboratories.

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## Appendix

Threshold values of t distribution at a 95% level of statistical significance in relation to degree of freedom f = m - 1 or n - 1

f	t two-sided	t one-sided
1	12.71	6.314
2	4.303	2.920
3	3.182	2.353
4	2.776	2.132
5	2.571	2.015
6	2.447	1.943
7	2.365	1.895
8	2.306	1.860
9	2.262	1.833
10	2.228	1.812
20	2.086	1.725
30	2.042	1.697

# Reporting of Analytical Results

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The governing factor for the reporting of analytical results in low concentration ranges is the limit of determination (LDM).

A finding that is above the limit of determination is expressed as a real numerical value.

A finding that is below the limit of determination but above the limit of detection is considered not to be numerically definable, and is expressed in accordance with convention as “< amount of the limit of determination”. It should be suffixed by the letters “ndm” to make perfectly clear that it was “not determinable under the given circumstances”. For example, a result of “~0.03 mg/kg” obtained at a limit of determination of 0.05 mg/kg is expressed as

<0.05 or <0.05 ndm.

An analytical result that is below the limit of detection (LDC) is expressed as “ndc” (meaning not detectable under the given circumstances), suffixed by the numerical value of the limit of detection,

e.g. ndc (LDC 0.01).

Blank signals can simulate the presence of residues and should, therefore, be as low as possible. Blank values can be neglected in the reporting of results provided they are distinctly below (about one power of ten) the actual residue values.

If, however, the blank values are of the same magnitude as the actual residue values, they must be taken into account in the reporting of results. A value corrected for the blank can then be given in addition to the blank value and the uncorrected residue value. Reasons for deduction of the blank value must be given.

# **Use of Forms in the Reporting of Analytical Results**

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In order to assess the degradational behaviour of pesticides, e.g. for the purpose of establishing maximum residue limits and safety intervals, other data are required in addition to the actual residue analysis results. Such additional information includes field trial data and details of climatic conditions at the trials location.

A recommended procedure is to use forms for compiling and reporting these data since it enables them to be evaluated more easily. The information presented in the forms should include the following items (and where applicable given separately for each parent compound or metabolite and for each sample material):

## **Field Trial**

### **Pesticide**

Product code (manufacturer's code used during registration procedure)

Trademark

Formulation

### **Active ingredient**

Common name (ISO)

Content (g/l or %)

### **Crop (or treated material)**

Species (name)

Cultivar (variety)

Seeding (date)

Planting (date)

Commencement of flowering (date)

End of flowering (date)

Harvest (date)

Growth stage at final treatment

Growth stage at final sampling

### **Application data**

Where applied

– in field

- under glass
- other situations (e.g. under plastic film cloche, plastic film tunnel)

Causal organism(s) (pest, disease pathogen, weed)  
(or state specific object of treatment, e.g. haulm destruction)

Method of application (e.g. spraying)

Location of trial (p.o. code, name of place, country)

Size of trials area (m<sup>2</sup>)

Details per treatment

Date (day, month, year)

Active ingredient dose rate (kg/ha)

Formulation dose rate (kg/ha, l/ha)

Water volume (l/ha)

Applied concentration (% formulation)

Use of other pesticides (which?)

- on the treated plants

- on the control plants

### **Sampling**

Growth stage at sampling

Sampled material (e.g. leaves)

Date (day, month, year)

Days after final application

Identity of sample

Size of field sample (no. of pieces, kg)

Size of laboratory sample (no. of pieces, kg)

Preparation of sample for analysis (e.g. trimming, brushing)

Storage until transport (duration, temperature)

### **Soil characteristics**

Soil texture

pH

Content of organic C (%)

### **Climatic data**

Location

Date/period

Mean temperature (°C)

Rainfall (mm)

Sunshine (hours)

General description of weather pattern during period of trial compared with long-term mean, and any additional comments (e.g. concerning treatments applied incl. irrigation, aeration, etc.; any unusual divergencies from long-term mean for location)

**Experimenter** (name, address)

### **Residue Analysis**

Active ingredient and/or metabolite(s)

Crop (or treated material)

#### **Data on sample**

Date of dispatch

Date of receipt in laboratory

Condition of sample on receipt

Storage temperature (°C)

Weight of analytical sample (g)

Date of analysis

#### **Analytical data**

Method (author, literature reference)

Principle of method

Limit of determination (mg/kg)

Metabolites determined (state which, if any)

Residues calculated as

(e.g. for dithiocarbamates expressed as CS<sub>2</sub>)

#### **Residue analysis values**

Calculated for (edible parts only or for total wt. of edible and inedible parts, e.g. for cherries less stones or for cherries plus stones and perhaps stalks)

Material analyzed/analyzed part of crop (e.g. leaves)

Mean value (mg/kg)

No. of individual analyses made

Range of values from ... to ... (mg/kg)

Blank value (mg/kg) (of untreated control sample)

Percent recovery

Analysis performed by (name of laboratory)

#### **Comments**

(include here any additional data or comments on above points)

## **Part 2**

# **Cleanup Methods**



# Cleanup Method 1

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Separation of organochlorine insecticides from hexachlorobenzene and polychlorinated biphenyls

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(German version published 1974)

## 1. Introduction

Polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) behave exactly like organochlorine insecticides when they are subjected to the usual cleanup procedures, and therefore appear in the extracts prepared for gas chromatography of organochlorine insecticides. The fact that commercial PCB products consist of a large number of single components is reflected in the gas chromatogram by the occurrence of several peaks scattered over a wide range. They may therefore superimpose the peaks of organochlorine insecticides and lead to misinterpretations. To obtain a satisfactory identification of organochlorine insecticides, it is therefore necessary to separate the PCBs. HCB which may interfere with the determination of HCH isomers can also be separated together with the PCBs. Given a choice of suitable solvents, separation is rendered possible by the fact that PCBs migrate in a rather compact front on chromatographic columns or on thin-layer plates (despite the differences in the chlorine content of the individual components), and that they differ distinctly in their speed of migration from a number of organochlorine insecticides.

Whenever a large number of peaks are observed to occur in gas chromatograms obtained with an ECD, it may always be suspected that PCBs are present. Suspicion of their presence may be strengthened by comparing the gas chromatogram with that of a commercial PCB product. PCBs that enter into consideration usually are those with a 60% content of chlorine although others containing less chlorine also may be encountered. Numerous methods have been published for their separation; some utilize column chromatography (Ref. 1, 2) while in others thin-layer chromatography is employed (Ref. 3, 4). The methods reported here are suitable especially for separation of the ubiquitous pattern of chlorinated hydrocarbons, i. e. separation of PCBs with an approx. 60% content of chlorine and HCB from p,p'-DDT, HCH isomers, heptachlor epoxide and dieldrin. The separation of o,p'-DDT and especially of DDE poses difficulties, as shown by experience.

## 2. Outline of method

### 2.1. Column-chromatographic separation

The cleaned-up extract is fractionated on a column packed with silica gel of given activity. The PCBs and HCB are eluted with n-pentane or preferably with n-heptane; the organochlorine insecticides are eluted with benzene (Ref. 2, 5).

## 2.2. Thin-layer chromatographic separation

The cleaned-up extract is streaked on thin-layer plates, and chromatographed by ascending development using n-heptane as solvent. The zone extending from the origin to just below (using aluminium oxide) or above (using silica gel) the  $R_f$  of p,p'-DDE is scraped off; the scraping is eluted with benzene. This eluate contains no HCB, no high chlorine content PCBs of the Clophen A 60 type and, when using aluminium oxide, also no DDE. The compounds mentioned are eluted in the same way from the remaining layer.

## 3. Apparatus

### 3.1. Column chromatography

Chromatographic tube, see Figure 1

### 3.2. Thin-layer chromatography

Graduated pipettes or  $\mu$ l-syringes (10 – 100  $\mu$ l)

Thin-layer chromatographic tank

Volumetric flask, 1-ml

Lamp for shortwave UV light

Small columns with funnel, see Figure 2

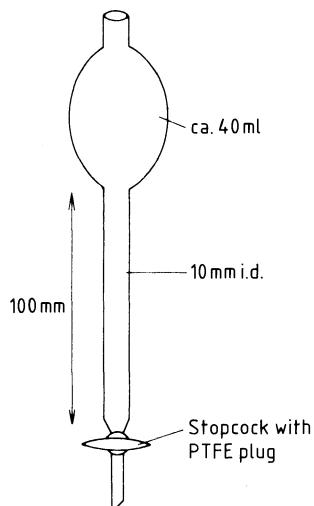


Figure 1. Chromatographic tube.

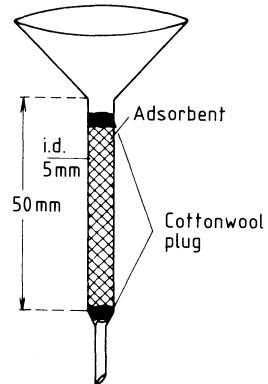


Figure 2. Small column with funnel.

## 4. Reagents

### 4.1. Column chromatography

Benzene distilled on sodium, or ECD pure  
n-Heptane distilled on sodium, or ECD pure  
Silica gel 60 (for column chromatography), 0.05–0.200 mm or 70–230 mesh (e.g. Merck No. 7754), activated for 8 h at 200°C

### 4.2. Thin-layer chromatography

Benzene distilled on sodium, or ECD pure  
n-Heptane distilled on sodium, or ECD pure  
Petroleum ether, boiling range 60–80°C, distilled on sodium, or ECD pure  
Thin-layer plates coated with silica gel or with aluminium oxide, each with fluorescent indicator for shortwave UV light, air-dried or activated at 100°C. Beforehand, ensure that no substances are present that respond to electron capture detection, by extracting a small amount of the adsorbent (about as much as in the analyses). If, however, the presence of such substances in large amounts is observed in the gas chromatogram, clean the adsorbent layer by passing over 1:1 acetone-water several times in the direction of travel. Then air-dry the plates or activate them at 100°C to remove acetone and excess water.

## 5. Procedure

Extracts yielded and cleaned up by one of the usual methods (see e.g. Ref. 7, 8) are used for the analysis.

### 5.1. Column chromatography

To silica gel add n-heptane in an Erlenmeyer flask. Tamp a plug of cottonwool into the bottom of the chromatographic tube, and fill up the tube with the silica gel mixture to a level of 8 cm. No air bubbles must be present in the column packing otherwise its separation efficiency will be reduced. After rinsing with an additional approx. 5-ml portion of n-heptane, drain the level of the solvent down to a few mm above the top of the silica gel. Dissolve the extract in 1 ml n-heptane and add to the column. Rinse the flask and column two times each with 1-ml portions of n-heptane. Elute the PCBs and HCB with 35 ml of the same solvent. Then elute the organochlorine insecticides with 40 ml benzene. Concentrate or dilute both solutions to a given volume, and inject into a gas chromatograph equipped with an ECD.

### 5.2. Thin-layer chromatography

Concentrate the extract to a small volume, and apply quantitatively by streaking (approx. 1 cm wide) on a TLC plate; rinse the flask and pipette with petroleum ether. Alongside the sample, apply a spot of about 20 µg p,p'-DDE as a reference compound. Chromatograph with n-heptane solvent. Inspect the plate under the UV lamp, mark the DDE reference spot and subdivide the

strip on the plate on which the analytical compound extract has been chromatographed. When aluminium oxide plates are used, the layer just below the DDE spot is scraped off down to the origin; when silica gel plates are used, the layer is scraped off from just above the DDE spot down to the origin. Transfer the adsorbent into a small column and, by adding about 2 ml benzene, elute into a 1-ml volumetric flask up to the mark. The major organochlorine insecticides are contained in this solution. In the same way, scrape off and elute the remaining layer containing the PCBs, HCB and DDE (in the case of aluminium oxide). Inject both solutions into a gas chromatograph equipped with an ECD.

## 6. Recovery

The recoveries determined for an added amount of 10 µg and calculated in relation to the amounts of compound present in the cleaned-up extract, exceeded 90%.

## 7. Important points

The following table gives the  $R_f$  values for the major chlorinated hydrocarbons separated by thin-layer chromatography on silica gel and aluminium oxide. The given  $R_f$  values were obtained by application of about 20 µg compound. They reflect the actual expansion of the spots (i. e. upper and lower limit) and therefore provide an indication of likely overlapping. However, they must be checked before using other adsorbent batches because of the likelihood of resultant variations in some  $R_f$  values. It is evident from the table that the low chlorine content PCBs of the Clophen A 30 type, which occur in extracts far less often than the higher chlorine content compounds, cannot be separated from some insecticides. Separation of Clophen A 60 from aldrin, heptachlor and o,p'-DDT, especially on aluminium oxide, also usually poses difficulties. If the extract has not been cleaned up sufficiently well, i. e. if large residual amounts of lipids are present, the spots may be distorted. In such cases, a more suitable cleanup method must be used.

If thin-layer plates without fluorescent indicator are used, the spots can also be visualized with silver nitrate spray reagent and UV irradiation (Ref. 6). In this case only a small portion (about 5%) of the residues present will be destroyed. With some stationary phases, some degree of separation can be achieved on packed gas-chromatographic columns without pre-fractionation. For example, p,p'-DDT can be isolated without any trouble from the commercial PCB product Clophen A 60 on a column (at least 2 m long; 2 mm i. d.) packed with 3% QF-1 on Chromosorb W-AW-DMCS, 80–100 mesh.

There is no need for pre-fractionation also when glass capillary columns are used for gas-chromatographic separation or when a gas chromatograph-mass spectrometer combination is used in the SIM mode.

Table.  $R_f$  values of major chlorinated hydrocarbons separated by thin-layer chromatography on silica gel and aluminium oxide, using n-heptane as solvent

Silica gel (air-dried)		Aluminium oxide (air-dried)	
HCB	0.54–0.65	HCB	0.69–0.82
Clophen A 60	0.32–0.50	Aldrin	0.63–0.73
Aldrin	0.31–0.38	Heptachlor	0.63–0.72
p,p'-DDE	0.24–0.34	p,p'-DDE	0.58–0.69
Heptachlor	0.24–0.33	o,p'-DDT	0.58–0.68
Clophen A 30	0.17–0.39	Clophen A 60	0.56–0.69
o,p'-DDT	0.23–0.32	Clophen A 30	0.50–0.65
p,p'-DDT	0.19–0.27	p,p'-DDT	0.49–0.59
p,p'-DDD	0.09–0.16	$\gamma$ -HCH	0.34–0.43
$\gamma$ -HCH	0.05–0.10	p,p'-DDD	0.29–0.39
$\alpha$ -HCH	0.02–0.09	Heptachlor epoxide	0.23–0.32
$\beta$ -HCH	0 –0.09	Dieldrin	0.18–0.25
Heptachlor epoxide	0 –0.06	$\beta$ -HCH	0 –0.13
Dieldrin	0 –0.03	$\alpha$ -HCH	0 –0.07

## 8. References

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## 9. Authors

Institute of Food Chemistry, University of Münster, *E. Schulte*  
 Federal State Control Laboratory of Food and Environmental Chemistry, Münster, *H.-A. Meemken* and *B. Habersaat*



# Cleanup Method 2

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Cleanup of crude extracts from plant and animal material by sweep co-distillation

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(German version published 1979)

## 1. Introduction

Sweep co-distillation is a method for the cleanup of crude extracts, by which the residues of numerous pesticides can be separated from the bulk of co-extractives. It is suitable for the separation of organophosphorus insecticides from plant material, of organochlorine pesticides from plant or fatty animal material, and of triazine herbicides from soil extracts.

Basically, the described cleanup procedure can be performed relatively fast and easily with little apparatus. However, commercial models are available which, apart from featuring ease of operation, permit reproducibility, cleanup of several samples and hence use of the method in routine analysis.

For determination of residues of the different pesticide groups, Part 4 of this Manual contains a number of multiresidue methods in which sweep co-distillation is used for extract cleanup.

## 2. Outline of method

The concentrated extract of the sample material is introduced into a continuous stream of inert gas and is carried into a hot zone where the dissolved components of the extract are condensed on a large surface. By passing a readily volatile solvent over the surface, the more volatile trace components are withdrawn from the condensate by sweep co-distillation and transferred to an intensely cooled receiver. For each sample solution, a new prepared scrubber tube is required. The cleaned-up distillate is concentrated and then analyzed for pesticide residues by gas chromatography as described in Multiresidue Methods S 12, S 13, and S 14.

## 3. Apparatus

Sweep co-distillation apparatus, optionally:

Laboratory constructed apparatus after Storherr and Watts (1965) or after Ramsteiner and Karlhuber (1972) or after Pflugmacher and Ebing (1973),

Commercial model (with preheater) of Kontes Glass Co.,

Automatic apparatus of Bender and Hobein Co.

Scrubber tube for sweep co-distillation apparatus, 5–10 mm i.d., 30–35 cm long, partly packed with quartz wool, end of constriction plugged with Teflon felt

Syringes, calibrated, 2-ml or 5-ml

## 4. Reagents

Cyclohexane, pure, dist.  
 Ethanol, absolute  
 Ethyl acetate, p. a., dist.  
 n-Hexane, p. a., dist. or for gas chromatography  
 Toluene, pure, dist.  
 Toluene + cyclohexane + ethanol mixture 4:1:1 v/v/v  
 Quartz wool for elementary analysis (Heraeus), extracted with ethyl acetate for 5 h in Soxhlet apparatus  
 Nitrogen, re-purified

## 5. Procedure

### 5.1. Organochlorine pesticides from plant material

Use a scrubber tube of 5 mm i. d.; pack middle 6-cm section not too tightly with quartz wool, leaving both ends free.

#### *Operating conditions*

Sample volume	1 ml, equivalent to 20 g plant matrix
Gas flow rate	Nitrogen, 70 ml/min
Solvent flow rate	n-Hexane, 3 ml/min, or 45 successive injections each of 1 ml n-hexane
Duration of injection	20–25 s; rinse twice with 1-ml portions of ethyl acetate
Time of sample exposure	15 min
Condensation temperature	230°C
Cold bath temperature	–5°C

### 5.2. Organophosphorus insecticides from plant material

Use a scrubber tube of 5–10 mm i. d.; pack two-thirds of tube length not too tightly with quartz wool, leaving about 4 cm free, measured from sidearm.

#### *Operating conditions I*

Sample volume	0.5 ml, equivalent to 20 g plant matrix
Gas flow rate	Nitrogen, 500 ml/min
Solvent flow rate	Ethyl acetate, 1.5 ml/min or 15 successive injections each of 1 ml ethyl acetate
Time of sample exposure	10 min
Condensation temperature	175–185°C
Cold bath temperature	–20 to –30°C

#### *Operating conditions II*

Sample volume	1 ml, equivalent to 20 g plant matrix
Gas flow rate	Nitrogen, 45 ml/min

Solvent flow rate	n-Hexane, 3 ml/min or 45 successive injections each of 1 ml n-hexane
Time of sample exposure	15 min
Condensation temperature	230°C
Cold bath temperature	-5°C

### 5.3. Triazine herbicides and their desalkyl metabolites from soil

Use a scrubber tube of about 8 mm i. d. packed with quartz wool.

#### *Operating conditions*

Sample volume	2 ml, equivalent to 25 g soil, injected within 1 min
Gas flow rate	Nitrogen, 1000 ml/min
Solvent flow rate	Toluene-cyclohexane-ethanol mixture, 4 ml/min or 10 successive injections each of 2 ml mixture
Time of sample exposure	5 min
Condensation temperature	200°C
Cold bath temperature	-10°C

## 6. Important points

Should the operating conditions require modification, the following points must be observed:

For the cleanup of organochlorine pesticides as set out in 5.1, it is advisable to add extract aliquots of 1 ml or less. For the cleanup of organophosphorus insecticides as set out in 5.2, the extract aliquot added should not exceed 1.5 ml. If bigger volumes are added, the described scrubber tube will become overloaded with the result that substantial amounts of co-extractives will be carried into the receiver. Addition of larger extract aliquots requires the use of tubes with a wider inner diameter.

Volatile sulphur-containing compounds present in an appreciable amount in sample material extracts cannot be removed by the described procedure.

For the cleanup of triazine herbicides from soil as set out in 5.3, it is not necessary to use a new scrubber tube for each sample solution. One tube can be used for approx. 10 sample solutions before interferences occur in the cleaned-up extract.

## 7. References

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Federal Biological Research Centre for Agriculture and Forestry, Department for Ecological Chemistry, Division for Pesticide Research, Berlin, *W. Ebing* and *J. Pflugmacher* (organophosphorus insecticides)

Federal State Control Laboratory of Food and Environmental Chemistry, Offenburg, *M. Eichner* (organochlorine and organophosphorus pesticides)

Ciba-Geigy AG, Agricultural Division, Basle, Switzerland, *B. Karlhuber* and *K. Ramsteiner* (triazine herbicides and desalkyl metabolites)

# Cleanup Method 3

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Cleanup of crude extracts from plant material by gel permeation chromatography on Sephadex LH-20

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(German version published 1979)

## 1. Introduction

Gel permeation chromatography is a generally applicable method for the cleanup of crude plant extracts. The method is suitable for use in the analysis of samples for residues of a wide range of pesticidal compounds, particularly those that are thermally sensitive and are prone to breakdown when subjected to other cleanup procedures.

## 2. Outline of method

The residues of organophosphorus compounds are separated by gel permeation chromatography on Sephadex LH-20 from the generally higher molecular weight constituents of crude plant extracts. The pesticide residues are eluted after the co-extractives. The column can be used any number of times.

## 3. Apparatus

Chromatographic tube for gel permeation chromatography, 25 mm i. d., 1 m long (e. g. Pharmacia No. SR 25/100), equipped with 2 adjustable plungers

Pump, solvent-resistant (e. g. Chromatronix Inc., or Spectra-Physics No. CMP 3)

Sample introduction valve (e. g. Chromatronix Inc., or Spectra-Physics No. SV-8031)

Outlet valve, e. g. Teflon needle valve (Serva No. 93017)

Syringes, calibrated, 2-ml

Flow-through UV-Vis filter photometer, 254 nm (e. g. Knauer No. 97.00), with potentiometric recorder

Fraction collector with timed flow (e. g. Pharmacia FRAC-100)

Rotary vacuum evaporator

## 4. Reagents

Ethanol, absolute, non-denatured

Pesticide standard solutions: 1–10 µg/ml in ethanol

Sephadex LH-20 (Pharmacia)

## 5. Procedure

### 5.1. Packing gel permeation column and assembly of system

A 118-g portion of Sephadex LH-20 is allowed to swell for 24 h in ethanol. The column is packed in the usual manner, from a loading reservoir. Ethanol is then pumped through the column at a flow rate of 45 ml/h until the level of the packing is constant. Next, the gel bed is settled in position by adjusting and tightening the plungers. The total volume of the gel bed is about 375 ml.

Gel permeation column, pump, sample introduction valve, etc. are assembled as illustrated in the Figure. The UV-Vis filter photometer can be connected between the outlet valve and the fraction collector.

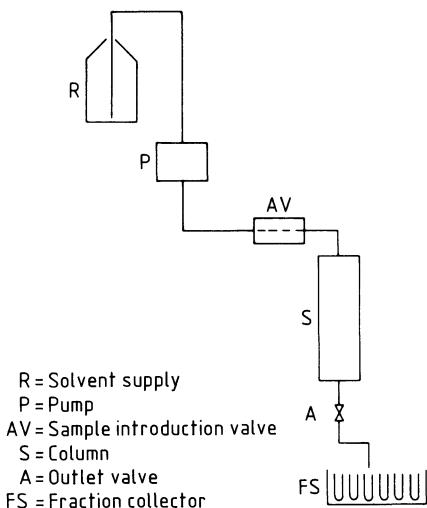


Figure. Diagrammatic representation of assembled system for gel permeation chromatography.

### 5.2. Determination of elution volumes

To determine the elution volumes of the sought compounds, 1 ml of the standard solution of an organophosphorus compound is added to the column using the sample introduction valve. The column is eluted with ethanol at a flow rate of 45 ml/h. The eluate is passed through the cell of the UV-Vis filter photometer; absorbance at 254 nm is recorded using a potentiometric recorder.

The elution volume of an organophosphorus compound is calculated from the flow rate and the time interval that elapses between sample introduction and attainment of the maximum measured for compound absorbance. If the absorbance of an organophosphorus compound at 254 nm is too low, the column is first eluted with about 200 ml and the following eluate is collected in 4-ml fractions. Each fraction is examined by gas chromatography, the concentration of the compound is determined, and the elution volume is calculated therefrom.

### 5.3. Cleanup of crude extracts

1 ml of a crude extract from plant material is added to the column through the sample introduction valve, and chromatographed with ethanol at a flow rate of 45 ml/h. That fraction of the column eluate is collected which corresponds to the elution volume range of the sought organophosphorus compounds in the sample.

## 6. Important points

The table gives the elution volumes of 25 organophosphorus compounds, determined by the procedure described in 5.2.

The accuracy of the determination of the elution volumes is 1 to 2%. Sephadex LH-20 columns were found to be still in a good unchanged working condition after 2 years of operation.

The solutions cleaned up by the procedure described in Section 5 are suitable for direct gas-chromatographic analysis with a thermionic phosphorus detector. Details of the appropriate procedure for the extraction of the samples and the gas-chromatographic determination are given in Multiresidue Method S 17.

## 7. References

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## 8. Authors

Federal Biological Research Centre for Agriculture and Forestry, Department for Ecological Chemistry, Division for Pesticide Research, Berlin, *W. Ebing* and *J. Pflugmacher*

Table. Elution volumes of organophosphorus compounds

Compound	Elution volumes	
	absolute (ml)	relative (to benzene = 1)
Azinphos-ethyl	412	1.268
Bromophos	316	0.972
Bromophos-ethyl	305	0.938
Chlorfenvinphos	265	0.816
Chlorthion	445	1.369
Diazinon	290	0.892
Dichlofenthion	325	1.000
Dichlorvos	306	0.942
Dimethoate	315	0.969
Disulfoton	330	1.015
Ethion	309	0.951
Fenchlorphos	380	1.169
Fenitrothion	396	1.218
Fensulfothion	360	1.108
Fenthion	399	1.228
Malathion	315	0.969
Mevinphos	298	0.917
Naled	355	1.092
Paraoxon	315	0.969
Parathion	376	1.157
Parathion-methyl	428	1.317
Phenkaptone	378	1.163
Phorate	345	1.062
Thionazin	318	0.978
Vamidothion	309	0.950

# Cleanup Method 4

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Cleanup of crude extracts from plant material by gel permeation chromatography on polystyrene gels

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(German version published 1979)

## 1. Introduction

Gel permeation chromatography can be used for cleanup of crude extracts from a wide variety of food crops and other commodities. It is recommended especially for the analysis of residues of such compounds that are thermally unstable and that are easily broken down or adsorbed irreversibly in other cleanup procedures. An advantage of using gels based on polystyrene over dextran gels like Sephadex LH-20 (see Cleanup Method 3) is the attainability of a faster eluant flow rate. Therefore, extract cleanup takes less time, added to which less solvent is required.

## 2. Outline of method

The residues of the compounds to be analyzed are separated by gel permeation chromatography on a polystyrene gel from the generally higher molecular constituents in the crude extracts. The residues of the compounds sought are eluted after the co-extractives. The column may be used any number of times.

## 3. Apparatus

Chromatographic tube for gel permeation chromatography, 25 mm i. d., 40 cm long (e.g. Pharmacia No. SR 25/45), see Figure 1

Four-way valve with sample loop, 1-ml, made of Teflon or the like, see Figure 2, or similar tool (e.g. Schoeffel No. 50-20)

Levelling vessel, e.g. separatory funnel, with adjustable holder

Fraction collector, volumetric control

Syringe, 1-ml

Rotary vacuum evaporator

## 4. Reagents

Ethyl acetate, p. a.

Pesticide standard solutions: 1–10 µg/ml in ethyl acetate

Styrene-divinylbenzene copolymer (2% DVB), e.g. Servachrom XAD-2, 50–100 µm (Serva) or Bio-Beads S-X2, 200–400 mesh (Bio-Rad Laboratories)

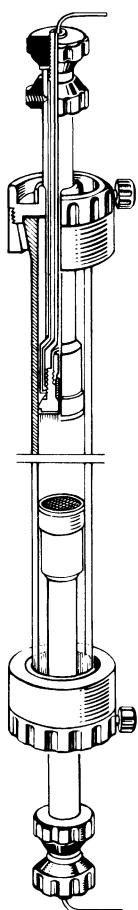


Figure 1. Chromatographic tube for gel permeation chromatography.

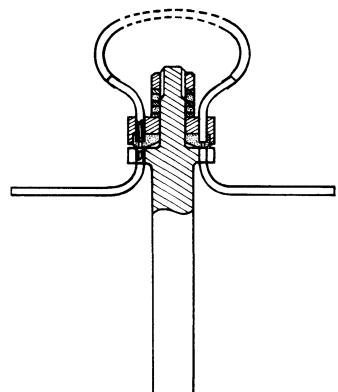


Figure 2. Four-way valve with sample loop (cross-section).

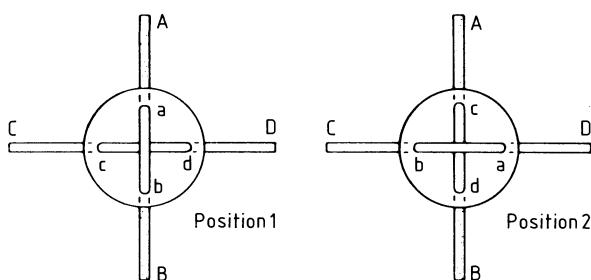


Figure 3. Four-way valve (schematic).

From the commercial products, isolate the 50–60 µm fraction (see Important points below) by air-sifting with a Multiplex zigzag sifter (Alpine). Whenever other fractions are used, column length and flow velocity must be adjusted accordingly

## 5. Procedure

### 5.1. Assembly of apparatus and packing of gel permeation column

The first step is to assemble the four-way valve (Figure 2). It consists of two metal disks; one is fastened to a metal bar holder and the second is screwed on so that it can be rotated. The tightly fastened disk has 4 bores with the connections A, B, C and D; opposite them are 4 bores in the rotatable disk with the connections a, b, c and d. The connections a and b on the rotatable disk are joined to a short length of tubing. A tube of 1-ml capacity positioned between the connections c and d forms the sample loop.

The column packing material is allowed to swell overnight in ethyl acetate. Then the suspension is poured all at once into the column (capacity of about 180 ml). As soon as the gel has settled, the plunger is inserted and screwed into place.

Next, the upper connection A on the four-way valve is joined to the levelling vessel, the lower connection B is joined to the upper end of the column, and the entire loading system is filled, free of bubbles, with ethyl acetate. With the valve set at position 1 (see Figure 3), the eluant flows through the valve via pathway A-a, passes through the connection a-b, and again flows through the valve along pathway b-B. The levelling vessel is then raised, and a pressure higher than the later working pressure is applied to compress the gel bed. The plunger is then lowered down to the gel bed (observe instructions of makers).

It is essential that the plunger is lowered also in the event of the gel bed becoming further compressed after prolonged operation. Provided the column is correctly loaded, ethyl acetate will flow through the column at a rate of 150 ml/h when the upper meniscus of the solvent in the levelling vessel is 1.3–1.4 m above the lower column outlet.

### 5.2. Determination of elution volumes

To determine the elution volumes of the compounds to be analyzed, 1 ml of the standard solution of an organophosphorus compound is injected, free of air bubbles, into connection C of the four-way valve so that the sample loop c-d is completely loaded. The rotatable disk of the valve is then turned 90° in a clockwise direction, or also anticlockwise, to position 2 (see Figure 3) so that the eluant flows via pathway A-c into the sample loop and emerges from it along pathway d-B. The column is eluted with ethyl acetate at a flow rate of 140–150 ml/h.

The eluate is collected in 5-ml fractions in a fraction collector. Each fraction is then analyzed for its content of the respective compound by a suitable analytical method. Usually, however, the first 60 ml of the eluate can be discarded.

With compounds featuring adequate UV absorbance, the eluate also can be passed firstly through the cell of an UV monitor and, by recording the absorbance, those fractions can be detected in which the respective compound is eluted.

### 5.3. Cleanup of crude extracts

The concentrated residue of a crude extract is dissolved in ethyl acetate. The solution must not contain any solid particles since these usually are retained undissolved in the gel bed of the column and reduce its cleanup efficiency. In cases of doubt, the solution should therefore be filtered or centrifuged.

A 1-ml portion of the solution is injected, free of air bubbles, through connection C of the four-way valve into the sample loop c-d and, by turning the rotatable disk through 90°, fed into the eluant stream as described in 5.2. The column is eluted with ethyl acetate at a flow rate of 140–150 ml/h. Those fractions of the column eluate are collected that correspond to the elution volumes of the sought compounds.

The respective fractions are combined and concentrated to a volume of 0.5–1 ml by rotary evaporation or by passing over a stream of purified air.

## 6. Important points

The gel permeation chromatographic cleanup method described herein was tried on the commodities and plant parts listed in the Table. The analytical samples were extracted with ethyl acetate, the extracts were chromatographed on polystyrene gel (2% DVB, 50–60 µm), and the quantity and distribution of the co-extractives were determined in the eluate. The results are also given in the Table, and show that the bulk of the co-extractives are eluted with 70–90 ml of ethyl acetate.

The examined residues showed, on the other hand, elution volumes of between 90 and 140 ml. For example, the following elution volumes were obtained for three organophosphorus compounds and three other compounds (column packed to a level of 30 cm):

Parathion-ethyl	90–100 ml
Pyrazophos	100–110 ml
Triazophos	90–110 ml
Binapacryl	90–110 ml
p,p'-DDT	110–130 ml
Quintozone	120–140 ml

To ensure satisfactory operation of the gel permeation column, it is important that the range of particle size distribution is as narrow as possible. This is especially the case when the required excess pressure is generated only by a levelling vessel so that there is no need to use an additional pump.

After lengthy use of the gel permeation column, the flow rate may slow down but without any change in the elution volumes of the compounds. It is, however, recommended to check the elution volumes from time to time. The gel permeation columns so far used were still in a good, unchanged working condition after several years of operation.

Table. Quantity of co-extractives (in mg) after extraction of different plant materials with ethyl acetate and gel permeation chromatography on polystyrene (2% DVB, 50–60 µm), in the eluate fractions

Plant material	Sample weight (in g)	Fraction of eluate (in ml)					
		0–50	50–70	70–90	90–110	110–130	130–180
Apples	50	7	105	165	36	—	—
Beans	50	3	14	32	10	2	—
Carrots	50	—	22	55	16	4	—
Cauliflower	50	3	2	65	11	—	—
Cauliflower, leaves	100	5	69	390	500	73	—
Celeriac, leaves	50	21	85	85	55	16	2
Celeriac, edible bulbs	50	4	10	75	16	1	—
Cherries	100	8	53	110	17	4	—
Cottonseed	23	5	80	2800	101	6	2
Cucumbers	50	2	2	7	2	1	—
Cucumber, peel	50	4	8	18	10	2	—
Grapes	100	15	850	180	51	22	—
Head cabbage	50	12	35	35	15	5	—
Hop cones	25	4	350	425	140	7	—
Onions	50	6	20	30	16	5	2
Onion, sprouts	50	8	55	85	31	5	2
Peas	50	6	29	115	10	—	—
Pea pods	50	31	28	42	50	2	—
Potatoes	50	—	26	8	—	—	—
Rice plants	20	24	72	68	30	9	4
Sugar beet, leaves	50	1	10	22	6	—	—
Sugar beet, edible roots	50	—	8	23	13	4	2
Tobacco	25	6	82	325	18	138	1
Tomatoes	50	2	18	60	24	10	1

## 7. References

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## 8. Authors

Hoechst AG, Analytical Laboratory, Frankfurt-Höchst, S. G. Gorbach, S. Winkler and E. Gaudernack



# Cleanup Method 5

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Cleanup of large quantities of fats for analysis of residues of organochlorine and organophosphorus compounds

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(German version published 1979)

## 1. Introduction

The usual methods employed for the determination of non-polar organochlorine and organophosphorus compounds in fats and fatty products permit the use of only a small amount of sample with the result that sensitivity is limited. More polar lipophilic pesticides usually cannot be determined by these methods or at least not without interferences because they cannot be separated sufficiently from fats and other lipids.

The principle of the method described herein is the removal of the bulk of lipids from a fatty solution by a suitable adsorbent. The yielded extracts are then further processed by Method S 9 or S 10 reported in this Manual. In this way, the fat sample weight can be raised to 30 g and sensitivity can be improved significantly. Additionally, more polar compounds and metabolites can also be determined substantially better than before when subsequent processing is performed by Method S 10.

## 2. Outline of method

The acetonic solution of the fat to be analyzed is mixed intensely with the suspension of a synthetic calcium silicate (tradename Calflo E). The mixture is filtered twice, the volume of the filtrate is measured, and the solution is rotary-evaporated to dryness.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor

Beaker, 100-ml

Buchner porcelain funnel, 12 cm dia., with vacuum filtration flask

Graduated cylinder, 200-ml, high form

Round-bottomed flasks, 500 and 250-ml, with ground joint

Rotary vacuum evaporator, 40°C bath temperature

Fluted filter papers, 20 cm dia., extracted with acetone

Round filter papers, 12 cm dia., fast or slow flow rate (Schleicher & Schüll)

#### 4. Reagents

Acetone, for residue analysis  
Acetonitrile, for residue analysis  
Isooctane, for residue analysis  
Calflo E (Johns-Manville Prod. Corp.), dried overnight at 130°C  
Filter aid, e.g. Celite 545 (Roth), dried overnight at 130°C

#### 5. Procedure

Dissolve 5 to 30 g of fat (G) in 25 ml acetone in a beaker. Stir the fat solution into a blender jar containing 200 ml acetonitrile, 20 g Calflo E and 10 g Celite 545. Rinse the beaker several times with small portions of acetonitrile (total amount of 25 ml). Blend intensively for 2 min, and filter the suspension with suction through a paper filter in a Buchner porcelain funnel. Apply gentle vacuum only, to ensure that no more than minimal portions of the filtrate can evaporate. Then filter the filtrate through a dry fluted filter paper covered with 3 g Calflo E into a graduated cylinder. Measure the volume of the filtrate (V), and transfer it, rinsing with acetone, into a round-bottomed flask. Add 2 ml isooctane, and rotary-evaporate at a bath temperature of 40°C to a volume of 0.5 to 1 ml. Remove the last traces of solvent with a gentle stream of air at room temperature.

#### 6. Evaluation

The residue after evaporation, derived from step 5, is equivalent to only a portion ( $G_{corr}$ ) of the fat sample weight.  $G_{corr}$  in g fat is calculated from the following equation:

$$G_{corr} = \frac{G \cdot V}{250}$$

where

G = sample weight (in g)

V = volume of filtrate after cleanup (in ml)

#### 7. Important points

To avoid loss of filtrate from evaporation, filtration with suction should be terminated timely even if it means obtaining filtrate volumes of only between 100 and 150 ml.

With sample weights of between 10 and 30 g of different fats and oils, residues after evaporation were found to amount to between 0.1 and 0.7 g. Somewhat larger amounts of lipids were obtained from milk fat and natural olive oil than from refined edible fats so that for these it is best to use sample weights of only 10 to 15 g. Using wool wax sample weights of 4 to 10 g, residues after evaporation amounted to 1 to 2 g.

Exploratory experiments have shown that dry, fatty foods (dry egg yolk, cocoa powder) and feeds as well as oilseeds (rape, poppy, groundnuts) also can be cleaned up directly by the described procedure. The samples are homogenized in the blender jar with 25 ml acetone, 225 ml acetonitrile, 20 g Calflo E and 10 g Celite 545. The suspension is then cleaned up as described in Section 5. The sample weight must be adjusted to the fat content and the amount of lipids left in the extract.

For further processing of the residue after evaporation, derived from Section 5, the Methods S 9 and S 10 have proved suitable. When samples need to be analyzed only for organochlorine and non-polar organophosphorus compounds, it is of advantage to use Method S 9 since it involves little work and time. By using Method S 10, the more polar organophosphorus compounds and several metabolites can be determined additionally. Further, the extracts are cleaned up more intensively so that Method S 10 is appropriate also for the analysis of materials from which Calflo E removes only a small portion of lipids.

Table. Organochlorine and organophosphorus compounds including several metabolites recovered at levels exceeding 70% (marked thus +) or not recovered (marked thus -) in recovery experiments following further extract cleanup by Method S 9 or S 10

	S 9	S 10		S 9	S 10
Aldrin	+	+	Azinphos-ethyl	-	+
$\gamma$ -Chlordane	+	+	Carbophenothion	+	+
Chlорfenson		+	Chlorfenvinphos	-	+
$\alpha,p'$ -DDD	+	+	Diazinon	-	+
$p,p'$ -DDD	+	+	Dioxathion		+
$\alpha,p'$ -DDE	+	+	Ethion	-	+
$p,p'$ -DDE	+	+	Fenchlorphos	+	+
$\alpha,p'$ -DDT	+	+	Malathion	-	+
$p,p'$ -DDT	+	+	Parathion-ethyl	-	+
Dieldrin	+	+	Parathion-methyl		+
$\alpha$ -Endosulfan	+	+	Phosalone	-	+
$\beta$ -Endosulfan	-	+			
Endosulfan sulphate	-	+			
Endrin	+	+			
Fenson		+			
$\alpha$ -HCH	+	+			
$\beta$ -HCH	+	+			
$\gamma$ -HCH (lindane)	+	+			
$\delta$ -HCH	+	+			
Heptachlor	+	+			
Heptachlor epoxide	+	+			
Hexachlorobenzene	+	+			
Methoxychlor					
PCB					
Quintozene	+	+			
Tetrasul					
Toxaphene (camphechlor)		+			

Recoveries of the compounds to be analyzed can be determined only in conjunction with further extract cleanup. In a collaborative study by 9 laboratories, recovery experiments were run in which control samples of refined sunflower oil were fortified with different pesticides and metabolites at concentrations ranging from 0.02 to 16 mg/kg. The Table lists those compounds for which total recoveries of between 70 and 100% (in most cases ranging from 80 to 95%) were obtained by the reported procedure in conjunction with either Method S 9 or S 10.

## 8. References

*Arbeitsgruppe "Pestizide", Zur Rückstandsanalytik der Pestizide in Lebensmitteln, 6. Mitteilung: Erfahrungen bei der Aufarbeitung größerer Fettmengen mit Calflo E, Lebensmittelchem. gerichtl. Chem. 32, 53–54 (1978).*

*W. M. Rogers, The use of a solid support for extraction of chlorinated pesticides from large quantities of fats and oils, J. Assoc. Off. Anal. Chem. 55, 1053–1057 (1972).*

*W. Specht, Methode zur raschen Aufarbeitung größerer Fettmengen für die Analyse von Pestizidrückständen, Lebensmittelchem. Gerichtl. Chem. 32, 51–53 (1978).*

## 9. Authors

Institute for Residue Analysis Dr. Specht and Dr. Winkelmann, Hamburg, *W. Specht*  
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# Cleanup Method 6

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Cleanup of crude extracts from plant and animal material by gel permeation chromatography on a polystyrene gel in an automated apparatus

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(German version published 1982)

## 1. Introduction

Gel permeation chromatography on polystyrene gels already has been described in Cleanup Method 4. For routine analyses, it is of particular benefit that the procedure takes little time and requires the use of only relatively small amounts of solvent and that the cleanup, performed as described below, can be done with commercial types of automated instruments.

## 2. Outline of method

In an instrument for automated gel permeation chromatography, the residues of the compounds to be analyzed are separated on Bio-Beads S-X3 from interfering substances, usually of higher molar mass, present in the crude extracts. The eluant is a mixture of equal parts by volume of cyclohexane and ethyl acetate, which provides efficient separation at a relatively low elution volume. The residues sought are eluted after the interfering substances. The column can be used any number of times.

## 3. Apparatus

Automated instrument for gel permeation chromatography, e.g. GPC Autoprep 1001 or 1002 (Analytical Bio-Chemistry Laboratories), equipped with chromatographic tube, 25 mm i.d., 40 cm long, and twenty-three 5-ml sample loops

Round-bottomed flask, 100-ml, with ground joint and 9-cm long neck

Test tube with ground stopper and graduated mark at 5 ml

Syringe, 10-ml

Rotary vacuum evaporator, 30–40°C bath temperature

Filter paper, 6 cm dia., fast flow rate (Schleicher & Schüll), exhaustively extracted with dichloromethane

## 4. Reagents

Cyclohexane, for residue analysis

Ethyl acetate, for residue analysis

Eluting mixture: cyclohexane + ethyl acetate 1:1 v/v

Standard solutions: 0.01–10 µg of pesticides in 10 ml eluting mixture, according to response of gas-chromatographic detectors

Bio-Beads S-X3, 200–400 mesh (Bio-Rad Laboratories)

Sodium sulphate, p. a., heated at 550 °C for at least 2 h

## 5. Procedure

### 5.1. Packing gel permeation column

The Bio-Beads (approx. 50 g) are allowed to swell overnight in the eluting mixture. Then the suspension is poured all at once into the column (capacity of about 180 ml). As soon as the gel bed has settled (free from air bubbles) to a level of about 32 cm, the plunger is inserted, lowered down to the bed level, and screwed into place. If the gel bed sinks to a still lower level after prolonged operation, the plunger must be adjusted accordingly (observe instructions of makers).

### 5.2. Checking elution volumes

When a new gel permeation column is used, the elution conditions must be checked on several compounds of the lower and upper elution volume range (see Table), and on appropriate crude extracts. To do so, load the sample loop with a mixture of standard solutions or crude extracts, elute as described in step 5.3, and determine by means of a suitable analytical method whether the added compounds are completely recovered or whether interferences are caused by non-separated impurities. The same check must be carried out on columns after they have been in use for a lengthy period.

### 5.3. Cleanup of crude extracts

To the concentrated residue of a crude extract add exactly 7.5 ml ethyl acetate, and dissolve by gentle swirling. Add 2 g sodium sulphate, swirl again, and add exactly 7.5 ml cyclohexane. Shake for about 20 s, filter through a fast flow-rate filter paper, and inject the filtrate into one of the sample loops of the gel permeation chromatograph.

To clean up fats, dissolve 5.0 g of the fat in the eluting mixture, make up with eluting mixture to a volume of 25.0 ml, and inject an aliquot of this solution into one of the sample loops.

Elute the gel permeation column with the eluting mixture at a flow rate of 5.0 ml/min. For multiresidue analyses, set the instrument switches as follows:

Dump switch to      20 min to discard 100 ml

Collect switch to      13 min to collect 65 ml

Wash switch to      2 min for column rinse with 10 ml

Concentrate the Collect volume to approx. 1 ml on a rotary evaporator (rotate slowly, immerse flask only slightly), pipette into a ground-stoppered graduated test tube, rinse with ethyl acetate, and make up to a volume of 5.0 ml.

## 6. Important points

For the determination of individual pesticide residues, the instrument can be set to a smaller Collect volume, matched to the respective compound, according to the values given in the Table.

Table. Elution volumes of a number of pesticides under the conditions of gel permeation chromatography set out in 5.3

Compound	Elution volume range in ml	Compound	Elution volume range in ml
<i>Organochlorine compounds</i>			
Aldrin	120-150	Azinphos-ethyl	130-160
Camphechlor	110-150	Bromophos	120-150
$\alpha$ -Chlordane	110-140	Bromophos-ethyl	110-140
$\gamma$ -Chlordane	100-130	Carbophenothion	120-140
Chlorfenson	120-150	Chlorfenvinphos	110-140
$\text{o},\text{p}'$ -DDD	110-140	Chlorpyrifos	110-140
$\text{p},\text{p}'$ -DDD	100-140	Diazinon	105-135
$\text{o},\text{p}'$ -DDE	120-150	Dicrotophos	130-160
$\text{p},\text{p}'$ -DDE	120-150	Dimefox	120-155
$\text{o},\text{p}'$ -DDT	120-150	Dimethoate	120-150
$\text{p},\text{p}'$ -DDT	110-140	Disulfoton sulphone	110-140
Dicofol	100-150	Disulfoton sulphoxide	120-150
Dieldrin	120-150	Ditalimfos	120-150
$\alpha$ -Endosulfan	110-150	Ethion	110-140
$\beta$ -Endosulfan	110-150	Fenchlorphos	120-150
Endosulfan sulphate	100-140	Fenitrothion	120-150
Endrin	130-160	Formothion	120-150
Fenson	130-160	Iodofenphos	120-150
$\alpha$ -HCH	120-150	Malaoxon	110-140
$\beta$ -HCH	100-130	Malathion	110-140
$\gamma$ -HCH (lindane)	110-140	Methamidophos	120-150
$\delta$ -HCH	100-130	Methidathion	130-165
Heptachlor	110-140	Mevinphos	120-150
Heptachlor epoxide	110-140	Omethoate	140-160
Hexachlorobenzene	140-165	Paraoxon	110-140
Isodrin	120-150	Parathion	110-140
Methoxychlor	125-155	Parathion-methyl	120-150
Oxychlordane	100-160	Phosalone	110-140
Quintozene	135-165	Pyrazophos	110-140
Tecnazene	130-160	Sulfotep	100-130
Tetradifon	120-150	Thionazin (zinophos)	120-150
Tetrasul	125-155	Triazophos	120-140
<i>Polychlorinated biphenyls</i>			
Clophen A 30	120-165	<i>Other compounds</i>	
Clophen A 60	120-160	Anilazine (zinochlor)	105-135
		Binapacryl	100-130

Table. (contd.)

Compound	Elution volume range in ml	Compound	Elution volume range in ml
Bitertanol	100–130	Rabenazazole <sup>a)</sup>	120–160
Captafol	120–150	Resmethrin	100–130
Captan	120–150	2,4,5-T <sup>b)</sup>	100–130
Dichlofluanid	100–140	Triadimefon	100–130
Dinocap	100–120	Triadimenol	100–130
Fluotrimazole	100–140	Trifluralin	95–120
Fuberidazole <sup>a)</sup>	120–160	Vinclozolin	100–130
Imazalil <sup>a)</sup>	120–150		
MCPA <sup>b)</sup>	100–130		
Metribuzin	110–140		
Pentachloroaniline	110–140		
Piperonyl butoxide	100–130		
Propoxur	110–130		
Pyrethrins	100–130		

<sup>a)</sup> with complete exclusion of light<sup>b)</sup> gel-chromatographed as acid, determined as methyl ester

## 7. Reference

*W. Specht and M. Tillkes, Gaschromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach Clean-up über Gel-Chromatographie und Mini-Kieselgel-Säulen-Chromatographie, 3. Mitt.: Methode zur Aufarbeitung von Lebensmitteln und Futtermitteln pflanzlicher und tierischer Herkunft für die Multirückstandsbestimmung lipoid- und wasserlöslicher Pflanzenbehandlungsmittel, Fresenius Z. Anal. Chem. 301, 300–307 (1980).*

## 8. Author

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# **Part 3**

## **Individual Pesticide Residue Analytical Methods**



# Acephate, Methamidophos

358-(365)

Apples, beans, carrots, grapes, lettuce, maize (grains, dried), plums, Savoy cabbage, tomatoes

Gas-chromatographic determination

Water

(German version published 1982)

## 1. Introduction

	<i>Acephate</i>	<i>Methamidophos</i>
Chemical name	O,S-Dimethyl acetylphosphoramidothioate (IUPAC)	O,S-Dimethyl phosphoramidothioate (IUPAC)
Structural formula		
Empirical formula	C <sub>4</sub> H <sub>10</sub> NO <sub>3</sub> PS	C <sub>2</sub> H <sub>8</sub> NO <sub>2</sub> PS
Molar mass	183.16	141.13
Melting point	93 °C	44.5 °C
Vapour pressure	2.3 · 10 <sup>-6</sup> mbar at 24 °C	4 · 10 <sup>-4</sup> mbar at 30 °C
Solubility	Readily soluble in water (70 g); readily soluble in acetone (15.1 g), ethanol, dichloromethane, slightly soluble in ethyl acetate (2.5 g), benzene (1.6 g), very sparingly soluble in n-hexane (0.01 g), in 100 ml each at room temperature	Miscible with water and methanol; very readily soluble in butanol, soluble to slightly soluble in solvents like acetone, acetonitrile, ether, chloroform, dichloromethane (2 to 5 g), practically insoluble in solvents like petroleum ether and n-hexane, in 100 ml each at room temperature
Other properties	Compared with many other insecticidal organophosphates, acephate and methamidophos are rather stable to hydrolysis and heat Half-lives in aqueous solution 60 h at pH 9 and 40 °C 710 h at pH 3 and 40 °C	120 h at pH 9 and 37 °C 140 h at pH 2 and 40 °C

## 2. Outline of method

Samples with a high water content are homogenized with acetone, those with a low water content are homogenized with a mixture of water and acetone. After evaporation of acetone, the aqueous residue is saturated with sodium chloride and extracted with dichloromethane. Water is extracted directly with dichloromethane. For column chromatographic cleanup, interfering substances are washed out with diethyl ether and a mixture of diethyl ether and methanol. Then

acephate and methamidophos are eluted with a second mixture of diethyl ether and methanol. Both compounds are determined by gas chromatography using a thermionic phosphorus detector.

### 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Buchner porcelain funnel, 9 cm dia.  
Filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Filtration flask, 500-ml  
Separatory funnel, 500-ml  
Fluted filter paper, 18.5 cm dia. (Schleicher & Schüll)  
Round-bottomed flasks, 1-l and 50-ml, with ground joints  
Rotary vacuum evaporator (vacuum source: water jet pump), 35°C bath temperature  
Liquid-liquid extractor for extraction with heavy solvents (Perforator), see Figure  
Chromatographic tube, 20 mm i. d., 30 cm long  
Volumetric flasks, various sizes ranging from 2 ml to 200 ml  
Gas chromatograph equipped with thermionic phosphorus detector  
Microsyringe, 10- $\mu$ l

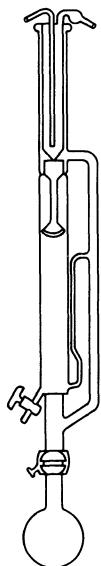


Figure. Liquid-liquid extractor for extraction with heavy solvents.

## 4. Reagents

Acetone, dist.  
Diethyl ether, dist.  
Dichloromethane, dist.  
Methanol, dist.  
Acetone + water mixture 2:1 v/v  
Eluting mixture 1: diethyl ether + methanol 95:5 v/v  
Eluting mixture 2: diethyl ether + methanol 9:1 v/v  
Acephate and methamidophos standard solutions: 1 and 5 µg/ml acetone  
Sodium chloride, p. a.  
Sodium sulphate, p. a., anhydrous  
Filter aid, e. g. Celite 545 (Roth)  
Silica gel 60, 0.063–0.200 mm (Merck No. 7734)  
Cottonwool  
Air, synthetic, re-purified  
Nitrogen, re-purified  
Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Samples with a high content of water (vegetables, fruit)

Homogenize 50 g of the largely comminuted sample (G) with 10 g Celite and 150 ml acetone in the blender for 3 min. Filter the homogenate with suction through a fast flow-rate filter paper in a Buchner funnel. Rinse the blender jar and filter cake portionwise with a total of 100 ml acetone. If the filtrate is cloudy, add 10 g Celite, swirl and re-filter. Transfer the combined filtrates to a 1-l round-bottomed flask, rotary-evaporate to an aqueous residue without further heating, and proceed as described in 6.2.1.

#### 6.1.2. Maize (grains)

Grind 50 g maize grains (G) in the blender, add 10 g Celite and 150 ml acetone-water mixture, and homogenize for 3 min. Filter the homogenate with suction through a fast flow-rate filter paper in a Buchner funnel. Return the filter cake to the blender, re-extract with 150 ml acetone-water mixture for 3 min, and suction-filter. Then proceed as described in 6.1.1.

### 6.1.3. Water

Dissolve 14 g sodium chloride in 50 ml water (G) in a separatory funnel, with vigorous shaking. Extract three times with 150-ml portions of dichloromethane for 2 min. Rotary-evaporate the combined organic phases almost to dryness. Transfer the residue with acetone to a 50-ml flask, rotary-evaporate the solution almost to dryness, and remove the residual solvent by swirling the flask in the hand. If the water used is not heavily contaminated, proceed to gas-chromatographic determination without any further cleanup. Otherwise continue to process as described in 6.2.2.

## 6.2. Cleanup

### 6.2.1. Liquid-liquid partition

Transfer the aqueous residue derived from 6.1.1 to a separatory funnel, saturate with sodium chloride (14 g NaCl for 50 ml solution) with shaking, and then extract three times with 150-ml portions of dichloromethane in the separatory funnel for 2 min. If a stubborn emulsion forms that cannot be broken also by centrifugation, it is recommended to extract it with dichloromethane for 1 h in a liquid-liquid extractor for extraction with heavy solvents.

Dry the combined organic phases on 50 g sodium sulphate, and filter through a fluted filter paper into a 1-l round-bottomed flask. Wash the sodium sulphate and the fluted filter paper portionwise with 50 ml dichloromethane. Rotary-evaporate the combined filtrates almost to dryness. Then continue as described in 6.2.2. For apples, column-chromatographic cleanup can be omitted.

### 6.2.2. Column chromatography

Fill a chromatographic tube with approx. 50 ml diethyl ether, tamp a plug of cottonwool to the bottom of the tube, slowly add 15 g silica gel, and let the supernatant liquid drain to the surface of the silica gel. Cover the silica gel with 5 g sodium sulphate. Dissolve the residue derived from 6.2.1 or 6.1.3 in 5 ml diethyl ether, and add to the column. Rinse the flask several times with 5-ml portions of diethyl ether, and elute part of the co-extractives with 100 ml diethyl ether. Then re-wash with 100 ml eluting mixture 1. Elute the compounds with 100 ml of eluting mixture 2, at a flow rate of 10 to 12 ml/min. To avoid obtaining false results, the mixture ratios must be strictly observed. It is also essential to check elution of the compounds from the column with the silica gel and eluting mixtures used.

Rotary-evaporate the eluate containing the compounds almost to dryness. Remove the residual solvent by swirling the flask in the hand.

## 6.3. Gas-chromatographic determination

Dissolve the residue derived from 6.2.2 or 6.1.3 or 6.2.1 in acetone, and make up to a given volume ( $V_{End}$ ) which should not be less than 2 ml. Inject an aliquot of this solution ( $V_i$ ) into the gas chromatograph.

*Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2150
Column	Glass, 2 mm i. d., 1 m long; packed with 1% Reoplex 400 on Gas Chrom Q, 100–120 mesh
Column temperature	170°C
Injection port temperature	225°C
Detector	Carlo Erba Model 793 PN Detector, bead heater, 730 scale divisions
Gas flow rates	Nitrogen carrier, 30 ml/min Air, 200 ml/min Hydrogen, 2 ml/min
Attenuation	10 · 512 or 10 · 16
Recorder	1 mV; chart speed 5 mm/min
Injection volume	1–3 µl
Retention times for methamidophos acephate	1 min 36 s 3 min 42 s

**7. Evaluation****7.1. Method**

Quantitation is performed by measuring the peak heights of sample solutions and comparing them with those of standard solutions. Equal volumes of sample solutions and standard solutions should be injected.

**7.2. Recoveries, limit of detection and limit of determination**

Recoveries from untreated control samples fortified at levels of 0.005–1.0 mg/kg usually ranged from 70–100%, averaging 87% for acephate and 80% for methamidophos. The limit of detection was 0.005 mg/kg for acephate and 0.001 mg/kg for methamidophos. The limit of determination was 0.01 mg/kg for acephate and 0.005 mg/kg for methamidophos.

**7.3. Calculation of residues**

The residue R, expressed in mg/kg acephate or methamidophos, is calculated from the following equation:

$$R = \frac{H_A \cdot V_{End} \cdot W_{St}}{H_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

V<sub>End</sub> = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu\text{l}$ )

$W_{St}$  = amount of compound injected with standard solution (in ng)

$H_A$  = peak height obtained from  $V_i$  (in mm)

$H_{St}$  = peak height obtained from  $W_{St}$  (in mm)

## 8. Important points

Acephate metabolizes partially in plant tissue. Methamidophos is formed as the main metabolite. The presence of acephate residues in analyzed plant material samples is an indication that methamidophos also is present.

All steps of the analytical procedure and the final determination should be completed on one and the same day otherwise substantial losses of compound may occur.

Plant samples to be analyzed should not be kept too long in a deep-frozen state for the same reason.

Sodium chloride p. a. and sodium sulphate p. a. may contain impurities which will interfere with the detection of acephate. If necessary, they must be cleaned prior to the analysis (e. g. by heating to 500°C, 2 h).

Prior to the gas-chromatographic determination, it is expedient to inject the compounds several times in large amounts (e. g. 300 ng) to achieve faster constancy and higher sensitivity of the gas-chromatographic system.

## 9. References

*J. B. Leary*, Gas-liquid chromatographic determination of acephate and Ortho 9006 residues in crops, *J. Assoc. Off. Anal. Chem.* 57, 189-191 (1974).

*C. E. Richmond, C. E. Crisp, J. E. Larson and G. R. Pieper*, Simple method for assessing acephate and methamidophos residues in plant tissues, *Bull. Environ. Contam. Toxicol.* 22, 512-516 (1979).

## 10. Authors

Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, *W. D. Weinmann and J. Siebers*

Strawberries (fruits and plants), sugar beet (edible root)  
Soil

Gas-chromatographic  
determination

(German version published 1984)

## 1. Introduction

Chemical name	2-Methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime (IUPAC)
Structural formula	$\begin{array}{c} \text{CH}_3 & & \text{O} \\   & & // \\ \text{CH}_3-\text{C}-\text{CH}=\text{N}-\text{O}-\text{C}-\text{NH}-\text{CH}_3 \\   \\ \text{S}-\text{CH}_3 \end{array}$
Empirical formula	C <sub>7</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S
Molar mass	190.27
Melting point	99–101 °C
Boiling point	225 °C at 6.7 mbar
Vapour pressure	1.3·10 <sup>-4</sup> mbar at 25 °C
Solubility	Sparingly soluble in water (0.6 g in 100 ml at 20 °C); readily soluble in most organic solvents, e. g. acetone (43 g), benzene (24 g), chloroform (44 g), methylisobutylketone (24 g), toluene (12 g), soluble in carbon tetrachloride (5 g), in 100 ml each at 30 °C
Other properties	Colourless crystals; sensitive to alkali and heat; undergoes oxidation to the sulphoxide (solubility > 33 g in 100 ml water) which is oxidized to the sulphone

## 2. Outline of method

Aldicarb and its metabolites aldicarb sulphoxide and aldicarb sulphone are extracted with an acetone-water mixture, partitioned into dichloromethane and then treated with peracetic acid whereupon aldicarb and aldicarb sulphoxide are oxidized to aldicarb sulphone. The extract is cleaned up on a silica gel/activated charcoal column, and analyzed by gas chromatography utilizing a flame photometric detector fitted with a sulphur-specific filter. The total residue is determined as aldicarb sulphone and calculated as aldicarb.

## 3. Apparatus

Homogenizer, e. g. Ultra-Turrax (Janke & Kunkel)  
Graduated cylinders, 500-ml and 100-ml  
Buchner porcelain filter funnel, 9 cm dia.

Filtration flask, 1-l  
 Round filter paper, 9 cm dia. (Schleicher & Schüll No. 595)  
 Round-bottomed flask, 500-ml  
 Rotary vacuum evaporator, 40°C bath temperature  
 Separatory funnel, 250-ml, with Teflon stopcock  
 Laboratory centrifuge (as required for strawberry leaves)  
 Kuderna-Danish concentrator, 1-l  
 Pear-shaped flask, 50-ml, for Kuderna-Danish concentrator  
 Inlet tube for Kuderna-Danish concentrator, approx. 25 cm long  
 Laboratory mechanical shaker  
 Funnel, large  
 Selecta fluted filter paper, 24 cm dia. (Schleicher & Schüll No. 1450 1/2)  
 Erlenmeyer flasks, 500-ml and 250-ml, with ground joint  
 Erlenmeyer flask, wide neck, 300-ml  
 Magnetic stirrer with Teflon stirring rod  
 Chromatographic tube, 25 mm i. d., 25 cm long, with sintered glass disk and Teflon stopcock  
 Volumetric flask, 1-ml  
 Gas chromatograph equipped with flame photometric detector and S-filter  
 Microsyringe, 10- $\mu$ l

#### **4. Reagents**

Acetone, for residue analysis  
 Acetonitrile, p. a. (Merck No. 3)  
 Dichloromethane, for residue analysis  
 Methanol, for residue analysis  
 Petroleum ether, p. a., boiling range 60–80 °C  
 Acetone + dichloromethane mixture 1:1 v/v  
 Acetone + water mixture 1:1 v/v  
 Methanol + water mixture 1:1 v/v  
 Petroleum ether + dichloromethane mixture 6:4 v/v  
 Standard solution: 1  $\mu$ g/ml aldicarb sulphone and 1  $\mu$ g/ml benzyl thiocyanate (internal standard) in acetone  
 Benzyl thiocyanate solution, 1  $\mu$ g/ml acetone  
 Peracetic acid solution: Add, with cooling, 0.5 ml conc. sulphuric acid to 50 ml hydrogen peroxide. Add, with continued cooling, 50 ml acetic anhydride p. a. and let stand overnight at room temperature. Freshly prepare the solution weekly and store in a refrigerator  
 Sodium chloride solution, 20 g/100 ml NaCl p. a.  
 Sodium hydrogen carbonate solution, 10 g/100 ml NaHCO<sub>3</sub> p. a.  
 Hydrogen peroxide, 30 g/100 ml H<sub>2</sub>O<sub>2</sub> p. a.  
 Sodium sulphate, p. a., anhydrous, heated at 400 °C  
 Activated charcoal, p. a. (Merck No. 2186)  
 Filter aid, e. g. Celite 545 (e. g. Roth)  
 Silica gel 60 for column chromatography, high purity, particle size 0.063 – 0.200 mm (Merck No. 7754)

Compressed air  
Oxygen, re-purified  
Nitrogen, re-purified  
Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material

Introduce 20 g of the analytical sample (G) into a 500-ml graduated cylinder, add 5 g Celite, and homogenize with 100 ml acetone-water mixture for 2 min using an Ultra-Turrax. Filter the homogenate with suction through the filter funnel into the filtration flask, and rinse the filter cake with 100 ml methanol-water mixture. Rotary-evaporate this filtrate or that derived from 6.1.2 in the round-bottomed flask of the evaporator to remove acetone and methanol. Extract the remaining aqueous phase five times with 30-ml portions of dichloromethane in a separatory funnel. To obtain better phase separation, add 25 ml sodium chloride solution. In the case of strawberry leaves, separate the phases by allowing to stand overnight or by centrifugation. Combine the dichloromethane phases in a 500-ml Erlenmeyer flask, dry for 1/2 h on sodium sulphate, filter through a fluted filter, rinse with a few ml of dichloromethane, and rotary-evaporate the filtrate to dryness in a Kuderna-Danish flask.

#### 6.1.2. Soil

Introduce 20 g of the analytical sample (G) into a 250-ml Erlenmeyer flask, add 1 g Celite, and extract with 100 ml acetone-water mixture for 1 h on the shaker. Filter the suspension with suction through the filter funnel into the filtration flask, and wash the filter cake with 100 ml methanol-water mixture. Then continue to process as in 6.1.1.

### 6.2. Oxidation

Transfer the residue derived from 6.1 with acetone (five 1-ml portions) into a 300-ml wide neck Erlenmeyer flask, and add 25 ml water to the solution. Set the magnetic stirrer in motion, add 2 ml of peracetic acid solution and 5 ml hydrogen peroxide, and continue to stir for 25 min. To neutralize the excess acid, add approx. 70 ml sodium hydrogen carbonate solution to the mixture, and continue to stir for 1/2 h. Extract the neutralized mixture five times with 20-ml portions of dichloromethane. Combine the organic phases in the 250-ml Erlenmeyer flask, and dry with sodium sulphate for 1/2 h. Then filter, rotary-evaporate the filtrate to dryness in a Kuderna-Danish flask, and immediately process the residue as described in 6.3 (see Important points in Section 8).

### 6.3. Cleanup

Slurry 5 g silica gel with 20 ml petroleum ether-dichloromethane mixture into the chromatographic tube. Then mix 15 g silica gel with 1 g activated charcoal, and add together with 40 ml petroleum ether-dichloromethane mixture to the silica gel layer with the stopcock open. Cover the packing with 5 g sodium sulphate.

Dissolve the residue derived from 6.2 portionwise in a total of 10 ml petroleum ether-dichloromethane mixture, and add the solution to the column. Rinse the flask with 20 ml of the same mixture and also add this solution to the column. Then pre-wash with 100 ml petroleum ether-dichloromethane mixture and discard the eluate. Next elute with 150 ml acetone-dichloromethane mixture, and rotary-evaporate the eluate to dryness in a Kuderna-Danish flask.

### 6.4. Gas-chromatographic determination

Dissolve the residue derived from 6.3 in acetone, transfer the solution to a 1-ml volumetric flask, and fill up to the mark with acetone ( $V_{\text{End}}$ ).

For material with a high content of wax (e.g. strawberry leaves), dissolve the residue derived from 6.3 in acetonitrile, pass the solution through a round filter paper into a 1-ml volumetric flask, evaporate to dryness with the aid of a stream of nitrogen, dissolve the residue in acetone, and fill up to the mark with acetone ( $V_{\text{End}}$ ).

Prior to gas chromatography (and following preliminary tests), add benzyl thiocyanate as internal standard in such amount that the peak areas of aldicarb sulphone and benzyl thiocyanate will be of about the same size.

Inject an aliquot of this solution ( $V_i$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Hewlett-Packard 7620 A
Column	Glass, 2 mm i. d., 2.05 m long, packed with 5% Carbowax 20 M on Chromosorb W-HP, 100–120 mesh
Column temperature	Programmed to rise from 100 to 210 °C at the rate of 8 °C/min; then hold for 10 min at 210 °C
Injection port temperature	240 °C
Detector	Flame photometric detector with sulphur-specific filter Temperature 180 °C, transfer line 220 °C
Gas flow rates	Nitrogen carrier, 20 ml/min Nitrogen, 40 ml/min Hydrogen, 100 ml/min Oxygen, 30 ml/min Air, 50 ml/min
Attenuation	$32 \cdot 10^3$
Recorder	1 mV; chart speed 2 mm/min
Injection volume	approx. 5–10 µl
Retention times for aldicarb sulphone benzyl thiocyanate	11 min 12 min 15 s

## 7. Evaluation

### 7.1. Method

Measure the peak areas and compare the ratio of the aldicarb sulphone peak area to the benzyl thiocyanate peak area for the sample solution with the corresponding ratio for the standard solution. Inject equal volumes of sample solution and standard solutions.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with aldicarb or its metabolites at levels of 0.05 to 0.25 mg/kg were 81 to 106% for strawberry fruit, 83 to 118% for strawberry plants, 81 to 104% for sugar beet and 80 to 115% for soil. The routine limit of determination was about 0.025 mg/kg.

### 7.3. Calculation of residues

The total residue R, expressed in mg/kg aldicarb, is calculated from the following equation:

$$R = \frac{Q_A \cdot V_{End} \cdot W_{St}}{Q_{St} \cdot V_i \cdot G} \cdot 0.856$$

where

G = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.4 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_{St}$  = amount of aldicarb sulphone injected with standard solution (in ng)

$Q_A$  = peak area ratio of aldicarb sulphone and internal standard obtained from  $V_i$  for the sample solution

$Q_{St}$  = peak area ratio of aldicarb sulphone and internal standard obtained from  $W_{St}$  for the aldicarb sulphone standard solution

0.856 = factor for conversion of aldicarb sulphone to aldicarb equivalent

## 8. Important points

To prevent any further oxidation, step 6.2 (oxidation) must be followed instantly by step 6.3 (cleanup).

To ensure that possible residues of the three compounds will be recovered at rates of more than 80%, it is essential that the procedure described above is conscientiously followed.

Aldicarb sulphone is difficult to prepare as a standard compound and often it is not obtainable. In this case, aldicarb should be used instead of its sulphone for preparing the standard solution and subjected to oxidation in pure solution. By this procedure, the conversion factor is omitted from the calculation of residues according to the equation given in 7.3.

## 9. Reference

*M. Galoux, J.-C. van Damme, A. Berne and J. Potvin, Gas-liquid chromatographic determination of aldicarb, aldicarb sulfoxide and aldicarb sulfone in soils and water using a Hall electrolytic conductivity detector, J. Chromatogr. 177, 245–253 (1979).*

## 10. Authors

Federal Biological Research Centre for Agriculture and Forestry, Department for Ecological Chemistry, Division for Pesticide Research, Berlin, *A. Koßmann and W. Ebing*

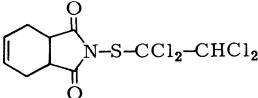
Apples, grapes, peaches, potatoes, small radishes,  
wheat (grains and straw)

Gas-chromatographic  
determination

Soil, water

(German version published 1982)

## 1. Introduction

Chemical name	N-(1,1,2,2-Tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide (IUPAC)
Structural formula	
Empirical formula	C <sub>10</sub> H <sub>9</sub> Cl <sub>4</sub> NO <sub>2</sub> S
Molar mass	349.06
Melting point	162 °C
Boiling point	Not distillable
Vapour pressure	<1.3 · 10 <sup>-9</sup> mbar at 20 °C
Solubility	Virtually insoluble in water; soluble in acetone (4.3 g) and in dichloromethane (10.2 g), slightly soluble in benzene (2.5 g), isopropanol (1.3 g) and toluene (1.7 g), in 100 ml each at 20 °C
Other properties	Slowly hydrolyzed in aqueous emulsion or suspension; rapidly hydrolyzed in acid and alkaline media

## 2. Outline of method

Plant and soil samples are extracted with acetone. After filtration and evaporation of acetone, captafol is partitioned from the remaining aqueous solution into dichloromethane. With wheat samples, the acetone extract is evaporated to dryness, and the residue is dissolved in dichloromethane. Water samples are extracted directly with dichloromethane. The extract is cleaned up by column chromatography on a Florisil-Attaclay-activated charcoal column. The concentration of captafol in the eluate is determined by electron capture gas chromatography.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Laboratory mechanical shaker  
Buchner porcelain funnel, 9 cm dia.

Round filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Filtration flask, 1-l  
Round-bottomed flasks, 1-l, 500-ml and 250-ml, with ground joints  
Rotary vacuum evaporator, 30°C bath temperature  
Separatory funnel, 1-l  
Erlenmeyer flask, 500-ml  
Chromatographic tube, 20 mm i. d., 40 cm long  
Dropping funnel, 250-ml  
Volumetric flask, 10-ml  
Gas chromatograph equipped with electron capture detector  
Microsyringe, 5- $\mu$ l

#### **4. Reagents**

Acetone, high purity  
Dichloromethane, high purity  
Toluene, p. a.  
Captafol standard solutions: 0.2–1  $\mu$ g/ml toluene  
Sodium chloride solution, saturated  
Sodium sulphate, p. a., anhydrous  
Activated charcoal, Nuchar C 190 N (Serva)  
Attaclay 325 (Chemie Mineralien KG)  
Celite 545 (Roth)  
Florisil, 60–100 mesh (Serva)  
Glass wool  
Nitrogen, re-purified

#### **5. Sampling and sample preparation**

The analytical sample is taken and prepared as described on p. 17ff and p. 21f. For water samples, observe the guidelines given on p. 23 ff.

#### **6. Procedure**

##### **6.1. Extraction**

###### **6.1.1. Material with high water content**

Homogenize 100 g of the analytical sample (G) with 10 g Celite and 200 ml acetone for 3 min in the blender. Suction-filter the homogenate through fast flow-rate filter paper in a Buchner porcelain funnel. Rinse the blender jar and the filter cake with 100 ml acetone. Transfer the filtrate with 25 ml acetone into a 1-l round-bottomed flask. Evaporate the acetone in a rotary evaporator (Caution! Solution foams readily. Let flask rotate rapidly!). Transfer the remaining

aqueous solution with 500 ml water and 100 ml dichloromethane to a 1-l separatory funnel. Add 50 ml saturated sodium chloride solution and shake for 2 min. Let the phases separate, and drain the dichloromethane layer into an Erlenmeyer flask. Re-extract with 100 ml dichloromethane. Dry the combined extracts for 1/2 h on approx. 20 g sodium sulphate, with occasional shaking. Then filter through a fluted filter paper overlaid with some sodium sulphate into a 500-ml round-bottomed flask, and rinse with 80 ml dichloromethane. Concentrate the extract to approx. 5 ml in a rotary evaporator.

#### **6.1.2. Wheat**

Grind 50 g of the dry analytical sample (G) for 1 min in the blender. Then add 20 g sodium sulphate, 5 g Celite and 200 ml acetone, and homogenize for 3 min. Suction-filter the homogenate through a fast flow-rate filter paper in a Buchner funnel. Wash the blender jar and the filter cake with 100 ml acetone. Transfer the filtrate with 25 ml acetone to a 1-l round-bottomed flask, and concentrate almost to dryness in the rotary evaporator. Remove the last traces of solvent with a stream of nitrogen. Dissolve the residue in 5 ml dichloromethane.

#### **6.1.3. Soil**

Extract 100 g of the soil sample (G) with 200 ml acetone for 1 h on the shaker. After filtration, rinse the shaker flask and filter cake with 100 ml acetone. Combine the filtrates and continue to process as described in 6.1.1. For very dry soil samples, it may be necessary to add some water to the filtrate prior to evaporation.

#### **6.1.4. Water**

To 200 g of the water sample (G) add 50 ml saturated sodium chloride solution. Extract captafol by shaking two times with 100-ml portions of dichloromethane. Combine the dichloromethane phases and continue to process as described in 6.1.1.

### **6.2. Cleanup**

Clean up the extract by chromatography on a mixed column. For packing two columns, thoroughly mix 15 g sodium sulphate, 15 g Florisil, 12 g Celite, 6 g Attaclay and 1.5 g activated charcoal by shaking for 2 min. Plug the bottom end of the chromatographic tube with glass wool and add 50 ml dichloromethane. Cover the glass wool with sodium sulphate to a level of 2 cm. Slurry 20 g column mixture with 40 ml dichloromethane, and carefully add the slurry, free of air bubbles, to the column. Rinse with 10 ml dichloromethane. After the mixture has settled, cover it carefully with sodium sulphate to a level of 2 cm. Drain the supernatant dichloromethane almost to the top of the column packing. Then rinse the column with 100 ml dichloromethane. Discard this forerun. Next add the extract from 6.1 to the column. Allow to percolate, and rinse the flask several times with 5-ml portions of dichloromethane until a total of 50 ml dichloromethane has been added. Continuously add another 50 ml from a dropping funnel. The column flow rate should be 1–2 drops per s. Collect the eluate in a 250-ml round-bottomed flask, and concentrate almost to dryness in the rotary evaporator. Remove the last traces of solvent by rotating the flask in the hand.

### 6.3. Gas-chromatographic determination

Dissolve the residue derived from 6.2 in toluene, and make up to a suitable volume ( $V_{End}$ ), e.g. 10 ml. Inject aliquots of this solution ( $V_i$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2150
Column	Glass, 2 mm i. d., 1.0 m long; packed with 7.5% DC-200 + 7.5% QF-1 on Gas Chrom Q, 80–100 mesh
Column temperature	200 °C
Injection port temperature	225 °C
Detector	Electron capture detector ECD HT-20 ( $^{63}\text{Ni}$ ), ECD Control Module 250 (Carlo Erba), voltage 50 V DC Temperature 300 °C
Carrier gas flow rate	Nitrogen, 150 ml/min
Attenuation	128 or 256
Recorder	1 mV; chart speed 5 mm/min
Injection volume	1–4 $\mu\text{l}$
Retention time for captafol	5 min 12 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the sample peak height and comparing it with the peak heights of captafol standard solutions. Equal volumes of sample solutions and standard solutions should be injected. For evaluation, the peaks of sample and standard solutions should exhibit comparable heights.

### 7.2. Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified at levels of 0.05 and 1 mg/kg ranged from 85 to 100%. The routine limit of determination was 0.05 mg/kg; for straw samples, it was 0.1 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg captafol, is calculated from the following equation:

$$R = \frac{H_A \cdot V_{End} \cdot W_{St}}{H_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu\text{l}$ )

$W_{St}$  = amount of captafol injected with standard solution (in ng)

$H_A$  = peak height obtained from  $V_i$  (in mm)

$H_{St}$  = peak height obtained from  $W_{St}$  (in mm)

## 8. Important points

The sensitivity of the detector is high enough for the determination of captafol residues in cereal grains when injection of 0.1 ng captafol produces a peak signal three times the noise level. For the analysis of slightly contaminated water, column-chromatographic cleanup can be omitted.

The carrier gas that is used must be very pure (e.g. supplemental purification by passage through molecular sieve).

## 9. References

Chevron Chemical Company, Ortho Division, Research and Development Department, Richmond, Calif., USA, Difolatan residue analysis by electron capture gas chromatography, Method RM-6 B, August 1965.

*W. W. Kilgore and E. R. White*, Determination of difolatan residues in fruits by electron-capture gas chromatography, *J. Agric. Food Chem.* *15*, 1118-1120 (1967).

## 10. Authors

Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, *W. D. Weinmann, H.-G. Nolting and A. Wolf*

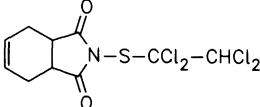


Barley, rye (green matter, grains and straw)

High-performance  
liquid chromatographic determination

(German version published 1984)

## 1. Introduction

Chemical name	N-(1,1,2,2-Tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide (IUPAC)
Structural formula	
Empirical formula	C <sub>10</sub> H <sub>9</sub> Cl <sub>4</sub> NO <sub>2</sub> S
Molar mass	349.06
Melting point	162 °C
Boiling point	Not distillable
Vapour pressure	<1.3 · 10 <sup>-9</sup> mbar at 20°C
Solubility	Virtually insoluble in water; soluble in acetone (4.3 g) and in dichloromethane (10.2 g), slightly soluble in benzene (2.5 g), isopropanol (1.3 g) and toluene (1.7 g), in 100 ml each at 20°C
Other properties	Slowly hydrolyzed in aqueous emulsion or suspension; rapidly hydrolyzed in acid and alkaline media

## 2. Outline of method

The analytical material is extracted with acetone. An aliquot of the filtered extract is diluted with water and saturated sodium chloride solution, and captafol is extracted with n-hexane. The n-hexane extract is washed with water, evaporated to dryness, and then cleaned up by column chromatography on silica gel. The eluate is evaporated to dryness and dissolved in conditioned mobile phase. Following high-performance liquid chromatographic separation, captafol is determined with a photoconductivity detector.

## 3. Apparatus

High-speed blender fitted with glass jar, and Ultra-Turrax (Janke & Kunkel)

Beater-cross mill

Wide neck bottle, 500-ml

Laboratory mechanical shaker  
Fluted filter paper  
Glass funnel  
Graduated cylinder, 250-ml  
Separatory funnel, 1-l  
Round-bottomed flasks, 300-ml, 100-ml and 25-ml  
Rotary vacuum evaporator, 40°C bath temperature  
Chromatographic tube with sintered glass disk, 2.3 cm i. d., 30 cm long  
High performance liquid chromatograph equipped with photoconductivity detector  
Microsyringe, 100- $\mu$ l

## 4. Reagents

Acetone, p. a.  
Diethyl ether, p. a.  
n-Hexane, p. a.  
Methanol, p. a.  
Propanol-(2) (isopropanol), p. a.  
2,2,4-Trimethyl pentane (isooctane), p. a.  
Eluting mixture 1: diethyl ether + n-hexane 1:2 v/v  
Eluting mixture 2: diethyl ether + n-hexane 1:1 v/v  
Mobile phase: isooctane + isopropanol + methanol 85:5:10 v/v/v; conditioned for several h by recirculation through ion exchange cartridge of detector  
Captafol standard solution: 100  $\mu$ g/ml toluene p. a.  
Sodium chloride solution, saturated  
Sodium sulphate, p. a., anhydrous  
Silica gel 60, 0.063–0.200 mm (Merck No. 7734)  
Dry ice  
Cottonwool, chemically pure

## 5. Sampling and sample preparation

To obtain a representative sample for the analysis, comminute the whole laboratory sample in a blender, and follow the guidelines given on p. 17 ff.

Chop green plant parts and straw. Grind chopped straw and grains in a beater-cross mill in the presence of dry ice.

## 6. Procedure

### 6.1. Extraction

Weigh 30 g green plant material and straw or 50 g ground grains (G) together with 50 g sodium sulphate into a wide neck bottle. Add 300 ml acetone ( $V_{\text{Ex}}$ ) and homogenize for 2 min with the

Ultra-Turrax. Tightly stopper the bottle and shake for 1 h on the shaker. Filter a 150-ml aliquot ( $V_{R1}$ ) through a fluted filter paper into a 250-ml graduated cylinder.

## 6.2. Liquid-liquid partition

Transfer the filtrate derived from 6.1 with 20 ml sodium chloride solution and 500 ml water to a separatory funnel. Shake vigorously three times with 75-ml portions of n-hexane. Drain each phase into a beaker. Discard the water phase. Return the hexane phases to the separatory funnel, and wash with 100 ml water and 20 ml sodium chloride solution. Discard the water phase. Filter the hexane phase through a cottonwool pad into a 300-ml round-bottomed flask, and rotary-evaporate to dryness.

## 6.3. Cleanup

Fill the chromatographic tube with 15 ml n-hexane. Slurry 30 g silica gel in 20 ml n-hexane, and pour the slurry, free from air bubbles, into the tube. Rinse with 10 ml n-hexane. Let the n-hexane drain to the top of the column packing. Transfer the residue derived from 6.2 with 5 ml n-hexane to the column. Allow to percolate through the column, and rinse the flask with two 5-ml portions of n-hexane. Rinse the column with 100 ml eluting mixture 1, and then elute captafol with 75 ml eluting mixture 2, using a flow rate of 1–2 drops per s. Collect the eluate in a 100-ml round-bottomed flask, dry on sodium sulphate, and rotary-evaporate to dryness.

## 6.4. High-performance liquid chromatographic determination

Dissolve the residue derived from 6.3 in the conditioned mobile phase, and dilute to a suitable volume ( $V_{End}$ ), at least 5 ml. Inject an aliquot of this solution ( $V_i$ ) into the high performance liquid chromatograph. Beforehand, always condition the mobile phase by recirculation through the ion exchange cartridge of the detector; see Important points in Section 8.

### *Operating conditions*

Chromatograph	High performance liquid chromatograph
Pump	Orlita
Injector	Valco injection valve fitted with 50- $\mu$ l sample loop
Column	Two stainless steel columns each 4.6 mm i. d. and 25 cm long; connected in series; packed with Zorbax CN, 6 $\mu$ m (Du Pont)
Mobile phase	Isooctane-isopropanol-methanol, conditioned by recirculation through ion exchange cartridge of detector
Column pressure	150 bar
Flow rate	1.6 ml/min
Detector	Photoconductivity detector (Tracor 965), halogen-specific, operated with an Hg lamp at 254 nm
Attenuation	1 · 10
Injection volume	50 $\mu$ l
Temperature	approx. 20°C
Retention time for captafol	9 min 48 s

## 7. Evaluation

### 7.1. Method

Prepare a calibration curve as follows. Transfer defined volumes of the captafol standard solution to a 25-ml round-bottomed flask, and rotary-evaporate to dryness. Dissolve each residue in appropriate amounts of conditioned mobile phase to produce captafol solutions of 0.2, 0.5, 1.0 and 2.0 µg/ml. Introduce 50-µl aliquots, equivalent to 10, 25, 50 and 100 ng captafol, onto the column through the sample loop. Plot a curve of measured peak heights vs. ng captafol.

### 7.2. Recoveries and lowest determined concentration

Untreated control samples of grains, green plant material and straw were fortified with captafol. Fortification levels (mg/kg) were 0.04–0.5 for grains, 0.1–0.5 for green plant material, and 0.1–0.5 for straw. The following recoveries were obtained: 90% from grains with an absolute standard deviation of 9% ( $n = 11$ ); 83% from green plant material with an absolute standard deviation of 10% ( $n = 24$ ); 85% from straw with an absolute standard deviation of 10% ( $n = 9$ ). The routine limit of determination was 0.02 mg/kg for grains, and 0.05 mg/kg for green plant material and straw.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg captafol, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{Ex} \cdot V_{End}}{V_{RI} \cdot V_i \cdot G}$$

where

$G$  = sample weight (in g)

$V_{Ex}$  = volume of acetone used for extraction of analytical sample (in ml)

$V_{RI}$  = portion of volume  $V_{Ex}$  used for cleanup in step 6.2 (in ml)

$V_{End}$  = terminal volume of sample solution from 6.4 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into high performance liquid chromatograph (in µl)

$W_A$  = amount of captafol for  $V_i$  read from calibration curve (in ng)

## 8. Important points

To obtain a stable baseline, the mobile phase must be conditioned for several hours, preferably overnight, by recirculation through the ion exchange cartridge of the detector, otherwise interfering peaks may occur. Therefore, the conditioned mobile phase must be used also for preparing the sample solutions in step 6.4 and the calibration solutions in step 7.1.

## 9. Reference

*B. Büttler and W. D. Hörmann*, High pressure liquid chromatographic determination of captan, captafol, and folpet residues in plant material, *J. Agric. Food Chem.* **29**, 257–260 (1981).

## 10. Authors

Ciba-Geigy AG, Agricultural Division, Basle, Switzerland, *B. Büttler and W. D. Hörmann*

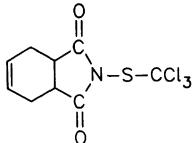


Apples, pears

High-performance  
liquid chromatographic determination

(German version published 1984)

### 1. Introduction

Chemical name	N-(Trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide (IUPAC)
Structural formula	
Empirical formula	C <sub>9</sub> H <sub>8</sub> Cl <sub>3</sub> NO <sub>2</sub> S
Molar mass	300.59
Melting point	176 °C; technical grade product 158–164 °C
Boiling point	Not distillable; undergoes decomposition above melting point
Vapour pressure	1.2 · 10 <sup>-4</sup> mbar at 50 °C
Solubility	Sparingly soluble in water and petroleum ether; soluble in acetone, chloroform, cyclohexane, dichloromethane and propanol-(2)
Other properties	Hydrolyzed in aqueous medium, especially under alkaline conditions

### 2. Outline of method

The analytical material is extracted with acetone. The filtered extract is diluted with water and saturated sodium chloride solution, and captan is extracted with n-hexane. The n-hexane extract is washed with water and then evaporated to dryness. The residue is cleaned up on a silica gel column. The eluate is evaporated to dryness, and the residue is dissolved in conditioned mobile phase. Following high-performance liquid chromatographic separation, captan is determined with a photoconductivity detector.

### 3. Apparatus

High-speed blender fitted with glass jar  
Wide neck bottle, 250-ml

Laboratory mechanical shaker  
Buchner porcelain funnel, 9 cm dia.  
Round filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Filtration flask, 1-l  
Separatory funnel, 1-l  
Round-bottomed flasks, 300-ml, 100-ml and 25-ml  
Rotary vacuum evaporator, 40°C bath temperature  
Chromatographic tube with sintered glass disk, 2.3 cm i. d., 30 cm long  
High performance liquid chromatograph equipped with photoconductivity detector  
Microsyringe, 100- $\mu$ l

#### **4. Reagents**

Acetone, p. a.  
Diethyl ether, p. a.  
n-Hexane, p. a.  
Methanol, p. a.  
Propanol-(2) (isopropanol), p. a.  
2,2,4-Trimethyl pentane (isooctane), p. a.  
Eluting mixture 1: diethyl ether + n-hexane 1 : 2 v/v  
Eluting mixture 2: diethyl ether + n-hexane 2 : 1 v/v  
Mobile phase: isooctane + isopropanol + methanol 85 : 5 : 10 v/v/v, conditioned for several h by recirculation through ion exchange cartridge of detector  
Captan standard solution: 100  $\mu$ g/ml toluene p. a.  
Sodium chloride solution, saturated  
Sodium sulphate, p. a., anhydrous  
Silica gel 60, 0.063–0.200 mm (Merck No. 7734)  
Cottonwool, chemically pure

#### **5. Sampling and sample preparation**

Comminute the whole laboratory sample in a blender and mix thoroughly. Observe the other guidelines given on p. 17 ff.

#### **6. Procedure**

##### **6.1. Extraction**

Weigh 50 g of the analytical sample (G) into a 250-ml wide neck bottle. Add 100 ml acetone, stopper the bottle tightly, and shake for 1 h on the mechanical shaker. Suction-filter the suspension through a fast flow-rate filter paper in a Buchner porcelain funnel. Rinse the bottle and filter cake each with 20 ml acetone. Combine the filtrate and washings.

## 6.2. Liquid-liquid partition

Transfer the filtrate from 6.1 with 20 ml sodium chloride solution and 500 ml water to a separatory funnel. Shake vigorously three times with 75-ml portions of n-hexane, draining each phase into a beaker. Discard the water phase. Pour the hexane phases back into the separatory funnel, and wash with 100 ml water and 20 ml sodium chloride solution. Discard the water phase. Filter the hexane phase through a cottonwool pad into a 300-ml round-bottomed flask, and rotary-evaporate to dryness.

## 6.3. Cleanup

Fill a chromatographic tube with 15 ml n-hexane. Slurry 30 g silica gel in 20 ml n-hexane, and pour the slurry, free from air bubbles, into the tube. Rinse with 10 ml n-hexane. Allow to settle, and then drain the n-hexane to the top of the column packing. Add the residue from 6.2 to the column, using 5 ml n-hexane to complete the transfer. Allow to percolate, and rinse the flask twice with 5-ml portions of n-hexane. Pre-rinse the column with 50 ml eluting mixture 1, and then elute captan with 75 ml eluting mixture 2 (flow rate of 1–2 drops per s). Collect the eluate in a 100-ml round-bottomed flask, dry on sodium sulphate, and rotary-evaporate to dryness.

## 6.4. High-performance liquid chromatographic determination

Dissolve the residue from 6.3 in the conditioned mobile phase, and make up to an appropriate known volume ( $V_{End}$ ), at least 5 ml. Inject an aliquot of this solution ( $V_i$ ) into the high performance liquid chromatograph. The mobile phase always must be conditioned beforehand by recirculation through the ion exchange cartridge of the detector; see Important points in Section 8.

### *Operating conditions*

Chromatograph	High performance liquid chromatograph
Pump	Orlita
Injector	Valco injection valve fitted with 50- $\mu$ l sample loop
Column	Two stainless steel columns, 4.6 mm i. d., each 25 cm long; connected in series; packed with Zorbax CN, 6 $\mu$ m (Du Pont)
Mobile phase	Isooctane-isopropanol-methanol, conditioned by recirculation through ion exchange cartridge of detector
Column pressure	150 bar
Flow rate	1.6 ml/min
Detector	Photoconductivity detector (Tracor 965), halogen-specific, operated with an Hg lamp at 254 nm
Attenuation	1 · 10
Injection volume	50 $\mu$ l
Temperature	approx. 20°C
Retention time for captan	9 min 5 s

## 7. Evaluation

### 7.1. Method

Prepare a calibration curve as follows. Transfer defined volumes of the captan standard solution to a 25-ml round-bottomed flask and rotary-evaporate to dryness. Dissolve each residue in appropriate amounts of conditioned mobile phase to produce captan solutions of 0.2, 0.5, 1.0 and 2.0 µg/ml. Introduce 50-µl aliquots, equivalent to 10, 25, 50 and 100 ng captan, onto the column through the sample loop. Plot a curve of the measured peak heights vs. ng captan.

### 7.2. Recoveries and limit of determination

The recoveries from untreated control samples fortified with captan at levels of 0.04 to 0.5 mg/kg amounted to 89% with an absolute standard deviation of 10%. The limit of determination was 0.02 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg captan, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{End}}{V_i \cdot G}$$

where

G = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.4 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into high performance liquid chromatograph (in µl)

$W_A$  = amount of captan for  $V_i$  read from calibration curve (in ng)

## 8. Important points

To obtain a stable baseline, the mobile phase must be conditioned for several hours, preferably overnight, by recirculation through the ion exchange cartridge of the detector, otherwise interfering peaks may occur. Therefore, the conditioned mobile phase must be used also for preparing the sample solutions in step 6.4 and the calibration solutions in step 7.1.

## 9. Reference

B. Büttler and W. D. Hörmann, High pressure liquid chromatographic determination of captan, captafol, and folpet residues in plant material, J. Agric. Food Chem. 29, 257–260 (1981).

## 10. Authors

Ciba-Geigy AG, Agricultural Division, Basle, Switzerland, B. Büttler and W. D. Hörmann

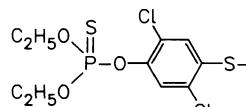
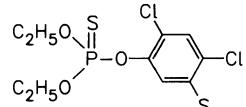
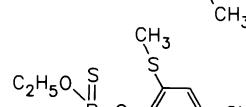
Apples, beans (green), Brussels sprouts, cauliflower, cherries, currants, fats (vegetable and animal), grapes (berries and must), lettuce, maize (green matter and kernels), mandarin oranges, meat (incl. liver and kidneys), peaches, plums, potatoes, rape (seeds and oil), Savoy cabbage, soybean oil, soybeans, spinach, sugar beet (foliage and edible root), tomatoes

Soil, water

Gas-chromatographic determination

(German version published 1984)

## 1. Introduction

Chemical name	Mixture of isomers with the main component O-2,5-dichloro-4-(methylthio)phenyl O,O-diethyl phosphorothioate (Ia) (IUPAC)
Structural formula and composition	 (1a) ca. 73%
	 (1b) ca. 14%
	 (1c) ca. 13%
Empirical formula	C <sub>11</sub> H <sub>15</sub> Cl <sub>2</sub> O <sub>3</sub> PS <sub>2</sub>
Molar mass	361.25
Specific gravity	d <sub>4</sub> <sup>20</sup> = 1.345
Boiling point	153–158 °C at 0.013 mbar
Vapour pressure	5.3 · 10 <sup>-6</sup> mbar at 25 °C
Solubility	Virtually insoluble in water; unlimited miscibility with acetone, cyclohexanone, dimethyl formamide, dioxane, isopropanol, toluene
Other properties	Dark brown liquid which, depending upon the ambient temperature, may contain crystals. These crystals can be melted by warming the compound in a water bath at approx. 40 °C

## 2. Outline of method

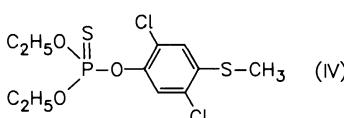
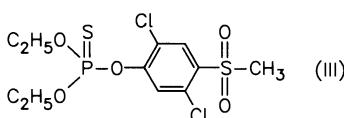
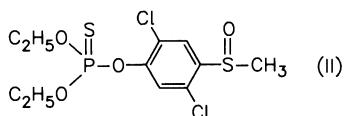
The sample is extracted with a solvent of a type depending upon the nature of the analytical material. With high-fat material, extraction is followed by a partition step with acetonitrile-petroleum ether. The residue is taken up in toluene, cleaned up on a Florisil column, and determined by gas chromatography using a thermionic phosphorus-specific detector.

Besides chlorthiophos (isomeric mixture of Ia, Ib and Ic), the following possible metabolites can also be determined by the reported method:

O-2,5-dichloro-4-(methylsulphinyl)phenyl O,O-diethyl phosphorothioate (chlorthiophos sulphoxide, p-isomer, II),

O-2,5-dichloro-4-(methylsulphonyl)phenyl O,O-diethyl phosphorothioate (chlorthiophos sulphone, p-isomer, III) and

O-2,5-dichloro-4-(methylthio)phenyl O,O-diethyl phosphate (chlorthiophos oxygen analogue, p-isomer, IV)



## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Glass filter funnel G 2, 13 cm dia.

Filtration flask, 1-l

Rotary vacuum evaporator, 50–60 °C bath temperature

Round-bottomed flasks, 1-l and 500-ml, with ground joints

Separatory funnels, 1-l, 500-ml and 250-ml

Mechanical shaker, suitable for holding separatory funnels

Chromatographic tube, 1.5 cm i. d., 40 cm long

Volumetric flasks, 500-ml, 50-ml and 10-ml

Gas chromatograph equipped with thermionic phosphorus-specific detector

Microsyringe, 10-μl

## 4. Reagents

Acetone, p. a.

Acetonitrile, p. a.

Dichloromethane, p. a.

Petroleum ether, technical grade, dist., boiling range 40–80°C

Toluene, p. a.

Acetone + water mixture 1 : 1 v/v

Acetone + toluene mixture 3 : 97 v/v

Acetonitrile, saturated with petroleum ether

Chlorthiophos standard solution: 50 µg/ml acetone; see Section 8, Important points

Chlorthiophos sulphoxide standard solution: 50 µg II/ml acetone

Chlorthiophos sulphone standard solution: 50 µg III/ml acetone

Chlorthiophos oxygen analogue standard solution: 50 µg IV/ml acetone

Bromophos-ethyl solution as internal standard: 2 mg/ml acetone

Sodium chloride solution, saturated

Sodium chloride, high purity

Sodium sulphate, p. a., anhydrous

Filter aid, e. g. Celite 545 (Roth)

Florisil, 60–100 mesh (e. g. Merck No. 12518), 5% water content. Determine the water content of commercial Florisil by heating a given amount for 1 h in a muffle furnace at 400°C, followed by re-weighing. Add an appropriate amount of water to the Florisil to adjust its water content to 5%; then thoroughly mix for 2 h using a mechanical shaker

Glass wool

Panzym KF (Boehringer Ingelheim)

Compressed air, re-purified

Nitrogen, re-purified

Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17ff and p. 21f. For water samples, observe the guidelines given on p. 23ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material

Homogenize 100 g of the finely comminuted sample\*) (G) with 100 ml acetone for 2 min in a blender. Suction-filter the mixture and wash with 50 ml acetone. Extract the filter cake a second time with acetone in the same manner. Combine the filtrates, transfer to a 1-l separatory funnel, and add 200 ml sodium chloride solution and 300 ml water. Extract the mixture twice with 100-ml portions of toluene for 5 min each time. Combine the organic phases, filter through sodium sulphate, and then concentrate to a few ml in a rotary evaporator. With oil-containing seeds and maize, proceed to 6.2.1, otherwise subject the solution directly to column-chromatographic cleanup in step 6.2.2.

#### 6.1.2. Vegetable and animal fats (vegetable oils, suet)

Directly process 25 g oil or melted fat (G) by the procedure described in step 6.2.1.

#### 6.1.3. Meat incl. liver and kidneys

To 50 g finely chopped material (G) add 10 g Celite and homogenize with 100 ml acetonitrile for 2 min. Suction-filter the mixture and wash the residue with 50 ml acetonitrile. Re-extract the filter cake with acetonitrile in the same manner. Transfer the combined filtrates to a 1-l separatory funnel, and add 100 ml sodium chloride solution and 450 ml water. Extract the mixture twice with 100-ml portions of toluene for 5 min each time. Combine the organic phases, filter through sodium sulphate, and concentrate to a few ml in a rotary evaporator. Then proceed to step 6.2.2. It is however recommended firstly to submit more fatty material to the partition step described in 6.2.1.

#### 6.1.4. Soil

Extract 100 g soil (insert 50 g for G in the equation used for calculation of residue in Section 7.3) with 150 ml acetone for 5 min in a blender. Suction-filter the mixture, and wash the filter cake with 50 ml acetone. Extract the soil a second time with 150 ml acetone-water mixture, draw off the liquid by suction, and wash with a further 50 ml acetone-water mixture. Transfer the extracts to a 500-ml volumetric flask, and make up to the mark with acetone. Take a 250-ml aliquot and shake it out with 200 ml dichloromethane for 5 min in a 500-ml separatory funnel. Drain the organic (lower) phase, filter through sodium sulphate, and evaporate almost to dryness in a rotary evaporator. Then proceed to step 6.2.2.

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\*) With currants and grape berries or must, add 5 to 10 drops of Panzym to each weighed sample on the evening prior to extraction, and let stand at room temperature to eliminate interferences from pectins.

### 6.1.5. Water

Extract 250 g water (G) twice with 75-ml portions of toluene for 5 min each time. Combine the separated organic phases, filter through sodium sulphate, and concentrate to a few ml in a rotary evaporator. With water that is only slightly contaminated, proceed directly to gas-chromatographic analysis, otherwise proceed firstly to step 6.2.2.

## 6.2. Cleanup

### 6.2.1. Petroleum ether-acetonitrile partition

Transfer fats and oils from 6.1.2 or high-fat extracts from 6.1.1 or 6.1.3 to a 250-ml separatory funnel, using 50 ml acetonitrile saturated with petroleum ether to complete the transfer. Rinse the flask with 50 ml petroleum ether, and add the rinsing to the separatory funnel. Shake for 5 min and then separate the lower phase (acetonitrile). Extract the petroleum ether two more times with 50-ml portions of acetonitrile saturated with petroleum ether. Dilute the combined acetonitrile phases with 50 ml sodium chloride solution and 250 ml water in a 500-ml separatory funnel, and extract twice with 75-ml portions of toluene for 5 min each time. Combine the organic phases, filter through sodium sulphate, and concentrate to a few ml in a rotary evaporator. Then proceed to step 6.2.2.

### 6.2.2. Column chromatography

Place a glass wool plug in the bottom end of a chromatographic tube about half-filled with toluene. Slowly add 20 g deactivated Florisil to the column, gently tapping the tube walls. Allow the adsorbent to settle, and then open the stopcock until the top of the Florisil is just covered with liquid. Then quantitatively rinse the rotary-evaporated solution from 6.1.1, 6.1.3, 6.1.4, 6.1.5 or 6.2.1 onto the column with toluene. Again open the stopcock a little so that the solution can percolate into the column (rate of 1–2 drops/s). Collect the solvent in a 500-ml round-bottomed flask. Elute chlorthiophos and its metabolites with 200 ml acetone-toluene mixture, and collect in the same round-bottomed flask. Concentrate the eluate to approx. 1 ml in a rotary evaporator.

## 6.3. Gas-chromatographic determination

Quantitatively rinse the residue from 6.2.2 or the concentrated solution from 6.1.5 into a 10-ml volumetric flask with acetone, and make up to the mark with acetone. Carry out a preliminary measurement (injection volume of 1  $\mu$ l) to estimate approximately the concentration of chlorthiophos and its metabolites. If the concentrations are higher than 5 ng/ $\mu$ l, dilute with acetone. If necessary, quantitate chlorthiophos and its metabolites in differently diluted solutions. Using a microsyringe, add to each of the thus prepared solutions a  $\mu$ l-amount of the internal standard bromophos-ethyl solution (shake!) equivalent to the ml-volume ( $V_{End}$ ) of the respective solution.

#### *Operating conditions*

Gas chromatograph

Varian 3700

Column

Glass, 2 mm i. d., 90 cm long; packed with 10% DC-200 on Gas Chrom Q, 80–100 mesh

Column temperature	230 °C
Injection port (glass-lined) temperature	240 °C
Detector	Thermionic specific detector (TSD) Temperature 320 °C
Gas flow rates	Nitrogen carrier, approx. 15 ml/min Hydrogen, approx. 4.5 ml/min Air, approx. 170 ml/min
Attenuation	$8 \cdot 10^{-11}$
Recorder	1 mV; chart speed 5 mm/min
Injection volume	1 µl
Retention times for	
bromophos-ethyl	2 min 30 s
chlorthiophos oxygen analogue	3 min 12 s
chlorthiophos	3 min 48 s
chlorthiophos sulfoxide	5 min 54 s
chlorthiophos sulphone	6 min 24 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Prepare a calibration curve as follows. Mix 1.0, 2.0, 3.0, 4.0 and 5.0 ml each of the standard solutions of chlorthiophos (Ia, Ib and Ic), chlorthiophos sulfoxide (II), chlorthiophos sulphone (III) and chlorthiophos oxygen analogue (IV) (equivalent to 50, 100, 150, 200 and 250 µg of each compound) in 50-ml volumetric flasks, and make up to the mark with acetone. Then add 50 µl bromophos-ethyl solution (equivalent to 100 µg bromophos-ethyl) as internal standard to each volumetric flask (shake!). Inject 1-µl aliquots of these solutions into the gas chromatograph under the conditions given in step 6.3. For graphic representation of the calibration curves, divide the peak heights of chlorthiophos or of the respective metabolite by the peak height of bromophos-ethyl. Plot each quotient vs. the concentration of the appropriate compound in the calibration solutions (ng/µl).

From the gas chromatogram of the sample solution measure the peak heights of chlorthiophos, its metabolites and bromophos-ethyl. From these measurements, calculate the quotients as described above, and read the concentrations of each compound in ng/µl ( $W_x$ ) from the calibration curves.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with chlorthiophos and its metabolites at levels of 0.05–2.0 mg/kg ranged from 85 to 105%.

Blanks usually did not occur in the analyzed control samples or, if so, they were less than 0.01 mg/kg. The routine limit of determination was 0.01–0.05 mg/kg.

### 7.3. Calculation of residues

The residues ( $R_x$ ) of chlorthiophos ( $R_1$ ), chlorthiophos sulphoxide ( $R_2$ ), chlorthiophos sulphone ( $R_3$ ) and chlorthiophos oxygen analogue ( $R_4$ ), expressed in mg/kg, are calculated from the following equation:

$$R_x = \frac{W_x \cdot V_{\text{End}}}{G}$$

where

$G$  = sample weight (in g) (for soil, insert only half of the sample weight (= 50 g) in the equation as stated in step 6.1.4)

$V_{\text{End}}$  = terminal volume of sample solution from 6.3 (in ml) (if necessary, take account of a dilution)

$W_x$  = concentration of the respective compound read from the calibration curve (in ng/ $\mu$ l)

## 8. Important points

The gas-chromatographic conditions are designed for the three isomers of chlorthiophos to produce only a single peak. However, especially in the presence of high residue levels, the peak may have a shoulder in the ascending part. Therefore, the isomeric mixture of technical chlorthiophos, and not the p-isomer (main component), should always be used for preparation of the calibration solutions and for recovery experiments. For metabolites, the respective p-isomer must be used. As their other isomers are of no significance as residue, they were not taken into account nor were the sulphoxide and the sulphone of chlorthiophos oxygen analogue.

## 9. Reference

*D. Eichler*, Provisional guideline for determining residues of S 2957 and the metabolites S 2957-sulfoxide, S 2957-sulfone and S 2957-O-isolog, C.H. Boehringer Sohn, Ingelheim, Analytische Forschung; Unpublished Report dated December 29, 1970.

## 10. Author

Celamerck GmbH & Co. KG, Chemical Research Department, Ingelheim/Rhein, *D. Eichler*



Bilberries, hay, mushrooms, potatoes, wheat (green matter, grains and straw)

Gas-chromatographic determination

Soil, water

(German version published 1982)

## 1. Introduction

Chemical name	2,2-Dichloropropionic acid (Sodium 2,2-dichloropropionate) (IUPAC)
Structural formula	$\text{CH}_3\text{CCl}_2\text{C}=\overset{\text{O}}{\text{O}}\text{ONa}$
Empirical formula	$\text{C}_3\text{H}_3\text{Cl}_2\text{NaO}_2$
Molar mass	164.95
Melting point	Decomposes above 191 °C
Solubility	Very readily soluble in water (>100 g/100 ml)
Other properties	No data

## 2. Outline of method

Dalapon residues are extracted from plant material and soil with a solution of phosphoric acid and phosphotungstic acid in water; dichloropropionic acid is liberated. The aqueous extracts and previously acidified water samples are extracted with ether. After evaporation of the ether, the remaining dichloropropionic acid is esterified with a mixture of methanol and sulphuric acid. The resultant methyl 2,2-dichloropropionate is determined by electron capture gas chromatography.

## 3. Apparatus

Homogenizer, e.g. Ultra-Turrax T 45 N (Janke & Kunkel)

Laboratory mechanical shaker

Rotary vacuum evaporator, <20 °C bath temperature

Glass funnel, 10–12 cm dia.

Fluted filter paper, 24 cm dia.

Graduated cylinders, 500-ml and 250-ml

Volumetric flask, 2-l

Erlenmeyer flask, 25-ml

Round-bottomed flask, 250-ml

Separatory funnels, 500-ml and 250-ml

Allihn condenser

Liebig condenser

Magnetic stirrer, heatable, with Teflon stirring rod, e.g. Ika-Combimag RCT (Janke & Kunkel)

Gas chromatograph equipped with electron capture detector

Microsyringe, 10- $\mu$ l

## 4. Reagents

Diethyl ether, dist.

n-Hexane, distilled from silver-jacketed column

Methanol, dist.

Extraction solution: dissolve 15 g phosphotungstic acid (Merck No. 583) and 30 ml conc. phosphoric acid in water, and make up to 2 l

Esterification mixture: methanol + conc. sulphuric acid 9 : 1 v/v

Dalapon standard solutions: 10 and 100  $\mu$ g/ml water

Calibration solutions: 0.1, 0.2 and 0.4  $\mu$ g methyl 2,2-dichloropropionate/ml n-hexane

Methyl 2,2-dichloropropionate: Reflux 14.3 g 2,2-dichloropropionic acid (Riedel-de Haen No. 63395) with 100 ml esterification mixture for 15 min. Allow to cool and dilute with 200 ml water. Extract the resultant methyl 2,2-dichloropropionate twice with 100-ml portions of diethyl ether. Wash the combined ether extracts once with 50 ml saturated aqueous sodium hydrogen carbonate solution and dry on approx. 10 g anhydrous sodium sulphate. Distill off the ether in vacuum at 30 °C bath temperature. Distill the remaining methyl 2,2-dichloropropionate using a descending Liebig condenser; b. p. 143 °C. The methyl dichloropropionate can be kept, without decomposing, in a brown glass bottle at room temperature

Phosphoric acid, conc.,  $H_3PO_4$  p.a.

Sulphuric acid, conc.,  $H_2SO_4$  p.a.

Sodium chloride, p.a.

Sodium sulphate, p.a., anhydrous

Argon + methane mixture 90:10 v/v

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17ff and p. 21f. For water samples, observe the guidelines given on p. 23ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material

Homogenize 25 g of the analytical sample (G) with 300 ml extraction solution for 5 min using an Ultra-Turrax. Transfer the homogenate to a 500-ml graduated cylinder, dilute with extraction solution to 500 ml ( $V_{Ex}$ ), and shake thoroughly. Then filter through a fluted filter paper.

#### 6.1.2. Soil

Shake 25 g of the soil sample (G) with 500 ml extraction solution ( $V_{Ex}$ ) for 30 min on the shaker. Then filter through a fluted filter paper.

#### 6.1.3. Water

To 250 g of the water sample (G) add 5 ml conc. phosphoric acid, and continue to treat as described in 6.2.

### 6.2. Extraction with diethyl ether

Dissolve 25 g sodium chloride in a 250-ml aliquot of the filtrate derived from 6.1.1 or 6.1.2 ( $V_{RI}$ ) or in the water sample prepared as described in 6.1.3. Then shake three times with 50-ml portions of diethyl ether. Discard the extracted water phase. Evaporate the combined extracts in a 250-ml round-bottomed flask by rotary evaporation until the ether has been completely removed. Any remaining drops of water in the round-bottomed flask must not be brought to dryness otherwise losses of dalapon will result from evaporation of dichloropropionic acid.

### 6.3. Esterification

To the residue derived from 6.2 add 1 g sodium sulphate, 10 ml of the esterification mixture and a Teflon stirring rod. Attach the flask to an Allihn condenser, and heat to boiling on the magnetic stirrer with gentle stirring. Reflux for 5 min and then cool the flask with ice water. Pour 20 ml water and 20.0 ml hexane ( $V_{End}$ ) through the condenser into the flask. Shake vigorously for 1 min and, after separation of the phases, decant the supernatant hexane phase into a 25-ml Erlenmeyer flask.

### 6.4. Gas-chromatographic determination

Inject an aliquot of the hexane phase from 6.3 (if necessary, after dilution with hexane) into the gas chromatograph ( $V_i$ ).

#### *Operating conditions*

Gas chromatograph

Becker Multigraph 409

Column

Glass, 2.5 mm i.d., 1.7 m long; packed with 10% SE-30 on Chromosorb W-AW, 60–80 mesh

Column temperature	70 °C
Injection port temperature	100 °C
Detector	$^{63}\text{Ni}$ electron capture detector
	Temperature 250 °C
Carrier gas flow rate	Argon-methane mixture, 50 ml/min
Attenuation	0.5 · 32
Recorder	1 mV; chart speed 2 cm/min
Injection volume	2–10 $\mu\text{l}$
Retention time for methyl 2,2-dichloropropionate	1 min 15 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak height and comparing it with a calibration curve constructed prior to each series of measurements, e.g. in the range from 0.2 to 2 ng, with the aid of methyl dichloropropionate calibration solutions. Equal volumes of sample solution and calibration solutions should be injected.

### 7.2. Recoveries and lowest determined concentration

Recoveries were determined by fortifying untreated control samples with dilutions of dalapon standard solutions. Values obtained are given in the following table.

Table. Recoveries from plant material and soil fortified at levels of 0.05–50 mg/kg and from water fortified at levels of 0.5–50  $\mu\text{g}/\text{kg}$ .

Analytical material	% Recoveries	
	Range	Average
Hay	74–104	85
Mushrooms	66– 94	78
Potatoes	78– 98	84
Wheat		
Green matter	69– 94	79
Grains	64– 82	73
Straw	67–103	81
Soil	81– 98	90
Water	76–106	90

The routine limit of determination was 0.5  $\mu\text{g}/\text{l}$  for water, and 0.05 mg/kg for all other analyzed materials.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg dalapon, is calculated from the following equation:

$$R = \frac{V_{Ex} \cdot V_{End} \cdot D_A}{V_i \cdot V_{RI} \cdot G} \cdot 1.051$$

where

G = sample weight (in g)

$V_{Ex}$  = volume of extract from 6.1.1 or 6.1.2 (in ml)

$V_{RI}$  = portion of volume  $V_{Ex}$  used for extraction with ether (in ml)

$V_{End}$  = volume of hexane used for extraction in 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$D_A$  = amount of derivative (methyl 2,2-dichloropropionate) for  $V_i$  read from calibration curve (in ng)

1.051 = factor for conversion of derivative to dalapon

## 8. Important points

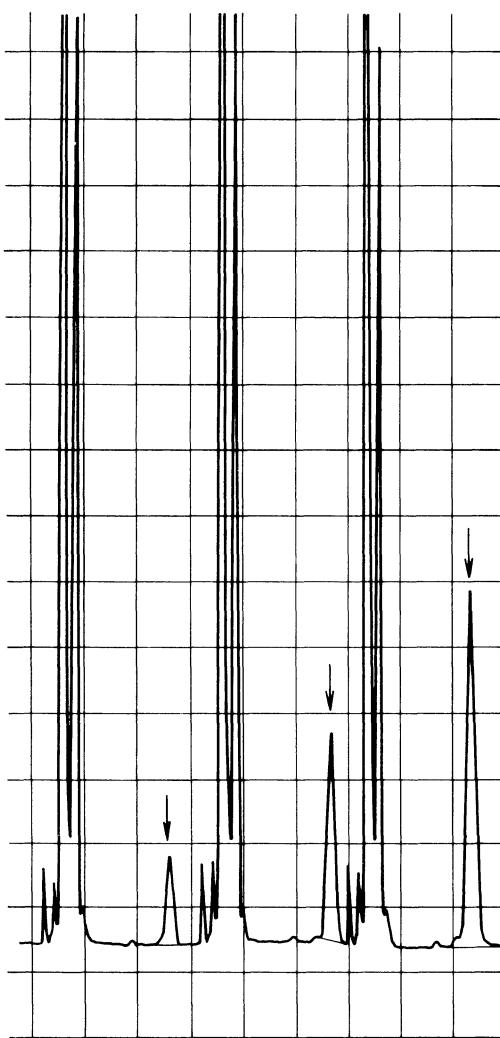
No data

## 9. References

No data

## 10. Authors

BASF, Agricultural Research Station, Limburgerhof, *W. Keller* and *S. Otto*



Chromatograms of 0.2, 0.4 and 0.8 ng methyl 2,2-dichloropropionate. Injected volume: 2  $\mu$ l.

Soil, water

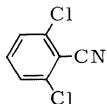
Gas-chromatographic determination

(German version published 1982)

## 1. Introduction

Chemical name                    2,6-Dichlorobenzonitrile (IUPAC)

Structural formula



Empirical formula                C<sub>7</sub>H<sub>3</sub>Cl<sub>2</sub>N

Molar mass                      172.02

Melting point                   144–145 °C

Boiling point                   270 °C

Vapour pressure                7.33 · 10<sup>-4</sup> mbar

Solubility                       Sparingly soluble in water (1.8 mg/100 ml at 20 °C);  
soluble in most organic solvents, e.g. acetone (5 g),  
toluene (4 g), ethyl alcohol (2.1 g), in 100 ml each at  
20 °C

Other properties                Hydrolyzed in strongly acid and alkaline media

## 2. Outline of method

A portion of the water sample or the aqueous suspension of the soil sample is distilled off. Thereupon the dichlobenil residue passes into the distillate and is thus separated from the less volatile interfering substances. The distillate is extracted with toluene, and the dichlobenil residue is determined by electron capture gas chromatography.

## 3. Apparatus

Distillation apparatus equipped with 1-l round-bottomed flask or 1-l Erlenmeyer flask as distillation flask, Liebig condenser and 500-ml round-bottomed flask as receiver

Ice bath for 500-ml round-bottomed flask

Heating mantle for 1-l round-bottomed flask

Hotplate with magnetic stirrer

Volumetric flask, 500-ml

Stopper clip for 500-ml volumetric flask

Laboratory mechanical shaker, e.g. W-2 Universal Shaker (Hormuth-Vetter)

Gas chromatograph equipped with electron capture detector  
Microsyringe, 5- $\mu$ l

## 4. Reagents

Toluene, dist.  
Water, distilled (not deionized)  
Dichlobenil standard solution: 100 ng/ml n-hexane  
Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 21 f. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Distillation and extraction

#### 6.1.1. Water

Attach a 1-l round-bottomed flask to the distillation apparatus, and fill the flask with 800 g of the water sample (G). Place the receiver in an ice bath. Distill approx. 400 ml. As distillation nears completion, stop cooling until the steam reaches the receiver adapter. Then stop distillation, transfer the distillate to a 500-ml volumetric flask, and rinse the receiver twice with 30-ml portions of water. If necessary, fill the volumetric flask with water up to the bottom end of the neck. Then add 5.0 ml toluene. Stopper the flask tightly, using a ground glass stopper secured with a clip. Next invert the flask and clamp it firmly on the shaker. Then shake for 20 min at 120 oscillations per min, remove the flask and let stand upright for 30 min. Then add water dropwise until the solvent phase can be reached with a microsyringe, and re-stopper the volumetric flask.

#### 6.1.2. Soil

To 100 g of the soil sample (G), add 800 ml water in a 1-l Erlenmeyer flask. Attach the flask to the distillation apparatus, and boil on the magnetic stirrer until approx. 400 ml water has been distilled. Then proceed as described in 6.1.1.

### 6.2. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the toluene phase from 6.1.1 ( $V_{End}$ ) into the gas chromatograph.

*Operating conditions*

Gas chromatograph	Varian Aerograph 1740
Column	Glass, 2 mm i.d., 1.5 m long; packed with 2% OV-101 and 3% QF-1 on Chromosorb W, AW-DMCS, 80-100 mesh
Column temperature	125 °C
Injection port temperature	170 °C
Detector	<sup>3</sup> H electron capture detector Temperature 190 °C
Carrier gas flow rate	Nitrogen, 30 ml/min
Attenuation	10 <sup>-12</sup> · 16
Recorder	1 mV; chart speed 10 inch/h (4.23 mm/min)
Injection volume	1-2 µl
Retention time for dichlobenil	2 min 18 s

**7. Evaluation****7.1. Method**

Quantitation is performed by measuring the peak height of the sample and comparing it with the peak heights obtained for diluted standard solutions of dichlobenil. Equal volumes of sample extract solutions and standard solutions should be injected. For evaluation, the peak heights of the sample extract and standard solutions should not differ greatly.

**7.2. Recoveries and lowest determined concentration**

The recoveries from untreated control samples of tapwater, surface water and soil fortified with dichlobenil at levels of 1 to 10 µg/kg ranged from 95 to 106% and averaged 98.5%. The routine limit of determination was 10 ng/kg.

**7.3. Calculation of residues**

The residue R, expressed in µg/kg dichlobenil, is calculated from the following equation:

$$R = \frac{H_A \cdot V_{End} \cdot W_{St}}{H_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

V<sub>End</sub> = terminal volume of sample solution (in ml; in this case 4.68 ml)\*

V<sub>i</sub> = portion of volume V<sub>End</sub> injected into gas chromatograph (in µl)

---

\*) see Important points in Section 8

$W_{St}$  = amount of dichlobenil injected with standard solution (in pg)

$H_A$  = peak height obtained from  $V_i$  (in mm)

$H_{St}$  = peak height obtained from  $W_{St}$  (in mm)

## 8. Important points

Expression of the terminal volume of sample solution ( $V_{End}$ ) must take account of the solubility of toluene in water, which is 0.64 ml per l water at room temperature.  
Dichlobenil may occur in soil also as a degradation product of chlorthiamid.

## 9. References

- K. I. Beynon, L. Davies, K. Elgar and A. N. Wright*, Analysis of crops and soils for residues of 2,6-dichlorobenzonitrile (dichlobenil) and 2,6-dichlorothiobenzamide (chlorthiamid), *J. Sci. Food Agric.* 17, 151-155 (1966).
- F. Herzel*, Extraktion organischer Spurenstoffe aus Wasser nach einer einfachen Schnellmethode, *Chemie f. Labor u. Betrieb* 27, 171-172 (1976).
- F. Herzel*, Zur Rückstandsbestimmung von 2,6-Dichlorbenzonitril aus Boden und Wasser, *J. Chromatogr.* 193, 320-321 (1980).

## 10. Author

Federal Health Office, Berlin, *F. Herzel*

Barley (grains), mangold, rape, red beet (foliage and edible root), soybeans, wheat (grains and straw)

Gas-chromatographic determination

Soil, sludge (mud)

(German version published 1984)

## 1. Introduction

Chemical name	Methyl (RS)-2-[4-(2,4-dichlorophenoxy)phenoxy]-propionate (IUPAC)
Structural formula	
Empirical formula	C <sub>16</sub> H <sub>14</sub> Cl <sub>2</sub> O <sub>4</sub>
Molar mass	341.19
Melting point	42 °C
Vapour pressure	0.72 · 10 <sup>-6</sup> mbar at 25 °C
Solubility	Virtually insoluble in water; readily soluble in ethanol, diethyl ether, n-hexane and pentane
Other properties	Stable under acid conditions; decomposes under alkaline conditions

## 2. Outline of method

The sample material is refluxed for 6 h with sodium hydroxide solution. Diclofop-methyl is saponified in the process to the sodium salt of 2-[4-(2,4-dichlorophenoxy)phenoxy]-propionic acid. Next, the reaction mixture is extracted with ethanol, with simultaneous homogenization, and then filtered to separate it from undissolved constituents. The filtrate is acidified with hydrochloric acid, and extracted with a mixture of n-hexane and diethyl ether. The organic phase is washed with water, and extracted with sodium hydrogen carbonate solution. The pH of this solution is then adjusted to 4.5 with hydrochloric acid, and the solution is re-extracted with the hexane-diethyl ether mixture. The organic phase is concentrated, and methylated with diazomethane. Next, diclofop-methyl is extracted with n-hexane from the methanolic solution. The residue remaining after evaporation is taken up in n-hexane, and diclofop-methyl is determined by electron capture gas chromatography.

Heavily contaminated extracts can be further cleaned up by thin-layer chromatography using a solvent consisting of a mixture of dichloromethane, tetrahydrofuran and acetic acid.

The method permits determination of diclofop-methyl and its free acid as total residue.

### 3. Apparatus

Homogenizer, e.g. Ultra-Turrax (Janke & Kunkel)  
Glass filter funnel, 10 cm dia., e.g. G 2  
Filtration flask, 1-l  
Round-bottomed flasks, 1-l, 500-ml, 100-ml and 50-ml, with ground joints  
Reflux condenser  
Separatory funnels, 1-l, 500-ml and 250-ml  
Beakers, 1-l and 500-ml  
pH meter  
Rotary vacuum evaporator, 40°C bath temperature  
Methylation apparatus, see Figure 1  
Magnetic stirrer  
Evaporating dish, 50-ml  
Infrared lamp  
Ventilator  
Tube with ground joint, 2.5-ml, graduated  
Equipment for thin-layer chromatography  
Glass syringe, 0.5-ml  
UV lamp  
Gel collector, see Figure 2  
Graduated cylinder, 2-ml  
Gas chromatograph equipped with electron capture detector  
Microsyringe, 10- $\mu$ l

### 4. Reagents

Acetone  
Dichloromethane  
Diethyl ether, technical grade  
Diethylene glycol, p.a.  
Ethanol  
Ethyl acetate, p.a.  
n-Hexane, pure, distilled  
Methanol, p.a.  
Tetrahydrofuran, high purity  
Dichloromethane + tetrahydrofuran + acetic acid mixture 95:4:1 v/v/v  
Ethanol + water mixture 1:1 v/v  
n-Hexane + diethyl ether mixture 1:1 v/v  
Methanol + water mixture 3:7 v/v  
Diclofop-methyl standard solution: 5  $\mu$ g/ml n-hexane  
Alkaline diethylene glycol solution: 10 g KOH p.a./100 ml diethylene glycol  
Methylating reagent: 50 g N-methyl-N-nitroso-p-toluenesulfonamide (Merck No. 808406)/100 ml diethyl ether  
Acetic acid, 9 mol/l CH<sub>3</sub>COOH p.a.

Hydrochloric acid, p. a., 5 mol/l  
Potassium hydroxide solution, 0.5 mol/l KOH p. a.  
Sodium hydroxide solution, 1 mol/l NaOH p. a.  
Sodium hydrogen carbonate solution, 5 g/100 ml NaHCO<sub>3</sub> p. a.  
Sodium sulphate, p. a., anhydrous  
Pre-coated silica gel G 1510/Ls 254 TLC plates with fluorescent indicator (254 nm) (Schleicher & Schüll No. 354103)  
Argon + methane mixture 95:5 v/v  
Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f.

## 6. Procedure

### 6.1. Saponification and extraction

Weigh 25 g of the analytical sample (G) into a 1-l round-bottomed flask, and reflux with 250 ml sodium hydroxide solution for 6 h. Allow to cool, and add 250 ml ethanol through the condenser. Transfer the mixture to a beaker, homogenize thoroughly with an Ultra-Turrax, and filter through a glass filter funnel. Dissolve the filter cake in 150 ml ethanol-water mixture, homogenize well with the Ultra-Turrax, and filter again. Combine the filtrates, adjust pH to 2.0 with hydrochloric acid, and extract three times with 200-ml portions of hexane-diethyl ether mixture.

### 6.2. Cleanup

Wash the combined hexane-diethyl ether extracts twice with 100 ml ethanol-water mixture. Discard the water phase. Extract the free acid (formed from diclofop-methyl) from the organic phase by extracting three times with 100-ml portions of sodium hydrogen carbonate solution. Combine the aqueous phases, wash with 100 ml n-hexane, and adjust pH to 4.5 with hydrochloric acid. Extract the water phase three times with 100-ml portions of hexane-diethyl ether mixture. Combine the organic phases, wash with 50 ml water, and dry over 20 g sodium sulphate. Then filter and re-wash. Concentrate the combined solutions to approx. 5 ml in a rotary evaporator.

### 6.3. Reaction with diazomethane

The apparatus for generating diazomethane (see Figure 1) consists of a small washbottle about half-filled with diethyl ether, a reaction vessel with dropping funnel, and a methylating vessel filled with the solution derived from step 6.2. The methylating vessel is followed by two more washbottles in which excess diazomethane is indicated and destroyed, respectively. These two washbottles are filled with 2 ml acetone and acetic acid, respectively. Add 1 ml diethyl ether, 2 ml

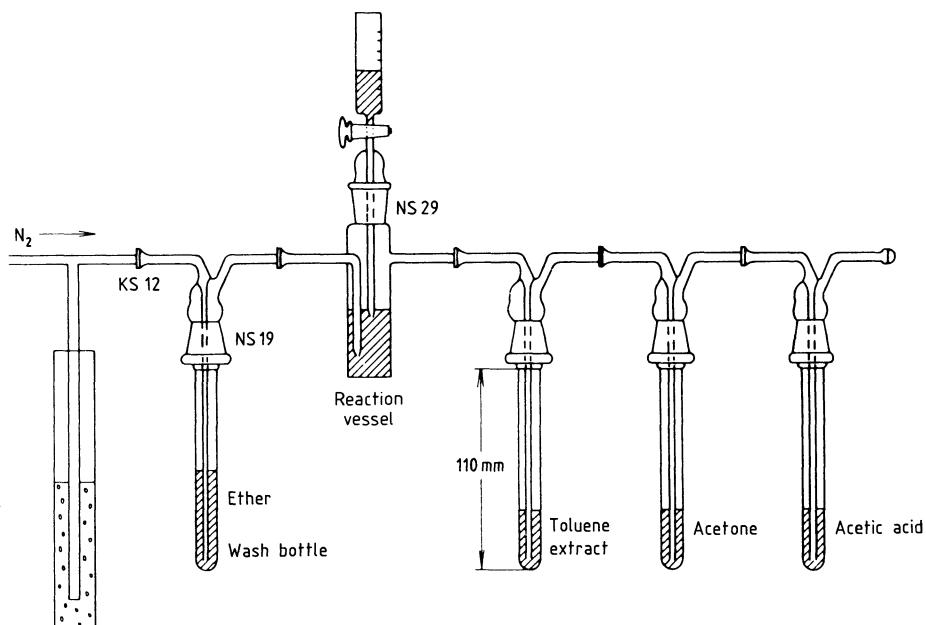


Figure 1. Methylation apparatus.

alkaline diethylene glycol solution and one small stirring rod into the reaction vessel. Place the reaction vessel in a beaker containing hot water, switch on the magnetic stirrer, and slowly pass nitrogen through the apparatus. Then slowly add the methylating reagent dropwise, and continue to sweep the diazomethane with a nitrogen current into the methylating vessel until the solution is coloured pale yellow.

#### 6.4. Isolation of reaction products

Transfer the solution from 6.3 to a 100-ml round-bottomed flask, and rotary-evaporate almost to dryness. Transfer the residue remaining after evaporation to a separatory funnel with successive washings of 75 ml methanol-water mixture and 75 ml n-hexane. Shake to partition diclofop-methyl into the hexane phase. Separate the lower phase, and re-extract with 75 ml n-hexane. Combine the hexane phases, wash with 50 ml water, transfer to a 500-ml round-bottomed flask, and concentrate to approx. 5 ml in the rotary evaporator. Quantitatively rinse the residual solution with n-hexane into an evaporating dish, and evaporate cautiously under infrared light and ventilation. Then rinse the residue with n-hexane into a graduated tube (with ground joint), and make up to 2.5 ml ( $V_{End}$ ).

#### 6.5. Thin-layer chromatographic cleanup

If the solution derived from 6.4 still contains too many interfering substances (usually the case in extracts from rape, soil and sludge (mud)), take an aliquot of  $V_{End}$  equivalent to 2.0 ml ( $V_{RI}$ ),

and streak along a 15-cm line on a silica gel plate. Spot diclofop-methyl as reference substance on the edge of the plate. Develop the prepared plate with the mixture of dichloromethane, tetrahydrofuran, and acetic acid. Using a needle, mark the diclofop-methyl zone, under UV light, at the level of the co-developed reference substance.

Raise the gel layer covering the diclofop-methyl zone with a spatula from the plate support, and remove it from the plate by suction using the gel collector (see Figure 2). Then slowly elute the gel with 50 ml ethyl acetate. Collect the eluate in a 100-ml round-bottomed flask, and evaporate almost to dryness. Using a 0.5-ml glass syringe, transfer the residue to a 2-ml graduated cylinder, rinsing with n-hexane. Use this solution for gas-chromatographic determination ( $V_{EndR} = 2.0 \text{ ml}$ ).

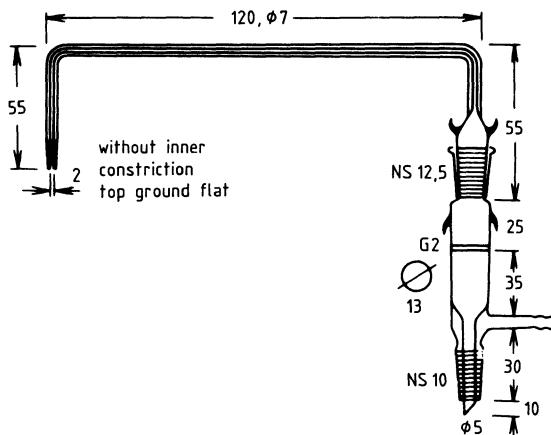


Figure 2. Gel collector.

## 6.6. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the solution of  $V_{End}$  from step 6.4 or of  $V_{EndR}$  from step 6.5 directly onto the column of the gas chromatograph.

### *Operating conditions*

Gas chromatograph	Hewlett-Packard 5050
Column	Glass, 1.8 mm i. d., 1.4 m long; packed with 3% OV-1 on Chromosorb W-HP, 100–120 mesh
Column temperature	210°C
Injection port temperature	275°C
Detector	Electron capture detector ( $^{63}\text{Ni}$ ) Temperature 275°C
Carrier gas flow rate	Argon-methane mixture, 45 ml/min
Attenuation	10 · 16
Recorder	1 mV; chart speed 75 cm/h
Injection volume	1–2 $\mu\text{l}$
Retention time for diclofop-methyl	2 min

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak areas or peak heights of the sample solutions and comparing them with peak areas or heights of standard solutions of known concentration. Equal volumes of the sample solutions and the standard solutions should be injected; additionally, the peaks of the solutions should exhibit comparable areas or heights.

### 7.2. Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified with diclofop-methyl at levels of 0.01–1.0 mg/kg ranged from 80 to 100% and averaged 90%. The routine limit of determination was about 0.01 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg diclofop-methyl, is calculated from the following equations:

$$R = \frac{F_A \cdot V_{End} \cdot W_{St}}{F_{St} \cdot V_i \cdot G} \quad (\text{without cleanup step 6.5})$$

$$R = \frac{F_A \cdot V_{EndR} \cdot W_{St} \cdot V_{End}}{F_{St} \cdot V_i \cdot V_{RI} \cdot G} \quad (\text{with cleanup step 6.5})$$

where

$G$  = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.4 (in ml)

$V_{RI}$  = portion of volume  $V_{End}$  used for cleanup in step 6.5 (in ml)

$V_{EndR}$  = terminal volume of cleaned-up sample solution from step 6.5 (in ml)

$V_i$  = portion of volume  $V_{End}$  (without cleanup) or of volume  $V_{EndR}$  (with cleanup) injected into gas chromatograph (in  $\mu\text{l}$ )

$W_{St}$  = amount of diclofop-methyl injected with standard solution (in ng)

$F_A$  = peak area or height obtained from  $V_i$  (in  $\text{mm}^2$  or in mm)

$F_{St}$  = peak area or height obtained from  $W_{St}$  (in  $\text{mm}^2$  or in mm)

## 8. Important points

A glass column of 2 mm i.d. and 1.0 m long, packed with 3% SE-30 on Varaport 30, 100–120 mesh, and having a temperature of 220°C, may also be used for gas-chromatographic determination.

Instead of the TLC procedure described in step 6.5, cleanup may be performed by gel permeation chromatography as specified in Cleanup Method 4 (see p. 65 ff).

The method permits determination not only of diclofop-methyl but also of its free acid which is contained in the total residue R and is calculated as diclofop-methyl.

## 9. References

No data

## 10. Authors

Hoechst AG, Analytical Laboratory, Frankfurt-Höchst, *S. Gorbach and K. Künzler*

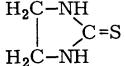


Apples, beans, beer, cauliflower, celeriac (leaves and bulbs), cherries, grape juice, grapes, hop cones, leek, lettuce, peas, potatoes, tobacco, tomatoes, tomato juice, wheat (grains and straw), wine

Water

(German version published 1982)

## 1. Introduction

Chemical name	Imidazolidine-2-thione (IUPAC)
Structural formula	
Empirical formula	C <sub>3</sub> H <sub>6</sub> N <sub>2</sub> S
Molar mass	102.16
Melting point	199–200 °C
Solubility	Sparingly soluble in water (0.2 g/100 ml at 30 °C); soluble in low-boiling alcohols
Other properties	No data

## 2. Outline of method

Ethylene thiourea is extracted from plant material with methanol. The methanol is evaporated from the extract. The remaining water phase is extracted with n-hexane and cleaned up on an aluminium oxide column. Liquid samples are cleaned up directly after evaporation. The aqueous eluate is concentrated and extracted with dichloromethane. The dichloromethane extract is concentrated and cleaned up on an aluminium oxide column. After concentration of the eluate, the residue is determined by gas chromatography using a sulphur-specific flame photometric detector.

## 3. Apparatus

Ultra-Turrax T 45 N homogenizer (Janke & Kunkel) and commercial-type mixer or meat mincer  
Wide neck bottle, 500-ml  
Round-bottomed flasks, 1-l, 500-ml and 10-ml  
Rotary vacuum evaporator, 50–60 °C bath temperature  
Separatory funnel, 250-ml  
Chromatographic tube, 18 mm i.d., 15 cm long

Glass funnel, 10 cm dia.

Gas chromatograph equipped with sulphur-specific flame photometric detector

Microsyringe, 5- $\mu$ l

## 4. Reagents

Dichloromethane, dist.

n-Hexane, dist.

Methanol, dist.

n-Octanol

Eluting mixture: dichloromethane + methanol 99:1 v/v

ETU standard solutions for preparation of calibration curve: 1, 2, 4 and 6  $\mu$ g/ml polyethylene glycol solution

ETU standard solution for recovery experiments: e.g. 5  $\mu$ g/ml methanol

Polyethylene glycol solution: 1 g/100 ml polyethylene glycol 400 (Merck No. 9726) in methanol

Ethylene urea solution: 1.5 g/100 ml imidazolidinone-(2) (Merck No. 804789) in methanol

Ammonium chloride solution, 1 g/100 ml  $\text{NH}_4\text{Cl}$  p.a.

Sodium ascorbate solution: dissolve 88 g L(+) -ascorbic acid (Merck No. 127) in ca. 300 ml water, neutralize to pH 7 with sodium hydroxide solution and dilute to 1 l with water

Thiourea solution, 0.1 g/100 ml thiourea (Merck No. 7979)

Sodium sulphate, p.a., anhydrous

Aluminium oxide 90, standardized, activity grade II-III (Merck No. 1097)

Quartz, fine-grained (e.g. Merck No. 7536)

Quartz wool

Oxygen, re-purified

Nitrogen, re-purified

Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken as described on p. 17 ff, comminuted in a meat mincer or in a mixer, and thoroughly mixed. The portion required for analysis is weighed from this mixture, if necessary after refrigeration at  $-20^\circ\text{C}$ . For water samples, the guidelines given on p. 23 ff should be observed.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Material containing water

Introduce 50 g of the analytical sample (G) into a wide neck bottle, add 10 ml sodium ascorbate solution, 10 ml thiourea solution, 10 ml ethylene urea solution and 125 ml methanol, and homogenize with the Ultra-Turrax for 10 min with ice water cooling. Then filter through a fluted

filter paper into a tared 1-l round-bottomed flask. Wash the filter cake three times with 25-ml portions of methanol, co-rinsing the wide neck bottle and the Ultra-Turrax each time.

#### **6.1.2. Dry material (wheat grains, hop cones, straw, tobacco)**

Introduce 10 g of the analytical sample (G) (50 g for wheat grains) into a wide neck bottle, add 30 ml water, 10 ml sodium ascorbate solution, 10 ml thiourea solution, 10 ml ethylene urea solution and 125 ml methanol, and homogenize with the Ultra-Turrax for 10 min with ice water cooling. Then continue as described in 6.1.1.

#### **6.1.3. Liquid samples (beer, tomato juice, grape juice, water, wine)**

To 50 g of the analytical sample (G) (up to 500 g for water) add 20 ml ammonium chloride solution, 10 ml sodium ascorbate solution, 10 ml ethylene urea solution and 10 ml thiourea solution. Rotary-evaporate the mixture in a tared 1-l round-bottomed flask to 20 g. For beer samples, beforehand add 1 drop of n-octanol to avoid excessive foaming during the concentration step. Then proceed to column-chromatographic cleanup step described in 6.2.2.

### **6.2. Cleanup**

#### **6.2.1. Hexane-water partition**

Add 20 ml ammonium chloride solution to the extract derived from 6.1.1, and rotary-evaporate to approx. 30 ml. Make up the extract to 40 g with water (weigh). Then add 50 ml hexane and shake vigorously for 1 min. Next transfer the liquid to a 250-ml separatory funnel. Rinse the round-bottomed flask with 10 ml ammonium chloride solution. Let the phases separate in the separatory funnel, and drain the lower aqueous phase into a round-bottomed flask. Discard the hexane phase.

#### **6.2.2. Cleanup by column chromatography**

Place a plug of quartz wool in the bottom of a chromatographic tube, and slowly add 10 g dry aluminium oxide. Cover the adsorbent with an approx. 2-cm layer of quartz sand.

Concentrate the aqueous phase derived from 6.2.1 to 20 g in the rotary evaporator (weigh). Add this concentrate or that derived from 6.1.3 to the prepared column and allow to percolate. Elute the column portionwise with ammonium chloride solution, and collect 50 ml eluate in a graduated cylinder (flow rate of 1 to 2 drops/s). If necessary, apply light pressure.

#### **6.2.3. Extraction with dichloromethane**

Concentrate the eluate derived from 6.2.2 to 20 g in a tared 500-ml round-bottomed flask in the rotary evaporator. Extract the aqueous concentrate three times with 100-ml portions of dichloromethane. Prepare a sodium sulphate filter by plugging the outlet of the glass funnel with some quartz wool and covering it with approx. 30 g sodium sulphate. Filter the dichloromethane phases successively through the sodium sulphate. Concentrate the filtrate to approx. 5 ml in a 500-ml round-bottomed flask in the rotary evaporator.

#### **6.2.4. Cleanup of dichloromethane extract by column chromatography**

Plug the bottom of a chromatographic tube with some quartz wool and approx. half-fill the tube with dichloromethane. Slowly add 5 g aluminium oxide and cover with an approx. 1-cm layer of sodium sulphate. Then drain the dichloromethane to a level of 1 mm above the sodium sulphate layer. Add the concentrated extract from 6.2.3 to the column, allow to percolate, and rinse the column three times with 5-ml portions of dichloromethane. Then elute ethylene thiourea with 50 ml of the eluting mixture. Concentrate the eluate to 1–2 ml in the rotary evaporator, and rinse quantitatively with a little methanol into a 10-ml round-bottomed flask. Evaporate to dryness, and dissolve the residue immediately (!) in 0.2–1.0 ml of polyethylene glycol solution ( $V_{End}$ ).

#### **6.3. Gas-chromatographic determination**

Inject 5 µl ( $V_i$ ) of the solution derived from 6.2.4 ( $V_{End}$ ) into the gas chromatograph. If possible, carry out the gas-chromatographic determination immediately. Otherwise, the extracts can be kept for one day at –20°C.

Note: After it has been packed, the column must not be fully heated but set at the operating temperature given below only while the measurements are in progress. During the remaining period, the oven temperature must be maintained at 180°C. The gas chromatograph must be fitted with a complete all-glass system between the injection port and the detector since ethylene thiourea undergoes decomposition on hot metals.

##### *Operating conditions*

Gas chromatograph	Becker Multigraph 409
Column	Glass, 2.5 mm i. d., 90 cm long; packed with 3% Versamid 940 (or Versamid 900) on Gas Chrom Q, 100–120 mesh
Column temperature	240°C
Injection port temperature	300°C
Detector	Flame photometric detector (FPD) incorporating a 394 nm filter specific for sulphur Temperature 200°C
Gas flow rates	Nitrogen carrier, 80 ml/min Hydrogen, 150 ml/min Oxygen, 50 ml/min
Recorder	1 mV; chart speed 5 mm/min
Injection volume	5 µl
Retention time for ethylene thiourea	2 min

When the Versamid column has not been used for some considerable time, it irreversibly retains small amounts of ethylene thiourea. Therefore, before carrying out the actual measurement, the column should be conditioned by making 2 or 3 injections of approx. 500 ng ethylene thiourea.

## 7. Evaluation

### 7.1. Method

Evaluation is performed by the calibration technique. Before each series of injections, prepare a fresh calibration curve by injecting 5- $\mu$ l aliquots of standard solutions, equivalent to e. g. 5, 10, 20 and 30 ng ethylene thiourea. Using log-log paper, plot a curve of the peak heights (measured in mm) vs. ng ethylene thiourea.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples of vegetables and fruit fortified with ethylene thiourea at levels of 0.02–0.1 mg/kg amounted to  $70 \pm 10\%$ . The routine limit of determination ranged from 0.01–0.02 mg/kg; for hops and tobacco, it was 0.1 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg ethylene thiourea, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{End}}{V_i \cdot G}$$

where

G = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.2.4 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_A$  = amount of ethylene thiourea for  $V_i$  read from calibration curve (in ng)

When the exact amounts and volumes given in the method are used, the residue R expressed in mg/kg ethylene thiourea is obtained by dividing the ng amount of ethylene thiourea read from the calibration curve by the mg aliquot of sample injected into the gas chromatograph.

Figure 1 presents an example of gas chromatograms for preparing a calibration curve, and Figure 2 illustrates gas chromatograms from recovery experiments in which untreated apple samples were fortified with ethylene thiourea at levels of 0.02 and 0.05 mg/kg.

## 8. Important points

The additions of sodium ascorbate, thiourea and ethylene urea serve the purpose of protecting the ethylene thiourea to be determined against oxidation and formation of a complex with heavy metal traces during the analytical procedure. As the mechanism of the protective action is not known exactly, it appeared expedient to use both ureas simultaneously. If, however, interferences from persistent sulphur signals are observed to occur when thiourea is added, as reported in one case, then the extraction should be performed by adding only ethylene urea and omitting thiourea.

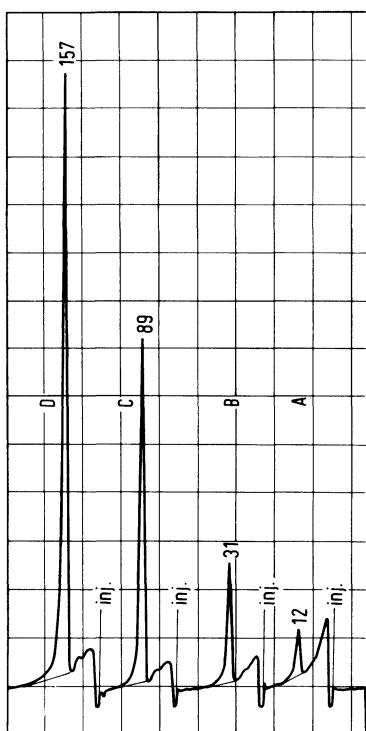


Figure 1. Gas chromatograms for preparing a calibration curve.  
 $A = 5$ ,  $B = 10$ ,  $C = 20$  and  $D = 30$  ng ethylene thiourea.  
 Volumes of 5  $\mu$ l each injected.

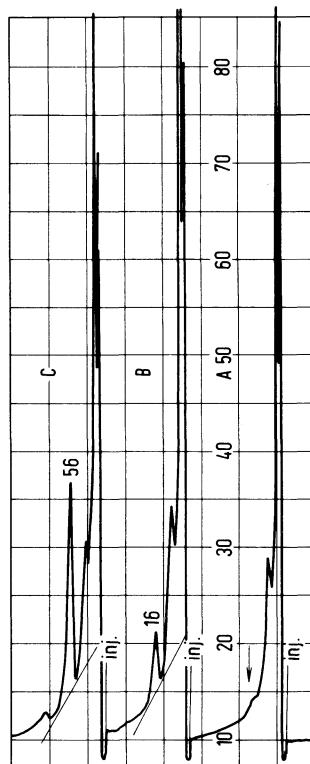


Figure 2. Gas chromatograms from recovery experiments.  
 $A$  = unspiked apple sample,  
 $B$  = apple sample fortified with 0.02 mg/kg ethylene thiourea,  
 $C$  = apple sample fortified with 0.05 mg/kg ethylene thiourea.

## 9. References

- J. H. Onley and G. Yip*, Determination of ethylene thiourea residues in foods, using thin layer and gas chromatography, *J. Assoc. Off. Anal. Chem.* **54**, 165–169 (1971).
- L. D. Haines and I. L. Adler*, Gas chromatographic determination of ethylene thiourea residues, *J. Assoc. Off. Anal. Chem.* **56**, 333–337 (1973).
- W. H. Newsome*, Determination of ethylene thiourea residues in apples, *J. Agric. Food Chem.* **20**, 967–969 (1972).

*R. G. Nash*, Improved gas-liquid chromatographic method for determining ethylene thiourea in plants, *J. Assoc. Off. Anal. Chem.* **57**, 1015–1021 (1974).

*S. Otto, W. Keller and N. Drescher*, New gas-chromatographic determination of ethylene thiourea (imidazolidine-2-thione) residues without derivatization, *J. Environ. Sci. Health, Part B* **12**, 179–191 (1977).

## 10. Authors

BASF, Agricultural Research Station, Limburgerhof, *S. Otto, W. Keller and N. Drescher*

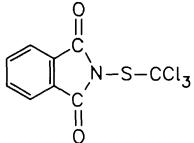


Grapes, lettuce, tomatoes, wine

High-performance  
liquid chromato-  
graphic determination

(German version published 1984)

## 1. Introduction

Chemical name	N-(Trichloromethylthio)phthalimide (IUPAC)
Structural formula	
Empirical formula	C <sub>9</sub> H <sub>4</sub> Cl <sub>3</sub> NO <sub>2</sub> S
Molar mass	296.56
Melting point	180°C
Boiling point	Not distillable; undergoes decomposition above melting point
Vapour pressure	<1.3 · 10 <sup>-9</sup> mbar at 20°C
Solubility	Very sparingly soluble in water; soluble in ketones, sparingly soluble in hydrocarbons
Other properties	Hydrolyzed in aqueous medium, especially under alkaline conditions

## 2. Outline of method

Plant material is extracted with acetone. The filtered extract is diluted with water and saturated sodium chloride solution, and folpet is extracted with n-hexane. The hexane phase is evaporated to dryness. The residue is cleaned up on a silica gel column. The eluate is evaporated to dryness, and the residue is dissolved in conditioned mobile phase. Following high-performance liquid chromatographic separation, folpet is determined with a photoconductivity detector. Wine samples are extracted continuously with n-hexane in a liquid-liquid extractor. The hexane phase is evaporated to dryness. The residue remaining after evaporation usually can be analyzed as it is by HPLC but if interfering peaks occur it too must be cleaned up firstly by column chromatography.

## 3. Apparatus

High-speed blender fitted with glass jar or Ultra-Turrax (Janke & Kunkel)  
Meat mincer

Wide neck bottle, 250-ml  
Laboratory mechanical shaker  
Buchner porcelain funnel, 9 cm dia.  
Round filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Filtration flask, 1-l  
Round-bottomed flasks, 300-ml, 100-ml and 25-ml  
Separatory funnel, 1-l  
Liquid-liquid extractor, 250-ml; apparatus after Kutscher and Steudel (Schott)  
Glass funnel  
Fluted filter paper  
Rotary vacuum evaporator, 40°C bath temperature  
Chromatographic tube with sintered glass disk, 2.3 cm i. d., 30 cm long  
High performance liquid chromatograph equipped with photoconductivity detector  
Microsyringe, 100- $\mu$ l

#### 4. Reagents

Acetone, p. a.  
Diethyl ether, p. a.  
Ethanol, p. a.  
n-Hexane, p. a.  
Methanol, p. a.  
Propanol-(2) (isopropanol), p. a.  
2,2,4-Trimethyl pentane (isoctane), p. a.  
Eluting mixture 1: diethyl ether + n-hexane 1 : 4 v/v  
Eluting mixture 2: diethyl ether + n-hexane 1 : 3 v/v  
Mobile phase: isoctane + isopropanol + methanol 85 : 5 : 10 v/v/v, conditioned for several h by recirculation through ion exchange cartridge of detector  
Folpet standard solution: 100  $\mu$ g/ml toluene p. a.  
Sodium chloride solution, saturated  
Sodium sulphate, p. a., anhydrous  
Silica gel 60, 0.063–0.200 mm (Merck No. 7734)  
Cottonwool, chemically pure

#### 5. Sampling and sample preparation

For lettuce and tomatoes, comminute the whole laboratory sample. Observe the other guidelines given on p. 17 ff. Remove grapes from the stalks and pass through a meat mincer.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Grapes, lettuce, tomatoes

Weigh 50 g of the analytical sample (G) into a 250-ml wide neck bottle. Add 100 ml acetone. Homogenize lettuce samples for 1 min with an Ultra-Turrax. Stopper the bottle tightly, and shake for 1 h on a mechanical shaker. Then suction-filter through a fast flow-rate filter paper in a Buchner porcelain funnel. Wash the bottle and filter cake each with 20 ml acetone. Combine the filtrate and washings, and proceed to step 6.2.

#### 6.1.2. Wine

Pour 50 ml wine (G) into the liquid-liquid extractor, add 20 ml ethanol, and extract with 150 ml n-hexane for 1 h. Separate the hexane phase, and dry on 20 g sodium sulphate. Then filter through a fluted filter paper into a 300-ml round-bottomed flask. Rinse the filter cake and flask each with 20 ml n-hexane. Rotary-evaporate the hexane phase to dryness. Then proceed to step 6.4 for HPLC determination of the residue but if interfering peaks occur interpose a column chromatographic cleanup as described in step 6.3.

## 6.2. Liquid-liquid partition

Transfer the solution from 6.1.1 with 20 ml sodium chloride solution and 500 ml water to a separatory funnel. Shake vigorously three times with 75-ml portions of n-hexane, draining each phase into a beaker. Discard the water phase. Pour the hexane phases back into the separatory funnel, and wash with 100 ml water and 20 ml sodium chloride solution. Discard the water phase. Filter the hexane phase through a cottonwool pad into a 300-ml round-bottomed flask, and rotary-evaporate to dryness.

## 6.3. Cleanup

Fill a chromatographic tube with 15 ml n-hexane. Slurry 30 g silica gel in 20 ml n-hexane. Pour the slurry, free from air bubbles, into the tube. Rinse with 10 ml n-hexane. Allow to settle and drain the hexane to the top of the column packing. Transfer the residue from 6.2 (if necessary, also from 6.1.2) to the column using 5 ml n-hexane to complete the transfer. Allow to percolate, and rinse the flask twice with 5-ml portions of n-hexane. Pre-rinse the column with 50 ml eluting mixture 1. Then elute folpet with 75 ml eluting mixture 2 (flow rate of 1–2 drops per s). Collect the eluate in a 100-ml round-bottomed flask, dry on sodium sulphate, and rotary-evaporate to dryness.

## 6.4. High-performance liquid chromatographic determination

Dissolve the residue from 6.1.2 or 6.3 in the conditioned mobile phase, and make up to an appropriate known volume ( $V_{End}$ ), at least 5 ml. Inject an aliquot of this solution ( $V_i$ ) into the high performance liquid chromatograph. Beforehand, always condition the mobile phase by recirculation through the ion exchange cartridge of the detector (see Important points in Section 8).

*Operating conditions*

Chromatograph	High performance liquid chromatograph
Pump	Orlita
Injector	Valco injection valve fitted with 50- $\mu$ l sample loop
Column	Two stainless steel columns, 4.6 mm i.d., each 25 cm long; connected in series; packed with Zorbax CN, 6 $\mu$ m (Du Pont)
Mobile phase	Isooctane-isopropanol-methanol, conditioned by recirculation through ion exchange cartridge of detector
Column pressure	150 bar
Flow rate	1.6 ml/min
Detector	Photoconductivity detector (Tracor 965), halogen-specific, operated with an Hg lamp at 254 nm
Attenuation	1 · 10
Injection volume	50 $\mu$ l
Temperature	approx. 20°C
Retention time for folpet	7 min 48 s

## 7. Evaluation

### 7.1. Method

Prepare a calibration curve as follows. Transfer defined volumes of the folpet standard solution to a 25-ml round-bottomed flask and rotary-evaporate to dryness. Dissolve each residue in appropriate amounts of conditioned mobile phase to produce folpet solutions of 0.2, 0.5, 1.0 and 2.0  $\mu$ g/ml. Introduce 50- $\mu$ l aliquots, equivalent to 10, 25, 50 and 100 ng folpet, onto the column through the sample loop. Plot a curve of measured peak heights vs. ng folpet.

### 7.2. Recoveries and limit of determination

The recoveries from untreated control samples fortified with folpet at levels of 0.04–0.5 mg/kg amounted to 97% with an absolute standard deviation of 10%. The limit of determination was 0.02 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg folpet, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{End}}{V_i \cdot G}$$

where

G = sample weight (in g) or volume (in ml)

$V_{End}$  = terminal volume of sample solution from 6.4 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into high performance liquid chromatograph (in  $\mu\text{l}$ )  
 $W_A$  = amount of folpet for  $V_i$  read from calibration curve (in ng)

## 8. Important points

To obtain a stable baseline, the mobile phase must be conditioned for several hours, preferably overnight, by recirculation through the ion exchange cartridge of the detector, otherwise interfering peaks may occur. Therefore, the conditioned mobile phase must be used also for preparing the sample solutions in step 6.4 and the calibration solutions in step 7.1.

## 9. Reference

*B. Büttler and W.D. Hörmann*, High pressure liquid chromatographic determination of captan, captafol, and folpet residues in plant material, *J. Agric. Food Chem.* 29, 257–260 (1981).

## 10. Authors

Ciba-Geigy, Agricultural Division, Basle, Switzerland, *B. Büttler and W.D. Hörmann*

Apples, carrots, cherries, currants (black and red), grapefruit, hop cones, kohlrabi, lettuce, maize (kernels, dried), oats (grains), oranges, sweet peppers, potatoes, small radishes, strawberries, sugar beet (edible root), tomatoes, wheat (grains)

Gas-chromatographic determination

Soil

(German version published 1982)

## 1. Introduction

Chemical name                    7-Chlorobicyclo[3.2.0]hepta-2,6-dien-6-yl dimethyl phosphate (IUPAC)



Empirical formula                 $C_9H_{12}ClO_4P$

Molar mass                      250.62

Boiling point                    90–91 °C at 0.03 mbar

Vapour pressure                 $1.7 \cdot 10^{-3}$  mbar at 25 °C

Solubility                        Sparingly soluble in water (250 mg/100 ml at 23 °C); very readily soluble in acetone, acetonitrile, alcohol, ether, benzene, chloroform, carbon tetrachloride

Other properties                No data

## 2. Outline of method

Heptenophos is extracted from plant material and soil samples with acetone. The acetone extract is diluted with an aqueous solution of sodium chloride and shaken with dichloromethane. The dichloromethane phases are dried on sodium sulphate. The solvent is removed by evaporation and the residue is dissolved in ethyl acetate. If it is necessary to clean up the extract, this is done by column chromatography on polystyrene gel using ethyl acetate as the mobile phase. The residue is determined by gas chromatography using a flame photometric detector.

## 3. Apparatus

Homogenizer, e.g. Ultra-Turrax (Janke & Kunkel)

Glass filter funnel, 10 cm i.d., 6 cm high, porosity 1

Beaker, 300-ml  
Separatory funnels, 3-l and 500-ml  
Mechanical shaker, suitable for holding separatory funnels  
Drying column: glass tube, 2 cm dia., 30 cm long, packed with anhydrous sodium sulphate to a level of 15 cm  
Rotary vacuum evaporator, 30°C bath temperature  
Round-bottomed flask, 500-ml, with ground joint  
Volumetric flasks, 10-ml and 5-ml  
Gas chromatograph equipped with flame photometric detector  
Microsyringe, 10- $\mu$ l

## 4. Reagents

Acetone, dist.  
Dichloromethane, dist.  
Dodecane, dist.  
Ethyl acetate, dist.  
Heptenophos standard solutions: 0.1, 1.0 and 10  $\mu$ g/ml ethyl acetate  
Sodium chloride solution, 2 g/100 ml NaCl p. a.  
Sodium sulphate, p. a., anhydrous  
Air, synthetic  
Nitrogen, special  
Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f.

## 6. Procedure

### 6.1. Extraction

Weigh 50 to 100 g of the analytical sample for plant material and 10 to 30 g for hop cones (G), and homogenize for 10 min with 150 ml acetone using an Ultra-Turrax. Then filter the homogenate with suction through a glass filter funnel. Extract the filter cake two more times in the same way. For dry sample material (oats, hops, maize, wheat) add approx. 50 to 60 ml water for the second extraction.

For soil samples, shake 50 to 100 g (G) three times with 150-ml portions of acetone for 30 min each time in a 500-ml separatory funnel attached to a mechanical shaker. Then filter with suction through a glass filter funnel. Add 50 to 60 ml water for the second shaking procedure.

On completion of the extractions, combine the extracts and add 2 l sodium chloride solution in a 3-l separatory funnel. Shake the mixture three times with 100-ml portions of dichloromethane. Add the dichloromethane phases successively to the drying column. Add ap-

prox. 1 ml dodecane to the combined phases, and concentrate almost to dryness in the rotary evaporator. Heptenophos has a relatively high vapour pressure; therefore, keep the concentration time as short as possible. Dissolve the residue in ethyl acetate, and dilute to a given volume of 5 to 10 ml ( $V_{\text{Ex}}$ ). For the analysis of apples, strawberries, currants, potatoes, cherries, kohlrabi, lettuce, small radishes, tomatoes and sugar beet, this solution can be subjected directly to gas chromatography. For the other crops and soil, proceed to step 6.2.

## 6.2. Cleanup

For samples of soil, grapefruit, oats, hops, maize, carrots, oranges, sweet peppers and wheat, clean up the extract by gel permeation chromatography on polystyrene gel as described in Cleanup Method 4 (see p. 65 ff).

## 6.3. Gas-chromatographic determination

Directly inject an aliquot ( $V_i$ ) of the solution from 6.1 ( $V_{\text{Ex}}$ ) or from 6.2 ( $V_{\text{End}}$ ) into the column of the gas chromatograph. The injection volume should not exceed 5  $\mu\text{l}$ .

### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2150
Column	Glass, 1.8 mm i.d., 1.2 m long; packed with 3% OV-1 on Chromosorb W-HP, 100–120 mesh
Column temperature	150 °C
Injection port temperature	250 °C
Detector	Flame photometric detector (FPD) Melpar 200 AT, fitted with 524 nm interference filter Temperature 200 °C
Gas flow rates	Nitrogen carrier, 58 ml/min Hydrogen, 150 ml/min Air, 40 ml/min
Attenuation	10 <sup>4</sup>
Recorder	1 mV; chart speed 30 mm/min
Injection volume	1–5 $\mu\text{l}$
Retention time for heptenophos	1 min 20 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak areas or peak heights of the sample solutions and comparing them with those obtained for dilutions of standard solutions of known concentration. Equal volumes of the sample solutions and standard solutions should be injected, and the peaks of the solutions should exhibit comparable areas or heights.

## 7.2. Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified with heptenophos at levels of 0.01 to 1.0 mg/kg ranged from 80 to 100% and averaged 90%. The routine limit of determination was approx. 0.01 mg/kg; for hop cones, it was 0.1 mg/kg.

## 7.3. Calculation of residues

The residue R, expressed in mg/kg heptenophos, is calculated from the following equations:

$$R = \frac{F_A \cdot V_{Ex} \cdot W_{St}}{F_{St} \cdot V_i \cdot G} \quad (\text{without cleanup step 6.2})$$

$$R = \frac{F_A \cdot V_{End} \cdot W_{St} \cdot V_{Ex}}{F_{St} \cdot V_i \cdot V_{R1} \cdot G} \quad (\text{with cleanup step 6.2})$$

where

G = sample weight (in g)

$V_{Ex}$  = volume of extract solution from 6.1 (in ml)

$V_{R1}$  = portion of volume  $V_{Ex}$  used for cleanup in step 6.2 (in ml)

$V_{End}$  = terminal volume of sample solution cleaned up in step 6.2 (in ml)

$V_i$  = portion of volume  $V_{Ex}$  (without cleanup) or volume  $V_{End}$  (with cleanup) injected into gas chromatograph (in  $\mu$ l)

$W_{St}$  = amount of heptenophos injected with standard solution (in ng)

$F_A$  = peak area or height obtained from  $V_i$  (in  $\text{mm}^2$  or mm)

$F_{St}$  = peak area or height obtained from  $W_{St}$  (in  $\text{mm}^2$  or mm)

## 8. Important points

A thermionic detector can be used instead of the flame photometric detector, except for the determination of residues in hop cones.

## 9. References

No data

## 10. Author

Hoechst AG, Analytical Laboratory, Frankfurt-Höchst, *S. Gorbach*

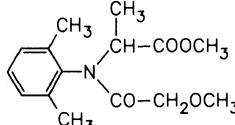
Apples, barley (grains), beer, broccoli, grapes, head cabbage, hop cones, lettuce, maize (whole plants, leaves, stalk and cobs), onions, oranges (pulp, juice and peel), peas (green), pepper (seeds), pineapples, potatoes, spinach, strawberries, sugar beet (foliage and edible root), sunflower seeds, tobacco, tomatoes, wine, youngberries

Soil, water

Gas-chromatographic determination

(German version published 1984)

## 1. Introduction

Chemical name	Methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate (IUPAC)
Structural formula	
Empirical formula	C <sub>15</sub> H <sub>21</sub> NO <sub>4</sub>
Molar mass	279.34
Melting point	71–72 °C
Boiling point	Not distillable
Vapour pressure	2.9 · 10 <sup>-6</sup> mbar at 20 °C
Solubility	Sparingly soluble in water (0.71 g/100 ml at 20 °C); soluble in most commonly used organic solvents, readily soluble in benzene (55 g), dichloromethane (75 g) and methanol (65 g), in 100 ml each at 20 °C, sparingly soluble in n-hexane (0.91 g/100 ml at 20 °C)
Other properties	Stable in neutral and acid media at 20 °C; distribution coefficient of 1.34 in n-hexane/water system

## 2. Outline of method

The necessary extraction and cleanup procedures depend upon the nature of the analytical material. Extraction is done with acetone, dichloromethane or methanol. The extracts are cleaned up by column chromatography on aluminium oxide but in some cases not until after precipitation of interfering substances with ferric chloride and cupric sulphate and cleanup with diluted sulphuric acid and sodium hydroxide solution. With some materials, column chromatography is preceded by liquid-liquid partition. Metalaxy is determined in the cleaned-up eluates by gas chromatography using a nitrogen-specific detector.

### 3. Apparatus

Homogenizer, e.g. Ultra-Turrax (Janke & Kunkel)  
Shaking flask, 500-ml, with stopper  
Laboratory mechanical shaker  
Buchner porcelain funnel, 9 cm dia.  
Filtration flask, 1-l  
Filter paper, 9 cm dia. (e.g. Macherey-Nagel No. 713)  
Graduated cylinder, 500-ml  
Fabric filter, 9 cm dia., cotton fabric  
Pear-shaped flasks, 1-l, 300-ml and 100-ml, with ground joints  
Rotary vacuum evaporator, 40°C bath temperature  
Hot extractor, see Figure, with extraction thimbles  
Separatory funnels, 1-l and 500-ml  
Ultrasonic bath

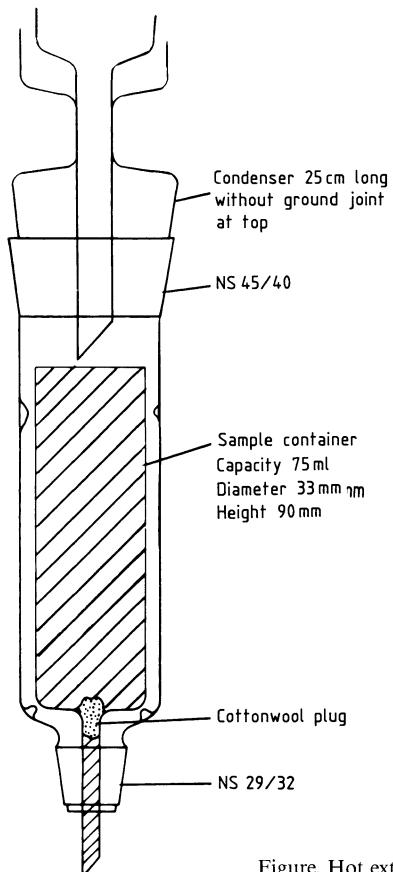


Figure. Hot extractor.

Chromatographic tube, 24 mm i. d., 30 cm long  
Test tubes, graduated, with ground stoppers  
Gas chromatograph equipped with nitrogen-specific detector  
Microsyringe, 5- $\mu$ l

## 4. Reagents

Acetone, dist.  
Dichloromethane, dist.  
Diethyl ether, p. a.  
Ethanol, p. a.  
n-Hexane, dist.  
Methanol, dist.  
Toluene, p. a.  
Diethyl ether + n-hexane mixture 1:1 v/v  
Ethanol + n-hexane mixture 1:1 v/v  
Metalaxyl standard solutions: 1, 2, 5 and 10  $\mu$ g/ml ethanol-hexane mixture  
Water, deionized  
Sulphuric acid, 0.1 mol/l  $H_2SO_4$  p. a.  
Sodium hydroxide solution, 0.1 mol/l NaOH p. a.  
Ferric chloride solution, 5 g/100 ml  $FeCl_3 \cdot 6 H_2O$  p. a.  
Cupric sulphate solution, 5 g/100 ml  $CuSO_4 \cdot 5 H_2O$  p. a.  
Sodium chloride solution, saturated  
Sodium hydrogen carbonate, p. a.  
Sodium sulphate, p. a., anhydrous  
Aluminium oxide: Alumina Woelm A Super I, activity grade V (add 19 ml water/100 g)  
Pre-packed columns for liquid-liquid chromatography, e.g. Extrelut (Merck No. 11737)  
Filter aid, e.g. Celite 545 (Roth)  
Universal indicator paper  
Compressed air, re-purified and dried  
Nitrogen, re-purified  
Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17ff and p. 21f. For water samples, observe the guidelines on p. 23ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material excl. hop cones, pepper, tobacco, grapes and head cabbage

Homogenize 100 g of the analytical sample (G) in 200 ml methanol for 5 min. Then shake the homogenate on a mechanical shaker for 2 h and suction-filter through a filter paper in a Buchner funnel. Wash shaking flask and filter cake twice with 50-ml portions of methanol. Make up the volume of the filtrate to 400 ml ( $V_{Ex}$ ) with methanol in the graduated cylinder. Thoroughly mix, and take 200 ml ( $V_{RI}$ ) for cleanup in step 6.2.2.1.

#### 6.1.2. Grapes

To a 100-g analytical sample of grapes (G) add approx. 5 g sodium hydrogen carbonate and 200 ml methanol, and homogenize for 5 min. After homogenization, the pH of the homogenate must be about 6; if not, add more sodium hydrogen carbonate. Then shake the homogenate on a mechanical shaker for 2 h, and continue to process as described in step 6.1.1.

#### 6.1.3. Hop cones, pepper, tobacco and head cabbage

Extract 15 g comminuted analytical sample (50 g for head cabbage) (G) with 200 ml acetone on a mechanical shaker for 2 h. Then suction-filter the mixture through a filter paper or a three-ply filter (fabric-paper-fabric) in a Buchner funnel. Wash shaking flask and filter cake three times with 50-ml portions of acetone. Rotary-evaporate the filtrate. Caution: Water-containing extracts from green plant parts tend to foam on evaporation. To the oily residue add 10 ml methanol and rotary-evaporate again. Then proceed to cleanup in step 6.2.1.

#### 6.1.4. Soil

Weigh 50 g soil (G) into an extraction thimble, and extract with 300 ml methanol in the hot extractor for 2 h. Moisten dry soil with 10 ml water prior to extraction. Then proceed to cleanup in step 6.2.1.

#### 6.1.5. Wine and beer

Weigh out 20 ml of the analytical sample (G) and add to the pre-packed column for liquid-liquid partition. After 20–30 min, the analytical sample will have dispersed as the stationary phase on the porous matrix of the column. Next elute metalaxyl with 60 ml dichloromethane, and rotary-evaporate the eluate to dryness. Then proceed to cleanup in step 6.2.3.

#### 6.1.6. Water

Extract 500 ml water (G) twice with 70-ml portions of dichloromethane in a 1-l separatory funnel. Discard the water phase. Rotary-evaporate the combined organic phases to dryness. For more heavily contaminated surface water, a cleanup as described in step 6.2.3 is necessary. Evaporation residues from clean surface water or groundwater samples usually can be submitted directly to gas-chromatographic determination as described in step 6.3.

## 6.2. Cleanup

### 6.2.1. Precipitation of interfering substances (hop cones, pepper, tobacco, head cabbage)

Dissolve the residue from step 6.1.3 in 15 ml methanol, immersing the flask in an ultrasonic bath for 1 min. Add 14 g filter aid and mix well. Next add, in the following order, 285 ml deionized water, 3 ml ferric chloride solution and 3 ml cupric sulphate solution. Briefly shake the flask, and then let stand for 30 min. Place a filter paper or a three-ply filter (fabric-paper-fabric) in a Buchner funnel. Wet the filter with water and cover the filter surface with a layer of 10 g filter aid. Wet the layer with water. Next again well mix the reaction mixture and filter with suction. Rinse the reaction flask with 3 ml methanol. Add 57 ml water, and wash the filter cake with the rinsing. Repeat this wash. Discard the filter cake. Do not keep the filtrate for a lengthy period (e.g. overnight). Next proceed to cleanup in step 6.2.2.2.

### 6.2.2. Liquid-liquid partition

#### 6.2.2.1. Plant material incl. grapes (excl. hop cones, pepper, tobacco and head cabbage) and soil

To the extract derived from 6.1.1 or 6.1.4 add 20 ml sodium chloride solution and 200 ml water in a 1-l separatory funnel. Then extract three times with 75-ml portions of dichloromethane. Discard the water phase. Dry the combined dichloromethane phases by filtration through sodium sulphate, and rotary-evaporate to dryness. Then proceed to a further cleanup in step 6.2.3.

#### 6.2.2.2. Hop cones, pepper, tobacco and head cabbage

To the filtrate derived from step 6.2.1 add 20 ml sodium chloride solution in a 500-ml separatory funnel. Extract the resultant aqueous solution three times with 75-ml portions of dichloromethane. Extract the combined dichloromethane phases with 100 ml sodium hydroxide solution for 2 min. Discard the aqueous alkaline phase. Then shake the dichloromethane phase with 100 ml sulphuric acid. Also discard this aqueous phase. Wash the organic phase with 30 ml water, and filter through sodium sulphate. Wash with 30 ml dichloromethane, and rotary-evaporate the filtrate to dryness. Then proceed to a further cleanup in step 6.2.3.

### 6.2.3. Column chromatography

Fill a chromatographic tube with n-hexane. Slowly add aluminium oxide to a level of 7 cm (approx. 30 g). Then drain the hexane to the top of the aluminium oxide.

Dissolve the residue derived from 6.1.5, 6.1.6, 6.2.2.1 or 6.2.2.2 in 5 ml toluene, immersing the flask for 30 to 60 s in an ultrasonic bath. Add the solution to the column and allow to percolate. Wash the flask twice with 5-ml portions of toluene, add these washings successively to the column and also allow to percolate. Next elute interfering substances firstly with 100 ml n-hexane and then with 30 ml diethyl ether-hexane mixture. Discard these eluates. Then elute metalaxyl with 80 ml diethyl ether-hexane mixture. Collect the eluate in a 300-ml pear-shaped flask, and evaporate to dryness in a rotary evaporator.

### 6.3. Gas-chromatographic determination

Dissolve the evaporation residue from step 6.2.3, if applicable also that from 6.1.6, in 2 ml ethanol-hexane mixture; for beer, wine and water, however, dissolve in 1 ml, and for hop cones, pepper and tobacco dissolve in 3 ml ( $V_{\text{End}}$ ). Inject an aliquot of this solution ( $V_i$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Hewlett-Packard 5710
Column	Glass, 2 mm i. d., 1.0 m long; packed with 3% Carbowax 20 M on Gas Chrom Q, 0.15–0.18 mm (80–100 mesh)
Column temperature	240 °C
Injection port temperature	250 °C
Detector	Hewlett-Packard P/N flame ionization detector Temperature 250 °C
Gas flow rates	Nitrogen carrier, 40 ml/min Hydrogen, 3 ml/min Air, 50 ml/min
Attenuation	$8 \cdot 10^{-12}$
Recorder	1 mV; chart speed 10 mm/min
Injection volume	2 µl
Retention time for metalaxyl	2 min 30 s

## 7. Evaluation

### 7.1. Method

Prepare a calibration curve as follows. Inject 2 µl of each metalaxyl standard solution into the gas chromatograph (equivalent to 2, 4, 10 and 20 ng). Plot graphs of the heights of the obtained peaks vs. ng metalaxyl. Prepare a new calibration curve for each sample series. Also inject 2-µl aliquots of the sample solutions. For the heights of the peaks obtained for these solutions, read the appropriate amounts of metalaxyl from the calibration curve.

### 7.2. Recoveries and lowest determined concentration

The recoveries from 47 different untreated control samples fortified with metalaxyl at levels of 0.002–1 mg/kg amounted to 103% with a relative standard deviation of 15%. The routine limit of determination was 0.2 mg/kg for hop cones, 0.1 mg/kg for pepper and tobacco, 0.001 mg/kg for drinking water and pond water, and 0.02 mg/kg for all other analytical materials.

The fortification levels in the recovery determinations generally were two to ten times the amount given for the routine limit of determination.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg metalaxyl, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{End} \cdot V_{Ex}}{V_i \cdot V_{RI} \cdot G}$$

where

G = sample weight (in g) or volume (in ml)

$V_{Ex}$  = volume of filtrate from 6.1.1 (in ml)

$V_{RI}$  = portion of volume  $V_{Ex}$  used for cleanup (in ml)

$V_{End}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_A$  = amount of metalaxyl for  $V_i$  read from calibration curve (in ng)

## 8. Important points

No data

## 9. Reference

In the reported method, improvements suggested by the Landesanstalt für Pflanzenbau und Tabakforschung Forchheim, Rheinstetten bei Karlsruhe, have been taken into account in the procedure for cleanup of tobacco and hop samples.

## 10. Authors

Ciba-Geigy AG, Agricultural Division, Basle, Switzerland, *K. Ramsteiner* and *W. D. Hörmann*



Apples, grapes, hop cones, lettuce, strawberries,  
tomatoes

Gas-chromatographic  
determination

Soil, water

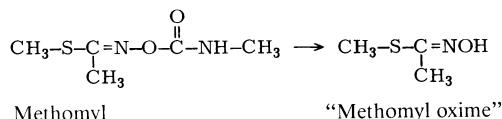
(German version published 1982)

## 1. Introduction

Chemical name	S-Methyl N-(methylcarbamoyloxy)thioacetimidate (IUPAC)
Structural formula	$\text{CH}_3-\text{S}-\underset{\text{CH}_3}{\text{C}}=\text{N}-\text{O}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{NH}-\text{CH}_3$
Empirical formula	$\text{C}_5\text{H}_{10}\text{N}_2\text{O}_2\text{S}$
Molar mass	162.21
Melting point	78–79 °C
Boiling point	No data
Vapour pressure	$9.3 \cdot 10^{-4}$ mbar at 25 °C
Solubility	Soluble in water (5.79 g/100 ml at 25 °C); very sparingly soluble in hydrocarbons, more readily soluble in polar organic solvents
Other properties	Largely stable in aqueous neutral solution; undergoes hydrolysis to oxime in alkaline solution

## 2. Outline of method

Methomyl is extracted from plant material and soil with ethyl acetate; for water samples, this step is omitted. After centrifugation and addition of water, the ethyl acetate is removed by distillation. Impurities are extracted from the acidified solution with n-hexane. Water samples are pre-cleaned up in the same way. Then methomyl is extracted with dichloromethane. After addition of water, the dichloromethane is removed by distillation. For hop cones, a supplemental chromatographic cleanup is performed on a Florisil column. Methomyl is then saponified in alkaline solution to yield S-methyl N-hydroxythioacetimidate (methomyl oxime):



After cooling and acidification, the oxime is extracted from the aqueous solution with ethyl acetate. The organic phase is dried and, after addition of triethyl amine, concentrated. Metho-

myl oxime is determined by flame photometric gas chromatography, using a sulphur-specific detector.

### **3. Apparatus**

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Laboratory mechanical shaker  
Centrifuge, 2000 r.p.m., with 250-ml glass tubes  
Buchner porcelain funnel, 9 cm dia.  
Round filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Filtration flask, 1-l  
Round-bottomed flasks, 1-l, 500-ml and 250-ml, with ground joints  
Rotary vacuum evaporator, 30°C bath temperature  
Separatory funnel, 250-ml  
Chromatographic tube with sintered glass disk, 10 mm i.d.  
Water bath, electrically heated, temperature 95°C  
Test tubes, 10-ml, graduated, with ground stoppers  
Gas chromatograph equipped with sulphur-specific flame photometric detector  
Microsyringe, 5- $\mu$ l

### **4. Reagents**

Dichloromethane, high purity  
Ethyl acetate, p.a.  
n-Hexane, boiling range 60–64 °C  
S-Methyl N-hydroxythioacetimidate (methomyl oxime) (Du Pont de Nemours)  
Methomyl oxime standard solutions: 1, 2, 3, 4, 5, 6 and 7  $\mu$ g/ml ethyl acetate  
Sulphuric acid, 0.5 mol/l  $H_2SO_4$  p.a.  
Sodium hydroxide solution, 1 mol/l NaOH p.a.  
Triethyl amine, p.a.  
Sodium sulphate, p.a., anhydrous  
Filter aid, e.g. Celite 545 (Roth)  
Florisil, 60–100 mesh, dried for 4 h at 130 °C  
Air, synthetic  
Nitrogen, re-purified  
Hydrogen, re-purified

### **5. Sampling and sample preparation**

The analytical sample is taken and prepared as described on p. 17ff and p. 21f. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material

Homogenize 25 g of the prepared sample (G) for 5 min with 150 ml ethyl acetate (200 ml for hop cones) in the blender. Quantitatively transfer the homogenate to a centrifuge tube, and centrifuge for 15 min at 2000 r. p. m. Suction-filter the liquid through a fast flow-rate filter paper overlaid with 5 g Celite in a Buchner porcelain funnel.

Shake the residue in the centrifuge tube twice for 2 min with 100-ml portions of ethyl acetate, centrifuge for 10 min, and filter. Combine the filtrates and add 50 ml water. Then remove the ethyl acetate by rotary evaporation (Caution! Solution foams readily).

#### 6.1.2. Soil

Shake 25 g soil (G) for 2 h with 200 ml ethyl acetate on the mechanical shaker. Filter the mixture through a fluted filter paper into a 500-ml flask. Wash the filter cake with 100 ml ethyl acetate. Add 50 ml water to the filtrate. Remove the ethyl acetate by rotary evaporation.

#### 6.1.3. Water

For extraction of water samples proceed as described in 6.2.1.

## 6.2. Cleanup

#### 6.2.1. Plant material (except hop cones), soil, water

Transfer 100 g of the water sample (G) to a 250-ml separatory funnel. Proceed likewise with the aqueous residue from 6.1.1 or 6.1.2, but adding 50 ml water used for rinsing the round-bottomed flask. After addition of 5 ml sulphuric acid, shake three times for 2 min with 50-ml portions of hexane. Discard the hexane phases. Then shake the aqueous phase three times for 2 min with 50-ml portions of dichloromethane. Combine the dichloromethane extracts, filter through sodium sulphate into a 500-ml flask, and re-wash with 50 ml dichloromethane. Add 50 ml water, and remove the dichloromethane by rotary evaporation (Caution! Solution foams very easily; add boiling chips).

#### 6.2.2. Hop cones

Transfer the aqueous residue derived from 6.1.1 with approx. 10 ml water to a centrifuge tube, and centrifuge for 10 min at 2000 r.p.m. Decant the aqueous phase into a 250-ml separatory funnel. Shake the residue for 1 min with 40 ml water, centrifuge for 10 min at 2000 r.p.m., and decant the aqueous phase into the same separatory funnel. Acidify the combined aqueous phases with 5 ml sulphuric acid, and shake three times for 2 min with 50-ml portions of hexane. Discard the hexane phases. Then extract the aqueous phase three times for 2 min with 50-ml portions of dichloromethane. Combine the dichloromethane extracts, dry on sodium sulphate, and filter into a 250-ml flask, rinsing with 50 ml dichloromethane. Concentrate the filtrate to 5 ml in the rotary evaporator.

Plug the chromatographic tube with glass wool to prevent the sintered glass plate from becoming clogged. Add Florisil to a level of 7 cm, and wash the column packing with 50 ml dichloromethane. Then add the concentrated dichloromethane extract. Wash with 100 ml dichloromethane and discard this forerun. Next elute methomyl with 150 ml ethyl acetate and collect in a 250-ml flask. Add 50 ml water to the eluate. Remove the ethyl acetate by rotary evaporation. Transfer the aqueous residue to a 250-ml separatory funnel, and shake twice for 1 min with 100-ml portions of hexane. Discard the hexane phases.

### 6.3. Saponification

Add 5 ml sodium hydroxide solution to the aqueous solution derived from 6.2.1 or 6.2.2, and heat for 30 min on the water bath at 95 °C. Let the solution cool, acidify with sulphuric acid to pH 2, and transfer to a 250-ml separatory funnel, rinsing with approx. 10 ml water. Then shake three times for 2 min with 50-ml portions of ethyl acetate. Combine the ethyl acetate extracts, dry on sodium sulphate, and filter through a fluted filter paper into a 250-ml flask. Rinse the filter cake with 50 ml ethyl acetate. Add 0.1 ml triethyl amine, and concentrate the filtrate to approx. 7 ml in the rotary evaporator. Transfer the flask contents with 3 ml ethyl acetate to a graduated test tube (with ground neck), add 0.1 ml triethyl amine, and concentrate to a given volume ( $V_{\text{End}}$ ) under a stream of nitrogen at 30 °C.

### 6.4. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the solution derived from 6.3 ( $V_{\text{End}}$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2101 A
Column	Glass, 2 mm i. d., 1.2 m long; packed with 3% Versamid 940 on Gas Chrom Q, 100–120 mesh
Column temperature	150 °C
Injection port temperature	250 °C
Detector	Flame photometric detector fitted with 394-nm sulphur-specific filter, Model FPD 100 AT (Tracor) Temperature 210 °C
Gas flow rates	Nitrogen carrier, 12 ml/min Hydrogen, 50 ml/min Air, 70 ml/min
Attenuation	$8 \cdot 10^4$ or $4 \cdot 10^4$
Chart speed	30 cm/h
Injection volume	1–3 µl
Retention time for methomyl oxime	2 min 45 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Construct a fresh calibration curve with methomyl oxime standard solutions for each set of analyses. Using log-log paper, plot the peak heights in mm vs. the injected amount of methomyl oxime in ng. As the peak height is dependent upon the injection volumes when using a flame photometric detector, equal volumes should be injected. Following lengthy intervals between measurements (e.g. overnight), the signals of methomyl oxime may increase during the first injections. Therefore, 2 or 3 injections each of approx. 50–100 ng methomyl oxime should be made prior to each set of measurements. It proved advantageous to inject the standard solutions and the sample solutions alternately rather than to construct the calibration curve separately.

### 7.2. Recoveries, limit of detection and limit of determination

The recoveries from untreated control samples fortified with methomyl at levels of 0.05 to 4.0 mg/kg ranged from 70–100%, and from 100–110% for apples. The limit of detection was 0.01 mg/kg, and the limit of determination was 0.05 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg methomyl, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{End}}{V_i \cdot G} \cdot 1.542$$

where

G = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_A$  = amount of methomyl oxime for  $V_i$  read from calibration curve (in ng)

1.542 = factor for conversion of methomyl oxime to methomyl

## 8. Important points

The hydrolysis product methomyl oxime is determined and not methomyl itself because the oxime is detected with substantially greater sensitivity by the flame photometric detector. On account of the high volatility of methomyl oxime, triethyl amine must be added prior to concentration in step 6.3. In all other operations in which solutions are concentrated, full account must be taken of the high volatility of both methomyl and its oxime. Therefore, special caution must be exercised when evaporating the last traces of solvent.

## 9. References

- H. L. Pease and J. J. Kirkland*, Determination of methomyl residues using microcoulometric gas chromatography, *J. Agric. Food Chem.* *16*, 554–557 (1968).
- R. F. Holt and H. L. Pease*, Determination of oxamyl residues using flame photometric gas chromatography, *J. Agric. Food Chem.* *24*, 263–266 (1976).

## 10. Authors

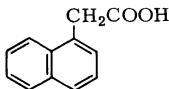
Du Pont de Nemours & Co., Wilmington, U.S.A., *H. L. Pease*, *J. J. Kirkland* and *R. F. Holt*

Apples, grapes, peaches, pears, plums

Gas-chromatographic determination

(German version published 1982)

## 1. Introduction

Chemical name	1-Naphthylacetic acid (IUPAC)
Structural formula	
Empirical formula	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>
Molar mass	186.21
Melting point	132–133 °C
Boiling point	Not distillable
Vapour pressure	No data
Solubility	Very sparingly soluble in water; readily soluble in alcohols and acetone, soluble in ether and dichloromethane
Other properties	No data

## 2. Outline of method

The sample material is extracted with acetone. The solvent is removed from the extract by evaporation, the aqueous residue is made alkaline, diluted with water, and extracted with ethyl acetate. The aqueous phase is acidified, potassium permanganate solution is added to it, and it is extracted with dichloromethane. The organic phase is cleaned up by a partition step.

Esterification of 1-naphthylacetic acid with a sulphuric acid-methanol mixture to 1-naphthyl-acetic acid methyl ester is followed by bromination. The resultant 4-bromo-1-naphthylacetic acid methyl ester is determined by electron capture gas chromatography.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Buchner porcelain funnel, 9 cm dia.  
Filtration flask, 500-ml  
Filter paper, fast flow rate, 9 cm dia. (Schleicher & Schüll)  
Round-bottomed flasks, 500-ml, 250-ml and 50-ml, with ground joints  
Rotary vacuum evaporator, 30–35 °C bath temperature

Separatory funnels, 500-ml and 50-ml  
Chromatographic tube, 10 mm i. d., 30–40 cm long  
Gas chromatograph equipped with electron capture detector  
Microsyringe, 10- $\mu$ l

#### 4. Reagents

Acetone, p. a.  
Dichloromethane, p. a.  
Ethyl acetate, p. a.  
Hexane, p. a.  
Petroleum ether, pure, boiling range 40–60°C  
Toluene, p. a.  
Petroleum ether + dichloromethane mixture 8:2 v/v  
Esterification mixture: methanol + conc. sulphuric acid 9:1 v/v  
1-Naphthylacetic acid standard solutions: 1, 10 and 100  $\mu$ g/ml methanol  
Calibration solution (4-bromo-1-naphthylacetic acid methyl ester in toluene): Treat 1 ml of the standard solution containing 10  $\mu$ g 1-naphthylacetic acid/ml, in exactly the same manner as the solution to be analyzed, with the esterification mixture as described in step 6.3 and then with the bromine-iodine mixture as outlined in step 6.5. When  $V_{\text{End}} = 10$  ml, 1  $\mu$ l of the calibration solution thus obtained corresponds to 1 ng 1-naphthylacetic acid  
Bromine + iodine mixture 95:5 w/w : dissolve 5 g finely powdered iodine p. a. in 95 g bromine p. a.  
Sulphuric acid, 5 mol/l  $\text{H}_2\text{SO}_4$  p. a.  
Sodium hydroxide solution, 2 mol/l NaOH p. a.  
Sodium hydrogen carbonate solution, 4 g/100 ml NaHCO<sub>3</sub>, p. a.  
Potassium permanganate solution, 0.2 mol/l KMnO<sub>4</sub> p. a.  
Sodium sulphate solution, saturated  
Sodium sulphate, p. a., anhydrous  
Florisil, 60–100 mesh: heat in 130°C oven for at least 5 h, let cool in desiccator and add 5% (w/w) water; shake this mixture for about 20 min, and then let equilibrate in tightly stoppered bottle for at least 24 h, with occasional shaking, before use  
Filter aid, e. g. Celite 545 (Roth)  
Universal indicator paper (e. g. Merck)  
Glass wool  
Nitrogen, re-purified

#### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Homogenize 50 g of the analytical sample (G) with 5 g Celite, 1 ml sulphuric acid and 150 ml acetone for 3 min in the blender. Suction-filter the homogenate through a fast flow-rate filter paper in a Buchner funnel and wash the filter cake with 50 ml acetone. Concentrate the filtrate in a rotary evaporator until only an aqueous residue (30–40 ml) remains. Add 50 ml sodium sulphate solution to this residue, adjust to pH 8–9 with sodium hydrogen carbonate solution, and make up with water to a volume of approx. 200 ml. Then add 5 g Celite, and filter with suction through a fast flow-rate filter paper in a Buchner funnel. Wash the filter cake with 50 ml water. Transfer the filtrate to a 500-ml separatory funnel, and shake twice for 2 min with 100-ml portions of ethyl acetate. Discard the ethyl acetate phase. Acidify the aqueous residue with 20 ml sulphuric acid, and add 20 ml potassium permanganate solution. After the solution becomes colourless or, if no decolorization occurs, after an interval of 10 min, shake three times for 2 min with 100-ml portions of dichloromethane. Filter the dichloromethane phases through a 2-cm layer of sodium sulphate retained by a plug of glass wool in a funnel.

### 6.2. Liquid-liquid partition

Extract 1-naphthylacetic acid from the combined organic phases derived from 6.1 with three 100-ml portions of sodium hydrogen carbonate solution, shaking 2 min each time. Combine the alkaline aqueous solutions, adjust to pH 1–2 with sulphuric acid added *dropwise*, and shake successively with 100-ml, 50-ml and 50-ml portions of dichloromethane for 2 min each time. Dry the combined organic phases on sodium sulphate, and rotary-evaporate just to dryness, finally in a 50-ml round-bottomed flask.

### 6.3. Esterification

To the residue remaining after evaporation in step 6.2 add 5 ml of the esterification mixture. Let stand for 10 min, transfer the flask contents with 20 ml hexane to a 50-ml separatory funnel, and shake vigorously with 10 ml water. Wash the organic phase with 10 ml sodium hydrogen carbonate solution, dry on sodium sulphate, and concentrate just to dryness in the rotary evaporator. Remove the last traces of solvent with a gentle stream of nitrogen.

### 6.4. Column chromatography

Tamp a plug of glass wool into the bottom of a chromatographic tube, half-fill the tube with petroleum ether-dichloromethane mixture, and slowly add 7 g Florisil. Let the Florisil settle for about 1 min to a level of 6–7 cm. Then top the column with about 1 cm sodium sulphate. Drain the supernatant solvent to a level of about 0.5 cm above the top of the column packing.

Transfer the residue remaining after evaporation in step 6.3 with 5 ml petroleum ether-dichloromethane mixture to the column, and follow with two 5-ml rinses of the same mixture. Allow to percolate into the column, elute with 150 ml petroleum ether-dichloromethane mixture, and again rinse the flask with the first portions of the eluant. Rotary-evaporate the eluate just to dryness, finally in a 50-ml round-bottomed flask.

### 6.5. Bromination

Dissolve the concentrate from 6.4 in 1 ml dichloromethane and add 1 drop (approx. 0.01 ml) of the bromine-iodine mixture. Swirl the flask, stopper it and let stand for 5 min at room temperature. Then transfer the solution with 20 ml dichloromethane into a 50-ml separatory funnel and extract with 5 ml sodium hydroxide solution. Wash the organic phase with 10 ml water, dry on sodium sulphate and rotary-evaporate just to dryness.

### 6.6. Gas-chromatographic determination

Dissolve the concentrate from 6.5 in 5 ml toluene ( $V_{End}$ ). Inject an aliquot of this solution ( $V_i$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2300
Column	Glass, 2 mm i. d., 2.0 m long; packed with 2% OV-210 + 1% OV-17 on Gas Chrom Q, 100–120 mesh
Column temperature	190 °C
Injection port temperature	250 °C
Detector	$^{63}\text{Ni}$ electron capture detector Temperature 250 °C
Carrier gas flow rate	Nitrogen, 66 ml/min
Attenuation	10 · 512
Recorder	1 mV; chart speed 30 cm/h
Injection volume	3 $\mu\text{l}$
Retention time for 4-bromo-1-naphthylacetic acid methyl ester	4 min 48 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Prepare a calibration curve before each set of determinations, e.g. in the range of 0.3 to 3 ng, with dilutions of the calibration solution. Measure the heights of the peaks obtained. Plot the peak heights vs. ng 1-naphthylacetic acid. Inject equal volumes of the sample solutions and the dilutions of the calibration solution.

### 7.2. Recoveries, limit of detection and limit of determination

Recoveries from untreated control samples of plant material fortified with dilutions of 1-naphthylacetic acid standard solutions at levels of 0.05 to 2.0 mg/kg ranged from 70 to 95% and averaged 80%. The limit of detection was 0.02 mg/kg, and the limit of determination was 0.05 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg 1-naphthylacetic acid, is calculated from the following equation:

$$R = \frac{V_{\text{End}} \cdot D_A}{V_i \cdot G}$$

where

G = sample weight (in g)

$V_{\text{End}}$  = terminal volume of sample solution from 6.6 (in ml)

$V_i$  = portion of volume  $V_{\text{End}}$  injected into gas chromatograph (in  $\mu\text{l}$ )

$D_A$  = amount of 1-naphthylacetic acid for  $V_i$  read from calibration curve (in ng)

## 8. Important points

By subjecting samples to the procedural steps described in 6.1 to 6.4, other compounds containing carboxyl groups are also detected, e. g. herbicides belonging to the class of chlorine-containing phenoxyalkanoic acids and benzoic acids. The extent to which such residues are responsible for the unexpected occurrence of interfering peaks can be determined easily by subjecting the cleaned-up extracts to gas chromatography prior to bromination. The compounds so far tested, however, showed retention times different from that of 4-bromo-1-naphthylacetic acid methyl ester and underwent no change also on being subjected to the gentle conditions of bromination described in step 6.5.

## 9. References

- H.-P. Thier, Nachweis und Bestimmung von Rückständen der herbiziden Säuren und Phenole in pflanzlichem Material, Dtsch. Lebensm. Rundsch. 66, 393–398 (1970).  
 H.-P. Thier, Analysegang zur Ermittlung von Pestizid-Rückständen in Pflanzenmaterial, Dtsch. Lebensm. Rundsch. 68, 345–350 and 397–401 (1972).  
 H. Y. Young, S. Shimabukuro and L. Aono, Spectrophotometric microdetermination of 1-naphthaleneacetic acid in pineapple, J. Agric. Food Chem. II, 132–133 (1963).

## 10. Authors

Institute of Food Chemistry, University of Münster, H.-P. Thier  
 Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, W. D. Weinmann, H.-G. Nolting and G. W. Richtarsky



Cauliflower, leeks, onions, wheat (grains and straw)  
Water

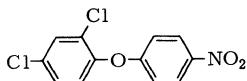
Gas-chromatographic  
determination

(German version published 1982)

## 1. Introduction

Chemical name                            2,4-Dichlorophenyl 4-nitrophenyl ether (IUPAC)

Structural formula



Empirical formula                        C<sub>12</sub>H<sub>7</sub>Cl<sub>2</sub>NO<sub>3</sub>

Molar mass                              284.10

Melting point                          70–71 °C

Vapour pressure                        1 · 10<sup>-5</sup> mbar at 40 °C

Solubility                                Very sparingly soluble in water;

readily soluble in acetone, methanol, petroleum ether  
and xylene (each approx. 25 g/100 ml at 20 °C)

Other properties                        Very stable in acid and alkaline media; decomposes  
under irradiation

## 2. Outline of method

Nitrofen is extracted from plant material with methanol and, after addition of water, partitioned into petroleum ether. Water samples are extracted directly with petroleum ether. The extract is cleaned up on a concentrated sulphuric acid-Celite column. After concentration of the eluate, the residue is determined by electron capture gas chromatography.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor

Buchner porcelain funnel, 9 cm dia.

Filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)

Filtration flask, 1-l

Separatory funnels, 2-l and 500-ml

Glass funnel

Fluted filter paper, 18.5 cm dia.

Round-bottomed flasks, 500-ml, 250-ml and 50-ml, with ground joints

Rotary vacuum evaporator, 35 °C bath temperature

Chromatographic tube, 18 mm i. d., 25 cm long  
Graduated cylinder, 100-ml  
Volumetric flask, 10-ml  
Gas chromatograph equipped with electron capture detector  
Microsyringe, 5- $\mu$ l

## 4. Reagents

Methanol, high purity  
Petroleum ether, for residue analysis, boiling range 40–60 °C  
Toluene, p. a.  
Nitrofen standard solutions: e. g. 0.1, 0.2, 0.5, 1.0 and 2.0  $\mu$ g/ml toluene  
Sodium chloride solution, saturated  
Sodium sulphate, p. a., anhydrous  
Celite 545 (Roth)  
Glass wool  
Sulphuric acid + Celite mixture: thoroughly mix 30 g Celite with 20 ml conc. sulphuric acid ( $H_2SO_4$  p. a., sp. gr.  $\approx$  1.84) in a beaker  
Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Extraction and partitioning

#### 6.1.1. Plant material

Homogenize 50 g (25 g for straw) of the analytical sample (G) with 5 g Celite and 100 ml methanol for 3 min in the blender. Suction-filter the homogenate through a fast flow-rate filter paper in a Buchner funnel. Rinse blender jar and filter cake with 100 ml methanol.

Transfer the filtrate to a 2-l separatory funnel, and shake for 1 min with 100 ml petroleum ether. Add 600 ml water and 40 ml sodium chloride solution, and shake for a further 3 min. Separate the upper organic phase and dry with approx. 10 g sodium sulphate.

#### 6.1.2. Water

To 200 g of the water sample (G) add 40 ml sodium chloride solution in a 500-ml separatory funnel, and shake for 3 min with 100 ml petroleum ether. Separate the upper organic phase and dry with approx. 10 g sodium sulphate.

## 6.2. Column chromatography

Filter the petroleum ether solution from 6.1.1 or 6.1.2 through a fluted filter paper, rinse the filter cake with approx. 30 ml petroleum ether, and rotary-evaporate the filtrate to near dryness.

Place a glass wool plug at the bottom of the chromatographic tube, and add the freshly prepared sulphuric acid-Celite mixture portionwise. Tamp the packing with a thick glass rod to a level of 10 cm. Transfer the concentrated extract from the round-bottomed flask to the column. Rinse the round-bottomed flask with petroleum ether, and add the rinsings portionwise to the column. Continue to elute the column with petroleum ether at a rate of 1–2 drops/s until 100 ml eluate has been collected. Concentrate the eluate to near dryness in a rotary evaporator. Remove the last traces of solvent with a gentle stream of nitrogen.

## 6.3. Gas-chromatographic determination

Dissolve the residue remaining after evaporation in step 6.2 in a suitable volume of toluene, e.g. 10 ml ( $V_{\text{End}}$ ). Inject an aliquot ( $V_i$ ) of the solution into the gas chromatograph.

### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2150
Column	Glass, 2 mm i. d., 1.5 m long; packed with 7.5% DC-200 + 7.5% QF-1 on Gas Chrom Q, 80–100 mesh
Column temperature	220 °C
Injection port temperature	250 °C
Detector	Electron capture detector ECD HT-20 ( $^{63}\text{Ni}$ ) ECD Control Mod. 250, voltage 50 V DC Temperature 300 °C
Carrier gas flow rate	Nitrogen, 55 ml/min
Attenuation	256
Recorder	1 mV; chart speed 5 mm/min
Injection volume	3 µl
Retention time for nitrofen	4 min 30 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Prepare a calibration curve by injecting equal volumes of standard solutions into the gas chromatograph. Plot the peak heights obtained (mm) vs. the amounts of nitrofen (ng) injected with the standard solutions. A calibration curve is obtained which is linear in the range of 0.3–6 ng.

The same volume of the sample solution must be injected. The amount of nitrofen appropriate to the peak height obtained is read from the calibration curve.

## 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with nitrofen at levels of 0.01 to 0.2 mg/kg ranged from 75 to 90%. The routine limit of determination was 0.002 mg/kg nitrofen.

## 7.3. Calculation of residues

The residue R, expressed in mg/kg nitrofen, is calculated from the following equation:

$$R = \frac{W_A \cdot V_{End}}{V_i \cdot G}$$

where

$G$  = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_A$  = amount of nitrofen for  $V_i$  read from calibration curve (in ng)

## 8. Important points

No data

## 9. References

Pesticide Analytical Manual, Vol. I, U.S. Food and Drug Administration, Washington D.C. (1971).

J. Kvalvåg, The detection and determination of residues of the herbicide nitrofen in vegetables, Analyst (London) 99, 666–669 (1974).

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Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, W. D. Weinmann, H. Krohn and H.-G. Nolting

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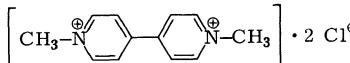
Soil, water

Photometric determination

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(German version published 1982)

## 1. Introduction

Chemical name	1,1'-Dimethyl-4,4'-bipyridinium dichloride (IUPAC) (see Section 8, Important points)
Structural formula	
Empirical formula	C <sub>12</sub> H <sub>14</sub> Cl <sub>2</sub> N <sub>2</sub>
Molar mass	257.16
Melting point	Decomposes above 230 °C
Vapour pressure	Not volatile as salt
Solubility	Very readily soluble in water; moderately soluble in alcohols, very slightly soluble in most other organic solvents
Other properties	Easily reduced to free radical by reducing agents; unstable in strongly alkaline medium

## 2. Outline of method

Soil samples are refluxed in strong sulphuric acid to extract paraquat residues. After filtration, a cation exchange resin is added to the diluted extract, and the suspension is stirred vigorously. The paraquat cation adsorbed on the exchange resin is eluted with saturated ammonium chloride solution. Sodium dithionite solution is added to an aliquot of the eluate resulting in reduction of the cation to the ion radical. The absorbance of this solution is measured at 396 nm.

Water samples are either directly stirred with the exchange resin and the adsorbed paraquat cation is eluted and further treated as described above or reduced directly with sodium dithionite. The absorbance of this solution is then measured.

## 3. Apparatus

Round-bottomed flask, 1-l, with reflux condenser  
Heating mantle for 1-l flask  
Glass filter funnel D 2, 9 cm dia.  
Filter paper, 9 cm dia., slow flow rate (Schleicher & Schüll)  
Filtration flask, 2-l

Magnetic stirrer  
Centrifuge  
Beaker, 400-ml  
Erlenmeyer flasks, 2-l and 500-ml  
Chromatographic tube, 10 mm i.d., 20 cm long, with glass stopcock  
Volumetric flasks, 100-ml, 50-ml and 25-ml  
Water bath, temperature 60°C  
Spectrophotometer, with 5-cm glass cells

## 4. Reagents

Octanol-(2), p.a.  
Paraquat stock solution, 200 µg/ml: Dissolve 55.3 mg pure paraquat dichloride (equivalent to 40 mg paraquat) in saturated ammonium chloride solution and dilute with same to a volume of 200 ml. As paraquat dichloride is hygroscopic, it must be dried for 5 h at 100°C and cooled in a desiccator prior to weighing  
Paraquat working standard solutions: Serially dilute the stock solution with saturated ammonium chloride solution to yield concentrations of 2, 1.5, 1.0, 0.75, 0.5, 0.25, 0.1, 0.05 and 0.025 µg paraquat/ml saturated ammonium chloride solution  
Sulphuric acid, 900 g/l H<sub>2</sub>SO<sub>4</sub> p.a.  
Ammonium chloride, p.a.  
Ammonium chloride solution, p.a., saturated  
Sodium hydroxide solution, 400 g/l NaOH p.a.  
Sodium dithionite solution: 1 g/100 ml sodium dithionite (Merck No. 6507) in sodium hydroxide solution (40 g/l NaOH p.a.). Prepare just prior to use and on no account use after letting stand for more than 2 h. Solid sodium dithionite decomposes in the presence of moisture; therefore, it should always be kept in small tightly stoppered bottles in a desiccator  
Ion exchange resin Dowex 50W-X8, 100–200 mesh (AG), H-form (e.g. Serva)  
Filter aid, e.g. Celite 545 (Serva)

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17f. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Soil

Weigh 100 g of sample (G) (correspondingly less if paraquat concentration of more than 2 mg/kg is anticipated) into a 1-l round-bottomed flask, add 200 ml sulphuric acid and 1 ml octanol, and boil under reflux for 5 h. Allow the solution to cool, and add three 100-ml portions

of water to the flask through the reflux condenser (caution!). Filter the flask contents as follows. Mount a glass filter funnel on a 2-l filtration flask, place a round filter paper in the funnel, and moisten the filter paper with water under suction. Pour 150 ml of a suspension of 10 g Celite 545 in water onto the filter paper, and allow the mass to pull dry. Discard the filtrate. Transfer the flask contents to the filter under gentle suction, and then allow to pull dry. Rinse the flask again with two 100-ml portions of water, and pass the rinsings through the filter. Wash the filter cake with three 100-ml portions of water. Transfer the filtrate to a 2-l Erlenmeyer flask with washings of water, and make up to 1.8 l with water. Then add 10 ml ion exchange resin. Stir the suspension vigorously for 30 min on a magnetic stirrer.

#### 6.1.2. Water

Filter water samples (volume of up to 1.8 l) (G), if necessary. Add 10 ml ion exchange resin to the sample in an Erlenmeyer flask, and stir for 30 min on a magnetic stirrer. The required water volume and Erlenmeyer flask size will depend upon the anticipated paraquat concentration. Groundwater and other water samples displaying little other contamination can also be subjected directly to further processing, i.e. without concentration of paraquat on an ion exchange resin, if their content of paraquat is greater than 50 µg/l. To this end, add 5 ml sodium hydroxide solution to 20 ml of the ammonium chloride-saturated water sample in a 50-ml volumetric flask, maintain for 15 h at 60°C, and then continue to process like an ion exchange resin eluate as described in 6.3.

### 6.2. Elution of paraquat

After stirring, let the solution derived from 6.1.1 or 6.1.2 stand in the Erlenmeyer flask for 30 min. Draw off the supernatant water to a volume of about 100 ml, transfer the remainder to a 400-ml beaker, and wash twice with a total of 400 ml water. Then introduce the ion exchange resin into the chromatographic tube, and drain the supernatant water almost to the top of the column. Cover the exchange resin with an approx. 1.5 cm layer of solid ammonium chloride, and elute with 50 ml saturated ammonium chloride solution at a flow rate of 1–2 ml/min. Collect the eluate in a 50-ml volumetric flask and make up to the mark with saturated ammonium chloride solution. Quantitatively transfer the flask contents to a 100-ml volumetric flask, add 12.5 ml sodium hydroxide solution and hold for 15 h at 60°C ( $V_{End}$ ). Then allow to cool to room temperature, and remove any turbidity by centrifuging at 3000 r.p.m. (see Section 8, Important points).

### 6.3. Colour development and measurement

Pipette 25 ml of the clear solution ( $V_A$ ) into a 25-ml volumetric flask, add 2.0 ml sodium dithionite solution, and mix thoroughly. For water samples processed without concentrating paraquat on an ion exchange resin (see 6.1.2), add the 2.0 ml of sodium dithionite solution to the 50-ml volumetric flask mentioned in 6.1.2. Within 5 min of adding sodium dithionite solution, measure the absorbance of the solution at 392, 396, 400 and 401 nm vs. a mixture of 20 ml saturated ammonium chloride solution and 5 ml sodium hydroxide solution heated at 60°C for 15 h and to which, after being allowed to cool, 2 ml sodium dithionite solution was added. Sodium dithionite solution must be added simultaneously to this blank solution and the sample

solution. The absorbance of the sample solution at 396 nm ( $A_{396}$ ) is corrected for irrelevant absorption with the following equations:

$$A_{396} (\text{corr.}) = 2.91 A_{396} - 1.61 A_{392} - 1.28 A_{401}$$

$$A_{396} (\text{corr.}) = 1.68 (2 A_{396} - A_{392} - A_{400})$$

The mean is computed from the two corrected values.

## 7. Evaluation

### 7.1. Calibration values

Evaluation is performed by preparing a calibration curve as follows. Pipette 20 ml each of the working standard solutions given in Section 4 (equivalent to 40, 30, 20, 15, 10, 5, 2, 1, 0.5 µg paraquat) and 5 ml sodium hydroxide solution into a 25-ml volumetric flask, hold for 15 h at 60°C, then allow to cool, and add 2.0 ml sodium dithionite solution. Thoroughly mix, and within 5 min measure the absorbance in 5-cm cells at 396 nm vs. a mixture of 20 ml saturated ammonium chloride solution and 5 ml sodium hydroxide solution heated at 60°C for 15 h and to which, after being allowed to cool, 2 ml sodium dithionite solution was added. Plot the measured absorbances vs. the µg amount of paraquat contained in 20 ml. The calibration curve obtained is linear in the given range. For example, it was seen that the absorbance measured in a 50-mm lightpath cell was 0.52 for 10 µg paraquat.

If the optical density of the sample solution is much higher than that of a standard solution containing 1.0 µg paraquat/ml, the eluate derived from 6.2 must be diluted still further with saturated ammonium chloride solution. The photometric measurement is then performed as described in 6.3; at the end of the calculation, however, the paraquat concentration must be corrected for the dilution made.

### 7.2. Recoveries, limit of detection and limit of determination

The recoveries from soil fortified with paraquat at levels of 0.05–50 mg/kg ranged from 82–99%. Recoveries from water ranged from 96–100% at fortification levels of 0.01–1 mg paraquat/kg. The limit of detection was 0.01 mg/kg. The limit of determination was 0.05 mg/kg in soil, and 0.01 mg/kg in water (0.05 mg/kg for direct determination).

### 7.3. Calculation of residues

The residue R, expressed in mg/kg paraquat, is calculated from the following equations:

$$\text{Soil, water: } R = \frac{W_{\text{AR}} \cdot V_{\text{End}}}{V_A \cdot G} \quad \begin{matrix} \text{Water:} \\ \text{(without addition of} \\ \text{ion exchange resin)} \end{matrix} \quad R = \frac{W_{\text{AR}}}{20 \cdot 0.78}$$

where

$G$  = sample weight (in g)

$V_{End}$  = terminal volume of cleaned-up extract solution from 6.2 (in ml)

$V_A$  = portion of volume  $V_{End}$  used for measurement of absorbance (in ml)

$W_{AR}$  = amount of paraquat for  $V_A$  read from calibration curve (in  $\mu\text{g}$ )

0.78 = correction of volume for saturation of water sample with ammonium chloride

## 8. Important points

The designation paraquat refers to the cation. The product traded in commerce is chiefly the dichloride so that the physical data and the weights of the standard solutions relate to paraquat dichloride.

The recoveries of paraquat depend very largely upon the ion exchange resin that is used. When an ion exchange resin is used that is different from the one given in the reported method, it is therefore essential to run recovery experiments with soil and water.

After elution of paraquat from the ion exchange resin, flocculent sedimentation may occur in the solution, and during colour development whitish turbidities may appear accompanied by  $\text{H}_2\text{S}$  odour. The former is eliminated by treatment with sodium hydroxide solution followed by centrifugation; the latter are avoided by complete removal of residual acid co-eluted from the ion exchange resin. Losses of paraquat caused by the alkali need not be feared because the pH remains below 10.

## 9. References

- A. Calderbank and S. H. Yuen, An ion-exchange method for determining paraquat residues in food crops, *Analyst* 90, 99–106 (1965).
- J. D. Pope, jr., and J. E. Benner, Colorimetric determination of paraquat residues in soil and water, *J. Assoc. Off. Anal. Chem.* 57, 202–204 (1974).
- P. F. Lott, J. W. Lott and D. J. Doms, The determination of paraquat, *J. Chromatogr. Sci.* 16, 390–395 (1978).
- B. V. Tucker, D. E. Pack and J. N. Ospenson, Adsorption of bipyridylium herbicides in soil, *J. Agric. Food Chem.* 15, 1005–1008 (1967).

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Federal Biological Research Centre of Agriculture and Forestry, Braunschweig, W. D. Weinmann and H.-G. Nolting  
 Federal Health Office, Berlin, F. Herzel and G. Schmidt



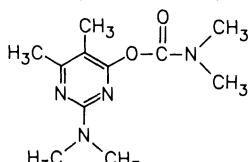
Apples, beans, Brussels sprouts, chives, head cabbage, lettuce

Gas-chromatographic determination

Soil, water

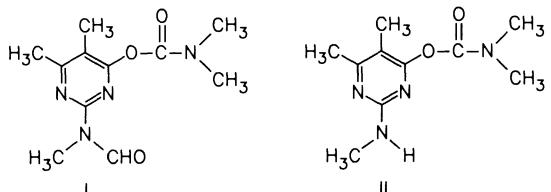
(German version published 1984)

## 1. Introduction

Chemical name	2-Dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (IUPAC)
Structural formula	
Empirical formula	C <sub>11</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>
Molar mass	238.29
Melting point	90.5 °C
Boiling point	Not distillable without decomposition
Vapour pressure	4 · 10 <sup>-5</sup> mbar at 30 °C
Solubility	Sparingly soluble in water (0.27 g/100 ml at 25 °C); readily soluble in alcohols, esters, ketones, aromatic hydrocarbons and chlorinated aliphatic hydrocarbons
Other properties	Hydrolyzed by concentrated acids and alkalies; forms well defined crystalline salts with diluted acids

## 2. Outline of method

The method permits the determination of pirimicarb and the sum of its two major metabolites 5,6-dimethyl-2-N-methylformamidopyrimidin-4-yl N,N-dimethylcarbamate (I) and 5,6-dimethyl-2-methylaminopyrimidin-4-yl N,N-dimethylcarbamate (II) in plant material and water. In soil, pirimicarb and desmethyl pirimicarb (II) can be determined by the reported method.



Plant material is extracted with methanol. After filtration, the concentrated extract is cleaned up by partitioning between n-hexane and water; metabolite I is converted to metabolite II by

treatment with acid, and the total residue is partitioned into the aqueous phase. Water samples are directly acidified and extracted with n-hexane. The respective organic phase is discarded. Pirimicarb and metabolite II are extracted with ethyl acetate at pH 6.7, and cleaned up by column chromatography on Fractosil 200. Soil samples are extracted with a mixture of acetone and aqueous ammonium chloride solution. After filtration and distillation of acetone, the aqueous residue is acidified and washed with diethyl ether. After neutralization of the aqueous solution with sodium hydrogen carbonate, pirimicarb and its metabolite II are extracted with diethyl ether. The residue is determined by gas chromatography using a nitrogen-specific detector.

### 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Buchner porcelain funnel, 9 cm dia.  
Filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Vacuum adapter  
Round-bottomed flasks, 1-l, 500-ml and 250-ml, with ground joints  
Rotary vacuum evaporator, 35°C bath temperature  
Erlenmeyer flasks, 500-ml and 250-ml, with ground stoppers  
Laboratory mechanical shaker  
Graduated cylinder, 1-l  
Separatory funnels, 500-ml and 250-ml  
Centrifuge equipped with 250-ml tubes  
Glass funnel, 9 cm dia.  
Fluted filter paper, 18.5 cm dia.  
Magnetic stirrer  
Chromatographic tube, 10 mm i. d., 40 cm long  
Centrifuge tubes, 10-ml, graduated  
Test tubes, graduated, 10-ml and 5-ml, with ground stoppers  
Gas chromatograph equipped with alkali flame ionization detector  
Microsyringe, 10- $\mu$ l

### 4. Reagents

Acetone, high purity, dist.  
Diethyl ether, high purity, dist.  
Ethyl acetate, high purity, dist.  
n-Hexane, high purity, dist., boiling range 60–64°C  
Methanol, high purity, dist.  
Eluting mixture 1: acetone + n-hexane 1:9 v/v  
Eluting mixture 2: acetone + n-hexane 2:8 v/v  
Extraction mixture: acetone + aqueous ammonium chloride solution 1:1 v/v  
Standard solutions: 1–10  $\mu$ g pirimicarb/ml acetone and 1–10  $\mu$ g metabolite II/ml acetone  
Hydrochloric acid, p. a., conc. and 0.1 mol/l HCl  
Sodium hydroxide solution, 1 mol/l NaOH p. a.

Ammonium chloride solution, 53.5 g/l NH<sub>4</sub>Cl p. a.

Phosphate buffer pH 6.7: Dissolve 136 g potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub> p. a.) and 174 g dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub> p. a.) in water and make up to 2 l

Sodium hydrogen carbonate, p. a.

Sodium sulphate, p. a., anhydrous

Glass wool

Fractosil 200 silica gel, 0.040–0.063 mm (Merck No. 9379)

Special indicator paper, pH 6.4–8.0 (Merck)

Air, synthetic, re-purified

Helium, dried with molecular sieve 5 Å

Hydrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material

Homogenize 50 g of the analytical sample (G) with 250 ml methanol for 10 min. Suction-filter the homogenate through two filter papers in a Buchner porcelain funnel into a 1-l round-bottomed flask. Wash the blender jar and filter cake portionwise with a total of 200 ml methanol, and concentrate the combined filtrates almost to dryness in a rotary evaporator. Remove the last traces of moisture with a gentle stream of nitrogen. Continue to treat the residue as described in step 6.2.1.

#### 6.1.2. Soil

Weigh 100 g soil (G) into a 500-ml Erlenmeyer flask, add 200 ml extraction mixture, and shake for 1 h on the mechanical shaker. Filter the extract with suction through a Buchner porcelain funnel, and wash the filter cake with 50 ml of the same extraction mixture. Next extract the soil sample for 20 min with a further 200 ml extraction mixture in the same manner as before, and wash the filter cake also with 50 ml. Then combine the filtrates and make up with the extraction mixture to a known volume (V<sub>Ex</sub>), e. g. to 600 ml. Mix thoroughly, take 1/10 of this extract (V<sub>RJ</sub>), concentrate to an aqueous residue in the rotary evaporator, and proceed to step 6.2.2.

#### 6.1.3. Water

Transfer 100 g of the filtered sample (G) to a 500-ml round-bottomed flask, acidify with 1 ml concentrated hydrochloric acid, and add 100 ml n-hexane. Then proceed to step 6.2.1.

## 6.2. Cleanup

### 6.2.1. Hexane-water partition

Dissolve the residue derived from 6.1.1 in 100 ml n-hexane and 100 ml diluted hydrochloric acid. Stopper the flask, and shake the mixture well for 2 min; proceed in the same way with the mixture from step 6.1.3. Then let stand overnight. (During this period, metabolite I is converted to metabolite II under the influence of the added acid.) Break any remaining emulsions by centrifuging. Next transfer the entire contents of the flask to a separatory funnel, rinse the flask with 20 ml diluted hydrochloric acid, and add the rinsing to the mixture in the separatory funnel. Draw the lower aqueous phase into a second separatory funnel, and discard the hexane phase. Shake the aqueous phase for 1 min with 100 ml ethyl acetate used earlier for rinsing the flask, and discard the organic phase. Neutralize the acid with approx. 15–20 ml sodium hydroxide solution, add 10 ml phosphate buffer, check that the pH is at 6.7, and extract the solution twice with 100-ml portions of ethyl acetate. Combine the extracts, dry on sodium sulphate, filter through a fluted filter paper into a 500-ml round-bottomed flask, wash the filter cake with 50 ml ethyl acetate, and concentrate carefully just to dryness on a rotary evaporator. Then proceed to step 6.2.3.

### 6.2.2. Diethyl ether-water partition

To the aqueous residue derived from 6.1.2 add 0.1 ml concentrated hydrochloric acid. Quantitatively transfer to a 250-ml separatory funnel, rinsing the flask several times with a total of 10 ml water in the process. Extract twice with 100-ml portions of diethyl ether, drain the aqueous solution into a 250-ml Erlenmeyer flask, and neutralize with 5 g solid sodium hydrogen carbonate added cautiously in small portions with stirring. The pH of the solution should be between 7 and 8. Next extract the neutralized aqueous solution four times with 100-ml portions of diethyl ether (Caution! Evolution of CO<sub>2</sub>). Combine the organic phases, dry on sodium sulphate, and filter through a fluted filter paper. Wash the filter cake with 50 ml diethyl ether. Concentrate the filtrate portionwise in a 250-ml round-bottomed flask to approx. 2 ml using a rotary evaporator. Remove the remaining solvent by rotating the flask manually. Then, without further cleanup, proceed to step 6.3.

### 6.2.3. Cleanup by column chromatography

Tamp a glass wool plug into the bottom of a chromatographic tube, and add 3 g Fractosil 200 in n-hexane. Drain the n-hexane to the top of the Fractosil. Transfer the residue derived from 6.2.1 to a graduated centrifuge tube with three 3-ml washings of acetone, concentrate the solution to 0.2 ml with a gentle stream of nitrogen, and add 0.8 ml n-hexane. Add this solution to the column. Allow to percolate to the top of the Fractosil. Rinse the centrifuge tube with 10 ml n-hexane, add the rinsing to the column, and allow to percolate.

Elute pirimicarb and metabolite II firstly with 50 ml of eluting mixture 1 and then with 30 ml of eluting mixture 2. Collect the eluates in a 250-ml round-bottomed flask, and rotary-evaporate to dryness.

### 6.3. Gas-chromatographic determination

Dissolve the residue derived from 6.2.2 or from 6.2.3 in 1 ml acetone. Transfer this solution to a graduated test tube (with ground joint), rinsing the flask several times with a total of 4 ml acetone in the process. Bring the solution to a known volume ( $V_{\text{End}}$ ) either by concentrating under a stream of nitrogen at room temperature or by diluting with acetone, and store in an airtight container in a refrigerator until it is used for gas-chromatographic analysis. For gas chromatography, inject an aliquot of this solution ( $V_i$ ) into the chromatograph. Unless glass-lined injection ports are used, inject the sample solutions directly onto the column to avoid decomposition of the analytical compounds on hot metal parts.

#### *Operating conditions*

Gas chromatograph	Perkin-Elmer F 33
Column	Glass, 2 mm i.d., 1 m long; packed with 3% OV-17 on Chromosorb W-HP, 100–120 mesh
Column temperature	200°C
Injection port temperature	250°C
Detector	Perkin-Elmer alkali flame ionization detector fitted with rubidium-silicate bead Temperature 250°C
Gas flow rates	Helium carrier, 50 ml/min Hydrogen, 2–3 ml/min Air, 100 ml/min
Attenuation	10 · 4
Recorder	1 mV; chart speed 5 mm/min
Injection volume	3–5 µl
Retention times for pirimicarb	2 min
metabolite II	2 min 30 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Prepare two calibration curves by injecting equal volumes of the differently concentrated standard solutions into the gas chromatograph. Plot curves of the peak heights obtained for pirimicarb and metabolite II vs. the amounts injected with the standard solutions.

Inject equal volumes of the sample solutions. From the calibration curves read the amounts of pirimicarb and metabolite II, respectively.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with pirimicarb and metabolite II ranged from 75 to 80% for plant material, from 70 to 90% for soil, and amounted to 90% for

pond water. The routine limit of determination was 0.05 mg/kg for plant material and soil, 0.1 mg/kg for Brussels sprouts, and 0.005 mg/kg for pond water.

### 7.3. Calculation of residues

The residue  $R_p$ , expressed in mg/kg pirimicarb, and the residue  $R_m$ , expressed in mg/kg metabolite II, are calculated for plant material and water from the following equation:

$$R_p \text{ or } R_m = \frac{W_A \cdot V_{End}}{V_i \cdot G}$$

The residues are calculated for soil samples by using the following equation:

$$R_p \text{ or } R_m = \frac{W_A \cdot V_{Ex} \cdot V_{End}}{V_{RI} \cdot V_i \cdot G}$$

To determine the total residue ( $R_{Tot}$ ), calculated as pirimicarb, the following equation should be used additionally:

$$R_{Tot} = R_p + 1.062 \cdot R_m$$

where

$G$  = sample weight (in g)

$V_{Ex}$  = total volume of extract (in ml)

$V_{RI}$  = portion of volume  $V_{Ex}$  used for cleanup in step 6.2.2 (in ml)

$V_{End}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_A$  = amount of pirimicarb or metabolite II for  $V_i$  read from calibration curve (in ng)

1.062 = factor for conversion of metabolite II to pirimicarb

## 8. Important points

For separate determination of pirimicarb and metabolite II, the fractions cleaned up by column chromatography in step 6.2.3 can be collected singly in 100-ml round-bottomed flasks, rotary-evaporated to dryness and analyzed in accordance with step 6.3.

When the solutions are concentrated in a rotary evaporator, care must be taken that the bath temperature does not exceed 40°C.

Bullock (1972) reports that the following gas-chromatographic conditions which differ from those given in Section 6.3, are also suitable:

Column

Glass, 2 mm i. d., 2 m long; packed with 3% phenyl diethanolamine succinate (PDEAS) on Gas Chrom Q, 100–120 mesh

Column temperature	200 °C
Carrier gas flow rate	Helium, 60 ml/min
Retention times for	
pirimicarb	2 min 48 s
metabolite II	8 min 24 s

With this column, the limit of detection for metabolite II may be higher than that obtained by using the column packed with OV-17.

## 9. References

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- D. J. W. Bullock*, Pirimicarb, in G. Zweig, Analytical methods for pesticides and plant growth regulators, Vol. VII, Thin layer and liquid chromatography and analyses of pesticides of international importance, p. 399-415, Academic Press, New York and London 1974.
- M. J. Edwards* and *J. P. Dick*, Pirimicarb: Crop extractability study, ICI Plant Protection Research Report No. TMJ 1166 A (1975).

## 10. Authors

ICI Plant Protection Ltd., Jealott's Hill Research Station, Bracknell, Berkshire, England, *D. J. W. Bullock*, *J. P. Dick* and *S. H. Kennedy*  
Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, *W. D. Weinmann*, *H.-G. Nolting*, *M. Blacha-Puller* and *J. Siebers*



Apples, barley (grains), beans, bran, bread, Brussels sprouts, carrots, celeriac, cucumbers, curly kale, currants (black and red), head cabbage, leeks, lettuce, mushrooms, olive oil, onions, oranges, peanuts (unshelled), pears, peas, raspberries, red cabbage, rice (grains), small radishes, strawberries, sweet peppers, tomatoes, wheat (grains)

Soil, water

Gas-chromatographic determination

(German version published 1982)

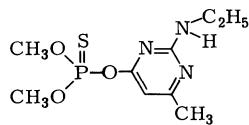
## 1. Introduction

Chemical name	O-2-Diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate (IUPAC)
Structural formula	
Empirical formula	C <sub>11</sub> H <sub>20</sub> N <sub>3</sub> O <sub>3</sub> PS
Molar mass	305.34
Melting point	Approx. 15 °C
Boiling point	Not distillable without decomposition
Vapour pressure	1.5 · 10 <sup>-4</sup> mbar at 30 °C
Solubility	Very sparingly soluble in water; readily soluble in acetone, acetonitrile, n-hexane and methanol
Other properties	Hydrolyzed by concentrated acids and alkalies

## 2. Outline of method

Plant samples are extracted with a mixture of n-hexane and acetone. Soil samples are extracted with a mixture of acetone and ammonium chloride solution; after addition of a buffer, pirimiphos-methyl is extracted with n-hexane. The extracts of high-fat materials are cleaned up by partitioning between acetonitrile and n-hexane. If need be, this can be followed by cleanup on a Florisil column. The residue of pirimiphos-methyl is determined by gas chromatography using a flame photometric detector or a thermionic detector.

Besides pirimiphos-methyl, the following metabolite is determined:



Desethyl-pirimiphos-methyl

### 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
 Laboratory mechanical shaker  
 Buchner porcelain funnel, 9 cm dia.  
 Filtration flask, 1-l  
 Filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
 Graduated cylinders, 500-ml and 100-ml  
 Round-bottomed flasks, 500-ml, 250-ml and 50-ml, with ground joints  
 Rotary vacuum evaporator, 30°C bath temperature  
 Separatory funnels, 1-l, 500-ml and 250-ml  
 Glass wool  
 Chromatographic tube, 20 mm i. d., 40 cm long  
 Volumetric flasks of different sizes ranging from 2 ml to 200 ml  
 Gas chromatograph equipped with flame photometric or thermionic detector  
 Microsyringe, 10- $\mu$ l

### 4. Reagents

Acetone, technically pure, dist.  
 Acetonitrile, technically pure, dist.  
 Diethyl ether, technically pure, dist.  
 n-Hexane, technically pure, dist.  
 Acetone + ammonium chloride solution mixture 1:1 v/v  
 Acetone + n-hexane mixture 2:8 v/v  
 Diethyl ether + n-hexane mixture 1:1 v/v  
 Pirimiphos-methyl and desethyl-pirimiphos-methyl standard solutions: 0.05, 0.1, 0.5, 1.0 and 5.0  $\mu$ g/ml n-hexane each  
 Ammonium chloride solution, 54 g/l NH<sub>4</sub>Cl p. a.  
 Sodium chloride solution, 2 g/100 ml NaCl p. a.  
 Phosphate buffer pH 6.8: Dissolve 68 g KH<sub>2</sub>PO<sub>4</sub> and 87 g K<sub>2</sub>HPO<sub>4</sub> in water and make up to 1 l  
 Sodium chloride, p. a.  
 Sodium sulphate, p. a., anhydrous  
 Filter aid, e. g. Celite 545 (Roth)  
 Florisil, 60–100 mesh: Dry for 12 h at 180°C, store in a desiccator, and add 1.5% water 1 h before use

Air, synthetic  
Hydrogen, re-purified  
Nitrogen, re-purified  
Oxygen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17ff and p. 21f. For water samples, observe the guidelines given on p. 23 ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material

Homogenize 50 g of the coarsely comminuted sample (G) with 5 g Celite and 200 ml acetone + hexane mixture for 3 min in the blender. Suction-filter the homogenate through a filter paper in a Buchner funnel. Wash the filter cake with 50 ml acetone + hexane mixture. Transfer the filtrate to a 500-ml graduated cylinder. Continue to add n-hexane, with careful mixing, until the upper organic phase has a volume of 250 ml ( $V_{Ex}$ ). Decant 100 ml ( $V_{R1}$ ) of this volume, clean up as described in 6.2.1 or dry for 10 min on 5 g sodium sulphate, and then rotary-evaporate to near dryness.

#### 6.1.2. Vegetable oils (e.g. olive oil)

Dissolve 10 g oil (G) in 100 ml n-hexane, if necessary with warming.

#### 6.1.3. Soil

Shake 50 g of the non-dried soil sample (G) with 200 ml acetone + ammonium chloride solution mixture for 60 min in a 500-ml round-bottomed flask on a laboratory shaker. Suction-filter the extract through a filter paper overlaid with moistened Celite. Wash with 50 ml acetone + ammonium chloride solution mixture. Re-extract the filter cake for 20 min. Then filter and wash the extract as above. Transfer the combined extracts to a 500-ml graduated cylinder, and make up to a volume of 500 ml ( $V_{Ex}$ ) with acetone + ammonium chloride solution mixture. Transfer 100 ml of the extract ( $V_{R1}$ ) to a 500-ml separatory funnel, add 50 ml phosphate buffer, and shake twice for 2 min with 100-ml portions of n-hexane. Dry the combined hexane phases for 10 min on 5 g sodium sulphate, and concentrate almost to dryness on a rotary evaporator.

### 6.1.4. Water

Add 1 g sodium chloride to 50 g water (G) in a 250-ml separatory funnel, and shake twice for 2 min with 50-ml portions of n-hexane. Dry the combined hexane phases for 10 min on 5 g sodium sulphate, and concentrate to near dryness on a rotary evaporator.

## 6.2. Cleanup

### 6.2.1. Hexane-acetonitrile partition for high-fat material (e.g. peanuts, olive oil)

Vigorously shake 100 ml hexane solution derived from 6.1.2 or 100 ml of extract ( $V_{R1}$ ) derived from 6.1.1 two times for 2 min with 100 ml acetonitrile. Shake the combined acetonitrile phases for 2 min with 50 ml hexane. Shake the upper hexane phase for 2 min with 30 ml acetonitrile. Concentrate the three combined acetonitrile phases to approx. 60 ml in a rotary evaporator. Transfer the acetonitrile to a 1-l separatory funnel, and wash the flask with 10 ml acetonitrile. Add 300 ml sodium chloride solution, and shake twice for 2 min with 100 ml hexane. Dry the combined hexane phases for 10 min on 5 g sodium sulphate, and concentrate to near dryness on a rotary evaporator.

### 6.2.2. Florisil column chromatography

The extracts derived from 6.1 or 6.2.1 can be cleaned up additionally on a Florisil column. This supplemental cleanup is recommended especially for samples of beans, bread, peas, barley, bran, oranges, peppers, leeks, radishes, rice, celery, wheat and onions. Proceed as follows. Tamp glass wool plug into bottom of chromatographic tube, add 50 ml diethyl ether + hexane mixture, slowly add 15 g Florisil, top the column with 3 g sodium sulphate, and drain the supernatant solvent to the top of the sodium sulphate. Dissolve the residue derived from 6.1 or 6.2.1 in 10 ml hexane, and add to the column. Elute pirimiphos-methyl and desethyl-pirimiphos-methyl with 100 ml diethyl ether + hexane mixture. Concentrate the eluate almost to dryness on a rotary evaporator, and remove the last traces of solvent by rotating the flask manually.

## 6.3. Gas-chromatographic determination

Dissolve the residue derived from 6.1 or 6.2 in n-hexane, transfer the solution to a volumetric flask, and make up to a given volume, e.g. 2 ml ( $V_{End}$ ). Inject an aliquot of this solution ( $V_i$ ) into the gas chromatograph.

### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2300
Column	Glass, 2 mm i. d., 1 m long; packed with 2% OV-101 on Gas Chrom Q, 100–120 mesh
Column temperature	180°C
Injection port temperature	250°C
Detector	Thermionic detector 793 PN (Carlo Erba) Temperature setting through 720 scale divisions (not in °C)

Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 2 ml/min Air, 200 ml/min
Attenuation	10 · 64
Recorder	1 mV; chart speed 5 mm/min
Injection volume	3 µl
Retention times for pirimiphos-methyl desethyl-pirimiphos-methyl	3 min 15 s 2 min 46 s

*Alternative operating conditions*

Gas chromatograph	Pye 104
Column	Glass, 3 mm i. d., 90 cm long; packed with 3% phenyl diethanolamine succinate (PDEAS) on Chromosorb W-HP, 80-100 mesh
Column temperature	180°C
Injection port temperature	195°C
Detector	Melpar flame photometric detector with 526-nm phosphorus-specific filter Temperature 200°C
Gas flow rates	Nitrogen carrier, 100 ml/min Hydrogen, 200 ml/min Oxygen, 35 ml/min Air, 25 ml/min
Attenuation	1 · 10 <sup>3</sup> – 1 · 10 <sup>5</sup>
Recorder	10 mV; chart speed 0.5 inch (12.7 mm)/min
Injection volume	5 µl
Retention times for pirimiphos-methyl desethyl-pirimiphos-methyl	1 min 54 s 3 min 54 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Prepare calibration curves prior to each set of determinations by injecting standard solutions of pirimiphos-methyl and desethyl-pirimiphos-methyl metabolite. Measure the heights of the peaks obtained. Plot the peak heights vs. ng pirimiphos-methyl and desethyl-pirimiphos-methyl metabolite. Equal volumes of sample and calibration solutions should be injected.

### 7.2. Recoveries, limit of detection and limit of determination

The recoveries at fortification levels of 0.01–5 mg/kg averaged 90% (with a relative standard deviation of ± 10%) for low-fat plant material, soil and water, and 80% (with a relative stan-

dard deviation of  $\pm 10\%$ ) for high-fat plant material. The limit of detection was 0.005 mg/kg, and the limit of determination was 0.01 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg pirimiphos-methyl (or desethyl-pirimiphos-methyl), is calculated from the following equation:

$$R = \frac{V_{\text{End}} \cdot V_{\text{Ex}} \cdot W_A}{V_i \cdot V_{\text{RI}} \cdot G}$$

where

G = sample weight (in g)

$V_{\text{Ex}}$  = volume of solvent in graduated cylinder from 6.1 (in ml)

$V_{\text{RI}}$  = portion of volume  $V_{\text{Ex}}$  used for cleanup (in ml)

$V_{\text{End}}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{\text{End}}$  injected into gas chromatograph (in  $\mu$ l)

$W_A$  = amount of pirimiphos-methyl or metabolite for  $V_i$  read from calibration curve (in ng)

## 8. Important points

Prior to the gas-chromatographic determination, several injections of large amounts (e.g. 300 ng) of pirimiphos-methyl and metabolite should be made to obtain faster constancy of the detector signal.

Sample and standard solutions should be kept in airtight containers in a cool, dark place.

Organic solvents containing pirimiphos-methyl and its metabolite should not be dried on large amounts of sodium sulphate otherwise adsorption of the compounds may occur.

Pirimiphos-methyl and its metabolite are adsorbed by plastic materials. Therefore, the sample material should be kept in glass or aluminium containers.

## 9. References

No data

## 10. Authors

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Apples, artichokes, barley (grains and straw), beans, carrots, cucumbers, grapes, hop cones, melons, peaches, potatoes, rice (grains), tobacco, wheat (grains and straw)

Gas-chromatographic determination

Soil

(German version published 1982)

## 1. Introduction

Chemical name	O-6-Ethoxycarbonyl-5-methylpyrazolo[1,5-a]pyrimidin-2-yl O,O-diethyl phosphorothioate (IUPAC)
Structural formula	
Empirical formula	C <sub>14</sub> H <sub>20</sub> N <sub>3</sub> O <sub>5</sub> PS
Molar mass	373.37
Melting point	50–51 °C
Boiling point	Not distillable without decomposition
Vapour pressure	Not measurable; extrapolated value approx. 1.3 · 10 <sup>-6</sup> mbar at 25 °C
Solubility (in 100 ml at 25 °C)	Virtually insoluble in water; very readily soluble in acetone (121.2 g); readily soluble in ethyl acetate (89.9 g) and in toluene (98 g); soluble in ethanol (9.5 g); slightly soluble in n-hexane (1.1 g)
Other properties	Hydrolyzed by acids and alkalies

## 2. Outline of method

Pyrazophos is extracted from the sample material with a mixture of dichloromethane and methanol in a Soxhlet extractor. The extract is shaken with an aqueous solution of sodium chloride, and the aqueous phase is extracted with dichloromethane. The combined dichloromethane phases are dried with sodium sulphate and concentrated. The residue is dissolved in ethyl acetate. If a cleanup is necessary, it is performed by gel permeation chromatography on polystyrene gel as described in Cleanup Method 4 (see p. 65 ff). The residue is determined by gas chromatography using a flame photometric detector.

### 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Soxhlet extractor  
Round-bottomed flasks, 1-l and 500-ml, with ground joints  
Separatory funnel, 2.5-l  
Steam bath  
Drying column: chromatographic tube, 2 cm i. d., 30 cm long, packed with anhydrous sodium sulphate to level of 15 cm  
Rotary vacuum evaporator, 40°C bath temperature  
Apparatus for gel permeation chromatography on polystyrene gels, see Cleanup Method 4, p. 65  
Gas chromatograph equipped with flame photometric detector  
Microsyringe, 10- $\mu$ l

### 4. Reagents

Dichloromethane, dist.  
Ethyl acetate, dist.  
Methanol, dist.  
Dichloromethane + methanol mixture 8:2 v/v  
Pyrazophos standard solutions: 0.1, 1.0 and 10.0  $\mu$ g/ml ethyl acetate  
Sodium chloride solution, 2 g/100 ml NaCl p. a.  
Sodium chloride, p. a.  
Sodium sulphate, p. a., anhydrous  
Quartz chips (Heraeus Quarzschemelze)  
Air, synthetic  
Hydrogen, ultrapure  
Nitrogen, special

### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f.

## 6. Procedure

### 6.1. Extraction

Weigh a 50-g sample (G) for dry, voluminous commodities (like barley grains and straw, hop cones, rice, tobacco, wheat grains and straw) or a sample of up to 100 g (G) for relatively high-density commodities (like apples, artichokes, beans, grapes, melons, peaches, potatoes) and for soil into a blender jar, mix with quartz chips, transfer the mixture to a Soxhlet extractor, and extract with 300 ml dichloromethane + methanol mixture for 8 h on a steam bath.

If the water content of the analytical sample is too low (e.g. as in soil, barley grains, cereal straw, hop cones, rice, tobacco, wheat grains), add 60 ml water to the sample in the extraction thimble after an extraction time of about 1 h. On completion of extraction, wash the dichloromethane-methanol extract with 2 l sodium chloride solution. Let the dichloromethane phase separate from the aqueous phase. Extract the aqueous phase with 200 ml dichloromethane. Pass the dichloromethane extracts successively through a drying column, and concentrate almost to dryness on a rotary evaporator. Dissolve the residue in ethyl acetate, and make up to a given volume ( $V_{Ex}$ ).

Extracts of apples, cucumbers, melons, peaches and potatoes can be analyzed directly by gas chromatography without further cleanup.

## 6.2. Cleanup by gel permeation chromatography

Extracts of artichokes, barley (grains and straw), beans, carrots, grapes, hop cones, rice, soil, tobacco and wheat (grains and straw) are cleaned up by the procedure described in Cleanup Method 4 (see p. 65).

## 6.3. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the extract solution ( $V_{Ex}$ ) derived from 6.1 or of the end solution ( $V_{End}$ ) cleaned up by gel permeation chromatography in step 6.2, into the gas chromatograph.

### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2150
Column	Glass, 4 mm i. d., 1.2 m long; packed with 3% OV-101 on Chromosorb W-HP, 100–120 mesh
Column temperature	230°C
Injection port temperature	250°C
Detector	Melpar flame photometric detector FPD 200 AT fitted with 524-nm phosphorus-specific filter; voltage 750 V Temperature 190°C
Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 100 ml/min Air, 80 ml/min
Attenuation	$2 \cdot 10^3$ or $4 \cdot 10^3$
Recorder	1 mV; chart speed 30 mm/min
Injection volume	1–5 µl
Retention time for pyrazophos	3 min 40 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak area or peak height of the sample solution and comparing it with the peak areas or peak heights obtained for standard solutions of pyrazophos. As the standard, it is best to use the extract from an untreated control sample mixed with differ-

ing amounts of the pyrazophos standard solution. Comparison of peak areas or peak heights with this standard makes for improved repeatability with the flame photometric detector and increases the accuracy of the analysis.

## 7.2. Recoveries and limit of determination

The recoveries from untreated control samples fortified with pyrazophos at levels of 0.01–1 mg/kg ranged from 80–100 %. The limit of determination was approx. 0.01 mg/kg; for hops and tobacco, it was 0.05 mg/kg.

## 7.3. Calculation of residues

The residue R, expressed in mg/kg pyrazophos, is calculated from the following equations:

Analytical procedure without cleanup step 6.2

$$R = \frac{F_A \cdot V_{Ex} \cdot W_{St}}{F_{St} \cdot V_i \cdot G}$$

Analytical procedure with gel permeation chromatographic cleanup step 6.2

$$R = \frac{F_A \cdot V_{Ex} \cdot V_{End} \cdot W_{St}}{F_{St} \cdot V_i \cdot V_{RI} \cdot G}$$

where

G = sample weight (in g)

$V_{Ex}$  = volume of extract solution obtained in step 6.1 (in ml)

$V_{RI}$  = portion of volume  $V_{Ex}$  used for cleanup in step 6.2 (in ml)

$V_{End}$  = terminal volume of sample solution from 6.2 (in ml)

$V_i$  = portion of volume  $V_{Ex}$  or  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_{St}$  = amount of pyrazophos injected with standard solution (in ng)

$F_A$  = peak area or height obtained from  $V_i$  (in  $\text{mm}^2$  or mm)

$F_{St}$  = peak area or height obtained from  $W_{St}$  (in  $\text{mm}^2$  or mm)

## 8. Important points

Instead of the flame photometric detector, a nitrogen/phosphorus-selective thermionic detector may be used.

## 9. Reference

*J. A. Burke, M. L. Porter and S. J. V. Young, Evaluation of two extraction procedures for pesticide residues resulting from foliar application and root absorption, J. Assoc. Off. Anal. Chem. 54, 142-146 (1971).*

## 10. Author

Hoechst AG, Analytical Laboratory, Frankfurt-Höchst, *W. Thier*



Apples, curly kale, grapes, maize cobs, peaches,  
strawberries

Gas-chromatographic  
determination

Soil, water

(German version published 1982)

## 1. Introduction

Chemical name	(Z)-2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (IUPAC)
Structural formula	
Empirical formula	C <sub>10</sub> H <sub>9</sub> Cl <sub>4</sub> O <sub>4</sub> P
Molar mass	365.97
Melting point	94–97 °C
Boiling point	No data
Vapour pressure	5.6 · 10 <sup>-8</sup> mbar at 20 °C
Solubility	Very sparingly soluble in water; readily soluble in acetone, benzene, chloroform and xylene
Other properties	Hydrolyzed slowly in neutral and acidic aqueous solu- tion, more rapidly in alkaline medium

## 2. Outline of method

Plant and soil samples are extracted with acetone. After filtration, most of the acetone is distilled in a rotary evaporator. Tetrachlorvinphos is extracted from the remaining aqueous phase by shaking with dichloromethane. Water samples are extracted directly with dichloromethane. The extract is cleaned up by Florisil column chromatography. The concentration of tetrachlorvinphos in the extracts is determined by electron capture gas chromatography.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Laboratory mechanical shaker  
Glass bottle, 1-l, with screw cap  
Buchner porcelain funnel, 9 cm dia.

Filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll)  
Fluted filter paper, 18.5 cm dia. (Schleicher & Schüll)  
Filtration flask, 1-l  
Separatory funnel, 1-l  
Round-bottomed flasks, 1-l, 500-ml and 250-ml, with ground joints  
Erlenmeyer flask, 300-ml, with ground joint  
Rotary vacuum evaporator, 30°C bath temperature  
Chromatographic tube, 15 mm i.d., 20 cm long  
Volumetric flask, 10-ml  
Gas chromatograph equipped with electron capture detector  
Microsyringe, 5- $\mu$ l

#### 4. Reagents

Acetone, p.a. (only for gas-chromatographic determination)  
Acetone, high purity, distilled  
Dichloromethane, high purity, distilled  
Petroleum ether, for residue analysis, boiling range 40–60°C  
Eluting mixture: petroleum ether + acetone 95:5 v/v  
Tetrachlorvinphos standard solution: 1  $\mu$ g/ml acetone p.a.  
Sodium chloride solution, saturated  
Sodium sulphate, p.a., anhydrous  
Filter aid, e.g. Celite 545 (Roth)  
Florisil, 100–120 mesh: Dry commercial Florisil overnight at 220°C, let cool in desiccator, add 6 g water per 100 g Florisil, and shake for 1 h on mechanical shaker  
Glass wool  
Nitrogen, re-purified

#### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f. For water samples, observe the guidelines given on p. 23 ff.

### 6. Procedure

#### 6.1. Extraction

##### 6.1.1. Plant material

Homogenize 100 g of the analytical sample (G) with 5 g Celite and 200 ml acetone for 3 min in the blender. Suction-filter the homogenate through a fast flow-rate filter paper in a Buchner funnel. Wash blender jar and filter cake with 100 ml acetone.

Quantitatively transfer the combined filtrates with 25 ml acetone into a 1-l round-bottomed flask. Distill most of the acetone in a rotary evaporator (Caution! Solution foams readily). Transfer the aqueous residue with 500 ml water and 100 ml dichloromethane to a 1-l separatory funnel. Add 50 ml sodium chloride solution and shake for 2 min. Allow phases to separate and drain the dichloromethane phase into an Erlenmeyer flask. Extract again with 100 ml dichloromethane. Dry the combined dichloromethane phases for 30 min on approx. 20 g sodium sulphate, with occasional shaking. Then filter through a fluted filter paper covered with a little sodium sulphate into a 500-ml round-bottomed flask, and rinse with 80 ml dichloromethane. Concentrate the solution to near dryness on a rotary evaporator. Remove the last traces of liquid with a gentle stream of nitrogen.

#### **6.1.2. Soil**

Extract 100 g of the sample (G) with 200 ml acetone in a screw cap glass bottle for 1 h on a mechanical shaker. For very dry soil samples, add 50 ml water prior to extraction. Filter the suspension and proceed as described in 6.1.1.

#### **6.1.3. Water**

To 200 g of the water sample (G), add 50 ml sodium chloride solution. Extract tetrachlorvinphos twice with 100-ml portions of dichloromethane by shaking. Combine the dichloromethane phases and proceed as described in 6.1.1.

### **6.2. Cleanup**

Tamp a plug of glass wool into the bottom of a chromatographic tube, and fill the tube with 10 ml petroleum ether. Tap tube to dislodge and eliminate all air bubbles.

Slurry 10 g Florisil in 30 ml petroleum ether and carefully pour the slurry, free from air bubbles, into the tube. Allow to settle, and then cover the Florisil layer with an approx. 1-cm layer of sodium sulphate. Drain the petroleum ether almost to the top of the column.

Dissolve the evaporation residue from 6.1 in a *little* eluting mixture and add to the column. Then elute the column with the eluting mixture at a flow rate of 1 to 2 drops/s. Discard the first 50-ml fraction collected. Collect the next 175 ml and evaporate almost to dryness on a rotary evaporator. Remove the last traces of solvent with a gentle stream of nitrogen.

### **6.3. Gas-chromatographic determination**

Dissolve the residue derived from 6.2 in acetone p.a., and dilute to an appropriate volume ( $V_{End}$ ), e.g. 10 ml. Inject aliquots of this solution ( $V_i$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2300
Column	Glass, 2 mm i.d., 2 m long; packed with 5% DC-200 and 7.5% QF-1 on Gas Chrom Q, 80–100 mesh
Column temperature	210°C
Injection port temperature	225°C

Detector	Electron capture detector ECD HT-20 ( <sup>63</sup> Ni), ECD Control Module 250, voltage 50 V DC Temperature 250 °C
Gas flow rates	Nitrogen carrier, 18 ml/min Nitrogen, 9 ml/min
Attenuation	10 · 128 or 10 · 256
Recorder	1 mV; chart speed 5 mm/min
Injection volume	1–3 µl
Retention time for tetrachlorvinphos	6 min 12 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak height of the sample solution and comparing it with the peak heights obtained for standard solutions of tetrachlorvinphos. Equal volumes of the sample solution and standard solutions should be injected. For evaluation, the peaks of the sample solution and standard solution should exhibit comparable heights.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with tetrachlorvinphos at a level of 0.1 mg/kg ranged from 85 to 100%. The routine limit of determination was approx. 0.01 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg tetrachlorvinphos, is calculated from the following equation:

$$R = \frac{H_A \cdot V_{End} \cdot W_{St}}{H_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

V<sub>End</sub> = terminal volume of sample solution from 6.3 (in ml)

V<sub>i</sub> = portion of volume V<sub>End</sub> injected into gas chromatograph (in µl)

W<sub>St</sub> = amount of tetrachlorvinphos injected with standard solution (in ng)

H<sub>A</sub> = peak height obtained from V<sub>i</sub> (in mm)

H<sub>St</sub> = peak height obtained from W<sub>St</sub> (in mm)

## 8. Important points

The column chromatographic cleanup can be omitted in analyses of slightly contaminated water.

## 9. References

- K. I. Beynon, M. J. Edwards and A. N. Wright, Residues of tetrachlorvinphos and its breakdown products on treated crops, Pestic. Sci. 1, 250–254 (1970).*
- J. E. Fahey, P. E. Nelson and D. L. Ballee, Removal of Gardona from fruit by commercial preparative methods, J. Agric. Food Chem. 18, 866–868 (1970).*

## 10. Authors

Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, *W. D. Weinmann, H.-G. Nolting and A. Wolf*



Apples, bananas, Brussels sprouts, cauliflower, Chinese cabbage, coffee (raw), field corn (green matter and kernels, dried), head cabbage, lemons, oranges, potatoes, rape (seed), small radishes, sweet corn, tomatoes, wheat (grains)

Soil

(German version published 1982)

Gas-chromatographic determination

## 1. Introduction

Chemical name	O,O-Diethyl O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate (IUPAC)
Structural formula	
Empirical formula	C <sub>12</sub> H <sub>16</sub> N <sub>3</sub> O <sub>3</sub> PS
Molar mass	313.31
Melting point	2–5 °C
Boiling point	Not measurable; decomposes above 200 °C
Vapour pressure	1.3 · 10 <sup>-5</sup> mbar at 38 °C
Solubility (in 100 ml at 20 °C)	Very sparingly soluble in water; readily soluble in ethanol (30 g) and toluene (30 g); sparingly soluble in n-hexane (0.7 g) and acetone (0.1 g); very sparingly soluble in dichloromethane (<0.1 g) and ethyl acetate (<0.1 g)
Other properties	Pale yellow to dark brown liquid; characteristic intrinsic odour of phosphoric acid esters; hydrolyzed by acids and alkalies

## 2. Outline of method

Triazophos is extracted from plant material by homogenizing with acetone using an Ultra-Turrax. Soil samples are shaken with acetone. The acetone extract is diluted with an aqueous solution of sodium chloride, and extracted with dichloromethane. The dichloromethane phases are dried with sodium sulphate. The solvent is removed by evaporation, and the residue is dissolved in ethyl acetate. If it is necessary to clean up the extract, this is done by column chromatography on polystyrene gel using ethyl acetate as the mobile phase. Triazophos is determined by gas chromatography using a flame photometric detector.

### 3. Apparatus

Homogenizer, e. g. Ultra-Turrax (Janke & Kunkel)  
Glass filter funnel, 10 cm i. d., 6 cm high, porosity 1  
Beakers, 300-ml  
Separatory funnels, 3-l and 500-ml  
Mechanical shaker, suitable for holding separatory funnels  
Drying column: chromatographic tube, 2 cm i. d., 30 cm long, packed with anhydrous sodium sulphate to a level of 15 cm  
Rotary vacuum evaporator, 30°C bath temperature  
Round-bottomed flask, 500-ml, with ground joint  
Volumetric flasks, 10-ml and 5-ml  
Gas chromatograph equipped with flame photometric detector  
Microsyringe, 5- $\mu$ l

### 4. Reagents

Acetone, dist.  
Dichloromethane, dist.  
Ethyl acetate, dist.  
Triazophos standard solutions: 0.1, 1.0 and 10  $\mu$ g/ml ethyl acetate  
Sodium chloride solution, 2 g/100 ml NaCl p. a.  
Sodium sulphate, p. a., anhydrous  
Air, synthetic  
Hydrogen  
Nitrogen, special

### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f.

## 6. Procedure

### 6.1. Extraction

Weigh 50 to 100 g (G) of the analytical sample, and homogenize with 150 ml acetone for 10 min using an Ultra-Turrax. Suction-filter through a glass filter funnel. Repeat extraction two more times. For dry sample material (coffee, rape, wheat), add approx. 50–60 ml water prior to second extraction.

For soil samples, extract 50–100 g (G) three times with 150-ml portions of acetone in a separatory funnel, using 30-min shaking periods for each extraction; before the second shaking period, add 50–60 ml water. Then suction-filter through a glass filter funnel.

On completion of the extraction, combine the extracts in a separatory funnel and add 2 l sodium chloride solution. Extract the mixture three times with 100-ml portions of dichloromethane. Pass the dichloromethane phases successively through a drying column. Concentrate the combined solutions almost to dryness on a rotary evaporator. Dissolve the residue in ethyl acetate, and bring to a known volume (5–10 ml) ( $V_{\text{End}}$ ).

For apples, bananas, field corn, lemons, oranges, potatoes, sweet corn and tomatoes, this final solution can be directly analyzed by gas chromatography. For the other crops and soil, it must first be cleaned up as described in step 6.2.

## 6.2. Cleanup

For soil, brassicas, coffee, radishes, rape and wheat, clean up the final solution derived from 6.1 by gel permeation chromatography on polystyrene gel as described in Cleanup Method 4 (see p. 65).

## 6.3. Gas-chromatographic determination

Inject an aliquot of the solution of volume  $V_{\text{End}}$  derived from 6.1 or of volume  $V_{\text{EndR}}$  derived from 6.2 ( $V_i$ ) directly onto the column of the gas chromatograph. The injection volume should not exceed 5 µl.

### *Operating conditions*

Gas chromatograph	Carlo Erba Fractovap 2150
Column	Glass, 2 mm i. d., 1.5 m long; packed with 3% OV-1 on Chromosorb W-HP, 80–100 mesh
Column temperature	215 °C
Injection port temperature	250 °C
Detector	Melpar flame photometric detector FPD 200 AT with 524-nm filter Temperature 170 °C
Gas flow rates	Nitrogen carrier, 56 ml/min Hydrogen, 160 ml/min Air, 40 ml/min
Attenuation	10 <sup>4</sup>
Recorder	1 mV; chart speed 30 mm/min
Injection volume	1–5 µl
Retention time for triazophos	1 min 54 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the sample solution peak height or area and comparing it with the peak areas or heights obtained for standard solutions of known concentration. Equal volumes of the sample solution and the standard solutions should be injected. Further, the peaks of the solutions should exhibit comparable areas or heights.

## 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with triazophos at levels of 0.01 to 1.0 mg/kg ranged from 80 to 100% and averaged 90%. The routine limit of determination was approx. 0.01 mg/kg.

## 7.3. Calculation of residues

The residue R, expressed in mg/kg triazophos, is calculated from the following equations:

$$R = \frac{F_A \cdot V_{End} \cdot W_{St}}{F_{St} \cdot V_i \cdot G} \quad (\text{without cleanup step 6.2})$$

$$R = \frac{F_A \cdot V_{EndR} \cdot W_{St} \cdot V_{End}}{F_{St} \cdot V_i \cdot V_{R1} \cdot G} \quad (\text{with cleanup step 6.2})$$

where

G      = sample weight (in g)

$V_{End}$       = terminal volume of sample solution from 6.1 (in ml)

$V_{R1}$       = portion of volume  $V_{End}$  used for cleanup in step 6.2 (in ml)

$V_{EndR}$       = terminal volume of sample solution cleaned up in step 6.2 (in ml)

$V_i$       = portion of volume  $V_{End}$  (without cleanup) or of volume  $V_{EndR}$  (with cleanup) injected into gas chromatograph (in  $\mu$ l)

$W_{St}$       = amount of triazophos injected with standard solution (in ng)

$F_A$       = peak area or height obtained from  $V_i$  (in  $\text{mm}^2$  or mm)

$F_{St}$       = peak area or height obtained from  $W_{St}$  (in  $\text{mm}^2$  or mm)

## 8. Important points

Instead of the flame photometric detector, a nitrogen/phosphorus-selective flame ionization detector may be used.

## 9. References

No data

## 10. Author

Hoechst AG, Analytical Laboratory, Frankfurt-Höchst, *W. Thier*

Apples, beans (green, pods and seeds), beer, blackberries, cherries, Chinese cabbage, corn salad, cucumbers, currants (black), garlic, gooseberries, grape juice, grapes, hop cones, jams, onions (bulbs and stalks), peaches, pears, plums, potatoes, radishes (small and large types), rape (seeds and straw), raspberries, sour cherries, strawberries, sugar (raw), sugar beet (edible root, syrup and dry chips), tomatoes (leaves and fruits), wine, witloof chicory

Soil

(German version published 1982)

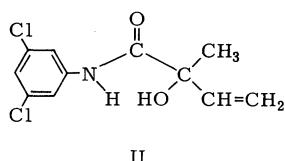
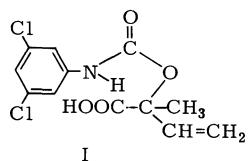
## 1. Introduction

Chemical name	3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione (IUPAC)
Structural formula	
Empirical formula	C <sub>12</sub> H <sub>9</sub> Cl <sub>2</sub> NO <sub>3</sub>
Molar mass	286.11
Melting point	108 °C
Solubility (in 100 g solvent at 20 °C)	Very sparingly soluble in water; readily soluble in acetone (43.5 g), acetonitrile (18.5 g), dichloromethane (13.0 g), ethyl acetate (25.3 g) and toluene (10.4 g); soluble in diethyl ether (6.3 g); slightly soluble in ethanol (1.4 g); sparingly soluble in n-hexane (0.4 g)
Other properties	Hydrolyzed readily, especially in alkaline medium

## 2. Outline of method

Upon alkaline hydrolysis, vinclozolin and its hitherto known metabolites split off 3,5-dichloroaniline. The main metabolites are (1-carboxy-1-methyl)-allyl 3,5-dichlorophenylcarbamate (I) and N-(3,5-dichlorophenyl) 2-hydroxy-2-methyl-3-butene amide (II).

Any liberated dichloroaniline present is distilled over with steam and reacted with chloroacetyl chloride to N-3,5-dichlorophenyl chloroacetamide. This derivative is then determined by electron capture gas chromatography.



### 3. Apparatus

Homogenizer, e. g. Ultra-Turrax (Janke & Kunkel)  
 Round-bottomed flasks, 1-l and 250-ml, with NS 29 joint  
 Buchner porcelain funnel, 9 cm dia.  
 Filter paper, 9 cm dia., fast flow rate (Schleicher & Schüll, No. 300 002)  
 Filtration flask, 1-l  
 Rotary vacuum evaporator, 40–50 °C bath temperature  
 Heating mantle, electrically heated, e. g. Pilz Type AS 634, 1-l (Heraeus-Wittmann)  
 Distillation apparatus, see Figure  
 Erlenmeyer flask, 250-ml  
 Separatory funnel, 1-l, with NS 29 joint and stopper  
 Volumetric flask, 50-ml  
 Gas chromatograph equipped with electron capture detector  
 Microsyringe, 5-μl

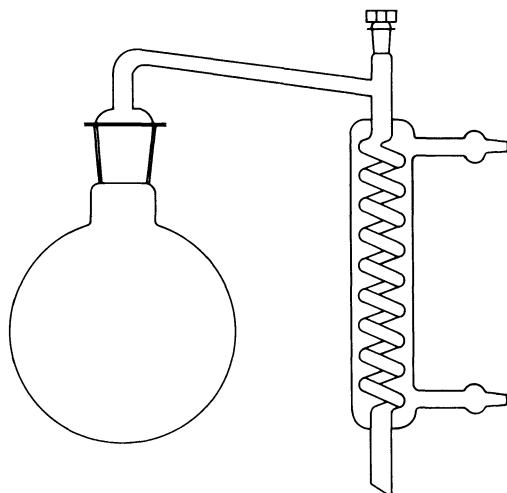


Figure. Distillation apparatus.

## 4. Reagents

Acetone, dist.

Dichloromethane, dist.

Ethyl acetate, dist.

Vinclozolin standard solution: 100 µg/ml acetonitrile

Derivative standard solutions: 10, 20, 30 and 50 ng/ml N-3,5-dichlorophenyl chloroacetamide in ethyl acetate

Chloroacetyl chloride solution: 2 g chloroacetyl chloride (Merck No. 802 411)/100 ml toluene

Acetic acid, 100% (Merck No. 63 E)

Sulphuric acid, 0.5 and 0.3 mol/l

Potassium hydroxide solution, 10 mol/l KOH p. a.

Sodium hydrogen carbonate solution, saturated at room temperature

N-3,5-Dichlorophenyl chloroacetamide: Dissolve 32.4 g 3,5-dichloroaniline (e. g. EGA-Chemie No. D 5.579-2) in 100 ml toluene. Let cool to approx. 5°C, and add 22.6 g chloroacetyl chloride dropwise with stirring. Add 20.2 g triethyl amine, and stir for 2 h at 60°C. Let cool, and then continue to add hydrochloric acid (4 mol/l HCl) and a mixture of ether and ethyl acetate (1 : 1 v/v) until all precipitate is completely dissolved. Wash the organic phase twice with hydrochloric acid (2 mol/l HCl) and five times with water, dry on anhydrous sodium sulphate, and evaporate the solvent under vacuum at 50°C. Yield: approx. 36 g of a brown substance. After recrystallization from ethyl acetate with activated charcoal decolorization followed by 4x recrystallization from toluene with activated charcoal decolorization, white crystals are obtained that are dried on paraffin and potassium hydroxide flakes at 40°C under vacuum. Yield: approx. 12 g white needles of N-3,5-dichlorophenyl chloroacetamide with a melting point of 143°C

Sodium sulphate, p. a., anhydrous

Silicone antifoam (Merck No. 7743)

Boiling chips

Quartz wool, 4–12 µm (Heraeus Quarzsenschmelze)

Nitrogen, special

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f.

## 6. Procedure

### 6.1. Acid extraction

Samples that foam heavily when boiled (e. g. beans, potatoes, soil) must be subjected firstly to an acid extraction as follows. To 50 g of the well mixed analytical material (G) add 2 to 3 ml acetic acid, and homogenize for 1 min with 150 ml acetone. Filter the mixture with suction through a filter paper in a Buchner porcelain funnel, and wash the filter cake with acetone. The amount of acetone required depends upon the nature of the residue. For heavy, compact material, e. g. soil, use 50 ml; for light, voluminous material, e. g. plant fibres, use 100 ml. Combine the acetone filtrates, add 25 ml sulphuric acid (0.3 mol/l), and concentrate on a rotary

evaporator until the acetone is completely evaporated. Then treat the aqueous residue as described in 6.2.

### 6.2. Hydrolysis and distillation

Weigh 50 g of the analytical sample (G) into a 1-l round-bottomed flask. If the sample has been subjected firstly to an acid extraction, transfer the residue from 6.1 into the 1-l round-bottomed flask. Add 450 ml water, 50 ml potassium hydroxide solution, boiling chips and a drop of silicone antifoam. Attach the flask to the distillation head. Heat the mixture to gentle boiling, and distill the 3,5-dichloroaniline formed, using a 250-ml Erlenmeyer flask filled with 25 ml sulphuric acid (0.5 mol/l) as receiver. Continue distillation until about 150–200 ml distillate is collected. Adjust the distillation rate so that it takes at least 90 min to obtain this amount of distillate. On completion of distillation, rinse the condenser with approx. 10 ml dichloromethane. Combine this washing later with the dichloromethane extracts obtained in step 6.3.

### 6.3. Extraction and derivatization

Transfer the distillate from step 6.2 to a separatory funnel, extract once with 25 ml dichloromethane, and neutralize by adding 100 ml saturated sodium hydrogen carbonate solution. Then extract the aqueous solution twice with 25-ml portions of dichloromethane. Combine the three dichloromethane extracts, add the washing from step 6.2, and filter through quartz wool and sodium sulphate into a 250-ml round-bottomed flask. Wash with approx. 10 ml dichloromethane, add 1 ml chloroacetyl chloride solution, and concentrate just to dryness on a rotary evaporator. Dissolve the residue in 50.0 ml ethyl acetate ( $V_{\text{End}}$ ).

### 6.4. Gas-chromatographic determination

Inject 2  $\mu\text{l}$  ( $V_i$ ) of the solution from 6.3 ( $V_{\text{End}}$ ) into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Varian Aerograph 1200
Column	Glass, 2.5 mm i. d., 2 m long; packed with 3% Dexsil 300 on Chromosorb G (AW-DMCS), 60–80 mesh
Column temperature	220 °C
Injection port temperature	250 °C
Detector	$^{63}\text{Ni}$ electron capture detector Temperature 250 °C
Carrier gas flow rate	Nitrogen, 40 ml/min
Attenuation	10 · 4
Recorder	1 mV; chart speed 10 mm/min
Injection volume	2 $\mu\text{l}$
Retention time for N-3,5-dichlorophenyl chloroacetamide	4 min

## 7. Evaluation

### 7.1. Method

Quantitation is performed by the calibration technique. Prepare a calibration curve by injecting 2- $\mu$ l aliquots of the derivative standard solutions, equivalent to 0.02, 0.04, 0.06 and 0.1 ng dichlorophenyl chloroacetamide, into the gas chromatograph. Measure the heights of the peaks obtained. Plot the peak heights vs. ng dichlorophenyl chloroacetamide.

The calibration curve must be checked after at least every third injection of sample solution by interposing injections of derivative standard solutions.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with vinclozolin at levels of 0.05 to 10 mg/kg ranged from 60–85% and averaged 75%. The routine limit of determination was approx. 0.05 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg vinclozolin, is calculated from the following equation:

$$R = \frac{D_A \cdot V_{\text{End}}}{V_i \cdot G} \cdot 1.20$$

where

G = sample weight (in g)

$V_{\text{End}}$  = terminal volume of sample solution from 6.3 (in ml)

$V_i$  = portion of volume  $V_{\text{End}}$  injected into gas chromatograph (in  $\mu$ l)

$D_A$  = amount of derivative (N-3,5-dichlorophenyl chloroacetamide) for  $V_i$  read from calibration curve (in ng)

1.20 = factor for conversion of N-3,5-dichlorophenyl chloroacetamide to vinclozolin

## 8. Important points

The reported method determines, in addition to unchanged vinclozolin, all metabolites containing the 3,5-dichloroaniline group. They are embodied in the final result calculated as vinclozolin.

In plants, the metabolites mentioned in Section 2 are not present in a free form but are conjugated to plant constituents. 3,5-Dichloroaniline also does not occur in a free form in plants, and in soil it occurs only in traces.

## **9. References**

No data

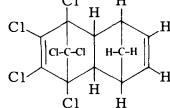
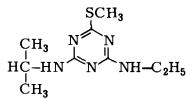
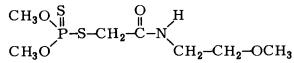
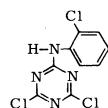
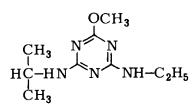
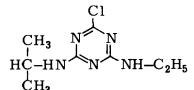
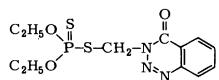
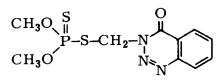
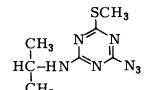
## **10. Authors**

BASF, Agricultural Research Station, Limburgerhof, *S. Otto* and *P. Beutel*

## **Part 4**

# **Multiple Pesticide Residue Analytical Methods**

## Pesticides, Chemically Related Compounds and Metabolites Determinable by the Multiresidue Methods (Table of Compounds)

Common name	Chemical name	Structural formula
Aldrin	(1R,4S,4aS,5S,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,-5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene	
Ametryn	2-ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine	
Amidithion	S-2-methoxyethylcarbamoylmethyl O,O-dimethyl phosphorodithioate	
Anilazine	2,4-dichloro-6-(2-chloroanilino)-1,3,5-triazine	
Arochlor 1242, 1248, 1260 see PCB		
Atraton	2-ethylamino-4-isopropylamino-6-methoxy-1,3,5-triazine	
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine	
Azinphos-ethyl	S-(3,4-dihydro-4-oxobenzo[d][1,2,3]triazin-3-ylmethyl) O,O-diethyl phosphorodithioate	
Azinphos-methyl	S-(3,4-dihydro-4-oxobenzo[d][1,2,3]triazin-3-ylmethyl) O,O-dimethyl phosphorodithioate	
Aziprotryne	2-azido-4-isopropylamino-6-methylthio-1,3,5-triazine	

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Common name	Chemical name	Structural formula
Azocyclotin	tri(cyclohexyl)-1H-1,2,4-triazol-1-yltin	
Binapacryl	2-sec-butyl-4,6-dinitrophenyl 3-methylbut-2-enoate	
Bitertanol	all-rac-1-(biphenyl-4-yloxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	
Bromacil	5-bromo-3-sec-butyl-6-methyluracil	
Bromophos	O-4-bromo-2,5-dichlorophenyl O,O-dimethyl phosphorothioate	
Bromophos-ethyl	O-4-bromo-2,5-dichlorophenyl O,O-diethyl phosphorothioate	
Bupirimate	5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate	
Buturon	3-(4-chlorophenyl)-1-methyl-1-(1-methylprop-2-ynyl)urea	
Camphechlor	A reaction mixture of chlorinated camphenes containing 67 to 69% chlorine	
Captafol	N-(1,1,2,2-tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide	
Captan	N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide	

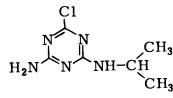
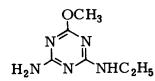
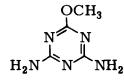
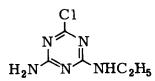
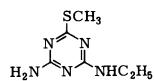
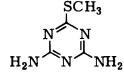
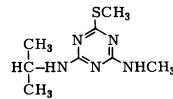
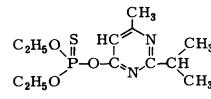
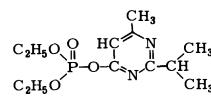
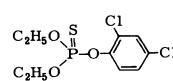
Common name	Chemical name	Structural formula
Carbophenothion	S-4-chlorophenylthiomethyl O,O-diethyl phosphorodithioate	
Carbophenothion sulphone	S-4-chlorophenylsulphonylmethyl O,O-diethyl phosphorodithioate	
Chlorbromuron	3-(4-bromo-3-chlorophenyl)-1-methoxy-1-methylurea	
Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	
Chlorfenson	4-chlorophenyl 4-chlorobenzenesulphonate	
Chlorfenvinphos	2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate	
Chlorotuluron	3-(3-chloro-p-tolyl)-1,1-dimethylurea	
Chloroxuron	3-[4-(4-chlorophenoxy)phenyl]-1,1-dimethylurea	
Chlorpropham	isopropyl 3-chlorophenylcarbamate	
Chlorpyrifos	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	
Chlorpyrifos-methyl	O,O-dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	
Chlorthal	tetrachloroterephthalic acid	

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Common name	Chemical name	Structural formula
Chlorthion	O-3-chloro-4-nitrophenyl O,O-dimethyl phosphorothioate	
Chlorthiophos	A reaction mixture of the three isomers: (i) O-2,4-dichloro-5-(methylthio)phenyl O,O-diethyl phosphorothioate (ii) O-2,5-dichloro-4-(methylthio)phenyl O,O-diethyl phosphorothioate (iii) O-4,5-dichloro-2-(methylthio)phenyl O,O-diethyl phosphorothioate	
Clophen A 30, A 60 see PCB		
Cyanofenphos	O-4-cyanophenyl O-ethyl phenylphosphonothioate	
Cyhexatin	tricyclohexyltin hydroxide	
Cypermethrin	(RS)- $\alpha$ -cyano-3-phenoxybenzyl (1RS,3RS)-(1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	
<i>o,p'</i> -DDD	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane	
<i>p,p'</i> -DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane	
<i>o,p'</i> -DDE	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene	
<i>p,p'</i> -DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene	
<i>o,p'</i> -DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane	

Common name	Chemical name	Structural formula
p,p'-DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane	
Deltamethrin	(S)- $\alpha$ -cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-carboxylate	
Demeton consists of: Demeton-O (appr. 60%) Demeton-S (appr. 40%)		
Demeton-O	O,O-diethyl O-2-ethylthioethyl phosphorothioate	
Demeton-O sulphone	O,O-diethyl O-2-ethylsulphonylethyl phosphorothioate	
Demeton-S	O,O-diethyl S-2-ethylthioethyl phosphorothioate	
Demeton-S sulphone	O,O-diethyl S-2-ethylsulphonylethyl phosphorothioate	
Demeton-S sulphoxide	O,O-diethyl S-2-ethylsulphinylethyl phosphorothioate	
Demeton-S-methyl	S-2-ethylthioethyl O,O-dimethyl phosphorothioate	
Demeton-S-methyl sulphone	S-2-ethylsulphonylethyl O,O-dimethyl phosphorothioate	
Demeton-S-methyl sulphoxide	S-2-ethylsulphinylethyl O,O-dimethyl phosphorothioate	
bis-(Desethyl)-simazine	2,4-diamino-6-chloro-1,3,5-triazine	

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Common name	Chemical name	Structural formula
Desethyl-atrazine	2-amino-4-chloro-6-isopropylamino-1,3,5-triazine	
Des-tert-butyl-terbumeton	2-amino-4-ethylamino-6-methoxy-1,3,5-triazine	
Des-tert-butyl-desethyl-terbumeton	2,4-diamino-6-methoxy-1,3,5-triazine	
Des-tert-butyl-terbutylazine	2-amino-4-chloro-6-ethylamino-1,3,5-triazine	
Des-tert-butyl-terbutyn	2-amino-4-ethylamino-6-methylthio-1,3,5-triazine	
Des-tert-butyl-desethyl-terbutyn	2,4-diamino-6-methylthio-1,3,5-triazine	
Desmetryn	2-isopropylamino-4-methylamino-6-methylthio-1,3,5-triazine	
Diazinon	O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate	
Diazoxon	diethyl 2-isopropyl-6-methylpyrimidin-4-yl phosphate	
Dichlobenil	2,6-dichlorobenzonitrile	
Dichlofenthion	O-2,4-dichlorophenyl O,O-diethyl phosphorothioate	

Common name	Chemical name	Structural formula
Dichlofuanid	N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulphamide	
Dichlorvos	2,2-dichlorovinyl dimethyl phosphate	
Dicofol	2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol	
Dicrotophos	(E)-2-(dimethylcarbamoyl)-1-methylvinyl dimethyl phosphate	
Dieldrin	(1R,4S,4aS,5R,6R,7S,8S,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene	
Dimefox	tetramethylphosphorodiamidic fluoride	
Dimethachlor	2-chloro-N-(2-methoxyethyl)acet-2',6'-xylidide	
Dimethoate	O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate	
Dinocap	An isomeric reaction mixture of 2,6-dinitro-4-octylphenyl crotonates and 2,4-dinitro-6-octylphenyl crotonates	
Dioxathion	S,S'-(1,4-dioxane-2,3-diyl) O,O,O',O'-tetraethyl bis(phosphorodithioate)	
Disulfoton	O,O-diethyl S-2-ethylthioethyl phosphorodithioate	
Disulfoton sulphone	O,O-diethyl S-2-ethylsulphonylethyl phosphorodithioate	

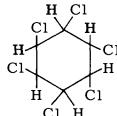
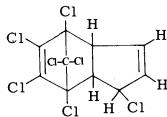
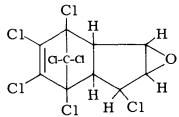
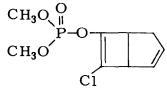
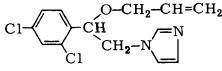
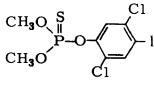
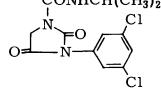
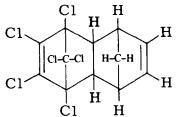
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Common name	Chemical name	Structural formula
Disulfoton sulphoxide	O,O-diethyl S-2-ethylsulphinylethyl phosphorodithioate	
Ditalimfos	O,O-diethyl phthalimidophosphonothioate	
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	
Endosulfan	C,C'-(1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene)(dimethyl sulphite)	
Endosulfan sulphate	C,C'-(1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene)(dimethyl sulphate)	
Endrin	(1R,4S,4aS,5S,6S,7R,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene	
Ethion	O,O,O',O'-tetraethyl S,S'-methylene bis(phosphorodithioate)	
Ethoprophos	O-ethyl S,S-dipropyl phosphorodithioate	
Etrimsfos	O-6-ethoxy-2-ethylpyrimidin-4-yl O,O-dimethyl phosphorothioate	
Fenamiphos	ethyl 4-methylthio-m-tolyl iso-propylphosphoramidate	
Fenamiphos sulphone	ethyl 4-methylsulphonyl-m-tolyl iso-propylphosphoramidate	

Common name	Chemical name	Structural formula
Fenamiphos sulphoxide	ethyl 4-methylsulphinyl-m-tolyl iso-propylphosphoramidate	
Fenbutatin-oxide	bis[tris(2-methyl-2-phenylpropyl)tin] oxide	
Fenchlorphos	O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate	
Fenitrothion	O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate	
Fenpropathrin	(RS)- $\alpha$ -cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate	
Fenson	4-chlorophenyl benzenesulphonate	
Fensulfothion	O,O-diethyl O-4-methylsulphinylphenyl phosphorothioate	
Fensulfothion sulphone	O,O-diethyl O-4-methylsulphonylphenyl phosphorothioate	
Fenthion	O,O-dimethyl O-4-methylthio-m-tolyl phosphorothioate	
Fenthion sulphone	O,O-dimethyl O-4-methylsulphonyl-m-tolyl phosphorothioate	
Fenthion sulphoxide	O,O-dimethyl O-4-methylsulphinyl-m-tolyl phosphorothioate	
Fenvalerate	(RS)- $\alpha$ -cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate	

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Common name	Chemical name	Structural formula
Fentin acetate	triphenyltin acetate	
Fentin chloride	triphenyltin chloride	
Fentin hydroxide	triphenyltin hydroxide	
Fenuron	1,1-dimethyl-3-phenylurea	
Fluometuron	1,1-dimethyl-3-( $\alpha,\alpha,\alpha$ -trifluoro-m-tolyl)urea	
Fluotrimazole	1-(3-trifluoromethyltrityl)-1H-1,2,4-triazole	
Folpet	N-(trichloromethylthio)phthalimide	
Fonofos	O-ethyl S-phenyl ethylphosphonodithioate	
Formothion	S-(N-formyl-N-methylcarbamoylmethyl) O,O-dimethyl phosphorodithioate	
Fuberidazole	2-(2-furyl)benzimidazole	
Gamma-HCH	(1,2,4,5/3,6)-1,2,3,4,5,6-hexachlorocyclohexane	
HCB see Hexachlorobenzene		

Common name	Chemical name	Structural formula
HCH	Mixed isomers of 1,2,3,4,5,6-hexachlorocyclohexane	
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane	
Heptenophos	7-chlorobicyclo[3.2.0]hepta-2,6-dien-6-yl dimethyl phosphate	
Hexachlorobenzene	hexachlorobenzene	
Hexachlorocyclohexane see HCH		
Imazalil	1-(β-allyloxy-2,4-dichlorophenethyl)imidazole	
Iodofenphos	O-2,5-dichloro-4-iodophenyl O,O-dimethyl phosphorothioate	
Iprodione	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	
Isobumeton see Secbumeton		
Isodrin	(IR,4S,5R,8S)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene	

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Common name	Chemical name	Structural formula
Isofenphos	O-ethyl O-2-isopropoxycarbonylphenyl isopropylphosphoramidothioate	
Lindane see Gamma-HCH		
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	
Malaoxon	S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorothioate	
Malathion	S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate	
Mancozeb	Complex of zinc and maneb containing 20% of manganese and 2,5% of zinc	
Maneb	manganese ethylenebis(dithiocarbamate) (polymeric)	
Metalaxyll	methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate	
Methamidophos	O,S-dimethyl phosphoramidothioate	
Methidathion	S-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl O,O-dimethyl phosphorodithioate	
Methoprotryne	2-isopropylamino-4-(3-methoxypropylamino)-6-methylthio-1,3,5-triazine	
Methoxychlor	1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane	
Methyl bromide	methyl bromide	$\text{CH}_3\text{Br}$
Methyl pentachlorophenyl sulphide	methyl pentachlorophenyl sulphide	

Common name	Chemical name	Structural formula
Metiram	mixture of the ammonia complex of zinc ethylenebis(dithiocarbamate) and poly[ethylenebis(thiuron disulphide)]	
Metobromuron	3-(4-bromophenyl)-1-methoxy-1-methylurea	
Metoxuron	3-(3-chloro-4-methoxyphenyl)-1,1-dimethylurea	
Metribuzin	4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one	
Mevinphos	2-methoxycarbonyl-1-methylvinyl dimethyl phosphate	
Monolinuron	3-(4-chlorophenyl)-1-methoxy-1-methylurea	
Monuron	3-(4-chlorophenyl)-1,1-dimethylurea	
Nabam	disodium ethylenebis(dithiocarbamate)	
Naled	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate	
Neburon	1-butyl-3-(3,4-dichlorophenyl)-1-methylurea	
Omethoate	O,O-dimethyl S-methylcarbamoylmethyl phosphorothioate	
Oxychlordane	1,2,4,5,6,7,8,8-octachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	
Oxydemeton-methyl see Demeton-S-methyl sulphoxide		

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Common name	Chemical name	Structural formula
Paraoxon	diethyl 4-nitrophenyl phosphate	
Paraoxon-methyl	dimethyl 4-nitrophenyl phosphate	
Parathion	O,O-diethyl O-4-nitrophenyl phosphorothioate	
Parathion-methyl	O,O-dimethyl O-4-nitrophenyl phosphorothioate	
PCB	mixture of isomers of polychlorinated biphenyls with 30–60% chlorine content	
PCCH	1,3,4,5,6-pentachlorocyclohex-1-ene	
Pentachloroaniline	pentachloroaniline	
Pentachlorobenzene	pentachlorobenzene	
Pentachlorocyclohexene see PCCH		
Pentachloronitrobenzene see Quintozene		
Permethrin	3-phenoxybenzyl (1RS,3RS)-(1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	
Perthane	1,1-dichloro-2,2-bis(4-ethylphenyl)ethane	
Phenkapton	S-2,5-dichlorophenylthiomethyl O,O-diethyl phosphorodithioate	

Common name	Chemical name	Structural formula
Phorate	O,O-diethyl S-ethylthiomethyl phosphorodithioate	
Phorate sulphone	O,O-diethyl S-ethylsulphonylmethyl phosphorodithioate	
Phosalone	S-6-chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl O,O-diethyl phosphorodithioate	
Phosphamidon	2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate	
Piperonyl butoxide	5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole	
Pirimiphos-methyl	O-2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate	
Polychlorinated biphenyls see PCB		
Procymidone	N-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide	
Profenofos	O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate	
Prometon	2,4-bis(isopropylamino)-6-methoxy-1,3,5-triazine	
Prometryn	2,4-bis(isopropylamino)-6-methylthio-1,3,5-triazine	
Propazine	2-chloro-4,6-bis(isopropylamino)-1,3,5-triazine	
Propham	isopropyl phenylcarbamate	

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Common name	Chemical name	Structural formula
Propineb	zinc propylenebis(dithiocarbamate) (polymeric)	
Propoxur	2-isopropoxyphenyl methylcarbamate	
Propyzamide	3,5-dichloro-N-(1,1-dimethylpropynyl)benzamide	
Prothiofos	O-2,4-dichlorophenyl O-ethyl S-propyl phosphorodithioate	
Pyrazophos	O-6-ethoxycarbonyl-5-methylpyrazolo[1,5-a]pyrimidin-2-yl O,O-diethyl phosphorothioate	
Pyrethrins	Pyrethrin I (1S)-2-methyl-4-oxo-3-[(Z)-penta-2,4-dienyl]cyclopent-2-enyl (1R)-trans-chrysanthemate	
	Pyrethrin II (1S)-2-methyl-4-oxo-3-[(Z)-penta-2,4-dienyl]cyclopent-2-enyl pyrethrinate	
	Cinerin I (1S)-3-[(Z)-but-2-enyl]-2-methyl-4-oxocyclopent-2-enyl (1R)-trans-chrysanthemate	
	Cinerin II (1S)-3-[(Z)-but-2-enyl]-2-methyl-4-oxocyclopent-2-enyl pyrethrinate	

Common name	Chemical name	Structural formula
	Jasmolin I (IS)-2-methyl-4-oxo-3-[(Z)-pent-2-enyl]cyclopent-2-enyl (IR)-trans-chrysanthemate	
	Jasmolin II (IS)-2-methyl-4-oxo-3-[(Z)-pent-2-enyl]cyclopent-2-enyl pyrethrinate	
Quintozene	pentachloronitrobenzene	
Rabenazole	2-(3,5-dimethylpyrazol-1-yl)benzimidazole	
Resmethrin	5-benzyl-3-furylmethyl (RS)-cis-trans-chrysanthemate	
Secbumeton	2-sec-butylamino-4-ethylamino-6-methoxy-1,3,5-triazine	
Siduron	1-(2-methylcyclohexyl)-3-phenylurea	
Simazine	2-chloro-4,6-bis(ethylamino)-1,3,5-triazine	
Simeton	2,4-bis(ethylamino)-6-methoxy-1,3,5-triazine	
Sulfotep	O,O,O'-tetraethyl dithiopyrophosphate	
o,p'-TDE see o,p'-DDD		
p,p'-TDE see p,p'-DDD		

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Common name	Chemical name	Structural formula
Tecnazene	1,2,4,5-tetrachloro-3-nitrobenzene	
Terbumeton	2-tert-butylamino-4-ethylamino-6-methoxy-1,3,5-triazine	
Terbutylazine	2-tert-butylamino-4-chloro-6-ethylamino-1,3,5-triazine	
Terbutryn	2-tert-butylamino-4-ethylamino-6-methylthio-1,3,5-triazine	
Tetrachloronitrobenzene see Tecnazene		
Tetrachlorvinphos	(Z)-2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate	
Tetradifon	4-chlorophenyl 2,4,5-trichlorophenyl sulphone	
Tetrasul	4-chlorophenyl 2,4,5-trichlorophenyl sulphide	
Thiometon	S-2-ethylthioethyl O,O-dimethyl phosphorodithioate	
Thionazin	O,O-diethyl O-pyrazin-2-yl phosphorothioate	
Thiram	tetramethylthiuram disulphide	
TMTD see Thiram		
Tolyfluanid	N-dichlorofluoromethylthio-N',N'-dimethyl-N-p-tolylsulphamide	
Toxaphene see Camphechlor		

Common name	Chemical name	Structural formula
Triadimefon	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butanone	
Triadimenol	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	
Triazophos	O,O-diethyl O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate	
Trichlorfon	dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate	
Trifluralin	$\alpha,\alpha,\alpha$ -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	
Vamidothion	O,O-dimethyl S-2-(1-methylcarbamoylethylthio)ethyl phosphorothioate	
Vamidothion sulphone	O,O-dimethyl S-2-(1-methylcarbamoylethylsulphonyl)ethyl phosphorothioate	
Vinclozolin	3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione	
Vondozeb	mixture of maneb and zineb containing 14.5% Mn and 2.1% Zn	
Zineb	zinc ethylenebis(dithiocarbamate) (polymeric)	
Zinophos sec Thionazin		



# Substituted Phenyl Urea Herbicides

S 6

Apples, asparagus, brassicas (curly kale, head cabbage and red cabbage), carrots, celeriac, cotton (leaves and seed), grapes, leeks, lettuce, maize (leaves, kernels and stalks), onions, pears, peas, potatoes, small-grain cereals (grains and straw), soybeans, spelt (grains), strawberries, sunflower oil, sunflower seeds, tobacco, tomatoes

Soil

Gas-chromatographic determination  
(Iodination of anilines)

(German version first published 1974, revised 1979)

## 1. Introduction

The method described below for the determination of residues of herbicidal phenyl ureas is based on determination of the total amount of the substituted aniline analogues yielded by alkaline hydrolysis of unchanged parent compound, the aniline-containing metabolites and certain conjugates. After they have been derivatized, the substituted anilines formed from different urea herbicides can be identified and determined by gas chromatography. This method describes the diazotization and iodination of the anilines. It combines high sensitivity with adequate specificity provided the phenyl ureas present in the analytical material are not hydrolyzed to identical anilines. The compounds measurable by the reported method, together with details of their properties, are given in Table 1.

Table 1. Substituted phenyl urea herbicides and their properties

Common name	Molar mass	Melting point (°C)	Vapour pressure (mbar)	Solubility in water (mg/kg)
Buturon	236.7	145–146	< $10^{-7}$ (20°C)	30 (20°C)
Chlorbromuron	293.6	94– 96	$5.3 \times 10^{-7}$ (20°C)	50 (20°C)
Chloroxuron	290.8	151–152	$2.9 \times 10^{-9}$ (20°C)	3.7 (20°C)
Chlorotoluron	212.7	147–148	$9.8 \times 10^{-8}$ (20°C)	70 (20°C)
Diuron	233.1	158–159	$4.1 \times 10^{-6}$ (50°C)	42 (25°C)
Fenuron	164.2	133–134	$2.1 \times 10^{-4}$ (25°C)	3850 (25°C)
Fluometuron	232.1	163–164.5	$6.7 \times 10^{-7}$ (20°C)	90 (25°C)
Linuron	249.0	93– 94	$2 \times 10^{-5}$ (24°C)	75 (25°C), Du Pont 220 (20°C), Hoechst
Metobromuron	259.1	95– 96	$4 \times 10^{-6}$ (20°C)	330 (20°C)
Metoxuron	228.7	126–127	$2.7 \times 10^{-7}$ (40°C)	650 (24°C)
Monolinuron	214.6	81– 82	$2 \times 10^{-4}$ (24°C)	580 (ca. 20°C)
Monuron	198.7	176–177	$6.7 \times 10^{-7}$ (25°C)	230 (25°C)
Neburon	275.2	102–103	no data	4.8 (24°C)
Siduron	232.2	133–138	no data	18 (25°C)

## 2. Outline of method

The herbicidal phenyl ureas are alkali hydrolyzed in the presence of the analytical material under reflux conditions. The resultant anilines are simultaneously steam-distilled and extracted from the distillate with isoctane. Following re-extraction with hydrochloric acid, the anilines are diazotized, and the diazonium moiety is exchanged for iodine (Sandmeyer reaction). The iodinated derivatives can be re-extracted from the aqueous solution with hexane. Interfering substances originating from soil or plant material can be removed by subsequently partitioning with alkaline potassium permanganate solution. The iodinated derivatives are determined by electron capture gas chromatography.

## 3. Apparatus

High-speed blender

Pyrex round-bottomed flask, 1-l, with NS 29 ground joint

Round-bottomed flask, 250-ml, with NS 29 ground joint

Bleidner apparatus, modified by W. Heizler, for hydrolysis, distillation and extraction; see Figure 1

Heating mantles, for 1-l and 250-ml round-bottomed flasks

Separatory funnel, 50-ml

Volumetric flasks, 50-ml and 10-ml

Ice bath

Water bath

Gas chromatograph equipped with electron capture detector

Microsyringe, 10- $\mu$ l

## 4. Reagents

n-Hexane, distilled in glass

Isooctane, pure

Standard solutions: 1  $\mu$ g/ml each of anilines listed in Table 2, Column 2, in hydrochloric acid (1 mol/l HCl)

Sulfamic acid solution, 10 g/100 ml  $H_2NSO_3H$  p. a.

Iodinating reagent: 5 g/100 ml  $I_2$  p. a. in potassium iodide solution (10 g/100 ml KI p. a.)

Potassium permanganate solution: 1 g/100 ml  $KMnO_4$  p. a. in sodium hydroxide solution (1 mol/l NaOH p. a.)

Sodium hydroxide solution, 10 mol/l NaOH p. a.

Sodium nitrite solution, 1 g/100 ml  $NaNO_2$  p. a., freshly prepared

Hydrochloric acid, 1 mol/l and 2 mol/l HCl

Sodium sulphite,  $Na_2SO_3$  p. a.

Antifoam liquid, NOPCO NXZ (Nopco or Serva)

Argon + methane mixture 95:5 v/v

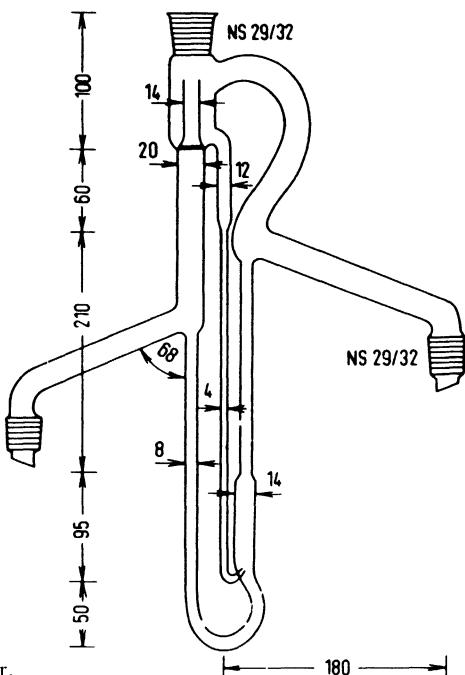


Figure 1. Bleidner apparatus, modified by W. Heizler.

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f. Two equal portions are used for a duplicate determination.

## 6. Procedure

### 6.1. Hydrolysis, distillation and extraction

Weigh 50 g of the comminuted analytical sample into a 1-l Pyrex round-bottomed flask. Then add 200 ml distilled water, 40 ml sodium hydroxide solution, 4 ml NOPCO Antifoam and a few boiling chips. Place the flask in a heating mantle, half-fill the U-tube of the Bleidner apparatus with water and isoctane, and connect the flask to the lower arm of the Bleidner apparatus. Attach a 250-ml round-bottomed flask filled with 100 ml isoctane to the upper arm of the Bleidner apparatus. Heat both flasks for 3 h (at least 15 h for chloroxuron) at a temperature that will ensure condensation of equal amounts of water and isoctane; this can be checked easily from the volumes of the immiscible solvent phases in the capillary of the Bleidner apparatus.

Extract an aliquot of the isoctane solution (that now contains the aromatic amine) two times with 5 ml hydrochloric acid (1 mol/l) in a 50-ml separatory funnel, by shaking vigorously for 2 min. Combine the acidified extracts (approx. 10 ml) in a 50-ml volumetric flask, and cool down to 0°C in an ice bath.

It is essential that starchy plant material (e.g. potatoes, peas, etc.) is pre-treated with hydrochloric acid to prevent excessive foaming in the Bleidner apparatus. In this case, proceed as follows: Weigh 50 g of the comminuted analytical sample into a 1-l Pyrex round-bottomed flask. Then add 200 ml distilled water, 50 ml hydrochloric acid (2 mol/l), 4 ml NOPCO Anti-foam and a few boiling chips. Attach the flask to the reflux condenser, and reflux the mixture for 30 min; the temperature of the heating mantle top should be between 340 and 350°C. Next allow the mixture to cool for 15 to 20 min, then pour in 80 ml sodium hydroxide solution through the reflux condenser, and wait another 15 min before removing the reflux condenser. Immediately afterwards, mount the Bleidner apparatus, and initiate the hydrolysis-distillation-extraction step as described above.

## 6.2. Diazotization

To the acidified extract derived from 6.1 add 2 ml sodium nitrite solution. Let the mixture stand for 25 min at 0°C in the ice bath and then destroy excess nitrite by adding 2 ml sulfamic acid solution. Continue to shake the solution vigorously until nitrogen generation stops.

## 6.3. Iodination

To the ice-cold diazonium salt solution add 0.5 ml iodinating reagent using a pipette. Allow to stand for 25 min at room temperature (20 to 25°C). Transfer the flask to a water bath and heat to boiling within 15 min. During this period, loosen the volumetric flask stopper several times to prevent excess pressure developing. Let the flask stand for 5 min in the boiling water. Remove the flask from the bath and allow to cool to room temperature. Reduce excess iodine in the flask by adding approx. 0.2 g sodium sulphite until the solution turns colourless. Then add 1.1 – 1.2 ml sodium hydroxide solution from a pipette, and shake the reaction mixture vigorously with 20 ml n-hexane. Let the phases separate, pipette 10 ml of the organic phase into a 50-ml separatory funnel, add the same amount of potassium permanganate solution, and shake. Let the mixture stand until the phases have separated, and collect the hexane fraction in a 10-ml flask.

## 6.4. Gas-chromatographic determination

Inject an aliquot of the solution derived from 6.3 into the gas chromatograph.

### *Operating conditions*

Gas chromatograph	F & M 402
Column	Glass, 3 mm i.d., 2 m long; packed with 10% QF-1-0065 on Gas Chrom Q, 80–100 mesh
Column temperature	150°C
Injection port temperature	160°C
Detector	Electron capture detector ( $^{63}\text{Ni}$ ) with pulsed voltage (30 V) Temperature 200°C
Gas flow rates	Argon-methane carrier, 23 ml/min Argon-methane, 37 ml/min
Attenuation	10 · 64 or 10 · 128

Linearity range

10 – 500 pg substituted aniline

Recorder

1 mV; chart speed 12.7 mm/min

Injection volume

5  $\mu$ l

Relative retention times of iodinated compounds derived from

aniline	1.0
3-(trifluoromethyl)aniline	1.2
4-chloroaniline	2.1
4-bromoaniline	3.0
3-chloro-4-methylaniline	3.4
3,4-dichloroaniline	4.3
3-chloro-4-bromoaniline	6.4
3-chloro-4-methoxyaniline	12.0

Examples of the gas-chromatographic separation of a number of urea herbicides following hydrolysis, diazotization and iodination are given in Figure 2.

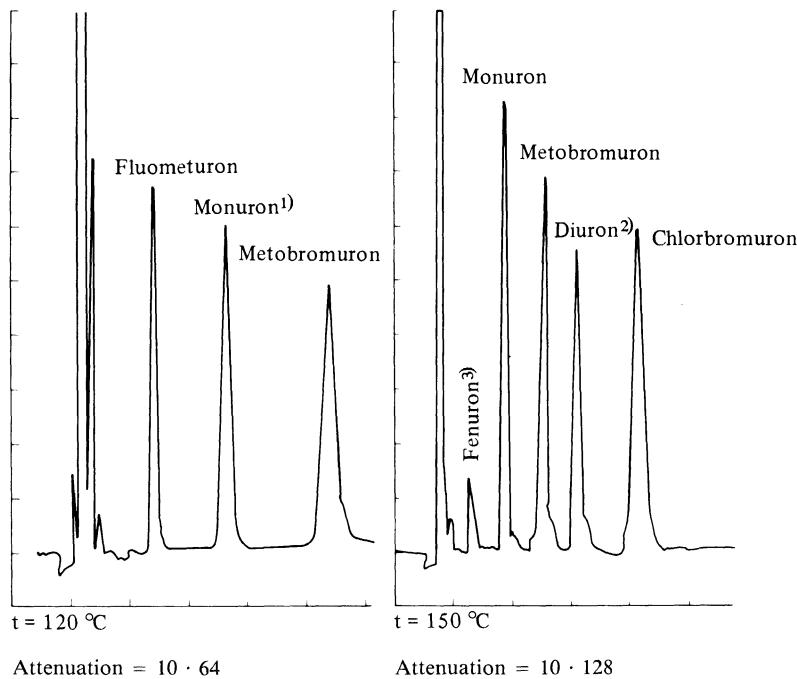


Figure 2. Gas chromatograms of different urea herbicides after hydrolysis to the respective anilines followed by derivatization to the corresponding substituted iodobenzenes (Baunok and Geissbühler, 1968). The produced peaks correspond to 50 pg (fluometuron) and 100 pg (all other ureas) aniline equivalents.  
1) Also buturon and monolinuron; 2) also linuron and neburon; 3) also siduron.

## 7. Evaluation

### 7.1. Method

Quantification is performed by reference to calibration curves prepared as follows. Submit 10 ml of the standard solution of the respective aniline (1 µg/ml) in hydrochloric acid (1 mol/l) to the diazotization and iodination procedures described above in 6.2 and 6.3, omitting the cleanup step with potassium permanganate solution. Dilute the final hexane solution so that the amount of substituted iodobenzene contained in 2–8 µl of the solution injected is equivalent to 20–500 pg aniline. Plot the peak heights (cm) vs. pg substituted aniline on a log-log scale. It is recommended to repeat the determination of some calibration values for each new set of analyses; to do so, diazotize, iodinate and inject known amounts of the respective aniline together with the extracts, concomitantly and under the same conditions.

### 7.2. Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified at levels of 0.05 to 0.5 mg/kg ranged from 80 to 110%. The routine limit of determination varied somewhat for the different urea herbicides. It generally ranged from 0.01 to 0.05 mg/kg.

### 7.3. Calculation of residues

The residues can be expressed in mg/kg of the unchanged parent compound or in mg/kg of the respective aniline.

To express the residues in mg/kg of a parent compound, the found amount of aniline is multiplied by a conversion factor calculated from the molar mass of the unchanged parent compound divided by the molar mass of the respective aniline. Such conversion factors are given in Table 2, Column 6. If it is assumed that several urea herbicides of the same aniline structure are present (e.g. monuron together with monolinuron or diuron together with linuron and/or neburon), this mode of calculation and the direct hydrolysis procedure cannot be employed. In such cases, the herbicides must be separated from each other by thin-layer chromatography prior to hydrolysis. See also Important points in Section 8 below. When foods are tested for conformity with the Federal German Regulation on Maximum Pesticide Residue Limits, the residues must be related to the respective aniline. As the anilines are used for the preparation of the calibration curve, no further recalculation is necessary in this case.

## 8. Important points

Besides those cases mentioned in 7.3, other instances in which it is not possible to employ the procedure of direct hydrolysis arise where it is suspected that samples contain phenyl carbamates, anilides or other compounds (e.g. chloridazon) which, upon hydrolysis, will yield the same anilines as phenyl ureas, e.g. propham or chloridazon together with fenuron or siduron, also monalide together with buturon, monolinuron or monuron. A source of error may be created also by the presence of differently substituted phenyl carbamates or anilides if their

iodinated hydrolysis products interfere with the gas-chromatographic determination of the iodinated derivatives from the phenyl ureas.

Also in these perhaps exceptional instances, the herbicides must be extracted and separated from each other or from interfering compounds by thin-layer chromatography, prior to hydrolysis.

Otherwise, the reported method can be used also for the determination of herbicidal phenyl carbamates and anilides.

Table 2. Substituted phenyl urea herbicides and their hydrolysis products

Parent compound	Hydrolysis product				Factor for conversion of aniline to phenyl urea
Common name	Chemical name	Structural formula	Empirical formula	Molar mass	
1	2	3	4	5	6
Buturon	4-Chloroaniline		C <sub>6</sub> H <sub>6</sub> ClN	127.57	1.86
Monolinuron					1.68
Monuron					1.56
Chlorbromuron	4-Bromo-3-chloroaniline		C <sub>6</sub> H <sub>5</sub> BrClN	206.47	1.42
Chloroxuron	4-(4'-Chlorophenoxy)aniline		C <sub>12</sub> H <sub>10</sub> ClNO	219.67	1.32
Chlorotuluron	3-Chloro-4-methylaniline		C <sub>7</sub> H <sub>8</sub> ClN	141.60	1.50
Diuron	3,4-Dichloroaniline		C <sub>6</sub> H <sub>5</sub> Cl <sub>2</sub> N	162.02	1.44
Linuron					1.54
Neburon					1.70
Fenuron	Aniline		C <sub>6</sub> H <sub>7</sub> N	93.13	1.76
Siduron					2.49
Fluometuron	3-(Trifluoromethyl)aniline		C <sub>7</sub> H <sub>6</sub> F <sub>3</sub> N	161.13	1.44
Metobromuron	4-Bromoaniline		C <sub>6</sub> H <sub>6</sub> BrN	172.02	1.51
Metoxuron	3-Chloro-4-methoxyaniline		C <sub>7</sub> H <sub>8</sub> ClNO	157.60	1.45

Table 3. List of analytical materials and compounds on which method has been tested at given concentrations

Analytical materials	Buturon	Chlor-bromuron	Chloroxuron	Chloroturon	Diuron
Apples/pears	+ 0.05				+ 0.05
Asparagus					+ 0.1
Brassicas (curly kale, head cabbage, red cabbage)					
Carrots	+ 0.05	+ 0.03	+ 0.05		
Celeriac		+ 0.04	+ 0.1		
Cereal grains, excl. maize	+ 0.05	+ 0.04		+ 0.1	
Cereal straw, excl. maize	+ 0.05	+ 0.04		+ 0.1	
Cotton (leaves and seed)			+ 0.1		
Grapes	+ 0.05				+ 0.01
Leeks			+ 0.1		
Lettuce			+ 0.1		
Maize (leaves, kernels, stalks)	+ 0.05	+ 0.05			
Onions			+ 0.1		
Peas		+ 0.03	+ 0.02		
Potatoes	+ 0.03	+ 0.03			
Soil	+ 0.05	+ 0.07	+ 0.05	+ 0.05	+ 0.05
Soybeans		+ 0.07			
Spelt (grains)	+ 0.05	+ 0.04		+ 0.1	
Strawberries			+ 0.1		
Sunflower seeds, sunflower oil					
Tobacco					
Tomatoes					

## Note

Plus sign (+) signifies that the compound, at the given concentration (expressed in mg/kg), was recovered at a rate of more than 70%. Where no figures are given for a compound alongside a substrate, this indicates that determination of the compound in the particular analytical material has not yet been tried.

Table 3. (contd.)

## 9. References

- W. E. Bleidner, H. M. Baker, M. Levitsky and W. K. Lowen*, Determination of 3-(p-chlorophenyl)-1,1-dimethylurea in soils and plant tissue, *J. Agric. Food Chem.* 2, 476-479 (1954).
- I. Baunok and H. Geissbühler*, Specific determination of urea herbicide residues by EC gas chromatography after hydrolysis and iodine derivative formation, *Bull. Environm. Contam. Toxicol.* 3, 7 (1968).
- R. E. Duggan, H. C. Barry, L. Y. Johnson and S. Williams*, Food and Drug Administration (U.S. Department of Health, Education and Welfare). 3-(p-Bromophenyl)-1-methoxy-1-methylurea, Method II; Pesticide Analytical Manual Vol. II (1969), Pesticide Reg. Sec. 120.250.

## 10. Author

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# **Substituted Phenyl Urea Herbicides**

**S 6-A**

Barley, oats, poppy seeds, tobacco, wheat (grains and straw)

Gas-chromatographic determination  
(Acetylation of anilines)

Soil

(German version first published 1976, revised 1979)

## **1. Introduction**

The method of analysis described below is an alternative to Method S 6 for substituted phenyl urea herbicides. The choice of method is dictated more by the availability of instruments and apparatus rather than by the nature of the commodity to be analyzed. The following method is recommended for use especially in analytical laboratories experienced in gas chromatography with nitrogen-specific detectors. It is based on determination of the total amount of the anilines obtained by alkaline hydrolysis of the unchanged parent compounds as well as their aniline-containing metabolites and certain conjugates.

After they have been derivatized, the differently substituted anilines formed from the different phenyl urea herbicides can be identified and determined by gas chromatography. This method describes the acetylation of the anilines. It combines high sensitivity with adequate specificity provided the phenyl ureas present in the analytical material are not hydrolyzed to identical anilines. The compounds measurable by the reported method are given in Table 1.

## **2. Outline of method**

The herbicidal phenyl ureas are alkali hydrolyzed in the presence of the analytical material under reflux conditions. The resultant anilines are simultaneously steam-distilled and extracted from the distillate with isoctane. Following cleanup by column chromatography, the anilines are acetylated with acetic anhydride. The final gas-chromatographic determination can be performed with either an alkali flame ionization detector or an electrolytic conductivity detector.

## **3. Apparatus**

Pyrex round-bottomed flask, 2-l, with NS 29 ground joint

Round-bottomed flasks, 250-ml and 100-ml, with NS 29 ground joint

Bleidner apparatus, modified by W. Heizler, for hydrolysis, distillation and extraction; see Figure

Heating mantles, for 2-l and 250-ml round-bottomed flasks

Chromatographic tube, 1 cm i.d., 20 cm long

Rotary vacuum evaporator, 45°C bath temperature

Table 1. Substituted phenyl urea herbicides and their acetylated hydrolysis products

Parent compound Common name	Chemical name	Structural formula	Hydrolysis product, acetylated			Molar mass	Relative retention time (acetanilide = 1)	Conversion factors
			Empirical formula	Hydrolysis product, acetated	Conversion			
1	2	3	4	5	6	7	8	
Buturon	4-Chloroacetanilide	c1ccc(NC(=O)C(C)C)c(Cl)c1	C <sub>8</sub> H <sub>8</sub> ClNO	169.61	2.0	1.39		
Monolinuron						1.27		0.75
Monuron						1.17		
Chlor-bromuron	4-Bromo-3-chloro-acetanilide	Brc1ccc(NC(=O)C(C)C)c(Cl)c1	C <sub>8</sub> H <sub>7</sub> BrClNO	248.51	3.8	1.18	0.83	
Chloroxuron	4-(4'-Chlorophenoxy)-acetanilide	c1ccc(Oc2ccc(NC(=O)C(C)C)c(Cl)c2)cc1	C <sub>14</sub> H <sub>12</sub> Cl <sub>2</sub> NO <sub>2</sub>	261.71	5.71	1.11	0.84	
Chlorotoluron	3-Chloro-4-methyl-acetanilide	c1ccc(NC(=O)C(C)C)c(Cl)c1	C <sub>9</sub> H <sub>10</sub> ClNO	183.64	2.37	1.16	0.77	
Diuron	3,4-Dichloro-acetanilide	c1ccc(NC(=O)C(C)C)c(Cl)c1	C <sub>8</sub> H <sub>7</sub> Cl <sub>2</sub> NO	204.06	3.27	1.14		
Linuron						1.22		0.79
Neburon						1.35		
Fenuron	Acetanilide	c1ccccc1N	C <sub>8</sub> H <sub>9</sub> NO	135.17	1.0	1.21		
Siduron						1.72		0.69
Fluometuron	3-(Trifluoromethyl)-acetanilide	c1ccc(NC(=O)C(C(F)(F)F)C)c1	C <sub>9</sub> H <sub>8</sub> F <sub>3</sub> NO	203.17	1.1	1.14	0.79	
Metobromuron	4-Bromoacetanilide	Brc1ccc(NC(=O)C(C)C)c1	C <sub>8</sub> H <sub>8</sub> BrNO	214.07	2.65	1.21	0.80	

Conversion factors I (Column 7): Conversion of acetanilides to the phenyl ureas  
 Conversion factors II (Column 8): Conversion of acetanilides to the anilines

Gas chromatograph equipped with alkali flame ionization detector or electrolytic conductivity detector

Microsyringe, 10- $\mu$ l

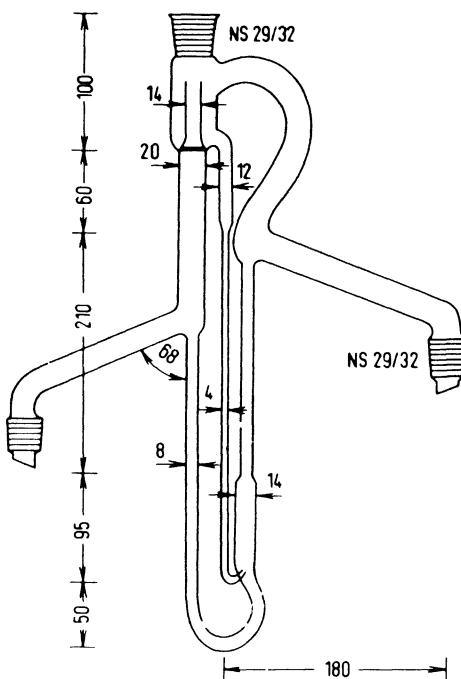


Figure. Bleidner apparatus, modified by W. Heizler.

#### 4. Reagents

Ethanol, distilled in glass

Dichloromethane, distilled in glass

n-Hexane, distilled in glass

Isooctane, pure

n-Hexane + ethanol mixture 1:1 v/v

Eluting mixture 1: n-hexane + dichloromethane 7:1 v/v

Eluting mixture 2: n-hexane + dichloromethane 1:4 v/v

Standard solutions: 10  $\mu$ g/ml each of substituted acetanilides listed in Table 1, Column 2, and 10  $\mu$ g/ml acetanilide in hexane-ethanol mixture

Acetic anhydride, p.a.

Sodium hydroxide solution, 40 g/100 ml NaOH p.a.

Aluminium oxide Woelm, basic, W 200, activity grade III: to 100 g aluminium oxide add 7 g water

Antifoam liquid, e.g. NOPCO NXZ (Nopco or Serva)

Compressed air, re-purified

Helium

Hydrogen, re-purified

Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff and p. 21 f. Two equal portions are used for a duplicate determination.

## 6. Procedure

### 6.1. Hydrolysis, distillation and extraction

Weigh 50 g of the comminuted analytical sample into a 2-l Pyrex round-bottomed flask. Then add 300 ml distilled water, 200 ml sodium hydroxide solution, 10 ml NOPCO Antifoam and a few boiling chips. Place the flask in a heating mantle, half-fill the U-tube of the Bleidner apparatus with water and isoctane, and connect the flask to the lower arm of the Bleidner apparatus. Attach a 250-ml round-bottomed flask filled with 100 ml isoctane to the upper arm of the Bleidner apparatus. Heat both flasks for 14 h at a temperature that will ensure condensation of equal amounts of water and isoctane (this can be checked easily from the volumes of the immiscible solvent phases in the capillary of the Bleidner apparatus). On completion of the distillation-extraction step, let the isoctane cool to room temperature.

### 6.2. Cleanup on an aluminium oxide column

Fill the chromatographic tube with isoctane, and slowly add aluminium oxide, activity grade III, to a level of approx. 12–14 cm (10 ml). Drain the supernatant isoctane to the level of the adsorbent. Next add the whole of the isoctane phase from the Bleidner extraction to the column. Wash the column with 40 ml of eluting mixture 1. Then elute the anilines with 40 ml of eluting mixture 2 into a 100-ml round-bottomed flask.

### 6.3. Acetylation

To the eluate collected from the aluminium oxide column add 10 ml acetic anhydride. Attach the flask to the rotary evaporator, and rotate without vacuum for 10 min in a water bath heated to 45°C. Next evaporate the eluate to dryness under vacuum. Rinse the flask walls with 5 ml of deionized water, and rotary-evaporate to dryness at a water bath temperature of 45°C.

### 6.4. Gas-chromatographic determination

Dissolve the residue in 1 ml of hexane-ethanol mixture. Inject an aliquot of the solution into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph

Varian Aerograph 1700

Column

Glass, 2 mm i. d., 1 m long; packed with 3% Carbowax  
20 M on Gas Chrom Q, 80–100 mesh

Column temperature	Programmed to rise from 150°C to 260°C at a rate of 8°C/min
Injection port temperature	240°C
Detectors:	a) Electrolytic conductivity detector
Carrier gas flow rate	Helium, 30 ml/min
Oven temperature	740°C
Catalyst	Granulated nickel
Absorber for acidic gases	Strontium hydroxide
Purge gas flow rate	Helium, 20 ml/min
Reactant gas flow rate	Hydrogen, 50 ml/min
Transfer line temperature	235°C
Voltage	30 V
Attenuation	4
Sensitivity	4 mV full-scale deflection
Gas flow rates	b) Alkali flame ionization detector (AFID) Nitrogen carrier, 30 ml/min Hydrogen, 35 ml/min Air, approx. 230 ml/min
Temperature	240°C
Sensitivity	$16 \cdot 10^{-12}$ A full-scale deflection
Recorder	1 mV; chart speed 1 cm/min
Injection volume	1–5 µl for electrolytic conductivity detector 0.1–1 µl for AFID
Retention time for acetanilide	6 min 48 s on electrolytic conductivity detector 2 min 27 s on AFID

The retention times of the substituted acetanilides, relative to the retention time of the unsubstituted acetanilide, are given in Table 1.

Presented on p. 257–262 are several typical gas chromatograms recorded for analytical materials fortified with different urea herbicides following hydrolysis to the associated anilines and derivatization to the corresponding acetanilides.

## 7. Evaluation

### 7.1. Method

Quantification is performed by reference to calibration curves prepared as follows. Inject different volumes of the standard solutions of the corresponding acetanilide (see Section 4) into the gas chromatograph. Using log-log paper, plot the peak heights or the peak areas vs. the injected amounts of acetanilide.

### 7.2. Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified at levels of 0.1 to 10 mg/kg ranged from 80 to 120%. The routine limit of determination varied somewhat for the different urea herbicides. It generally ranged from 0.02 to 0.05 mg/kg according to the type of detector used (see Table 2).

Table 2. Examples of recovery rates and routine limit of determination (RLD) for different analytical materials

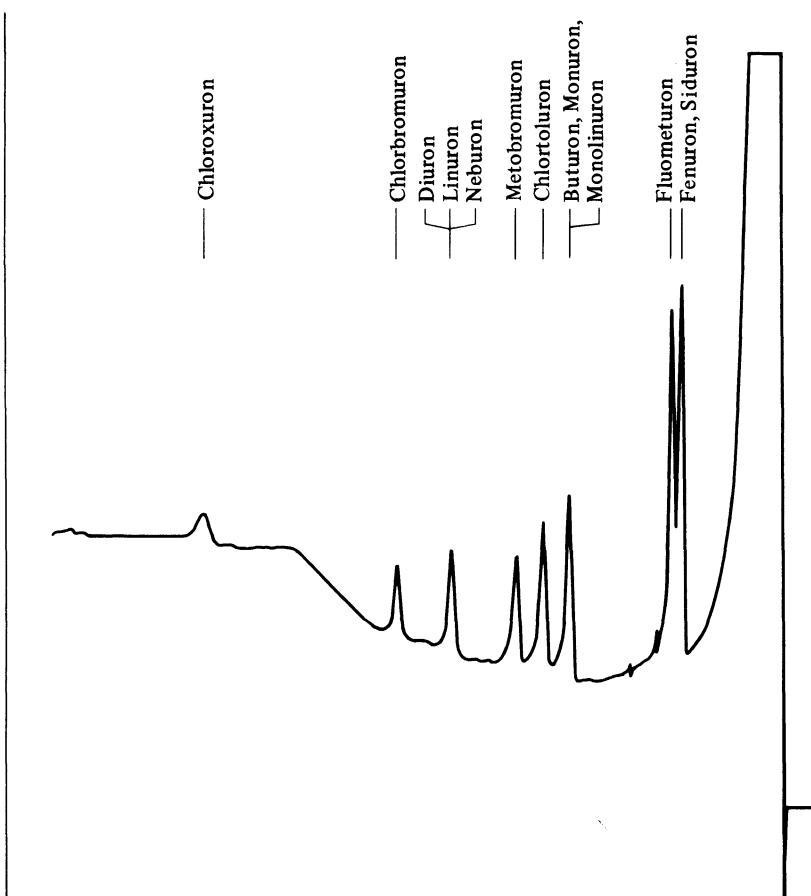
Compound	Added in mg/kg	Recovery %	Analytical material	RLD in mg/kg
Chlorbromuron	5.0	94	Soil	0.025
Metobromuron	0.2	118	Tobacco	0.025
	0.5	80	Tobacco	0.025
	0.2	100	Soil	0.04
	0.5	104	Soil	0.04
Chlorotoluron	0.4	97	Poppy seeds	0.05
	0.1	100	Wheat grains	0.03
	0.1	80	Wheat grains	0.03
	0.1	94	Straw	0.03
	0.5	92	Straw	0.03
	0.5	90	Soil	0.03
	1.0	87	Soil	0.03
	2.0	104	Soil	0.03
	5.0	92	Soil	0.03
	10.0	106	Soil	0.03
	0.5	110	Oats	0.05

### 7.3. Calculation of residues

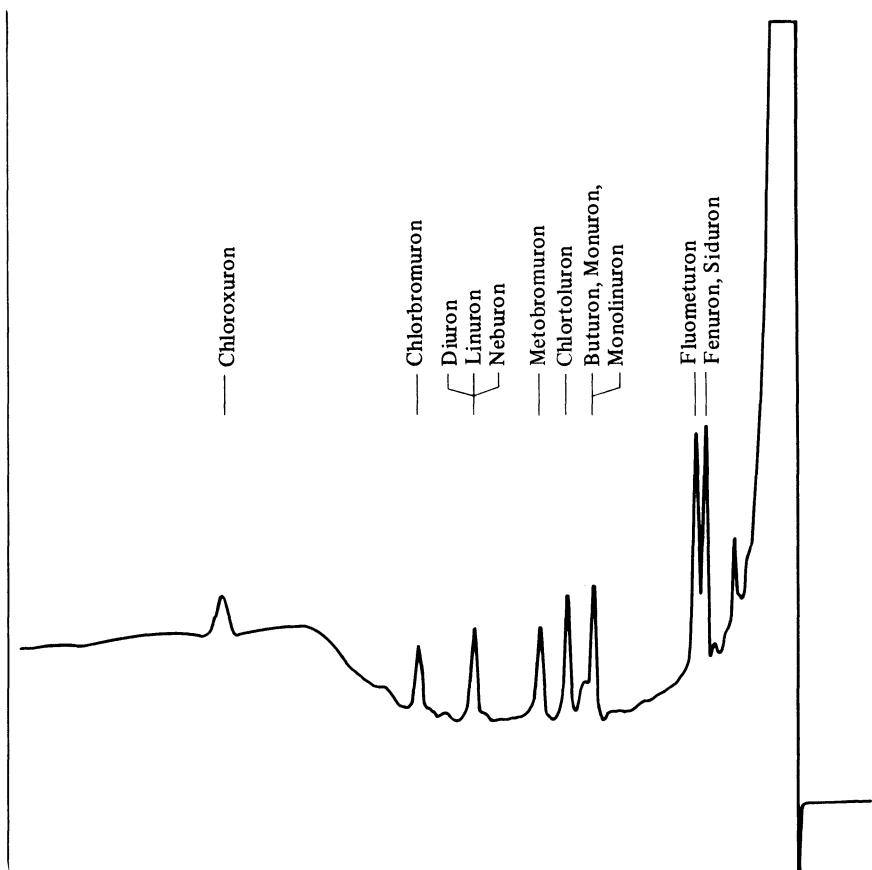
The residues can be expressed in mg/kg of the unchanged parent compound or in mg/kg of the respective aniline.

To express the residues in mg/kg of a parent compound, the found amount of acetanilide is multiplied by a conversion factor calculated from the molar mass of the unchanged parent compound divided by the molar mass of the respective acetanilide. Such conversion factors are given in Table 1, Column 7. If it is assumed that several urea herbicides of the same aniline structure are present (e.g. monuron together with monolinuron or diuron together with linuron and/or neburon), this mode of calculation and the direct hydrolysis procedure cannot be employed. In such cases, the herbicides must be separated from each other by thin-layer chromatography prior to hydrolysis. See also Important points in Section 8 below.

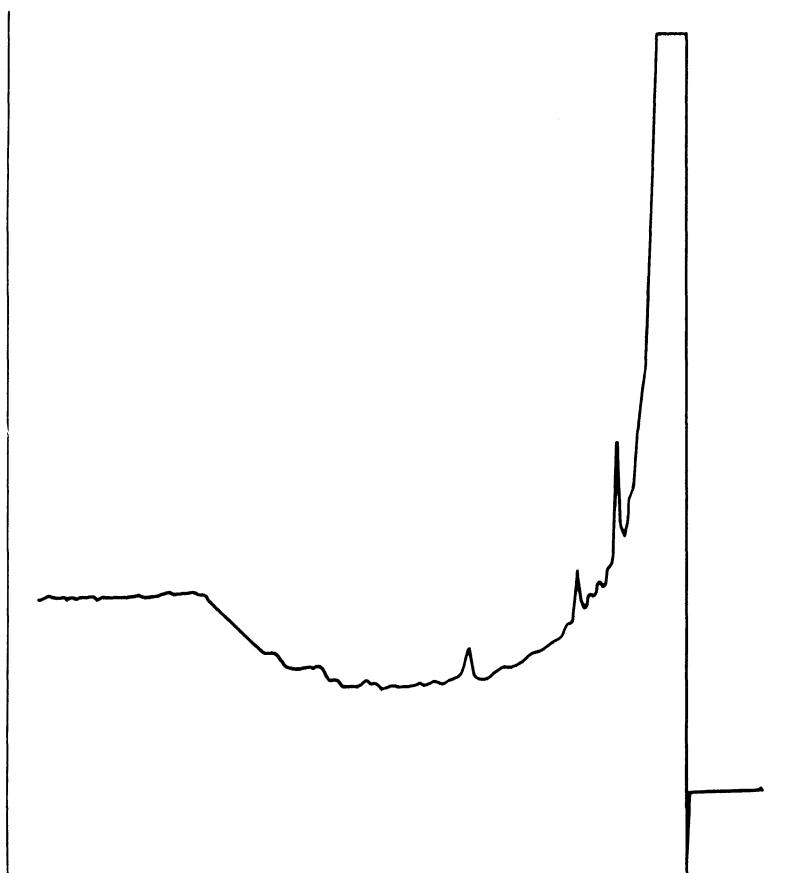
When foods are tested for conformity with the Federal German Regulation on Maximum Pesticide Residue Limits, the residues must be related to the respective aniline. Multiply the found amount of the acetanilide by a conversion factor given by the molar mass of the aniline divided by the molar mass of the acetanilide. Such conversion factors are presented in Table 1, Column 8.



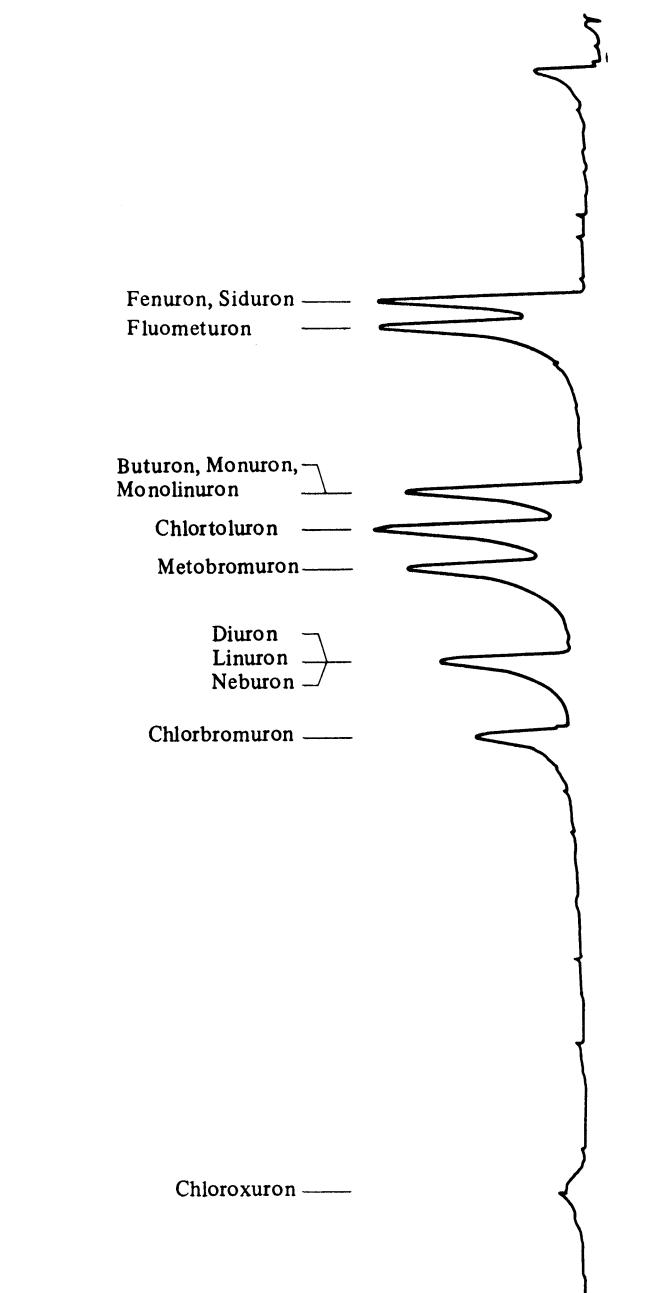
Gas chromatogram 1. Standard mixture of 5 ng each of 8 different acetanilides, using an alkali flame ionization detector. Sensitivity:  $16 \cdot 10^{-12}$  Ampère full-scale deflection.



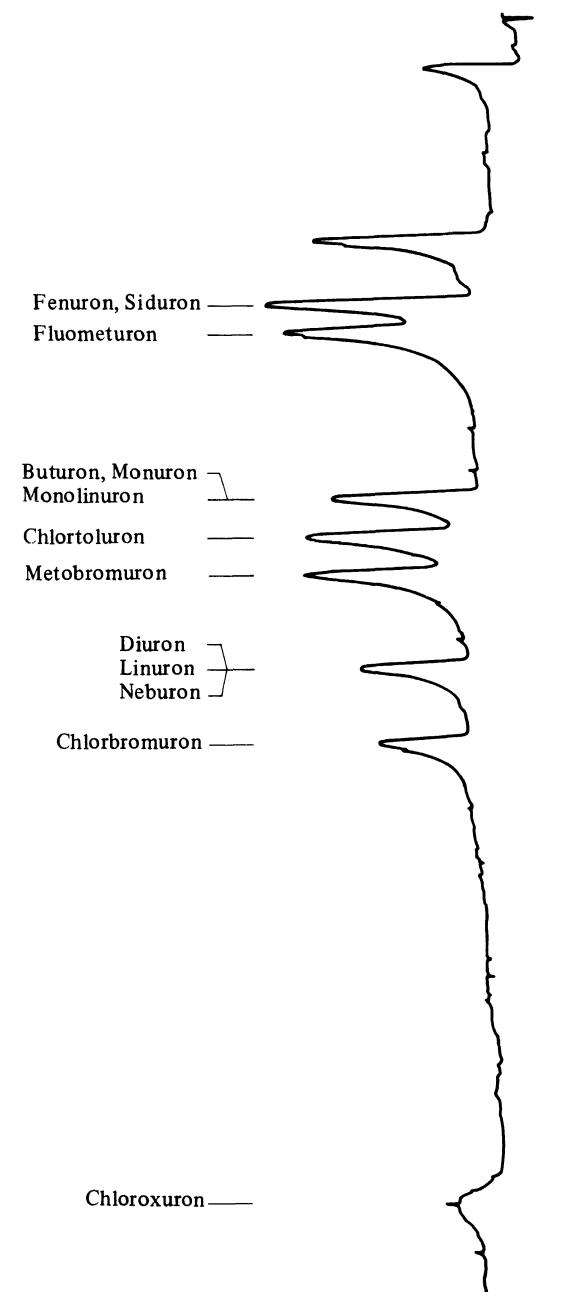
Gas chromatogram 2. Cereal sample (50-mg aliquot of barley) fortified with 0.5 mg/kg each of different urea herbicides, using an alkali flame ionization detector. Sensitivity:  $16 \cdot 10^{-12}$  Ampère full-scale deflection.



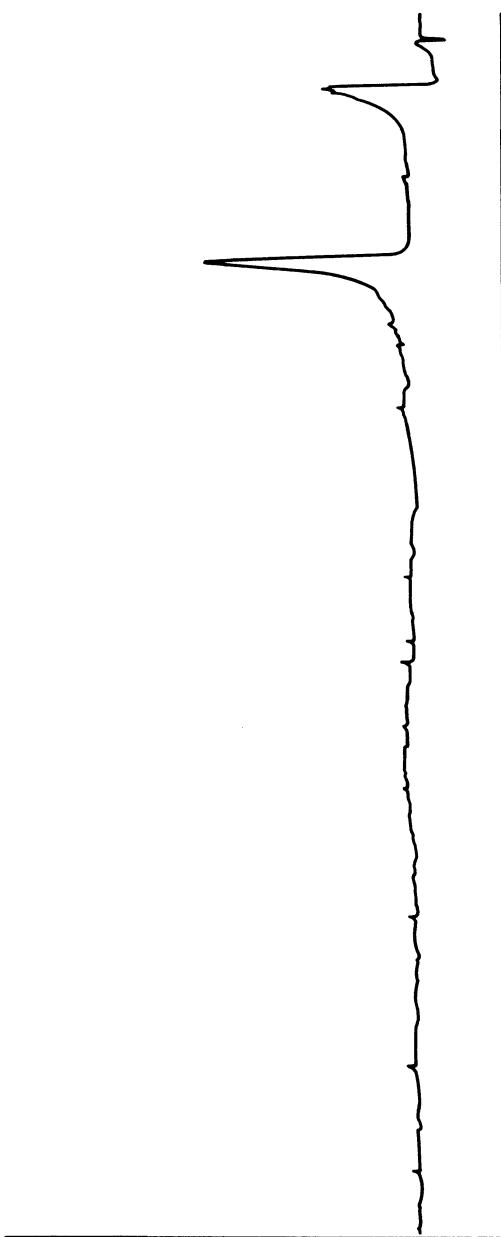
Gas chromatogram 3. Cereal blank sample (50-mg aliquot of barley), using an alkali flame ionization detector. Sensitivity:  $16 \cdot 10^{-12}$  Ampère full-scale deflection.



Gas chromatogram 4. Standard mixture of 50 ng each of 8 different acetanilides, using an electrolytic conductivity detector. Sensitivity: 4 mV full-scale deflection.



Gas chromatogram 5. Soil sample (250-mg aliquot) fortified with 0.25 mg/kg each of different urea herbicides, using an electrolytic conductivity detector. Sensitivity: 4 mV full-scale deflection.



Gas chromatogram 6. Soil blank sample (250-mg aliquot), using an electrolytic conductivity detector. Sensitivity: 4 mV full-scale deflection.

## 8. Important points

Besides those cases mentioned in 7.3, other instances in which it is not possible to employ the procedure of direct hydrolysis arise where it is suspected that samples contain phenyl carbamates, anilides or other compounds (e.g. chloridazon) which, upon hydrolysis, will yield the same anilines as phenyl ureas, e.g. propham or chloridazon together with fenuron or siduron, also monalide together with buturon, monolinuron or monuron. A source of error may be created also by the presence of differently substituted phenyl carbamates if their acetylated hydrolysis products interfere with the gas-chromatographic determination of the acetylated derivatives from the phenyl ureas.

Also in these perhaps exceptional instances, the herbicides must be extracted and separated from each other or from interfering compounds by thin-layer chromatography, prior to hydrolysis. Otherwise, the reported method can be used also for the determination of herbicidal phenyl carbamates and anilides.

## 9. Reference

*W. E. Bleidner, H. M. Baker, M. Levitsky and W. K. Lowen, Determination of 3-(p-chlorophenyl)-1,1-dimethylurea in soils and plant tissue. J. Agric. Food Chem. 2, 476–479 (1954).*

## 10. Author

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Alfalfa, apples, apricots, asparagus, brassicas (curly kale, head cabbage and red cabbage), Brussels sprouts, carrots, celeriac, cereal grains, citrus fruit, cocoa, coffee, cotton (leaves and seed), currants, grapes, grass, leeks, lettuce, maize (kernels, leaves and stalks), onions, peaches, pears, peas, pineapples, plums, potatoes, radishes (large and small types), raspberries, sorghum, soybeans, spinach, straw, strawberries, sugar beet, sugarcane, sunflowers

Gas-chromatographic determination

Soil

---

(German version published 1974)

## 1. Introduction

The method described below for the determination of residues of triazine herbicides is based on gas-chromatographic determination of the unchanged parent compounds, using different element-specific detectors. This method combines high sensitivity and good selectivity. The compounds measurable by the reported method, together with details of their properties, are given in Table 1.

## 2. Outline of method

Following extraction and cleanup by procedures tailored to the materials to be analyzed, the samples are examined for the presence of triazine herbicides by gas chromatography using a nitrogen-specific detector. Quantitation is performed versus a number of external standards, using a Cl-, N- or S-specific detector according to the type of triazine involved (chloro-, methoxy- or methylthiotriazine).

## 3. Apparatus

Beater-cross mill

High-speed blender

Mixer for soil (e.g. dough kneader)

Sample divider

Wide neck bottle, 500-ml, with glass stopper

Homogenizer, e.g. Ultra-Turrax T 45 (Janke & Kunkel)

Laboratory mechanical shaker

Buchner porcelain funnel, 9 cm dia.

Round filter paper, 9 cm dia.

Filtration flask, 1-l

Table 1. Triazine herbicides and their properties

Common name and code number	Empirical formula	Molar mass	Melting point (°C)	Vapour pressure (mbar at 20°C)	Solubility at 25°C (w/v)	pK value
Ametryn G 34162	C <sub>9</sub> H <sub>17</sub> N <sub>5</sub> S	227.3	84–86	1.1 · 10 <sup>-6</sup>	water xylene water	185 ppm 30%
Atraton G 32293	C <sub>9</sub> H <sub>17</sub> N <sub>5</sub> O	211.3	94–96	3.9 · 10 <sup>-6</sup>		1650 ppm
Atrazine G 30027	C <sub>8</sub> H <sub>14</sub> ClN <sub>5</sub>	215.7	173–175	4.0 · 10 <sup>-7</sup>	water chloroform ethyl acetate n-pentane	33 ppm 5.2% 2.8% 360 ppm
Desmetryn G 34360	C <sub>8</sub> H <sub>15</sub> N <sub>5</sub> S	213.3	84–86	1.3 · 10 <sup>-6</sup>	water	580 ppm
Isobumeton (Secbumeton) GS 14254	C <sub>10</sub> H <sub>19</sub> N <sub>5</sub> O	225.3	86–88		water isopropanol dioxane xylene	620 ppm 30–50% > 50% 30–50%
Methoprotryne G 36393	C <sub>11</sub> H <sub>21</sub> N <sub>5</sub> OS	271.4	68–70	3.8 · 10 <sup>-7</sup>	water acetone benzene xylene	320 ppm 20% 23% 14%
Prometon G 31435	C <sub>10</sub> H <sub>19</sub> N <sub>5</sub> O	225.3	91–92	3.1 · 10 <sup>-6</sup>	water acetone benzene xylene	750 ppm > 50 % > 25 % 14%
Prometryn G 34161	C <sub>10</sub> H <sub>19</sub> N <sub>5</sub> S	241.4	118–120	1.3 · 10 <sup>-6</sup>	water trichloroethylene DMF xylene	48 ppm 25% 50% 15%
Propazine G 30028	C <sub>9</sub> H <sub>16</sub> ClN <sub>5</sub>	229.7	212–214	3.9 · 10 <sup>-8</sup>	water	8.6 ppm
Simazine G 27692	C <sub>7</sub> H <sub>12</sub> ClN <sub>5</sub>	201.7	225–227	8.1 · 10 <sup>-9</sup>	water chloroform methanol n-pentane ethyl acetate	5.0 ppm 900 ppm 400 ppm 3 ppm 1200 ppm
Terbutylazine GS 13529	C <sub>9</sub> H <sub>16</sub> ClN <sub>5</sub>	229.7	177–179	1.5 · 10 <sup>-6</sup>	water ethyl acetate isopropanol xylene	8.5 ppm 4% 1% 1%
Terbutryn GS 14260	C <sub>10</sub> H <sub>19</sub> N <sub>5</sub> S	241.3	104–105	1.3 · 10 <sup>-6</sup>	water isopropanol xylene	58 ppm 25% 10%

Graduated cylinder, 500-ml  
Hot extractor as illustrated in Figure on p. 154, or equivalent  
Separatory funnels, 1-l and 500-ml  
Rotary vacuum evaporator, 40°C bath temperature  
Chromatographic tube, 18 mm i. d., at least 20 cm long  
Gas chromatographs each equipped with a nitrogen-, chlorine- and sulphur-specific detector  
Microsyringes, 10- $\mu$ l and 50- $\mu$ l

## 4. Reagents

Benzene, redistilled  
Chloroform, redistilled  
Diethyl ether, redistilled  
Ethanol, redistilled  
n-Hexane, redistilled  
Methanol, redistilled  
n-Hexane + ethanol mixture 1:1 v/v  
Eluting mixture: n-hexane + diethyl ether 2:1 v/v  
Standard solutions: 10  $\mu$ g/ml each of compounds listed in Table 1, in hexane-ethanol mixture  
Sodium chloride solution, saturated  
Hydrochloric acid, 1 mol/l  
Sodium hydroxide solution, 1 mol/l  
Aluminium oxide Woelm, basic, W 200, activity grade V: to 100 g aluminium oxide add 19 g water  
Universal indicator paper  
Air, re-purified  
Helium  
Hydrogen, re-purified  
Nitrogen, re-purified  
Oxygen, re-purified

## 5. Sampling and sample preparation

The portions of the laboratory sample taken for analysis are prepared as follows. Vegetables, fruit, leaves, grass, alfalfa, potatoes and straw are cut into small pieces, mixed and deep-frozen until required for analysis. The laboratory sample of grain crops is reduced to the analytical sample by using a sample divider. Large stones are removed from soil samples which are then homogenized in a mixer and deep-frozen. Very stony soil samples are dried, ground in a beater-cross mill and then likewise deep-frozen. An aliquot of the non-dried soil samples is used to measure the water content.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Grain crops

Weigh 100 g of the sample into a wide-neck bottle and add 400 ml methanol. Insert the homogenizer in the bottle, and switch on for 2 to 5 min until the grains are ground and blended with the methanol. Next stopper the bottle and shake for 2 h on a mechanical shaker. Filter off a 200-ml aliquot (equivalent to 50 g sample material).

#### 6.1.2. Fruit, vegetables, leaves, grass, alfalfa, potatoes

Weigh 100 g of the sample into a wide-neck bottle and add 400 ml methanol. Using the homogenizer, comminute and blend for 2 to 5 min. Then stopper the bottle and shake for 2 h on a mechanical shaker. Filter with suction through a Buchner funnel to remove solid constituents. Wash the residue with methanol. Measure the total volume of the filtrate, and retain half of it (equivalent to 50 g sample material) for further processing.

#### 6.1.3. Straw

Weigh 50 g of the chopped sample into a wide-neck bottle, and add 400 ml methanol. Using the homogenizer, comminute and blend for 2 to 5 min. Then stopper the bottle and shake for 2 h on a mechanical shaker. Filter with suction through a Buchner funnel to remove solid constituents, and wash the filter cake twice with methanol. Collect and retain the whole filtrate for further processing.

#### 6.1.4. Soil

Weigh 50 g of the prepared sample into an extraction thimble, and extract with 100 ml methanol for 2 h on the hot extractor.

## 6.2. Cleanup

The two cleanup steps 6.2.1 and 6.2.3 usually are sufficient. For cleanup of sample materials which have a particularly high content of interfering substances, like alfalfa or straw, employ step 6.2.2 instead of step 6.2.1.

#### 6.2.1. Re-extraction with chloroform

To the methanolic extract derived from 6.1 add an equal volume of water and 20 ml sodium chloride solution. Shake two times with portions of chloroform each equivalent to 20% of the total volume. Drain the combined chloroform extracts through a cottonwool plug, and evaporate to dryness on a rotary evaporator at 40°C water bath temperature.

### **6.2.2. Extraction with n-hexane (necessary only for extracts that are difficult to clean up; see 6.2)**

To the methanolic extract derived from 6.1 add an equal volume of water and 20 ml sodium chloride solution. Adjust to pH 3–4 with hydrochloric acid (indicator paper). Then extract three times with portions of n-hexane each equivalent to one-third of the total volume. Collect the hexane phases; they contain the chlorotriazines. Adjust the aqueous-methanolic phase with sodium hydroxide solution to pH 7–8 (indicator paper). Extract two times with portions of chloroform each equivalent to 20% of the total volume. Drain the combined chloroform phases which contain the methoxy- and methylthiotriazines, through a cottonwool plug, and evaporate to dryness on a rotary evaporator at 40°C water bath temperature. Carry the hexane phases through the same procedure.

### **6.2.3. Column chromatography on aluminium oxide**

Fill the chromatographic tube with n-hexane, and slowly add aluminium oxide to a level of 7 cm. Then drain the hexane to the level of the aluminium oxide.

Dissolve the residue derived from 6.2.1 or 6.2.2 in 5 ml benzene. Add the solution to the column and allow to trickle in. Wash the flask two times with 5-ml portions of n-hexane. Add these washings successively to the column and allow to percolate. Then rinse the column with 80 ml n-hexane, and discard this forerun. Next elute with 75 ml eluting mixture, collect the eluate, and evaporate on a rotary evaporator at 40°C water bath temperature.

## **6.3. Gas-chromatographic determination**

Dissolve the residue derived from 6.2.3 in 1 ml hexane-ethanol mixture. Inject an aliquot of this solution into the gas chromatograph.

Firstly, a trial gas chromatogram is run using a nitrogen-specific Coulson electrolytic conductivity detector. The column used separates all the listed triazine herbicides from one another apart from the two pairs terbutylazine/secbumeton and simazine/terbutryn. If peaks occur at those positions where, according to the standard chromatogram, terbutylazine, secbumeton, terbutryn and simazine are to be expected, the analytical solution is injected into a gas chromatograph equipped with a Dohrmann microcoulometric detector using a chloride-specific titration cell and/or a flame photometric detector fitted with an S-filter. If peaks occur at the same positions on this chromatogram, they are evaluated quantitatively. The content of secbumeton is then calculated by subtraction. If no peaks occur at the above-mentioned positions in the nitrogen-specific or in the sulphur- and chlorine-specific gas chromatogram, the quantitative evaluation for the 8 other possible triazines can be performed directly on the nitrogen-specific gas chromatogram.

### *Operating conditions*

#### **6.3.1. Coulson electrolytic conductivity detector**

Gas chromatograph	Varian Aerograph 1700
Column	Glass, 2 mm i. d., 2 m long; packed with 3% Carbowax 20 M on Chromosorb G-AW-DMCS, 80–100 mesh

Column temperature	220 °C isothermal for 16 min, then programmed to rise to 240 °C at the rate of 15 °C/min
Injection port temperature	240 °C
Carrier gas flow rate	Helium, 30 ml/min
Detector	Coulson electrolytic conductivity detector (nitrogen-specific)
Catalyst	Nickel granules
Absorber for acidic gases	Strontium hydroxide
Purge gas flow rate	Helium, 20 ml/min
Reactant gas flow rate	Hydrogen, 50 ml/min
Transfer line temperature	235 °C
Block temperature	235 °C
Furnace temperature	800 °C
Voltage	30 V
Attenuation	4
Recorder	1 mV; chart speed 1 cm/min
Injection volume	max. 10 µl
Retention times for	
prometon	5 min 36 s
atraton	6 min 48 s
propazine	7 min 18 s
terbutylazine	7 min 54 s
secbumeton	8 min 6 s
atrazine	9 min 36 s
prometryn	10 min 48 s
terbutryn	12 min
simazine	12 min 24 s
ametryn	13 min 24 s
desmetryn	14 min 48 s
methoprotryne	28 min 24 s

### 6.3.2. Dohrmann microcoulometric detector

Gas chromatograph	Varian Aerograph 1400
Column	Glass, 3 mm i.d., 1 m long; packed with 3% Carbowax 20 M on Chromosorb G-AW-DMCS, 80–100 mesh
Column temperature	200 °C isothermal for 8 min, then programmed to rise to 240 °C at the rate of 8 °C/min
Injection port temperature	240 °C
Carrier gas flow rate	Nitrogen, 60 ml/min
Detector	Dohrmann microcoulometric detector GTS 20
Cell	Chloride titration cell T-300-S
Bias voltage	240 mV
Reactant gas flow rate	Oxygen, 60 ml/min
Transfer line temperature	240 °C
Furnace temperature, centre	850 °C
Furnace temperature, outlet	800 °C
Gain	600

Range	50 ohms
Recorder	1 mV; chart speed 1 cm/min
Injection volume	max. 20 µl
Retention times for	
propazine	10 min
terbutylazine	10 min 42 s
atrazine	11 min 36 s
simazine	13 min 12 s

### 6.3.3. Flame photometric detector

Gas chromatograph	Varian Aerograph 1700
Column	Glass, 2 mm i.d., 1 m long; packed with 3% Carbowax 20 M on Chromosorb G-AW-DMCS, 80–100 mesh
Column temperature	200°C isothermal for 13 min, then programmed to rise to 240°C at the rate of 10°C/min
Injection port temperature	240°C
Detector	Melpar flame photometric detector equipped with 394-nm filter (specific for sulphur) Temperature 200°C
Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 80 ml/min Oxygen, 30 ml/min
Attenuation	$512 \cdot 10^{-11}$ A full-scale deflection
Recorder	1 mV; chart speed 1 cm/min
Injection volume	max. 10 µl
Retention times for	
prometryn	8 min 54 s
terbutryne	9 min 54 s
ametryn	11 min 36 s
desmetryn	12 min 30 s
methoprottryne	20 min 12 s

Several typical gas chromatograms recorded for fortified analytical materials are presented on pages 276–281.

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring and comparing the peak height of the sample solution with peak heights measured for standard solutions of known concentration. Equal volumes of sample solution and standard solutions should be injected, and the peaks of the solutions should display comparable heights.

## 7.2. Recoveries

Recoveries from untreated control samples fortified at levels of between 0.05 and 1 mg/kg ranged from 80 to 120%. Table 2 lists the analytical materials on which the method has been tried, together with details of the success of the tryout and the concentration range.

## 7.3. Calculation of residues

From the standard injections, a calibration curve is constructed on a log-log scale either graphically or by using a computer. The ng amounts equivalent to the peak heights of the samples are read from the calibration curve. The mg/kg content of the compound is computed from the injected sample aliquot. Results for soil samples are corrected for the water content, i.e. the content of the compound is related to dry soil.

## 8. Important points

Instead of the flame photometric detector, the microcoulometric detector fitted with the sulphur-specific cell can be used equally well. In this case, the conditions are the same as those given above apart from the bias voltage (150 mV with a sulphur-specific cell) and temperature programming.

In many cases, it is possible to use an  $\text{Rb}_2\text{SO}_4$  alkali flame ionization detector instead of the nitrogen-specific Coulson electrolytic conductivity detector. The operating conditions for alkali flame ionization detection are as follows:

Gas chromatograph	Varian Aerograph 1700
Column	Glass, 2 mm i.d., 2 m long; packed with 3% Carbowax 20 M on Chromosorb G-AW-DMCS, 80–100 mesh
Column temperature	200°C isothermal for 16 min, then programmed to rise to 240°C at the rate of 10°C/min
Injection port temperature	240°C
Detector	Alkali flame ionization detector with $\text{Rb}_2\text{SO}_4$ pellet Temperature 250°C
Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 35 ml/min Air, 230 ml/min
Attenuation	$16 \cdot 10^{-12} \text{ A}$ full-scale deflection
Recorder	1 mV; chart speed 1 cm/min
Injection volume	2 $\mu\text{l}$
Retention times for	
prometon	3 min 54 s
atraton	4 min 54 s
propazine	5 min 18 s
secbumeton/terbutylazine	5 min 54 s
atrazine	7 min 6 s
prometryn	8 min 6 s
terbutrym/simazine	9 min 12 s

ametryn	10 min 24 s
desmetryn	11 min 18 s
methoprottryne	21 min 48 s

The principal criterion governing the choice of the gas-chromatographic conditions is the separation of the 12 single components. Due to the different instrument parameters, the GLC parameters in the method are not constant for all detection modes.

## 9. References

- R. Delley, K. Friedrich, B. Karlhuber, G. Székely and K. Stammbach*, The identification and determination of various triazine herbicides in biological materials, *Fresenius Z. Anal. Chem.* 228, 23–38 (1967).
- A. M. Mattson, R. A. Kahrs and R. I. Murphy*, Quantitative determination of triazine herbicides in soils by chemical analysis, *Residue Rev.* 32, 371–390 (1970).
- K. Ramsteiner, W. D. Hörmann and D. O. Eberle*, Multiresidue method for the determination of triazine herbicides in field-grown agricultural crops, water, and soils, *J. Assoc. Off. Anal. Chem.* 57, 192–201 (1974).

## 10. Author

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Table 2. Analytical materials and compounds on which method has been tested at given concentrations (mg/kg)

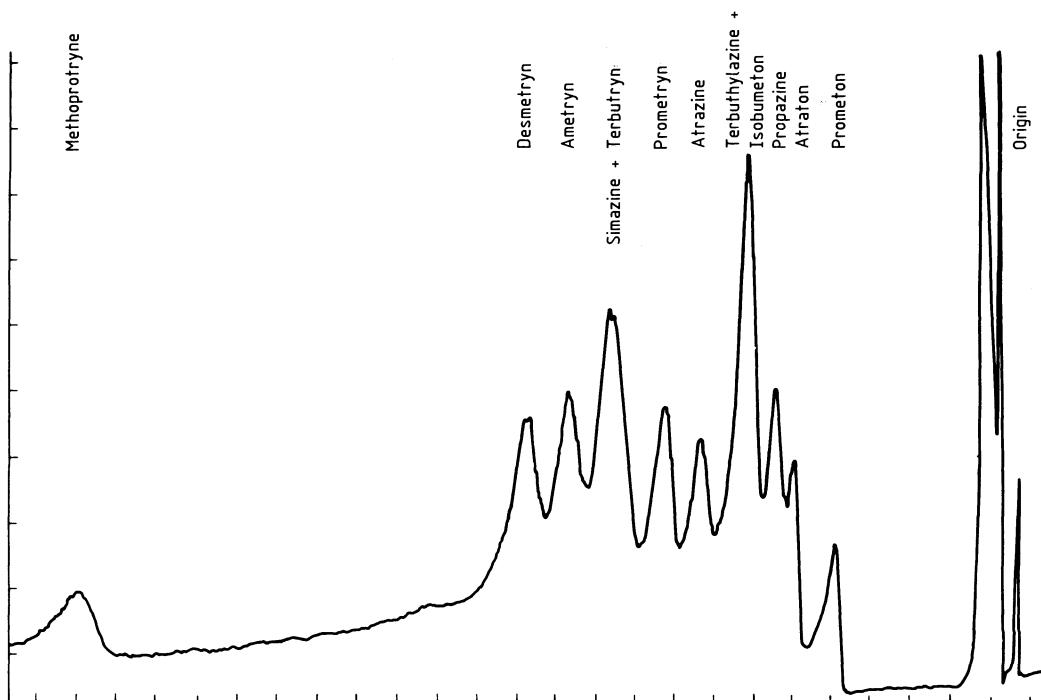
Analytical materials	Ametryn	Araton	Atrazine	Desmetron	Isobutumeton (Secbumeton)	Metho-proryne	Promecton	Propazine	Simazine	Tributyl azine	Tributyrin
Alfalfa	-0.05 + 0.1	+ 0.1 + 0.02	+ 0.1 + 0.02	-0.05 + 0.1	+ 0.02	+ 0.02	+ 0.02	-0.05 + 0.1 + 0.02	+ 0.1 + 0.02	+ 0.02	+ 0.02
Apples/pears											
Apricots											
Asparagus											
Brassicaceae (curly kale, head cabbage and red cabbage)	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.1	+ 0.1 + 0.05	+ 0.05	+ 0.05
Brussels sprouts	+ 0.1	+ 0.05	+ 0.1	+ 0.05	+ 0.1	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Carrots	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Celeriac	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Cereal grains	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02
Citrus fruit	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02
Cocoa/coffee	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Cotton leaves and seed											
Currents											
Grapes	+ 0.05	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02
Grass	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Leeks											
Lettuce											
Maize (kernels, leaves and stalks)	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02
Onions <sup>1</sup>	+ 0.1	+ 0.1	+ 0.1	+ 0.1	+ 0.1	+ 0.1	+ 0.1	+ 0.1 + 0.1	+ 0.1 + 0.1	+ 0.1	+ 0.1
Peaches											
Peas	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02
Pineapples	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Plums	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02
Potatoes	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05	+ 0.05 + 0.05	+ 0.05 + 0.05	+ 0.05	+ 0.05
Radishes											
Raspberries											
Soil	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02	+ 0.02 + 0.02	+ 0.02 + 0.02	+ 0.02	+ 0.02

Sorghum	+ 0.02	+ 0.02	+ 0.02	+ 0.02
Soybeans	+ 0.05	+ 0.05	+ 0.05	+ 0.05
Spinach	+ 0.05	+ 0.05	+ 0.05	+ 0.05
Straw	+ 0.05	+ 0.05	+ 0.05	+ 0.05
Strawberries	+ 0.05	+ 0.05	+ 0.05	+ 0.05
Sugar beet	+ 0.05	+ 0.05	+ 0.05	+ 0.05
Sugarcane	+ 0.05	+ 0.05	+ 0.05	+ 0.05
Sunflowers	+ 0.05	+ 0.05	+ 0.05	+ 0.05

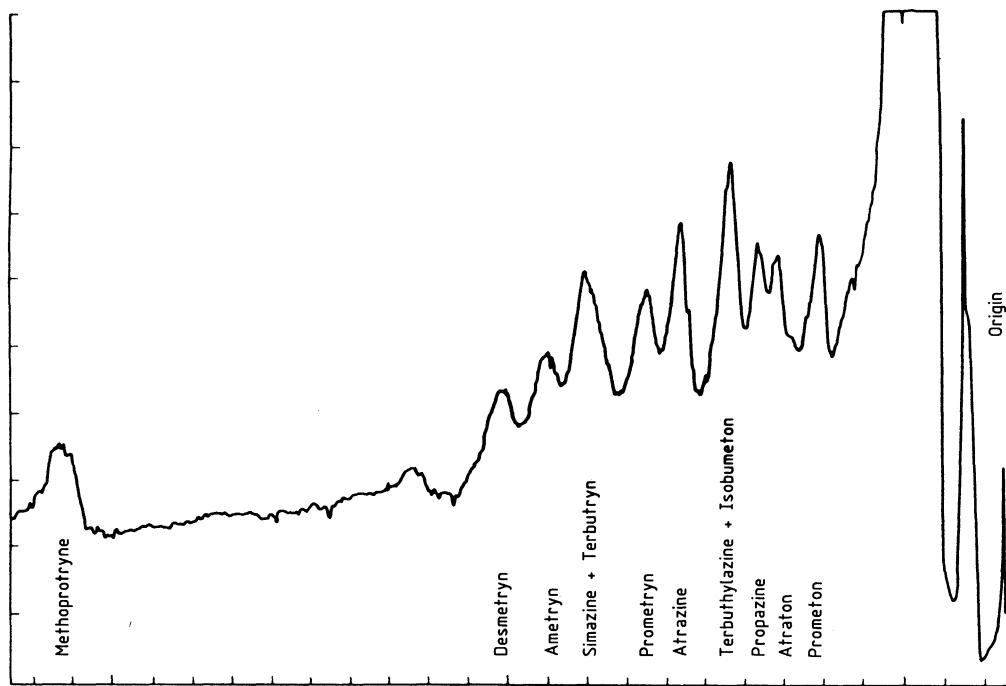
<sup>1</sup> with sulphur-specific detector at 0.1 mg/kg

*Note*

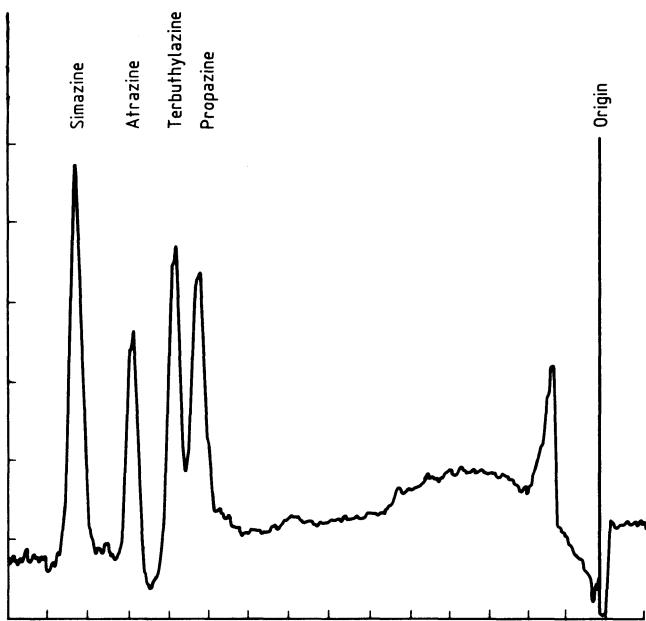
- + Signifies that the compound, at the given concentration (expressed in mg/kg), was recovered at a rate of more than 70%
- Signifies that at the given concentration the compound was not determinable or that the recovery rate was less than 70%
- Where no figures are given for a compound alongside a substrate, this indicates that determination of the compound in the particular analytical material has not yet been tried



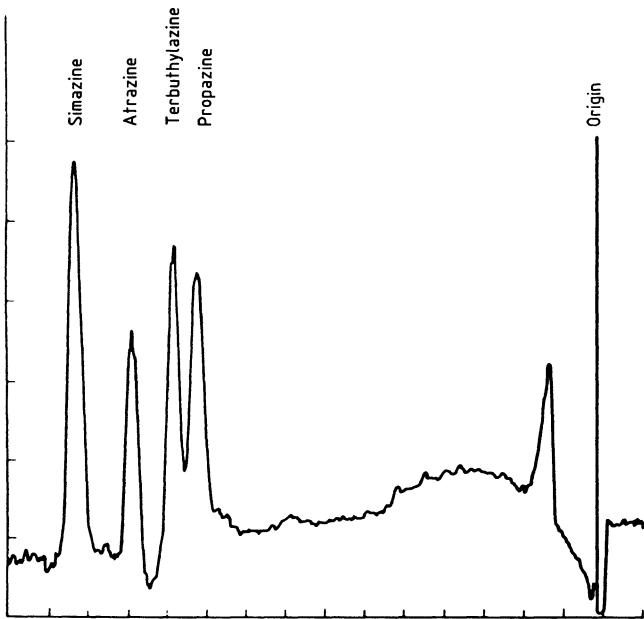
Gas chromatogram 1. Standard mixture of 60 ng each of all twelve triazine herbicides, using Coulson nitrogen-specific electrolytic conductivity detector. Attenuation 8; for other conditions, see text.



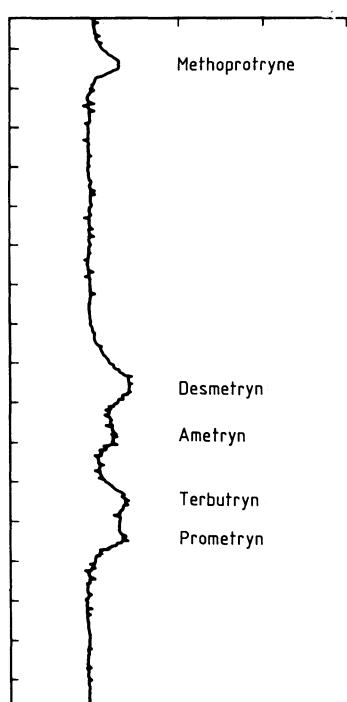
Gas chromatogram 2. Alfalfa sample (500-mg aliquot) fortified with 0.05 mg/kg each of all twelve triazine herbicides, using Coulson electrolytic conductivity detector. Attenuation 4 for 27 min after injection, then 2; for other conditions, see text.



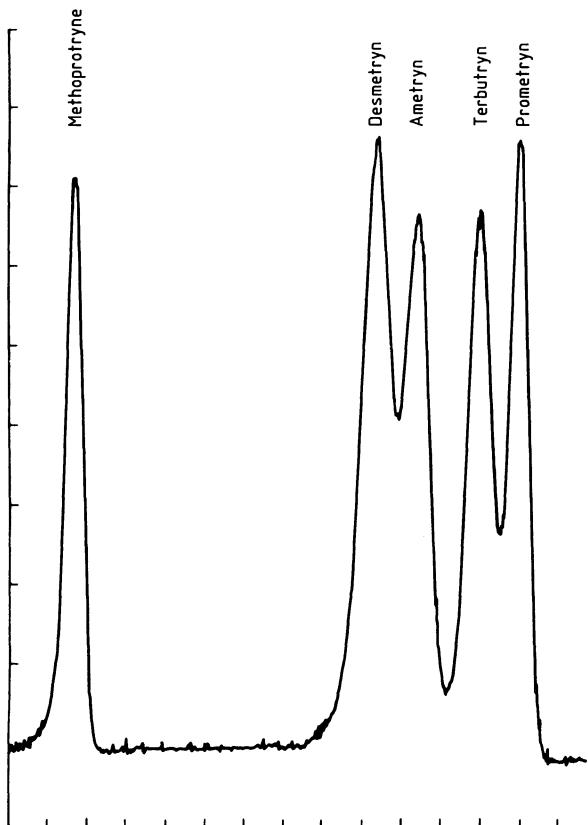
Gas chromatogram 3. Standard mixture of 200 ng each of all twelve triazine herbicides, using Dohrmann microcoulometric detector with Cl cell. For conditions, see text.



Gas chromatogram 4. Soil sample (1-g aliquot) fortified with 0.05 mg/kg each of all twelve triazine herbicides, using Dohrmann microcoulometric detector. For conditions, see text.



Gas chromatogram 5. Maize cob sample (500-mg aliquot) fortified with 0.05 mg/kg each of all twelve triazine herbicides, using flame photometric detector with S-specific filter. For conditions, see text.



Gas chromatogram 6. Maize cob sample (1-g aliquot) fortified with 0.5 mg/kg each of all twelve triazine herbicides, using flame photometric detector with S-specific filter. For conditions, see text.

# **Organohalogen, Organophosphorus and Triazine Compounds**

**S 8**

Apples, beans, carrots, celeriac, cherries, Chinese cabbage, corn salad, cucumbers, dandelion, endives, grapes, head cabbage, kohlrabi, leeks, lettuce, mushrooms, oranges, parsley, peaches, pears, pineapples, plums, radishes (large and small types), red cabbage, spinach, strawberries, sweet peppers, tomatoes, witloof chicory

Gas-chromatographic determination

(German version first published 1974, revised 1982 and 1985)

## **1. Introduction**

Use of the reported method enables plant material to be extracted and cleaned up for gas-chromatographic determination of residues of 91 compounds of the organohalogen, organophosphorus and triazine groups by one and the same procedure. Table 1 lists the compounds that can be determined by the reported method, and also the crops (harvested commodities) on which the method has been tested with the listed compounds.

## **2. Outline of method**

The compounds are extracted from the prepared plant sample with acetone. The extract is filtered. After dilution with water, the compounds are extracted with dichloromethane from an aliquot of the filtrate. The organic phase is dried and rotary-evaporated, and the residue is dissolved in dichloromethane. Next, interfering substances are separated on an activated carbon-silica gel column. The compounds are eluted with a mixture of dichloromethane, toluene and acetone. The eluate is rotary-evaporated, and made up with n-hexane to a given volume. The compound residues in this solution are identified and quantitated by gas chromatography using an electron capture detector and thermionic phosphorus and nitrogen detectors.

## **3. Apparatus**

Homogenizer, e.g. Ultra-Turrax (Janke & Kunkel)

Buchner porcelain funnel, 11 cm dia.

Filter paper, 11 cm dia., e.g. Ederol No. 15 (Binzer)

Fluted filter paper

Beakers, 1-l and 50-ml

Separatory funnel, 1-l, with ground stopper

Round-bottomed flasks, 250-ml and 50-ml, with ground joints

Table 1. Tested combinations of compounds and crops (harvested commodities) for which the recovery from control samples fortified with each of the listed compounds at the given concentration was more than 70%, see also Section 8.

Compound	Concentration mg/kg	Crop (harvested commodity)
Aldrin	0.05	Apples, beans, cherries, grapes, head cabbage, lettuce, peaches, pears, plums, red cabbage, strawberries
	0.004	Cucumbers, witloof chicory
Ametryn	1.0	Apples, carrots, corn salad, cucumbers, dandelion, endives, grapes, head cabbage, kohlrabi, leeks, peaches, pears, peppers, red cabbage, tomatoes, witloof chicory
	0.2	Cucumbers
Atrazine	0.5	Apples, carrots, corn salad, cucumbers, dandelion, endives, grapes, head cabbage, kohlrabi, leeks, lettuce, peaches, pears, peppers, red cabbage, tomatoes, witloof chicory
	0.5	Grapes
Azinphos-ethyl	1.25	Apples, pears, witloof chicory
Azinphos-methyl	0.5	Apples, pears, witloof chicory
Aziprotryne	0.5	Apples, carrots, dandelion, lettuce, radishes, spinach
Bromacil	0.5	Apples, grapes, kohlrabi, pears, peppers, radishes
Bromophos	1.25	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
	0.1	Carrots
Bromophos-ethyl	0.025	Apples, carrots, pears, witloof chicory
	0.2	Apples, pears
Bupirimate	0.05	Lettuce
	0.2	Beans
Captafol	0.1	Apples, pears
	5.0	Apples, cherries, grapes, peaches, pears
Captan	0.5	Apples, pears
	0.2	Apples, beans, carrots, cherries, grapes, head cabbage, lettuce, peaches, pears, plums, red cabbage, strawberries
Carbophenothion	0.25	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
	0.25	Carrots, celeriac, parsley
Chlorfenvinphos	0.25	Cucumbers
	0.1	Carrots
Chlorpyrifos	0.05	Cucumbers
	0.5	Cucumbers, lettuce, radishes, strawberries, tomatoes, witloof chicory
Chlorpyrifos-methyl	0.1	Cucumbers
	0.01	Lettuce
Chlorthal	0.05	Chinese cabbage
Chlorthiophos	0.1	Apples, cucumbers, grapes, peppers, radishes, strawberries
Cyanofenphos	0.04	Apples, cucumbers, endives, lettuce, pears, tomatoes, witloof chicory
	0.01	Cucumbers, head cabbage, peaches
p,p'-DDD	0.025	Mushrooms
		Apples, cherries, pears, peppers, spinach, strawberries

Table 1. (contd.)

Compound	Concentration mg/kg	Crop (harvested commodity)
p,p'-DDE	0.025	Apples, cherries, pears, peppers, spinach, strawberries
p,p'-DDT	0.25	Apples, beans, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
	0.02	Cucumbers, witloof chicory
Desmetryn	0.02	Cucumbers
Diazinon	0.05	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Dichlobenil	0.025	Apples, endives, grapes, pears, radishes
Dichlofenthion	0.25	Cherries, lettuce, peppers, radishes, strawberries, tomatoes
	0.1	Beans
Dichlofluanid	0.1	Apples, beans, carrots, celeriac, cherries, grapes, lettuce, peaches, pears, plums, strawberries
Dichlorvos	0.1	Beans, cucumbers, lettuce
Dicofol	0.5	Apples, cherries, peaches, spinach, strawberries, tomatoes
Dieldrin	0.1	Apples, beans, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
	0.008	Cucumbers, witloof chicory
Dimethachlor	0.5	Apples, cucumbers, grapes, head cabbage, peaches, pears, radishes, red cabbage, tomatoes
Dimethoate	1.0	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, peaches, pears, plums, red cabbage, strawberries
	0.1	Apples, cucumbers, pears
Dioxathion	0.5	Grapes
Disulfoton	0.05	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Ditalimfos	0.5	Apples, corn salad, dandelion, endives, grapes, head cabbage, lettuce, red cabbage, spinach, witloof chicory
$\alpha$ -Endosulfan	0.125	Apples, beans, cherries, grapes, head cabbage, lettuce, pears, plums, red cabbage, strawberries
	0.01	Cucumbers, witloof chicory
Ethion	0.05	Pears, witloof chicory
Ethoprophos	0.2	Lettuce
	0.1	Beans, mushrooms
Etrimfos	0.5	Peaches, plums
	0.2	Cherries, grapes
Fenchlorphos	0.125	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Fenitrothion	0.5	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Fensulfothion	0.1	Apples, pears

Table 1. (contd.)

Compound	Concentration mg/kg	Crop (harvested commodity)
Fenthion	0.125	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Folpet	0.15	Apples, beans, carrots, cherries, grapes, head cabbage, lettuce, peaches, pears, plums, red cabbage, strawberries
Fonofos	0.1	Apples, corn salad, endives, grapes, head cabbage, lettuce, radishes, red cabbage, spinach
Formothion	0.125	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
$\alpha$ -HCH	0.005	Apples, cucumbers, dandelion, kohlrabi, pears, radishes, strawberries
$\beta$ -HCH	0.025	Apples, cucumbers, dandelion, kohlrabi, pears, radishes, strawberries
Heptachlor	0.05	Apples, beans, carrots, cherries, grapes, head cabbage, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
	0.004	Cucumbers, witloof chicory
Heptachlor epoxide	0.025	Apples, pears, radishes, spinach, strawberries
	0.005	Cucumbers
Heptenophos	0.1	Apples, cucumbers, endives, lettuce, pears, tomatoes, witloof chicory
Iprodione	0.5	Cherries, lettuce, peppers, radishes, spinach, strawberries, witloof chicory
Isofenphos	0.1	Apples, kohlrabi, lettuce
Lindane	0.025	Apples, beans, carrots, cherries, grapes, head cabbage, lettuce, peaches, pears, plums, red cabbage, strawberries
Malaoxon	0.25	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Malathion	0.25	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Metalaxyl	2.0	Grapes, oranges
Methidathion	0.2	Cucumbers, peppers
Methoprotryne	0.5	Apples, grapes, kohlrabi, lettuce, peppers, radishes, tomatoes
Methoxychlor	1.0	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Mevinphos	0.5	Lettuce
	0.1	Apples, cucumbers
Naled	2.5	Cucumbers, lettuce, radishes, tomatoes, witloof chicory
	0.1	Cucumbers
Paraoxon	0.2	Cucumbers

Table 1. (contd.)

Compound	Concentration mg/kg	Crop (harvested commodity)
Parathion	0.25	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Parathion-methyl	0.02 0.25	Carrots, corn salad, head cabbage Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Perthane	0.05 15.0	Apples, pears, witloof chicory Apples, corn salad, grapes, kohlrabi, pears, peppers, spinach, witloof chicory
Phenkapton	0.1 0.5	Apples Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, peppers, plums, red cabbage, strawberries
Phorate	0.1 0.05	Cucumbers, lettuce, radishes, strawberries, tomatoes Cucumbers, peppers
Phosalone	0.15 0.05	Apples, pears, strawberries Apples
Pirimiphos-methyl	0.1	Apples, cucumbers, endives, lettuce, pears, tomatoes, witloof chicory
Procymidon	0.2	Grapes, strawberries
Profenofos	0.2	Cherries, lettuce, strawberries
Prometryn	0.1	Apples, cucumbers, dandelion, endives, pears, radishes, tomatoes
Propazine	0.5	Apples, cucumbers, dandelion, endives, leeks, pears, tomatoes, witloof chicory
Propyzamide	0.05	Cherries, endives, lettuce
Prothifos	0.04	Apples, grapes, pineapples
Pyrazophos	1.5	Apples, endives, grapes, head cabbage, lettuce, spinach
Pyrethrum	1.0 0.4	Strawberries Grapes, lettuce
Quintozene	0.025	Apples, beans, carrots, cherries, grapes, head cabbage, lettuce, peaches, pears, plums, red cabbage, strawberries
Simazine	0.1	Apples, carrots, cherries, corn salad, cucumbers, dandelion, endives, grapes, head cabbage, kohlrabi, pears, peppers, red cabbage, tomatoes, witloof chicory
Sulfotep	0.1	Lettuce, radishes, spinach, strawberries
Tecnazene	0.05	Apples, beans, carrots, cherries, grapes, head cabbage, lettuce, peaches, pears, plums, red cabbage, strawberries
Terbutryn	0.05	Cucumbers
Tetrachlorvinphos	2.5 0.5 0.1	Grapes, pears Apples Cucumbers
Tetradifon	0.25	Apples, beans, cherries, grapes, head cabbage, leeks, lettuce, peaches, pears, peppers, plums, red cabbage, strawberries

Table 1. (contd.)

Compound	Concentration mg/kg	Crop (harvested commodity)
Tetrasul	0.25	Apples, beans, cherries, grapes, head cabbage, leeks, lettuce, peaches, pears, plums, red cabbage, strawberries
	0.05	Cucumbers
Thionazin	0.05	Apples, beans, carrots, celeriac, cherries, grapes, head cabbage, leeks, lettuce, parsley, peaches, pears, plums, red cabbage, strawberries
Tolylfluanid	0.2	Grapes, strawberries
Triadimefon	0.2	Grapes, strawberries
Triazophos	0.2	Apples
Vinclozolin	0.05	Apples, cherries, peppers, strawberries, tomatoes

Chromatographic tube with sintered glass disk and stopcock, 2.5 cm i. d., 40 cm long

Rotary vacuum evaporator, 40°C bath temperature

Graduated cylinders, 10-ml, with ground stoppers

Gas chromatograph equipped with electron capture detector and phosphorus and nitrogen-specific detectors

Microsyringe, 10- $\mu$ l

#### 4. Reagents

Acetone, chemically pure, distilled in rotary evaporator at 40°C

Dichloromethane, p. a. (e.g. Merck No. 6050)

n-Hexane, for residue analysis (e.g. Merck No. 4371)

Toluene, for residue analysis (e.g. Merck No. 8389)

Eluting mixture: dichloromethane + toluene + acetone 10:2:2 v/v/v

Pesticide standard solutions: 0.05 to 5  $\mu$ g/ml n-hexane

Sodium chloride solution, saturated

Sodium chloride, p. a.

Sodium sulphate, p. a., anhydrous, heated at 600°C

Activated carbon, p. a. (Merck No. 2186)

Silica gel 60 for column chromatography, 0.063 – 0.200 mm (Merck No. 7734)

Air, synthetic (Messer Griesheim)

Argon + methane mixture 95:5 v/v (PR Gas, Messer Griesheim)

Helium (He 99.995, Messer Griesheim)

Hydrogen, special (Messer Griesheim)

#### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Weigh a 100-g portion of the coarsely comminuted sample (G) into a 1-l beaker, add 200 ml acetone and homogenize for about 30 s. Rinse the homogenizer with 50 ml acetone, and reserve the washing for rinsing the beaker and the Buchner funnel later. Filter the homogenate with suction through a moistened round filter paper in the Buchner funnel. Rinse the filter cake with the 50-ml portion of acetone used earlier as washing liquid.

Thoroughly shake the filtrate and measure its volume. Take exactly one-fifth of this filtrate and shake it vigorously (!) for at least 2 min with 250 ml water, 25 ml sodium chloride solution and 50 ml dichloromethane in a 1-l separatory funnel. (It has been found that if the filtrate is not shaken sufficiently well, the recovery obtained in experiments on fortified samples will be reduced substantially.) — Repeat this extraction with 50 ml dichloromethane. Combine the dichloromethane phases, and dry on 30 g sodium sulphate for 30 min. Filter the dried extract through a fluted filter paper. Rinse the flask and filter paper with 30 ml dichloromethane applied in 3 portions. Rotary-evaporate the filtrate to about 2 ml, and remove the last traces of solvent by swirling the flask manually. Dissolve the residue in 10 ml dichloromethane.

### 6.2. Cleanup

Fill the chromatographic tube with dichloromethane to a level of 1 cm. Slurry 5 g silica gel in 15 ml eluting mixture, and pour the slurry into the column. Drain off the supernatant. Next, thoroughly mix 15 g silica gel and 1 g activated carbon in a 50-ml beaker, and slowly add 35 ml eluting mixture (Caution! Generation of heat). Do not add more than 35 ml eluting mixture otherwise the suspension will become separated into phases, resulting in poor passage of material through the column.

Add the activated carbon-silica gel mixture onto the silica gel in the chromatographic column, by pouring it through a funnel, at first slowly and then in a gush, at the same time stirring constantly and with the column stopcock open. Use any eluate that has already passed through the column for rinsing the flask. Drain the eluting mixture to a level 2 cm above the packing, and top the column with a total of 5 g sodium sulphate added in small portions. Next pre-wash the column with 50 ml eluting mixture. Transfer the dichloromethane solution derived from 6.1 quantitatively to the thus prepared column, completing the transfer with a total of 5 ml dichloromethane. Collect liquid already flowing through the column and subsequent eluate in a 250-ml round-bottomed flask. Elute the column with 140 ml of eluting mixture. Rotary-evaporate the eluate to about 30 ml. Transfer it to a 50-ml round-bottomed flask and rotary-evaporate again to about 2 ml. Beforehand, empty the receiver. Do not on any account rotary-evaporate the solution to dryness (see Important points in Section 8).

### 6.3. Gas-chromatographic determination

Transfer the solution derived from 6.2 to a graduated cylinder, and make up with n-hexane to 5.0 ml ( $V_{End}$ ). Inject an aliquot of this solution ( $V_i$ ) — for routine analysis, 2  $\mu$ l — into the gas chromatograph.

*Operating conditions***6.3.1. Organochlorine and other compounds detectable by electron capture**

Gas chromatograph	Hewlett-Packard 5755 G
Column	Glass, 1/4 inch (6.35 mm) o.d., 2 mm i.d., 6 feet (1.83 m) long; packed with 3% SE-30 on Chromosorb W-AW-DMCS, 80-100 mesh
Column temperature	210°C
Injection port temperature	230°C
Detector	Hewlett-Packard electron capture detector (ECD, $^{3}\text{H}$ ) Temperature 230°C
Gas flow rates	Helium carrier, 30 ml/min Argon-methane, 120 ml/min
Attenuation	$16 \cdot 10^2$ (0.1 ng lindane = half-scale deflection)
Recorder	1 mV; chart speed 0.5 inch/min (12.5 mm/min)
Injection volume	2 $\mu\text{l}$
Retention time for aldrin	3 min 40 s

**6.3.2. Organophosphorus compounds****6.3.2.1.**

Gas chromatograph	Hewlett-Packard 5755 G
Column	Glass, 1/4 inch (6.35 mm) o.d., 2 mm i.d., 6 feet (1.83 m) long; packed with 2% FS-1265 (QF-1) on Chromosorb W-AW-DMCS, 60-80 mesh
Column temperature	210°C
Injection port temperature	230°C
Detector	Hewlett-Packard alkali flame ionization detector (AFID) Temperature 220°C
Gas flow rates	Helium carrier, 45 ml/min Hydrogen, 40 ml/min Air, 390 ml/min
Attenuation	$8 \cdot 10^2$ (0.5 ng parathion = half-scale deflection)
Recorder	1 mV; chart speed 0.5 inch/min (12.5 mm/min)
Injection volume	2 $\mu\text{l}$
Retention time for parathion	1 min 50 s

**6.3.2.2.**

Gas chromatograph	Carlo Erba 4200
Column	Glass, 6 mm o.d., 2 mm i.d., 2 m long; packed with 3% OV-210 on Gas Chrom Q, 80-100 mesh
Column temperature	220°C
Injection port temperature	230°C
Detector	Alkali flame ionization detector Temperature 240°C

Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 2 ml/min Air, 240 ml/min
Attenuation	256 (0.5 ng parathion = half-scale deflection)
Recorder	2 mV; chart speed 1 cm/min
Injection volume	2 µl
Retention time for parathion	2 min 6 s

### 6.3.3. Triazine compounds

Gas chromatograph	Perkin-Elmer F-22
Column	Glass, 1/4 inch (6.35 mm) o.d., 2 mm i.d., 6 feet (1.83 m) long; packed with 3% SE-30 on Chromosorb W-AW-DMCS, 80–100 mesh
Column temperature	190°C
Injection port temperature	200°C
Detector	Perkin-Elmer nitrogen-specific detector (PND) Temperature 250°C
Gas flow rates	Helium carrier, 30 ml/min Hydrogen, 7 ml/min Air, 125 ml/min
Attenuation	64
Recorder	1 mV; chart speed 0.5 inch/min (12.5 mm/min)
Injection volume	2 µl
Retention time for parathion	4 min 50 s

The relative retention times for the different compounds are given in Table 2.

## 7. Evaluation

### 7.1. Method

The peaks are quantitated by measuring their area (or height if they are very narrow).

If the presence of compound residues is indicated in the gas chromatogram, qualitative assignment is followed by quantitatively comparing the sample solution peaks with those of standard solutions of the detected compound. The concentration of the standard solutions should be such that the peaks of sample and standard are of approximately the same size for equal volumes of injected solution.

### 7.2. Recovery and lowest determined concentration

To check the reproducibility of the method and to determine the recovery, experiments were run on control samples fortified with the compounds listed in Table 1. Each compound was applied as a solution to the surface of untreated plant material and, after evaporation of the solvent, determined as described above. The successfully tested compound-substrate combinations with recovery rates of more than 70% are listed in Table 1. The routine limit of determination is given for each compound in Table 2.

Table 2. Routine limit of determination and relative retention times

No.	Compound	Routine limit of determination mg/kg	Relative retention time			
			6.3.1	Column		
				Aldrin = 1	6.3.2.1 Parathion = 1	6.3.2.2
1	Aldrin	0.004	1.00			
2	Ametryn	0.1				0.75
3	Atrazine	0.05				0.46
4	Azinphos-ethyl	0.1	6.4	5.6	4.8	
5	Azinphos-methyl	0.2	4.90	4.6	4.2	
6	Aziprotryne	0.05				0.59
7	Bromacil	0.05	0.83			
8	Bromophos	0.02	1.06	0.51		
9	Bromophos-ethyl	0.01	1.38			0.68
10	Bupirimate	0.05	1.67			1.3
11	Captafol	0.5	3.95			
12	Captan	0.01	1.18			1.1
13	Carbophenothion	0.03	2.63	1.23	1.1	
14	Chlorfenvinphos	0.03		0.95	0.95	
15	Chlorpyrifos	0.05	0.93	0.48	0.46	
16	Chlorpyrifos-methyl	0.01	0.71			0.42
17	Chlorthal	0.01	1.0			
18	Chlorthiophos	0.01	2.46	1.25	1.22	
19	Cyanofenphos	0.01	2.37			2.4
20	p,p'-DDD	0.01	2.18			
21	p,p'-DDE	0.01	1.84			
22	p,p'-DDT	0.01	3.1			
23	Desmetryn	0.02				0.66
24	Diazinon	0.01		0.20	0.28	
25	Dichlobenil	0.01	0.13			
26	Dichlofenthion	0.03		0.56	0.48	
27	Dichlofluanid	0.01	0.87			0.84
28	Dichlorvos	0.02				0.11
29	Dicofol	0.05	0.99			
30	Dieldrin	0.01	1.84			
31	Dimethachlor	0.05	0.70			
32	Dimethoate	0.1	0.44	0.64	0.61	
33	Dioxathion	0.05		0.31	0.39	
34	Disulfoton	0.01		0.27	0.32	
35	Ditalimfos	0.05	1.49	1.15	1.18	
36	$\alpha$ -Endosulfan	0.01	1.56			
37	Ethion	0.03	2.4	1.4	1.32	
38	Ethoprophos	0.02	0.32			0.27
39	Etrimfos	0.04	0.56			0.31
40	Fenchlorphos	0.02	0.77	0.41	0.44	
41	Fenitrothion	0.05	0.79	0.86	0.88	
42	Fensulfothion	0.1	2.21	4.26	3.37	
43	Fenthion	0.01		0.51	0.59	

Table 2. (contd.)

No.	Compound	Routine limit of determination mg/kg	Relative retention time			
			6.3.1 Aldrin = 1	Column		
				6.3.2.1 Parathion = 1	6.3.2.2	6.3.3 Para-thion = 1
44	Folpet	0.01	1.27			
45	Fonofos	0.01	0.49	0.28	0.32	
46	Formothion	0.01	0.61	0.82	0.80	
47	$\alpha$ -HCH	0.01	0.39			
48	$\beta$ -HCH	0.01	0.42			
49	Heptachlor	0.004	0.79			
50	Heptachlor epoxide	0.005	1.22			
51	Heptenophos	0.01		0.22	0.25	
52	Iprodione	0.05	2.24			
53	Isofenphos	0.02	1.57		0.80	
54	Lindane	0.002	0.47			
55	Malaoxon	0.025		0.94	0.90	
56	Malathion	0.02	0.85	0.72	0.72	
57	Metalaxyl	0.4			0.65	
58	Methidathion	0.1	1.3	1.13	1.12	
59	Methoprotryne	0.05				2.0
60	Methoxychlor	0.1	4.56			
61	Mevinphos	0.1		0.21	0.20	
62	Naled	0.1		0.09		
63	Paraoxon	0.1		1.33	1.24	
64	Parathion	0.02	0.96	1.00	1.00	1.00
65	Parathion-methyl	0.02	0.67	0.79	0.80	
66	Perthane	0.1	2.15			
67	Phenkaption	0.05	4.25	1.18	1.93	
68	Phorate	0.01		0.30	0.23	
69	Phosalone	0.02	6.0	4.65	4.10	
70	Pirimiphos-methyl	0.01		0.41	0.45	
71	Procymidon	0.02	1.30		1.47	
72	Profenofos	0.04	1.56		1.05	
73	Prometryn	0.01				0.77
74	Propazine	0.05				0.47
75	Propyzamide	0.01	0.50			
76	Prothiofos	0.01	1.61		0.78	
77	Pyrazophos	0.2	6.8	5.41	4.67	
78	Pyrethrum	0.2	1.8; 2.35			
79	Quintozene	0.002	0.49			
80	Simazine	0.01				0.44
81	Sulfotep	0.01		0.23	0.26	
82	Tecnazene	0.01	0.24			
83	Terbutryn	0.05				0.88
84	Tetrachlorvinphos	0.1	1.47	1.22	1.12	
85	Tetradifon	0.03	4.70			
86	Tetrasul	0.05	2.45			

Table 2. (contd.)

No.	Compound	Routine limit of determination mg/kg	Relative retention time			
			6.3.1	6.3.2.1 Aldrin = 1	6.3.2.2 Parathion = 1	6.3.3 Parathion = 1
87	Thionazin	0.01	0.27	0.19		
88	Tolylfluanid	0.02	1.20			
89	Triadimefon	0.04	0.93		0.62	
90	Triazophos	0.1		2.0		
91	Vinclozolin	0.01	0.69			

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, of an identified compound is calculated from the following equation:

$$R = 5 \cdot \frac{F_A \cdot V_{End} \cdot W_{St}}{F_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

$V_{End}$  = terminal volume of sample solution from 6.2 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_{St}$  = amount of compound injected with standard solution (in ng)

$F_A$  = peak area obtained from  $V_i$  (in  $\text{mm}^2$ )

$F_{St}$  = peak area obtained from  $W_{St}$  (in  $\text{mm}^2$ )

## 8. Important points

With the reported method, up to six samples can be analyzed per manday. A single sample takes 2 h to analyze.

The earlier employed procedure has been modified as follows. In step 6.1 (extraction), washing of the combined dichloromethane phases with water and sodium chloride solution has been omitted thus improving the recoveries of compounds readily soluble in water. Due to the continued presence of acetone traces, the amount of sodium sulphate required for drying has been raised to 30 g. The eluate collected after elution of the column in step 6.2 must not be concentrated to dryness because toluene has a higher boiling point than the earlier used benzene and thus requires application of a higher vacuum which results in compound losses.

In analyses of carrots, parsley, leeks and celeriac using an electron capture detector, interfering peaks may appear in the gas chromatogram of organochlorine compounds. Therefore, in

this case an additional cleanup is necessary e.g. on a sulphuric acid-Celite column. Use of modern thermionic detectors equipped with electrically heated alkaline beads creates difficulties when toluene is injected due to formation of carbon black. Therefore, the concentrated solution derived from 6.2 should always be made up to the terminal volume ( $V_{End}$ ) only with n-hexane.

Hexachlorobenzene, pentachloroaniline, chlordecon,  $\beta$ -endosulfan, endosulfan sulphate, phosphamidon and triamiphos are not detected by the reported method.

Due to the big increase in the number of compounds now detectable by the reported method, there is considerably greater likelihood of mutual interferences and especially of misinterpretations in the gas-chromatographic determination. Therefore, it is essential to ensure that the detected compounds are unequivocally identified.

## 9. References

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## 10. Author

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# **Organochlorine and Organophosphorus Pesticides**

**S 9**

Butter, cheese (hard, medium, soft, spread), chocolate, cocoa butter, cocoa powder, coconut oil, condensed milk, dried egg yolk, dried whole egg, eggs (fresh), fish, infant foods based on milk, lard, margarine, meat, milk (fresh), olive oil, peanut oil, poultry meat, sausage, skim milk powder, soybean oil, suet (tallow), sunflower oil, whole (full cream) milk powder

Gas-chromatographic determination and thin-layer chromatographic confirmation

(German version published 1976)

## **1. Introduction**

The method is applicable to samples of fatty foods of plant or animal origin like meat, fish, fats and oils, eggs and dried egg products, milk and dairy products, cocoa powder and chocolate for the determination of residues of 35 organochlorine and organophosphorus compounds (predominantly insecticides and their fat-soluble metabolites) listed in Tables 1 and 2.

A particular advantage of the method is that it permits a rapid screening analysis of a series of samples. As only one step is required for cleanup, recoveries of the analyzed compounds are high. However, an extra cleanup of the extract may be necessary when determining residues at very low concentrations. In such cases, it is more expedient to use Method S 10.

## **2. Outline of method**

The fat is extracted from meat, meat products, fish and cheese by a suitable procedure. The solution of a maximal 1-g amount of the extracted fat or of pure fats and oils is cleaned up by chromatography on a column packed with partly deactivated Florisil.

For the analysis of milk, dairy products, eggs, egg powder, cocoa powder, chocolate or the like, the analytical sample containing an amount of fat not exceeding 1 g is mixed, in the presence of water, with enough Florisil to form a homogeneous, free-flowing powder. In the process, the Florisil is completely deactivated, and the surface of the adsorbent is coated with a thin film of all the fat contained in the food sample so that it becomes readily accessible to extracting solvents. The mixture is added to the top of a column packed with partly deactivated Florisil. Extraction and cleanup are performed in one and the same step by eluting the column with a mixture of petroleum ether and dichloromethane.

The cleaned-up extracts are analyzed by electron capture gas chromatography, and for organophosphorus compounds by additionally using a thermionic or flame photometric phosphorus-specific detector. Confirmation of the result can be obtained by thin-layer chromatography.

### 3. Apparatus

Chromatographic tube, 22 mm i. d., 25 cm long, fitted with a glass or Teflon stopcock and an approx. 300-ml reservoir at upper end  
Round-bottomed flasks, 1-l, 250-ml and 100-ml, with ground joints  
Erlenmeyer flask, 250-ml, with ground joint  
Volumetric flask, 5-ml  
Reflux condenser  
Rotary vacuum evaporator  
Gas chromatograph (dual column instrument) equipped with electron capture detector and with thermionic or flame photometric phosphorus-specific detector  
Microsyringe, 10- $\mu$ l  
Equipment for thin-layer chromatography  
Ultraviolet lamp for photochemical uses, e. g. Philips HPK 125 W/L quartz lamp with VG I/HP 125 W transformer

### 4. Reagents

Acetone, p. a.  
Dichloromethane, pure, dist.  
Diethyl ether, p. a.  
Ethanol, 96%, p. a.  
n-Hexane, p. a.  
Isooctane, pure  
Petroleum ether, pure, distilled over a 1-m long Raschig column, boiling range 40 – 60 °C  
Water, dist., extracted with petroleum ether  
Eluting mixture: petroleum ether + dichloromethane 8 : 2 v/v  
Extraction mixture: dichloromethane + acetone 2 : 1 v/v  
Standard solutions in isooctane for gas chromatography: HCB, all HCH isomers, aldrin, heptachlor, heptachlor epoxide, chlordane isomers, DDE, dieldrin 0.005 µg/ml; DDT and TDE isomers, endrin 0.01 µg/ml; PCBs (Aroclor 42, 48, 54, 60 or 62) 0.05 – 0.1 µg/ml; camphechlor (toxaphene) 0.5 µg/ml; organophosphorus pesticides 0.02 – 0.05 µg/ml (electron capture detector) or 0.05 – 0.2 µg/ml (phosphorus-specific detectors)  
Standard solutions in isooctane for thin-layer chromatography: organochlorine pesticides 0.05 mg/ml; organophosphorus pesticides 0.01 mg/ml  
Silver nitrate solution: dissolve 0.5 g AgNO<sub>3</sub> p. a. in 1 ml water, and make up with ethanol to 100 ml  
Bromine p. a.  
 $\beta$ -Naphthyl acetate solution:  $\beta$ -naphthyl acetate, naphthol-free, 0.25 g/100 ml in ethanol; prepare fresh  
Fast blue salt solution: Merck No. 3191 Echtblaualsalz B, 0.1 g/100 ml in ethanol-water mixture 1 : 3 v/v; prepare fresh  
Lyophilized human blood serum (e. g. Nutritional Biochemical Corp.)  
Sodium sulphate, anhydrous, p. a., heated at 500 °C  
Florisil, 60 – 100 mesh: Heat overnight at 550 °C, let cool, and store in a tightly stoppered container. Prior to use, heat the Florisil for at least 5 h at 130 °C and add 3% (w/w) water. Shake

this mixture for at least 20 min, and then store stoppered for at least 10 to 12 h. The standardized Florisil may be used as such only during the next 3 days, after which time it must again be heated at 130°C and deactivated.

Glass wool, washed with dichloromethane and dried at 130°

Hydrogen, re-purified

Nitrogen, re-purified

Oxygen, re-purified

## 5. Sampling and sample preparation

For the foods of interest here, the laboratory sample is comminuted and reduced to the analytical sample according to the guideline given in the 3. Empfehlung (Third Recommendation) of the *GDCh-Arbeitsgruppe "Pestizide"*. The guideline also gives examples of which food parts (e. g. bones, rind) must be removed prior to the analysis. The size of the analytical sample depends upon the fat content of the material to be analyzed.

## 6. Procedure

For some of the foods, the contained amount of pesticide residue is expressed on fat basis. The procedure described in step 6.1 permits simultaneous determination of the fat content of the samples by evaporating the extract or an aliquot of it. In the procedure described in step 6.2, the fat is not isolated from the food. In this case, it is necessary to determine the fat content separately by one of the customary procedures.

### 6.1. Extraction and cleanup for meat, fish and their products, butter, cheese, cheese spread, fats and oils

#### 6.1.1. Extraction of fat from meat, fish and their products

Grind 25 g of the coarsely comminuted sample with 100 g sodium sulphate in a mortar. Extract the mixture successively with four 100-ml portions of boiling petroleum ether for 10 min under reflux. Evaporate the combined extracts on a rotary evaporator. Weigh the remaining fat, unless it is required to determine the fat content of the sample by other means, and proceed to 6.1.4.

#### 6.1.2. Extraction of fat from cheese and cheese spread

Thoroughly mix 10 to 30 g (according to fat content) of the finely grated or otherwise comminuted sample with a two or three times bigger amount by weight of sodium sulphate. Transfer the mixture to an Erlenmeyer flask, and extract successively with four 100-ml portions of the extraction mixture by heating for 15 min under reflux. Evaporate the combined extracts. Dissolve the residue remaining after evaporation in 20 ml petroleum ether, decant the solution carefully through a glass wool plug into a small round-bottomed flask, and evaporate the solution. Weigh the remaining fat, unless it is required to determine the fat content of the sample by other means, and proceed to 6.1.4.

**6.1.3. Isolation of fat from butter**

Heat the sample in a beaker at about 60°C until the fat clearly separates. Decant the melted fat through a small glass wool plug, and proceed to 6.1.4.

**6.1.4. Column chromatographic cleanup of fats and oils and of the fat extracted from other foods in 6.1.1–6.1.3**

Fill a chromatographic tube with 100 ml petroleum ether, and slowly add 25 g Florisil. Allow the adsorbent to settle, and drain the petroleum ether to a level of about 1 cm above the Florisil layer. Then dissolve 0.5 g to max. 1 g of the fat or oil to be analyzed or of the fat portion extracted in step 6.1.1, 6.1.2 or 6.1.3 in 10 ml petroleum ether, and quantitatively transfer the solution to the column. Allow the solution to percolate, elute with 300 ml of the eluting mixture, and collect the eluate in a 1-l round-bottomed flask (rate of flow should not exceed 5 ml/min). Rotary-evaporate the eluate, transfer to a 100-ml round-bottomed flask, and concentrate to about 5 ml. Remove the last traces of solvent with the aid of a gentle stream of air. Transfer the residue remaining after evaporation with small portions of petroleum ether into a 5-ml volumetric flask, and dilute to the mark.

**6.2. Extraction and cleanup for other fat-containing foods****6.2.1. Pre-treatment of the different foods**

Directly use 10 g of fresh milk and unsweetened condensed milk for analysis. Dilute 10 g of sweetened condensed milk with 5 ml water.

Completely dissolve 3 g of milk powder and milk-based infant food in 10 ml water having a temperature of 40°C. Avoid formation of lumps.

Triturate 2 to 5 g of homogenized whole egg with 8 to 5 ml water. Thoroughly blend 1 to 3 g (according to fat content) of egg powder, cocoa powder and chocolate with 10 ml warm water.

**6.2.2. Column chromatographic extraction and cleanup**

Fill a chromatographic tube with 100 ml petroleum ether, and slowly add 25 g of standardized Florisil. Allow the adsorbent to settle, and drain the petroleum ether to a level of about 50 ml above the top of the adsorbent.

Add a total of 25 g Florisil in small portions to the mixture or solution prepared in step 6.2.1. While adding the Florisil, stir continuously with a glass rod until a homogenous, free-flowing powder is obtained. Then add this powder to the column.

Drain the supernatant petroleum ether and collect in a 1-l round-bottomed flask. Then elute the column with 300 ml of the eluting mixture at a flow rate not exceeding 5 ml/min, and collect the eluate in the same flask. Rotary-evaporate the eluate, transfer to a 100-ml round-bottomed flask, and concentrate to about 5 ml. Remove the last traces of solvent with the aid of a gentle stream of air. Transfer the residue remaining after evaporation with small portions of petroleum ether into a 5-ml volumetric flask, and dilute to the mark.

### 6.3. Gas-chromatographic determination

Inject an aliquot of the solution derived from step 6.1.4 or 6.2.2 into the gas chromatograph.

#### *Operating conditions*

Gas chromatographs	Perkin-Elmer F-11 and Tracor 550
Column 1	Pyrex glass, 3 mm i. d., 1.6 m long; packed with 1.5% OV-17 + 1.95% QF-1 on Chromosorb W-HP, 100–120 mesh
Column temperature 1	210°C
Column 2	Pyrex glass, 3 mm i. d., 1.6 m long; packed with 2% DEGS + 0.5% H <sub>3</sub> PO <sub>4</sub> on Chromosorb W-HP, 100–120 mesh
Column temperature 2	185°C
Injection port temperature	240°C
Detectors	Electron capture detector (ECD, <sup>63</sup> Ni) Temperature 250°C Flame photometric detector (Micro Tek) fitted with 520-nm phosphorus filter Temperature 170°C
Gas flow rates	Nitrogen carrier, 40 ml/min Hydrogen, 150 ml/min Oxygen, 30 ml/min
Recorder	1 mV; chart speed 10 mm/min
Injection volume	2–5 µl
Retention times for aldrin	<i>Column 1</i> 5 min <i>Column 2</i> 1 min 45 s
parathion	8 min 45 s 8 min

The retention times, relative to aldrin = 1.00 and parathion = 1.00 on Columns 1 and 2 are given in Tables 1 and 2.

The analysis should be performed wherever possible on both columns using both the electron capture detector and one of the phosphorus-specific detectors. If only one column is available, then Column 1 should preferably be used. A sensitivity should be selected at which 25 µg lindane produces at least half full-scale deflection using the electron capture detector, and the same response by 1 ng parathion or 1 ng bromophos using the phosphorus-specific detectors. Where it is required to determine, in addition to the main components, other pesticides present at very low concentrations, proceed as follows. After analysis, cautiously evaporate the solution derived from 6.1.4 or 6.2.2 to dryness, dissolve the residue in 0.5–1.0 ml isoctane, and re-inject into the gas chromatograph.

### 6.4. Thin-layer chromatographic confirmation

#### 6.4.1. Organochlorine pesticides

Concentrate the solution derived from 6.1.4 or 6.2.2 and analyzed by gas chromatography in step 6.3, to approx. 0.2 ml. Spot aliquots of this solution, which should contain not more than 0.25 µg of the compound assumed to be present, onto a thin-layer plate coated with neutral

Table 1. Retention times of organochlorine compounds relative to aldrin = 1.00

Pesticide or degradation product	Column 1	Column 2
Pentachlorobenzene	0.23	0.25
$\gamma$ -PCCH*	0.27	0.34
$\delta$ -PCCH*	0.32	0.82
$\alpha$ -PCCH*	0.40	1.10
HCB	0.45	0.40
$\alpha$ -HCH	0.49	1.19
$\gamma$ -HCH	0.66	1.90
Quintozone	0.68	0.84
$\beta$ -HCH	0.75	5.90
Heptachlor	0.80	1.00
Pentachloroaniline	0.86	2.10
$\delta$ -HCH	0.86	5.40
$\epsilon$ -HCH	0.97	5.28
Aldrin	1.00	1.00
Methyl pentachlorophenyl sulfide	1.08	1.35
Oxychlordane	1.35	1.92
Dicofol	1.50	3.50
Heptachlor epoxide	1.51	2.76
$\gamma$ -Chlordane	1.64	2.70
$\alpha$ -Chlordane	1.80	2.95
p,p'-DDE	2.20	3.50
Dieldrin	2.36	4.20
$\alpha$ , $\rho$ '-TDE (DDD)	2.53	6.20
Endrin	2.81	4.70
$\alpha$ , $p$ '-DDT	3.07	4.85
p,p'-TDE (DDD)	3.26	10.70
p,p'-DDT	3.95	8.85
Methoxychlor	8.25	—
Camphechlor	irregular deviations from the baseline of 0.7 to 7; maxima at 2.3, 3.25, 4.2 and 5.8	

\* PCCH = pentachlorocyclohex-1-ene

Table 2. Retention times of organophosphorus pesticides relative to aldrin = 1.00 or parathion = 1.00

Pesticide	Column 1		Column 2	
	Aldrin = 1	Parathion = 1	Aldrin = 1	Parathion = 1
Fenchlorphos	1.07	0.66	1.75	0.37
Chlorpyrifos	1.37	0.77	1.75	0.42
Bromophos	1.56	0.90	2.78	0.65
Iodofenphos	2.73	1.54	5.37	1.20
Ethion	4.43	2.40	6.8	1.50
Phenkaptone	8.2	4.70	16.8	3.24

aluminium oxide. Good results have been obtained on Aluminium oxide 60 F<sub>254</sub> neutral TLC Sheets (Merck No. 5550). For reference, spot 1 to 6-µl volumes of standard solutions of those compounds having retention times similar to that of the suspected compound.

Petroleum ether is used as a mobile phase in a pre-saturated tank. If the R<sub>f</sub> values obtained with petroleum ether are too low, a petroleum ether + acetone mixture 99 : 1 v/v should be used.

After it has been developed, air-dry the plate, spray it vigorously with silver nitrate solution, and expose it to the UV lamp (for photochemical uses). After 10 min, spray the plate lightly and evenly with water, and again expose it to the UV lamp.

After about 1 min, the spots appear greyish-black on an almost colourless background; the limit of detection is approx. 0.02 µg. Examples of the R<sub>f</sub> values obtained are given in Table 5. Camphechlor appears as a band between R<sub>f</sub> 0 and 0.6. PCBs appear as indistinctly separated spots between R<sub>f</sub> 0.65 and 0.75. Besides the organochlorine pesticides, halogen-containing or- ganophosphorus pesticides also are detected. Their R<sub>f</sub> values are as follows:

Phenkaption	0.09
Chlorpyrifos	0.10
Iodofenphos	0.15
Bromophos	0.17
Fenchlorphos	0.19

#### 6.4.2. Organophosphorus pesticides

Concentrate the solution derived from 6.1.4 or 6.2.2 and analyzed by gas chromatography in step 6.3, to approx. 0.2 ml. Spot aliquots of this solution onto a thin-layer plate coated with silica gel G. Good results have been obtained with pre-coated Silica Gel 60 TLC Plates (Merck No. 5721). For reference, standard solutions of the compounds assumed to be present are applied onto the plate. A mixture of n-hexane + dichloromethane + diethyl ether 8:2:2 v/v/v is used as solvent.

After the plate has been developed, expose it to bromine vapour in a glass tank, and then remove excess bromine by exposing the plate to a stream of cold air for 20 min. Subsequently spray the plate vigorously with a freshly prepared solution of 0.25 g lyophilized human blood serum in 50 ml water.

Transfer the plate to an incubator (38°C) containing a dish of warm water to saturate it with water vapour. After 30 min, spray the plate with a freshly prepared mixture of 2 ml β-naphthyl acetate solution and 20 ml fast blue salt solution. Transfer the plate back to the incubator (38°C).

After about 5 min, the organophosphates appear as white spots on a reddish-violet background. The limit of detection is between 1 and 10 ng. The following R<sub>f</sub> values were obtained:

Ethion	0.75
Iodofenphos	0.80
Phenkaption	0.80
Bromophos	0.82
Fenchlorphos	0.84
Chlorpyrifos	0.87

Unlike gas chromatography which gives excellent separations, thin-layer chromatographic resolution of these nonpolar organophosphorus pesticides is not satisfactory. However, the method is reliable enough to confirm that a peak obtained by gas chromatography actually is that of an organophosphorus pesticide. Furthermore, it is unlikely that residues of two of these organophosphorus pesticides could occur simultaneously in one sample.

## 7. Evaluation

### 7.1. Method

Identification is achieved by determination of the retention times relative to aldrin or parathion (Tables 1 and 2). Quantitation is performed by measuring the peak area and comparing it with the peak areas of the same compound from standard solutions. Equal volumes of the sample solution and the standard solutions should be injected. When using an electron capture detector, it is especially important to ascertain that the detector is indeed operating within its linear range and then to inject amounts of both residue and reference standard which fall within that range.

To avoid misinterpretations, it is important to remember that the electron capture detector responds also to halogen-containing organophosphorus pesticides which, if they have similar retention times, may be mistaken for organochlorine pesticides (e. g. heptachlor epoxide/bromophos), especially if only one column is used. On the other hand, these organophosphorus pesticides can also be determined with an electron capture detector if a phosphorus-specific detector is not available. In that case, however, the finding definitely must be confirmed by thin-layer chromatography.

### 7.2. Recoveries and lowest determined concentration

Recoveries from samples of the various fatty foods that were fortified with pesticidal compounds at levels of 0.01 to 1.0 mg/kg were consistently in the range of 80 to 100%.

Seven laboratories of the *GDCh-Arbeitsgruppe "Pestizide"* each conducted 9 recovery experiments on pesticide-free samples of commercial edible oil fortified with 5 organochlorine pesticides. The results were as follows:

Added	Range	Recoveries		Mean of standard deviations
		Mean		
0.05 mg/kg HCB	79–102%	90%		3.4%
0.05 mg/kg $\alpha$ -HCH	87–100%	94%		4.0%
0.10 mg/kg dieldrin	79–99%	95%		6.1%
0.20 mg/kg $\beta$ -HCH	84–100%	94%		4.8%
0.50 mg/kg p,p'-DDT	89–108%	97%		4.0%

The routine limit of determination depends upon the level of the blank value from solvents, adsorbents, etc., upon the design of the detectors and electrometers used, upon the degree of detector contamination, and upon the quality of the column packing.

Experience has shown that the routine limit of determination for most organochlorine pesticides is 0.01 mg/kg. However, by concentrating the cleaned-up extract, still lower concentrations often can be detected. The routine limit of determination for PCBs and organophosphorus pesticides is 0.05 mg/kg, and for methoxychlor and camphechlor it is about 0.3 mg/kg.

### 7.3. Collaborative studies

The *GDCh-Arbeitsgruppe "Pestizide"* already mentioned the described method of analysis in its 1. Empfehlung (First Recommendation), and had it tested in five collaborative studies in which the laboratories analyzed samples of edible oil, condensed milk, whole milk powder and dried whole egg containing organochlorine pesticides at concentrations ranging from 0.02 mg/kg to 1.8 mg/kg.

Details of the comparative statistical evaluation for HCB,  $\gamma$ -HCH and total DDT, the coefficients of variation obtained and the deviations of the results from the actual concentrations contained in the samples are given in the 4. Mitteilung (Fourth Communication).

## 8. Important points

The list of pesticides and their metabolites which can be detected by the reported method is not complete. To date, only the compounds listed in Tables 1 and 2 have been analyzed. Other pesticides as well as polychlorinated biphenyls (PCBs) and naphthalenes also may appear in the eluate from the Florisil column.

The presence of PCBs may interfere with the quantitation of some pesticides (in particular DDT, its isomers and metabolites) through superposition of the peaks. But as a rule, the interferences are of minor significance. For example, a PCB concentration of 1 mg/kg (calculated as Aroclor 1260 or Clophen A 60) simulates no more than a concentration of about 0.1 mg/kg DDT. Consequently, there is usually no need to take the PCB concentrations in milk and dairy products into account because they are mostly below 0.2 mg/kg (relative to fat).

When PCBs are present in fatty foods, their chlorine content is nearly always in the range of 54 to 62%. Those with a lower chlorine content (e.g. Aroclor 1242 or 1248) usually are not residues but constitute contaminations often attributable to the sample packing material or to the reagents. Indications of the PCB type present can be obtained from the distribution of the peaks in the gas chromatogram (Tables 3 and 4).

If large amounts of PCBs are present in a sample, as is often the case in fish, they can be separated from most pesticides by a suitable method. An appropriate one is Masumoto's modification of the procedure developed by Armour and Burke, which involves fractionated elution from a silicic acid column, or the alkali treatment procedure reported by Young and Burke, in which the PCBs, in contrast to most pesticides, remain unchanged.

Furthermore, it should be pointed out that some of the analyzed compounds occur only exceptionally in fatty foods. Pentachloroaniline and methyl pentachlorophenyl sulfide, for example, are found only in the milk of cows that have been fed quintozene-containing vegetable waste for a lengthy period.

Table 3. Retention times of chlorinated biphenyls relative to aldrin = 1.00, and area of peaks in % of total peak area, measured on Column 1

Retention times	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.4	1.6	1.8	2.3	2.5	2.8	3.2	3.6	4.1	4.4	5.4	5.8	6.5	7.0	8.5	11.4	13.0
Aroclor	% of total peak area																								
1242	3.7	9.0	5.8	21.6	9.6	7.6	7.7	8.7	6.9	10.8	8.1														
1248		3.0	1.6	9.7	3.3	3.8	10.5	9.3	17.7	13.2	4.9	6.6	8.2	1.7	5.7										
1254		2.1	1.2	4.0	2.5	4.3	2.8	1.9	7.5	8.6	5.3	9.8	13.2	9.5	11.3	2.2	2.2	1.5	1.1						
1260											1.4	2.4	6.7	6.1	10.0	7.4	11.5	13.2	7.7	3.0	15.8	13.4		4.0	
1262											0.4	0.6	4.4	4.1	6.1	5.4	6.0	13.5	6.8	7.0	15.3	4.8	17.8	2.4	5.8

Table 4. Retention times of chlorinated biphenyls relative to aldrin = 1.00, and area of peaks in % of total peak area, measured on Column 2

Retention times	0.6	0.7	0.9	1.0	1.1	1.2	1.3	1.5	1.6	1.8	1.9	2.0	2.3	2.8	2.9	3.4	3.7	4.0	4.3	4.7	5.3	6.0	6.3	7.3	7.9	8.4	10.6	15.7
Aroclor	% of total peak area																											
1242	3.5	10.4	2.3	6.9	20.6	8.6	13.9	7.2	3.8		11.0	5.0																
1248		3.4	1.7	4.8	1.8	5.7		3.4	1.6	11.4	6.1	4.9	26.5	12.2	2.9	2.7	8.9											
1254		1.3	2.3							0.9	16.9	4.6	5.9	1.3	0.2	13.5	4.3											
1260										1.3	1.8		5.0	5.6	2.6	12.1	16.6	12.5	4.3	3.7	1.3	1.1	0.7					
1262										0.4	0.3	3.5	3.3	2.7	6.8	1.6	16.1	6.6	6.8	5.2	0.6	17.9	13.7	5.6	8.9			

Table 5.  $R_f$  values for organochlorine compounds

Compound	$R_f$
Methoxychlor	0.00
$\delta$ -HCH	0.00
$\beta$ -HCH	0.03
$\alpha$ -PCCH	0.08
$\varepsilon$ -HCH	0.11
Dieldrin	0.13
Endrin	0.16
Heptachlor epoxide	0.18
$\gamma$ -HCH	0.20
Pentachloroaniline	0.21
p,p'-TDE (DDD)	0.23
o,p'-TDE (DDD)	0.26
$\alpha$ -HCH	0.31
$\delta$ -PCCH	0.35
$\gamma$ -Chlordane	0.39
$\alpha$ -Chlordane	0.45
p,p'-DDT	0.48
$\gamma$ -PCCH	0.50
Oxychlordane	0.54
o,p'-DDT	0.57
Heptachlor	0.60
Dicofol	0.00, 0.60
p,p'-DDE	0.65
Quintozene	0.68
Aldrin	0.72
Methyl pentachlorophenyl sulfide	0.72
Pentachlorobenzene	0.76
HCB	0.77

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# **Organochlorine and Organophosphorus Pesticides**

**S 10**

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Butter, cheese (hard, medium, soft, spread), cocoa butter, coconut oil, condensed milk, dried egg yolk, dried whole egg, eggs (fresh), fish, lard, margarine, meat, milk (liquid, fresh), olive oil, peanut oil, poultry meat, sausage, soybean oil, suet (tallow), sunflower oil, whole (full cream) milk powder

Gas-chromatographic determination

(German version published 1976)

## **1. Introduction**

The method is applicable to samples of meat, fish, fats and oils, eggs and dried egg products, milk and dairy products for the determination of residues of 26 organochlorine and organophosphorus pesticides (predominantly insecticides). The compounds determined by the reported method are listed in Table 1.

The method is suitable especially for the determination of very low residue concentrations because very well cleaned-up extracts are obtained and high recovery levels are achieved for the analyzed pesticides. However, the rigorous cleanup procedure takes a correspondingly large amount of time to complete. Therefore, for rapid screening analyses in which highest sensitivity is not a stringent requirement, Method S 9 will be more appropriate.

## **2. Outline of method**

Fats and oils (up to 5 g) and the fat portion of other foods extracted quantitatively beforehand, are partitioned between dimethylformamide and petroleum ether. The partition step, patterned after the procedure reported by de Faubert Mauder, has been modified so that hexachlorobenzene can now be determined considerably better. The extract is then subjected to an extra cleanup on a Florisil column.

The organochlorine pesticides are analyzed by gas chromatography on at least two columns of different polarity with electron capture detection. For residues of lipid-soluble organophosphorus pesticides, phosphorus-specific detectors like the thermionic or flame photometric phosphorus detector are used additionally.

## **3. Apparatus**

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Mincer, e. g. fish chopper (Weisser No. 81 K)  
Soxhlet extractor

## 310      Method S 10

Separatory funnels, 500-ml and 250-ml, with ground stoppers and Teflon stopcocks  
Chromatographic tube, 20 mm i.d., 40–50 cm long, with Teflon stopcock and sintered glass disk

Rotary vacuum evaporator

Round-bottomed flasks, 500-ml and 100-ml, with ground joints

Volumetric flasks, 50-ml and 10-ml, or test tubes with ground stoppers, graduated

Gas chromatograph equipped with electron capture detector and with thermionic or flame photometric phosphorus-specific detector

Microsyringe, 10-ml

*Important:* All glassware must be rinsed with acetone immediately before use.

## 4. Reagents

Acetone, p.a., dist.

Diethyl ether, p.a., dist.

Dimethylformamide, p.a., saturated with petroleum ether

Ethyl acetate, p.a., dist.

n-Hexane, p.a., dist.

Methanol, p.a., dist.

Petroleum ether, pure, dist., boiling range 30–60°C

Petroleum ether, pure, dist., boiling range 30–60°C, saturated with dimethylformamide

Water, distilled from glass apparatus

Eluting mixture I: petroleum ether + diethyl ether 94:6 v/v

Eluting mixture II: petroleum ether + ethyl acetate 6:4 v/v

Standard solutions for gas chromatography: Organochlorine pesticides – 0.1 µg/ml lindane and 0.5 µg/ml p,p'-DDT in n-hexane. Organochlorine and organophosphorus pesticides – 0.1 µg/ml lindane, aldrin and diazinon and 0.5 µg/ml p,p'-DDT in acetone

Sulphuric acid, p.a., conc.

Eosin solution: 2 mg eosin, yellowish, in 100 ml water

Sodium sulphate solution, 2 g/100 ml Na<sub>2</sub>SO<sub>4</sub> · 10 H<sub>2</sub>O

Sodium oxalate, p.a., finely ground

Sodium sulphate, anhydrous, heated for at least 2 h at 550°C

Celite 545 (e.g. Roth)

Florisil, 60–100 mesh: Heat for at least 2 h at 550°C, let cool, and store in a tightly stoppered container. Prior to use, heat the Florisil for at least 5 h at 130°C, let cool in a desiccator, and then add 5% (w/w) water. Shake this mixture for at least 20 min, and then store in a tightly stoppered container for at least 10 h

Filter paper, extracted exhaustively with petroleum ether, for making thimbles for Soxhlet extraction

Cottonwool, extracted exhaustively with petroleum ether

Air, re-purified

Argon + methane mixture 9:1 v/v

Helium, re-purified

Hydrogen, re-purified

Nitrogen, re-purified

## 5. Sampling and sample preparation

The laboratory sample is comminuted and reduced to the analytical sample, for the foods of interest here, according to the guideline given in the 3. Empfehlung (Third Recommendation) of the *GDCh-Arbeitsgruppe "Pestizide"*. The guideline also gives examples of which food parts (e.g. bones, rind) must be removed prior to analysis. Meat, fish and other animal tissues are finely comminuted in a mincer; heating of the sample during comminution is avoided by briefly chopping several times. The size of the analytical sample depends upon the fat content of the analytical material.

## 6. Procedure

For some of the foods, the content of pesticide residues relates to the fat content of the analytical material. The processing procedure described in step 6.1 permits simultaneous determination of the fat content of the samples, by evaporating the extract or an aliquot of it.

### 6.1. Extraction of fat and processing of samples

#### 6.1.1. Meat, fish and other animal tissues, cheese, eggs

Triturate a sample of the analytical food material, containing about 5 g fat, with sodium sulphate to a dry, powdery mixture; with the aid of a "home-made" extraction thimble (made from pre-extracted filter paper), extract the mixture exhaustively with petroleum ether in a Soxhlet apparatus.

If a Soxhlet extractor is not available, extract the trituration of the sample with a mixture of equal parts of sodium sulphate and quartz sand by the method described by Ernst et al. for extraction of fat from marine animals.

In both cases, concentrate the extract solution on a rotary evaporator, and dilute to the mark with petroleum ether in a 50-ml volumetric flask.

If, in addition to organochlorine pesticides, lipoid-soluble organophosphorus pesticides are also required to be determined in the analysis, then diethyl ether should be used instead of petroleum ether for the extraction.

Determine the fat content of the extract by evaporating an aliquot of the extract solution (e.g. 10 ml) in a weighed platinum crucible. Rotary-evaporate the remaining extract solution just to dryness at 30 to 40 °C. Dissolve the residue remaining after evaporation in 25 ml petroleum ether saturated with dimethylformamide.

#### 6.1.2. Milk (modified procedure according to AOAC)

Place 100 g milk in a 500-ml separatory funnel, add 1 g finely ground sodium oxalate, and shake with 100 ml methanol for 15 s. Add 100 ml diethyl ether, and shake cautiously but thoroughly for 1 min. Then add 100 ml petroleum ether, and again shake cautiously but well for 1 min.

Let the phases separate. Drain off the lower, aqueous-methanolic phase and discard. Measure the volume of the organic phase. Dry on sodium sulphate, and then determine the fat content of the extract in an aliquot of the solution, containing 0.2–0.5 g fat, by evaporation in a weighed

platinum crucible. Rotary-evaporate the remaining extract solution to a few ml. Remove the last traces of solvent with the aid of a gentle stream of air or nitrogen. Dissolve the residue remaining after evaporation in 25 ml petroleum ether saturated with dimethylformamide.

#### **6.1.3. Condensed milk**

Dilute condensed milk with water to a fat content of 3.5%. Process 100 g of this dilution as described for milk in step 6.1.2.

#### **6.1.4. Whole (unskimmed) milk powder**

Dissolve whole milk powder in water of 40°C. Dilute the solution to a fat content of 3.5%, and stir for 30 min at 40°C, e.g. with a magnetic stirrer. Process 100 g of this dilution as described for milk in step 6.1.2.

#### **6.1.5. Butter**

Heat butter at 50 to 60°C until the fat phase separates. Dissolve 3 to 5 g of the isolated butter fat in 25 ml petroleum ether saturated with dimethylformamide.

#### **6.1.6. Dried egg products**

Triturate 15 g dried whole egg or 10 g dried egg yolk with 10 ml water having a temperature of 40°C. Then add a five or six times bigger amount by weight of sodium sulphate, and triturate to a dry, powdery mixture. Extract this mixture as described in step 6.1.1.

#### **6.1.7. Fats and oils**

Dissolve 3 to 5 g of a fat or oil in 25 ml petroleum ether saturated with dimethylformamide.

### **6.2. Cleanup**

#### **6.2.1. Dimethylformamide-petroleum ether partition**

Transfer the solution of fat (dissolved in 25 ml petroleum ether saturated with dimethylformamide) derived from step 6.1.1, 6.1.2, 6.1.5 or 6.1.7, with the aid of a pipette, to a 250-ml separatory funnel. Rinse the flask with small portions of a previously measured amount of 75 ml dimethylformamide. Then add the remainder of the dimethylformamide to the separatory funnel, and shake vigorously for 1 min. Drain the dimethylformamide phase, and again extract the petroleum ether phase with 10 ml dimethylformamide.

Transfer the combined dimethylformamide phases to a 500-ml separatory funnel, and add 200 ml sodium sulphate solution. Add a few drops of eosin solution to achieve better recognition of phase separation in the subsequent partition. Then extract successively with a 40-ml portion and three 25-ml portions of petroleum ether for 1 min each time. Wash the combined petroleum ether phases with 10 ml water, dry on sodium sulphate, filter through a cottonwool plug, add 5 ml n-hexane, and concentrate on a rotary evaporator to approx. 5 ml.

### 6.2.2. Florisil column chromatography

About half-fill a chromatographic tube with petroleum ether, and sprinkle in 30 g Florisil in small portions through a funnel with stopcock open, tapping the column in the process. Avoid formation of bubbles in the column packing! Cover the Florisil with an approx. 2-cm layer of sodium sulphate. Drain the supernatant solvent to the top of the column packing.

Pipette the solution derived from 6.2.1 onto the column. Let the solution percolate to a level of 1–2 mm above the top of the column. Then rinse the flask with small portions of eluting mixture I, add the rinsings to the column, and also let them percolate to a level of 1–2 mm above the top of the column. Next elute the column with the remainder of the total 200-ml amount of eluting mixture I, at a flow rate of about 5 ml/min.

Add 5 ml n-hexane to the eluate, concentrate the eluate on a rotary evaporator to approx. 5 ml, quantitatively rinse with n-hexane into a volumetric flask or test tube with ground joint (graduated), and dilute with n-hexane to 10 ml (= eluate I).

If, in addition to the organochlorine compounds, it is required also to determine the organophosphorus pesticides, stop the elution just before the last traces of eluting mixture I have infiltrated into the column packing. Then change the receiver, and continue to elute with 300 ml eluting mixture II. Process the first eluate as described above (= eluate I). Concentrate the second eluate on a rotary evaporator to approx. 5 ml, quantitatively rinse with ethyl acetate into a volumetric flask or test tube with ground joint (graduated), and dilute with ethyl acetate to 10 ml (= eluate II).

## 6.3. Gas-chromatographic determination

Inject aliquots of the solutions derived from 6.2.2 (eluate I and eluate II) into the gas chromatograph.

The determinations were performed with different gas chromatographs suitable for simultaneous operation with an electron capture detector (ECD) and thermionic phosphorus detector (AFID) or flame photometric detector (FPD).

### *Operating conditions*

#### 6.3.1.

Gas chromatograph	Hewlett-Packard 5755 G
Column tubes	Glass, 1.8 m long, 4 mm i. d. (1.2 m long for column [XE-60])
Column [OV-101]	5% OV-101 on Gas Chrom Q, 80–100 mesh; conditioned for 24 h at 250°C Temperature isothermal for 5 min at 180°C, programmed to rise at 2°C/min to 200°C, then isothermal for 5 min at 200°C
Column [OV-17/QF-1]	1.5% OV-17 + 1.95% QF-1 on Chromosorb W-HP, 100–120 mesh; conditioned for 36 h at 250°C Temperature isothermal for 5 min at 200°C, programmed to rise at 2°C/min to 220°C, then isothermal for 5 min at 220°C

Column [XE-60]	2.5% XE-60 on Chromosorb G-AW-DMCS, 80–100 mesh; conditioned for 12 h at 230°C
Column [DEGS]	2% Diethylene glycol succinate (DEGS) + 0.5% H <sub>3</sub> PO <sub>4</sub> on Chromosorb W-HP, 100–120 mesh; conditioned for 1 h at 200°C Temperature 180°C
Injection port temperature	230°C
Detectors	Electron capture detector ( <sup>63</sup> Ni ECD), pulse interval 50 µs Temperature 245°C (280°C with column [DEGS]) Alkali flame ionization detector (Hewlett-Packard 15161 B) (AFID) Temperature 380°C Flame photometric detector (Micro-Tek) fitted with 520-nm phosphorus filter (FPD) Temperature 190°
Gas flow rates	Helium carrier, 90 ml/min, splitted at column outlet in ECD : P-detector ratio of 1:2 (glass splitters supplied by Reiss) Argon-methane 9:1 v/v, 60 ml/min (purge gas for ECD) Hydrogen, 30 ml/min (for AFID), 110 ml/min (for FPD) Air, 200 ml/min (for AFID), 150 ml/min (for FPD)
Recorder	1 mV; chart speed 10 mm/min
Injection volume	3–5 µl

### 6.3.2.

Gas chromatograph	Perkin-Elmer F-20 FE
Column tubes	Glass, 2 mm i. d., 2 m long
Column [QF-1/DC-200]	Mixture of equal parts by weight of 15% QF-1 on Gas Chrom Q, 80–100 mesh, and 10% DC-200 (12500 cst) on Gas Chrom Q, 80–100 mesh; conditioned for 36 h at 250°C Temperature 200°C
Column [XE-60]	2.5% XE-60 on Chromosorb G-AW-DMCS, 80–100 mesh; conditioned for 12 h at 230°C Temperature 190°C
Injection port temperature	250°C
Detectors	Electron capture detector ( <sup>3</sup> H ECD), pulse interval 50 µs Temperature 200°C Alkali flame ionization detector (KCl, own construction) (AFID) Temperature 200°C

Gas flow rates	Nitrogen carrier, 60 ml/min, splitted at column outlet in ECD : AFID ratio of 1 : 2 (glass splitters supplied by Reiss)
	Nitrogen, 120 ml/min (purge gas for ECD)
	Hydrogen, 30 ml/min (for AFID)
	Air, 320 ml/min (for AFID)
Recorder	1 mV; chart speed 10 mm/min
Injection volume	3–5 µl

### 6.3.3. Comments

The retention times for aldrin on the different columns and under the conditions described in Sections 6.3.1 and 6.3.2 were as follows:

Column [OV-101]	6 min 15 s
Column [QF-1/DC-200]	3 min 30 s
Column [OV-17/QF-1]	4 min
Column [XE-60]	1 min 50 s
Column [DEGS]	1 min 45 s

The retention times relative to aldrin = 1.00 for the analyzed compounds on the five different columns are given in Table 1. Camphechlor gives several peaks on all columns; the position of these peaks must each be determined by recording a reference chromatogram.

A proven practice for the simultaneous determination of organochlorine and organophosphorus pesticides is to use a combination of one column and two detectors. For identification and quantitation, however, the analysis must be performed on at least two different columns one of which should be a polar column (Column [XE-60] or Column [DEGS]).

Detector sensitivity should be set at a level at least high enough for injection of 3 µl of one of the standard solutions to give peaks of at least half FSD.

Prior to injection of the first sample solution, a standard solution should be injected at least three times to check the operating conditions and the constancy of the detector signals. Further, linearity of the ECD signal should be checked by injecting serial dilutions preferably of solutions of HCB and p, p'-DDT. A standard solution should be injected after at least every other sample solution so that any alterations of the gas-chromatographic system can be recognized (e.g. conversion of p,p'-DDT to p,p'-DDD due to column contamination).

When other instruments are used, the operating conditions chosen should be such that optimal column efficiencies will be achieved and that the retention times will be 2 to 4 min for lindane and aldrin, 10 to 16 min for p,p'-DDT, and 1.5 to 3 min for diazinon.

Table 1. Retention times of organochlorine and organophosphorus compounds relative to aldrin = 1.00

Pesticide	OV-101	QF-1/ DC-200	OV-17/ QF-1	XE-60	DEGS
$\alpha$ -HCH	0.41	0.46	0.58	0.94	1.20
$\beta$ -HCH	0.44	0.59	0.79	3.67	5.18
HCB	0.45	0.44	0.54	0.43	0.56
$\gamma$ -HCH	0.48	0.58	0.72	1.36	1.75
$\delta$ -HCH	0.50	0.67	0.92	3.80	4.82
Heptachlor	0.81	0.82	0.85	0.95	0.98
Aldrin	1.00	1.00	1.00	1.00	1.00
Oxychlordane			1.08		1.52
Heptachlor epoxide	1.24	1.45	1.45	2.20	2.42
$\gamma$ -Chlordane	1.40	1.51	1.56		2.39
$\alpha$ -Chlordane	1.56	1.65	1.68	2.45	2.60
p,p'-DDE	1.75	1.80	1.92	3.91	2.95
Dieldrin	1.75	2.10	2.13	3.48	3.54
Endrin	1.93	2.44	2.54	3.83	4.04
p,p'-DDD (TDE)	2.13	2.53	2.70	6.52	8.95
$\alpha$ ,p'-DDT	2.20	2.39	2.60	3.64	4.19
p,p'-DDT	2.61	3.07	3.16	5.90	7.57
Methoxychlor	3.36	4.37		10.8	
Diazinon		0.55	0.70	0.65	
Fenchlorphos		0.92	1.08	1.50	
Bromophos		1.25	1.45	2.21	
Malathion		1.35	1.44	2.72	
Parathion-ethyl		1.73	1.61	3.70	
Chlorgenvinphos		1.83	1.87	3.45	
Carbophenothion		3.09	3.37	6.93	

## 7. Evaluation

### 7.1. Method

Identification is achieved by determination of the retention times relative to aldrin (Table 1). Further indications of the identity of the assumed compound are provided by the selective response of the different detectors and by the occurrence of the compound in the two eluates collected from the Florisil column. Eluate I contains all organochlorine pesticides as well as the organophosphorus pesticides bromophos, carbophenothion and fenchlorphos. Diazinon and parathion-ethyl occur in eluates I and II; chlorgenvinphos and malathion occur only in eluate II.

Quantitation is performed by measuring the peak area and comparing it with the peak areas of the same compound from standard solutions of known concentration. Equal volumes of the sample solution and the standard solutions should be injected. When using an ECD, it is especially important to ascertain that the detector is indeed operating within its linear range and then to inject into the gas chromatograph weights of sample residue and reference standard

which fall within that range. Calculation of the residue must take account of the extract aliquot taken for determination of fat.

## 7.2. Recoveries

Recoveries from untreated control samples of butter fat, lard and unskimmed milk powder fortified with the analyzed compounds at levels of 0.1 to 1.0 mg/kg (3.0 mg/kg for camphechlor) are given in Table 2; they ranged from 80 to 100%.

Table 2. Recoveries from butterfat, lard, and unskimmed milk fortified with organochlorine and organophosphorus pesticides

Pesticide	Recovery (%)	Pesticide	Recovery (%)
HCB	82– 96	Bromophos	96–100
α-HCH	95–101	Carbofenothon	88– 98
β-HCH	95–100	Chlorfenvinphos	81– 86
γ-HCH	93–100	Diazinon	95–100
δ-HCH	91–100	Fenchlorphos	93– 99
Aldrin	86– 92	Malathion	98–100
Dieldrin	94–102	Parathion-ethyl	98–100
Endrin	85– 91		
Heptachlor	96–100		
Heptachlor epoxide	98–100		
α-Chlordane	92–100		
γ-Chlordane	86– 91		
Oxychlordane	89– 93		
Camphechlor	95– 97		
p,p'-DDD (TDE)	98–100		
p,p'-DDE	95–101		
o,p'-DDT	98–100		
p,p'-DDT	92–100		
Methoxychlor	85– 94		

## 7.3. Collaborative studies

The *GDCh-Arbeitsgruppe "Pestizide"* checked the described method of analysis in five collaborative studies in which the laboratories analyzed samples of edible oil, condensed milk, whole (unskimmed) milk powder and dried whole egg containing organochlorine pesticides at concentrations ranging from 0.02 mg/kg to 1.8 mg/kg.

Details of the comparative statistical evaluation for HCB, γ-HCH and total DDT, the coefficients of variation obtained and the deviations of the results from the actual concentrations contained in the samples are given in the 4. Mitteilung (Fourth Communication).

## 8. Important points

### 8.1. Other alternatives for cleanup of eluate I

After foods containing volatile, nonpolar constituents and some oils and fats have been cleaned up by the described procedure, they may still give interfering peaks in the gas chromatogram. In these cases, eluate I can be further cleaned up, the choice of the procedure used depending upon the analytical material. However, after this additional cleanup, some pesticides may no longer be detectable.

Often, it is sufficient simply to shake eluate I with concentrated sulphuric acid as described by Murphy or to carry out an appropriate column-chromatographic cleanup by the method of Hahn and Thier, which is suitable especially for determination of HCH isomers, HCB, DDT, DDD, DDE and PCBs. Treatment with sulphuric acid, however, results in decomposition of aldrin, dieldrin, endrin, methoxychlor and the organophosphorus pesticides.

For additional cleanup of eluate I and for identification of some pesticides, proven procedures are those described by Holmes and Wood (especially for the removal of interfering carotenoids and sulphides), by Zimmerli and Marek (especially for determination of HCB), by Masumoto (for separation of PCBs), by Hadorn and Zürcher (especially for dieldrin), and in the 1. Empfehlung (First Recommendation) of the *GDCh-Arbeitsgruppe "Pestizide"* for thin-layer chromatographic separation, followed where appropriate by gas-chromatographic identification.

### 8.2. Possible interferences

With some foods of animal origin, e. g. intensely dried or roasted products, the extracted portion of fat and pesticide may happen to be smaller than the content of fat determined by the customary methods. If this difference exceeds 10%, quantitative extraction of the pesticide residues will fail to be assured. In such cases, the extraction must be modified to obtain a fat yield equivalent to at least 90% of the directly determined value.

The presence of polychlorinated biphenyls (PCBs) may interfere with the quantitation of organochlorine pesticides through superposition of the peaks. In order to recognize the interference, reference chromatograms of the different types (Aroclor and Clophen of different degrees of chlorination) must be recorded on all columns used. PCBs can be separated from most other pesticides by the method of Masumoto.

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# Potato Sprout Suppressants Propham and Chlorpropham

S 11

Potatoes

Gas-chromatographic  
determination

(German version published 1976)

## 1. Introduction

Propham and chlorpropham are used, sometimes also in combination, to inhibit sprouting of potatoes. Wherever both compounds occur together, quantitative colorimetric determination of their residues by compound-specific methods is not readily feasible, added to which it involves high work input. On the other hand, the method reported herein permits residues of both these N-phenyl carbamates to be identified and quantified together by gas chromatography. The ability to achieve this is of importance also in the light of the Federal German Regulation on Maximum Pesticide Residue Limits, in which a tolerance is established for the sum of propham and chlorpropham.

## 2. Outline of method

The sample is extracted with acetonitrile. The extract is diluted with water, and shaken with a mixture of petroleum ether and dichloromethane. The organic phase is cleaned up by chromatography on a Florisil column. By treating the residue with bromine, isopropyl 2,4-dibromo-phenylcarbamate is derived from propham, and isopropyl 2,4-dibromo-5-chlorophenylcarbamate is derived from chlorpropham. Both bromine derivatives can be determined separately without decomposition by electron capture gas chromatography at high sensitivity.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Buchner porcelain funnel, approx. 10 cm dia.

Filtration flask, 500-ml

Separatory funnels, 500-ml and 100-ml

Round-bottomed flasks, 500-ml and 100-ml, with ground joints

Rotary vacuum evaporator

Chromatographic tube, 10 mm i. d., 30–40 cm long, fitted with stopcock and with 100 to 150-ml reservoir at upper end

Pear-shaped flask, 25-ml, long form, with ground joint and stopper

Gas chromatograph equipped with electron capture detector

Microsyringe, 10- $\mu$ l

## 4. Reagents

Acetonitrile, for residue analysis (e.g. Merck or Mallinckrodt)  
 Benzene, for residue analysis (e.g. Merck or Mallinckrodt)  
 Dichloromethane, for residue analysis (e.g. Merck or Mallinckrodt)  
 Glacial acetic acid, pure  
 Petroleum ether, for residue analysis, boiling range 40–60°C (e.g. Merck or Mallinckrodt)  
 Eluting mixture: petroleum ether + dichloromethane 8 : 2 v/v  
 Standard solution: Place 1.00 ml of a solution containing 2 µg propham and 2 µg chlorpropham/ml acetone in a pear-shaped flask, carefully evaporate to dryness, and continue to process as for the sample solution in step 6.3. 1 µl of the thus obtained standard solution is equivalent to an amount of 0.1 ng propham and 0.1 ng chlorpropham  
 Bromine + iodine mixture (95 : 5): dissolve 5 g finely powdered iodine in 95 g bromine p. a.  
 Sodium acetate solution, 20 g/100 ml CH<sub>3</sub>COONa · 3H<sub>2</sub>O  
 Sodium metaarsenite solution, 5 g/100 ml NaAsO<sub>2</sub> (Merck No. 6287)  
 Sodium chloride solution, saturated  
 Sodium sulphate, p. a., anhydrous, heated at 500 °C  
 Florisil, 60–100 mesh: Heat for at least 5 h at 130 °C, let cool in a desiccator, and add 5% (w/w) water. Shake this mixture for at least 20 min, and then store in a stoppered container for at least 24 h with occasional shaking  
 Filter aid, e.g. Celite 545 (Roth)  
 Glass wool  
 Nitrogen, re-purified

## 5. Sampling and sample preparation

To obtain a representative analytical sample, aliquots of the laboratory sample are comminuted and thoroughly mixed. The portions required for analysis are weighed out from this mixture. See p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Weigh 100 g of the analytical sample (G) into the blender jar, add 100 ml acetonitrile, and homogenize at high speed for about 2 min. Then add 10 g Celite, and re-blend briefly. Suction-filter the homogenate through an acetonitrile-moistened filter paper in a Buchner porcelain funnel. Press the filter cake with a glass stopper until it is completely dry.

Transfer the filtrate to a 500-ml separatory funnel, and add 50 ml eluting mixture. Shake vigorously, add 20 ml sodium chloride solution and 30 ml water, and again shake vigorously. If three phases form in the process, continue to process the two upper phases jointly. Extract the lower aqueous phase again with two 50-ml portions of eluting mixture. Wash the combined organic phases with two 250-ml portions of water. Dry for 15 min on approx. 10 g sodium sulphate, decant, rinse the sodium sulphate with approx. 10 ml eluting mixture, and rotary-

evaporate the solution to near dryness. Remove the last traces of solvent with the aid of a gentle stream of nitrogen.

## 6.2. Cleanup

Tamp a small plug of glass wool into the bottom of a chromatographic tube. About half-fill the tube with eluting mixture. Slowly add 5 g Florisil. Allow the Florisil to settle to a height of 6–7 cm (takes about 1 min). Then cover the Florisil with an approx. 2-cm layer of sodium sulphate. Drain the supernatant solvent to a level of 0.5 cm above the column packing.

Dissolve the evaporation residue from 6.1 in 10 ml eluting mixture, and add the solution to the column. Allow to percolate. Rerinse the flask with the first portions of eluant. Elute the column with 150 ml eluting mixture at a flow rate of approx. 5 ml/min. Never allow the column to run dry. Concentrate the eluate to about 5 ml on a rotary evaporator. Transfer the solution to a pear-shaped flask, with rinsing, and cautiously concentrate to near dryness. Remove the last traces of solvent with a gentle stream of nitrogen.

## 6.3. Preparation of bromine derivatives

Dissolve the evaporation residue from 6.2 in 0.5 ml glacial acetic acid, add 6 drops of bromine-iodine mixture from a dropping pipette, mix rapidly by swirling, close the flask with a ground stopper, and let stand stoppered for 10 min at room temperature.

Then add successively 5 ml sodium acetate solution and 5 ml sodium metaarsenite solution, and quantitatively transfer the colourless, slightly cloudy solution to a small separatory funnel. Extract vigorously with two 10-ml portions of benzene. Between the two extractions, drain the aqueous phase into the pear-shaped flask to rinse it out. Dry the combined benzene phases on approx. 2 g sodium sulphate for 15 min ( $V_{\text{End}} = 20 \text{ ml}$ ).

## 6.4. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the solution derived from step 6.3 into the gas chromatograph.

If the amounts found exceed 0.5 ng prophan or chlorprophan (equivalent to 0.05 mg/kg), these may already be above the linear range of response of some electron capture detectors. In this case, take  $V_A$  ml from the solution derived from step 6.3, and dilute it with benzene to an appropriate total volume of  $V_B$  ml.

### *Operating conditions*

Gas chromatograph	Varian Aerograph 1840
Column 1	Glass, 1.5 mm i. d., 1.5 m long; packed with 1.5% QF-1 + 1% DC-200 on Chromosorb G-AW-DMCS, 80–100 mesh
Column 2	Glass, 3 mm i. d., 1.5 m long; packed with 2.5% XE-60 on Chromosorb G-AW-DMCS, 80–100 mesh
Column temperature	200 °C
Injection port temperature	230 °C
Detector	Electron capture detector ( $^3\text{H}$ ECD) Temperature 200 °C

Gas flow rate	Nitrogen carrier, 22 ml/min (column 1) or 85 ml/min (column 2)
Attenuation	16–64 · 10 <sup>-10</sup>
Recorder	1mV; chart speed 10 inches (25.4 cm)/h
Injection volume	2–4 µl
Retention times for bromine derivatives of propham	3 min 45 s (column 1) or 3 min 50 s (column 2)
chlorpropham	7 min 40 s (columns 1 and 2)

The retention times of the bromine derivatives relative to aldrin = 1.00 were 0.80 for propham and 1.49 for chlorpropham on column 1; 1.00 for propham and 1.95 for chlorpropham on column 2. The peak height given by 0.1 ng propham and 0.2 ng chlorpropham was about the same as that given by 0.1 ng aldrin.

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak height of the sample solution and comparing it with the peak heights obtained for the brominated compounds from the standard solution or from the diluted standard solution. Equal volumes of sample solution and standard solution should be injected.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with the compounds at levels of 0.1 to 1.0 mg/kg ranged from 92 to 95% and averaged 94% for propham, and ranged from 87 to 100% and averaged 91% for chlorpropham. The routine limit of determination is approx. 0.01 mg/kg.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, is calculated for each of the two compounds from the following equation:

$$R = \frac{H_A \cdot V_{End} \cdot V_B \cdot W_{St}}{H_{St} \cdot V_i \cdot V_A \cdot G}$$

where

- G           = sample weight (in g)
- V<sub>End</sub>     = terminal volume of sample solution from 6.3 (in ml)
- V<sub>A</sub>       = portion of volume V<sub>End</sub> used for dilution in 6.4 (in ml)
- V<sub>B</sub>       = total volume of dilution prepared from V<sub>A</sub> in 6.4 (in ml)

$V_i$	= portion of volume $V_{End}$ or of volume $V_B$ injected into gas chromatograph (in $\mu\text{l}$ )
$W_{St}$	= amount of compound injected with standard solution (in ng)
$H_A$	= peak height obtained from $V_i$ (in mm)
$H_{St}$	= peak height obtained from $W_{St}$ (in mm)

## 8. Important points

Special attention must be given to the purity of the solvents and reagents used. If interfering peaks occur, solvents and reagents must be tested for purity by a blank analysis.

Extraction and cleanup by the procedures described in step 6.1 and 6.2 result in the detection also of other less polar pesticides if they are eluted with the eluting mixture from the Florisil column. However, most of them, especially the organophosphates, are altered to such an extent during preparation of the bromine derivatives in step 6.3 that they then fail to elicit any response from the electron capture detector. On the other hand, the organochlorine insecticides are not influenced by bromination.

To what extent organochlorine insecticides are responsible for unexpected occurrence of interfering peaks can be established easily by subjecting the cleaned-up extracts to gas chromatography prior to bromination. If present, organochlorine insecticides can be separated by eluting them from the Florisil column with approx. 100 ml petroleum ether. They can then be determined quantitatively in the eluate. Subsequently, the residues of prophan and chlorprophan are then eluted with eluting mixture as described in step 6.2.

## 9. References

*H.-P. Thier*, Gaschromatographische Rückstandsanalyse der Keimhemmungsmittel Prophan und Chlorprophan in Kartoffeln, Mitteilungsbl. GDCh-Fachgruppe Lebensmittelchem. gerichtl. Chem. 26, 220–221 (1972).

*H.-P. Thier*, Analysengang zur Ermittlung von Pestizid-Rückständen in Pflanzenmaterial, Dtsch. Lebensm. Rundsch. 68, 345–350 and 397–401 (1972).

## 10. Authors

Institute of Food Chemistry, University of Münster, *H.-P. Thier*  
Municipal Laboratory of Food and Environmental Control, Nürnberg, *H. Hahn*



Apples, asparagus, cauliflower, cherries, endives, greengages, kohlrabi, lemons, mandarin oranges, melons, potatoes, strawberries, sweet peppers, tomatoes

Gas-chromatographic determination after cleanup by sweep co-distillation

(German version published 1979)

## 1. Introduction

The method permits determination of residues of 24 organochlorine compounds used as insecticides or fungicides or that occur as their metabolites. The extracts from the plant material are cleaned up by sweep co-distillation as described in Cleanup Method 2 in this Manual.

## 2. Outline of method

The plant material is extracted with acetone. An aliquot of the extract is diluted with water and shaken with dichloromethane. The evaporation residue of the organic phase is cleaned up by sweep co-distillation in an appropriate apparatus, and the distillate is analyzed by electron capture gas chromatography.

## 3. Apparatus

High-speed blender fitted with leak-proof jar and explosion-proof motor, or homogenizer (e.g. Ultra-Turrax)

Buchner porcelain funnel, 12 cm dia.

Filtration flask, 500-ml

Fluted filter paper, 24 cm dia. (Schleicher & Schüll, No. 1450 1/2); extracted with ethyl acetate for 5 h in a Soxhlet apparatus

Round filter paper, 12 cm dia. (Schleicher & Schüll, No. 602 eh)

Separatory funnel, 500-ml

Round-bottomed flask, 500-ml, with NS 29 ground joint

Pear-shaped flask, 50-ml, with NS 14.5 ground joint

Rotary vacuum evaporator

Sweep co-distillation apparatus

Gas chromatograph equipped with electron capture detector

Microsyringe, 10- $\mu$ l

## 4. Reagents

Acetone, high purity, dist., boiling point 56–56.5 °C  
Dichloromethane, p. a.  
Ethyl acetate, p. a.  
n-Hexane, p. a., dist.  
Pesticide standard solutions: 0.1–0.5 µg/ml n-hexane  
Sodium chloride solution, saturated  
Sodium sulphate, p. a., anhydrous  
Filter aid, e. g. Celite 545 (Roth)  
Argon + methane mixture 95 : 5 v/v  
Helium

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Cut 100 g of the analytical sample (G) into small pieces, and homogenize with 200 ml acetone for 3 min with an Ultra-Turrax or in the blender jar. Mix the homogenate with 5 g Celite, and suction-filter through an acetone-moistened round filter paper in a Buchner porcelain funnel. Rinse homogenizer and filter cake with two 25-ml portions of acetone. Measure the volume of the filtrate ( $V_{Ex}$ ).

Transfer one fifth of the filtrate volume ( $V_{R1}$ ) to a 500-ml separatory funnel, and dilute with 200 ml water and 35 ml saturated sodium chloride solution. Add 50 ml dichloromethane, and shake vigorously for 2 min. Separate the dichloromethane phase, extract the aqueous phase once more with 50 ml dichloromethane and discard. Wash the combined dichloromethane phases with a mixture of 200 ml water and 25 ml saturated sodium chloride solution, and dry on 10 g sodium sulphate. Filter through a fluted filter paper, and rinse the filter cake with three 10-ml portions of dichloromethane. Concentrate the filtrate to approx. 20 ml on a rotary evaporator, transfer to a 50-ml pear-shaped flask, add 5 ml n-hexane, and concentrate to 1 ml.

### 6.2. Cleanup

Cleanup is performed by sweep co-distillation as described in Section 5.1 of Cleanup Method 2, using the whole of the solution derived from step 6.1. The distillate obtained from this cleanup is concentrated to 1 ml ( $V_{End}$ ) on a rotary evaporator.

### 6.3. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the cleaned-up solution derived from step 6.2 into the gas chromatograph.

#### *Operating conditions*

##### 6.3.1. Column 1

Gas chromatograph	Hewlett-Packard 5730 A
Column	Glass, 2 mm i. d., 3.8 m long; packed with 5% QF-1 on Chromosorb W-AW-DMCS, 80–100 mesh
Column temperature	200 °C
Injection port temperature	300 °C
Detector	$^{63}\text{Ni}$ electron capture detector (Hewlett-Packard 18713 A); $5.55 \cdot 10^8 \text{ s}^{-1}$ , linear Temperature 300 °C
Carrier gas flow rate	Argon-methane, 30 ml/min
Attenuation	1 · 64
Recorder	Hewlett-Packard 7130 A
Integrator	Hewlett-Packard 3380 A
Injection volume	1–5 µl

##### 6.3.2. Column 2

Gas chromatograph	Hewlett-Packard 5750
Column	Glass, 2 mm i. d., 1.9 m long; packed with 2% SE-30 on Chromosorb W-AW-DMCS, 80–100 mesh
Column temperature	190 °C
Injection port temperature	320 °C
Detector	$^{63}\text{Ni}$ electron capture detector (F. & M. Scientific, Hewlett-Packard Model 2 · 6195); $7.4 \cdot 10^7 \text{ s}^{-1}$ Pulse interval 150 µs Temperature 300 °C
Gas flow rates	Helium carrier, 40 ml/min Argon-methane, 90 ml/min
Attenuation	10 · 16
Recorder	Hewlett-Packard 7128 A
Injection volume	1–5 µl

The retention times obtained are given in Table 1.

## 7. Evaluation

### 7.1. Method

Identification is achieved by determining the retention time and comparing it with that of compounds from appropriately diluted standard solutions. Quantitation is performed by measur-

Table 1. Retention times  
Column 1 (QF-1)

Compound	Retention time		Compound	Retention time	
	min	s		min	s
Hexachlorobenzene	2	56	$\alpha$ -Chlordane	8	24
Tecnazene	3	10	p,p'-DDE	8	31
$\alpha$ -HCH	3	30	Endosulfan	9	49
$\gamma$ -HCH	4	7	Dichlofluanid	10	25
Quintozene	4	17	$\alpha$ , $\beta$ -DDT	10	28
Heptachlor	4	30	Dieldrin	11	26
$\beta$ -HCH	4	34	Folpet	12	49
$\delta$ -HCH	4	58	Endrin	13	11
Aldrin	5	6	p,p'-DDD	13	14
Heptachlor epoxide	7	4	Captan	13	20
Dicofol	7	52	p,p'-DDT	14	26
$\gamma$ -Chlordane	8	10	Methoxychlor	20	12

Column 2 (SE-30)

Compound	Retention time		Compound	Retention time	
	min	s		min	s
Tecnazene	43		Folpet	2	28
$\alpha$ -HCH	55		Heptachlor epoxide	2	29
$\beta$ -HCH	59		$\gamma$ -Chlordane	2	47
Hexachlorobenzene	60		$\alpha$ -Chlordane	3	2
$\delta$ -HCH	1	5	Endosulfan	3	4
Quintozene	1	5	p,p'-DDE	3	20
$\gamma$ -HCH	1	6	Dieldrin	3	33
Heptachlor	1	42	Endrin	3	59
Dicofol	1	58	p,p'-DDD	4	20
Aldrin	2	3	$\alpha$ , $\beta$ -DDT	4	42
Dichlofluanid	2	8	p,p'-DDT	5	45
Captan	2	23	Methoxychlor	8	19

ing the peak area and comparing it with the peak areas from appropriately diluted standard solutions. Equal volumes of the sample solution and the standard solutions should be injected.

## 7.2. Recoveries and lowest determined concentration

Table 2 lists the combinations of compounds and crops on which the method has been tested. The figures given in this table represent the residue levels in mg/kg tested in recovery experiments. In about 90% of the cases, the recoveries ranged from 80 to 100% (marked thus +); in other cases they were lower (marked thus -). The routine limit of determination generally was 0.0025–0.005 mg/kg.

Table 2. Combinations of compounds and crops on which method has been tested

The figures give the concentration in mg/kg at which the recovery experiments were performed.

- + Signifies that the compound, at the given concentration (expressed in mg/kg), was recovered at a rate of more than 80%.

- Signifies that at the given concentration the compound was not determinable or that the recovery rate was less than 80%.

Where no symbols are given for a compound alongside a substrate, this indicates that determination of the compound in the particular analytical material has not yet been tried.

Table 2. (contd.)

Table 2. (contd.)

Crop	Compounds							
	$\gamma$ -HCH 0.0025	$\delta$ -HCH 0.0025	Heptachlor 0.0025	Heptachlor epoxide 0.0025	Hexachloro- benzene 0.0025	Methoxychlor 0.025	Quintozone 0.0025	Tecnazine 0.00125
Apples	+	+	+	+	+	-	-	
Asparagus	+	+	+	+	+	+	+	+
Cauliflower	+	-	+	+	+	-	+	+
Cherries	+	+	+	+	+	+	-	+
Endives	-	-	+	+	+	-	+	+
Greengages	+	+	+	+	+			
Kohlrabi	+	+	+	+	+	-	+	+
Lemons	+	+	+	+	+	-	+	-
Mandarin oranges	+	+	+	+	+	-	+	+
Melons	+	+	+	+	+	+	+	+
Peppers	+	+	+	+	+	-	+	+
Potatoes	+	-	+	+	+	+	+	+
Strawberries	+	+	+	+	+	+	+	+
Tomatoes	+	+	+	+	+	-	+	+

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, of an identified compound is calculated from the following equation:

$$R = \frac{F_A \cdot V_{\text{End}} \cdot V_{\text{Ex}} \cdot W_{\text{St}}}{F_{\text{St}} \cdot V_i \cdot V_{\text{R1}} \cdot G}$$

where

G = sample weight (in g)

$V_{\text{Ex}}$  = volume of total extract from sample (in ml)

$V_{\text{R1}}$  = portion of volume  $V_{\text{Ex}}$  used for extraction with dichloromethane (in ml)

$V_{\text{End}}$  = terminal volume of sample solution from 6.2 (in ml)

$V_i$  = portion of volume  $V_{\text{End}}$  injected into gas chromatograph (in  $\mu$ l)

$W_{\text{St}}$  = amount of compound injected with standard solution (in ng)

$F_A$  = peak area obtained from  $V_i$  (in  $\text{mm}^2$ )

$F_{\text{St}}$  = peak area obtained from  $W_{\text{St}}$  (in  $\text{mm}^2$ )

### 8. Important points

It is recommended to use an automatic sampler and to employ peak integration for the gas-chromatographic determination.

## 9. Reference

*J. Pflugmacher and W. Ebing, Reinigung Pestizidrückstände enthaltender Rohextrakte mit einer automatisch arbeitenden Apparatur nach dem Prinzip der kombinierten Spül- und Co-destillation (Sweep Co-Distillation), Fresenius Z. Anal. Chem. 263, 120–127 (1973).*

## 10. Author

Federal State Control Laboratory of Food and Environmental Chemistry, Offenburg, *M. Eichner*



Apples, asparagus, beans, carrots, cauliflower, celeriac, cherries, cucumbers, grapes, head cabbage, kohlrabi, lettuce, pears, pineapples, potatoes, red cabbage, Savoy cabbage, small radishes, spinach, strawberries, sweet peppers, tomatoes

Gas-chromatographic determination after cleanup by sweep co-distillation

(German version published 1979)

## 1. Introduction

The method permits determination of residues of 35 organophosphorus insecticides and three of their metabolites. The extracts from the plant material are cleaned up by sweep co-distillation as described in Cleanup Method 2 in this Manual.

## 2. Outline of method

The sample material of some of the listed crops is extracted with dichloromethane, and the extract is evaporated. Other plant material is extracted with acetone. The extract is diluted with water, shaken with dichloromethane, and the organic phase is evaporated. The extracts are cleaned up by sweep co-distillation in an appropriate apparatus, and the distillate is analyzed by gas chromatography using a phosphorus-specific thermionic detector.

## 3. Apparatus

High-speed blender fitted with leak-proof glass jar and explosion-proof motor, or homogenizer (e.g. Ultra-Turrax)

Buchner porcelain funnel, 12 cm dia.

Filtration flask, 500-ml

Fluted filter paper, 24 cm dia. (Schleicher & Schüll, No. 1450 1/2); extracted with ethyl acetate for 5 h in a Soxhlet apparatus

Round filter paper, 12 cm dia. (Schleicher & Schüll, No. 602 eh)

Separatory funnels, 1-l and 500-ml

Round-bottomed flask, 500-ml, with NS 29 ground joint

Kuderna-Danish evaporative concentrator flask, see Figure

Volumetric flasks of different sizes

Rotary vacuum evaporator

Sweep co-distillation apparatus

Gas chromatograph equipped with thermionic detector

Microsyringe, 10- $\mu$ l

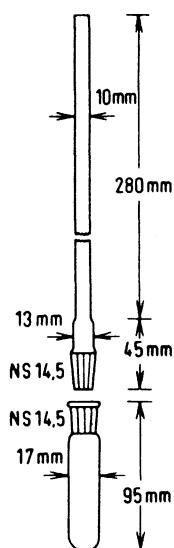


Figure. Kuderna-Danish evaporative concentrator flask.

#### 4. Reagents

Acetone, high purity, distilled on silver nitrate p. a., boiling point 56–56.5 °C  
Dichloromethane, p. a.

Ethyl acetate, p. a., distilled on silver nitrate p. a., boiling point 74.5–75.5 °C

Insecticide standard solutions: 1–5 µg/ml acetone

Sodium chloride solution, saturated

Sodium sulphate, p. a., anhydrous, heated

Filter aid, e. g. Celite 545 (Roth)

Compressed air

Hydrogen, re-purified

Nitrogen, re-purified, freed from residual oxygen in an Oxsorb cartridge (Messer Griesheim)

#### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Choice of the extraction procedure is determined by the nature and quality (water content, constituents) of the crops. For samples with a predominantly low water content, e.g. beans, carrots, celeriac, head cabbage, lettuce and spinach, extraction is best performed by the procedure described in step 6.1.1. Samples of apples, cherries, cucumbers, radishes and tomatoes should be extracted by step 6.1.2.

#### 6.1.1. Extraction of crop samples with low water content

Homogenize 100 g of the sample (G) with 250 ml dichloromethane for 3 min. Rinse the blender jar with two 25-ml portions of dichloromethane, measure the total volume ( $V_{EX}$ ), filter through a fluted filter paper, and continue to process half ( $V_{R1}$ ) of this volume.

Dry the extract solution on 8 g sodium sulphate, and again filter. Wash the sodium sulphate remaining on the filter paper four times with 5-ml portions of dichloromethane. Then concentrate the filtrate in a Kuderna-Danish evaporative concentrator flask on a rotary evaporator to 2–3 ml, add 5 ml ethyl acetate, and concentrate again. Repeat this procedure two or three times, transfer the extract to a small volumetric flask, and dilute with ethyl acetate to a volume ( $V_{R2}$ ) amenable to the operating conditions chosen for sweep co-distillation in step 6.2.

#### 6.1.2. Extraction of crop samples with high water content

Homogenize 100 g of the sample (G) with 200 ml acetone for 3 min. Mix the homogenate with 5 g Celite, and suction-filter through an acetone-moistened round filter paper in a Buchner porcelain funnel. Rinse blender jar and filter cake with two 25-ml portions of acetone. Measure the volume of the filtrate ( $V_{EX}$ ).

Transfer one fifth of the filtrate volume ( $V_{R1}$ ) to a separatory funnel, and dilute with 250 ml water and 35 ml saturated sodium chloride solution. Add 60 ml dichloromethane, and shake vigorously for 2 min. Collect the dichloromethane phase, then extract the aqueous phase once more with 50 ml dichloromethane and discard. Wash the combined dichloromethane phases with a mixture of 250 ml water and 35 ml saturated sodium chloride solution, and dry on 10 g sodium sulphate. Filter the dried extract, and rinse the filter cake three times with 10-ml portions of dichloromethane. Continue to process the filtrate as described in step 6.1.1, repeating the procedure two or three times. Dilute the remaining extract with ethyl acetate to a volume ( $V_{R2}$ ) amenable to the operating conditions chosen for sweep co-distillation in step 6.2.

## 6.2. Cleanup

Cleanup is performed by sweep co-distillation as described in Section 5.2 of Cleanup Method 2, using an appropriate aliquot ( $V_{R3}$ ) of the extract solution ( $V_{R2}$ ) derived from step 6.1. The operating conditions for this cleanup are also described in the above-mentioned Section; choice of the given conditions (I or II) is optional.

The distillate obtained from this cleanup is dissolved in acetone, and concentrated to 1–5 ml ( $V_{End}$ ) in a rotary evaporator.

### 6.3. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the cleaned-up solution derived from step 6.2 into the gas chromatograph.

#### *Operating conditions*

##### 6.3.1. Columns 1 and 2

Gas chromatograph	Hewlett-Packard 5750
Column tubes	Glass, 1.7 mm i. d., 2.1 m long
Column 1	4% OV-1 on Chromosorb W-HP, 100–120 mesh
Column temperature 1	140°C isothermal for 18 min, then programmed to rise at 8°C/min to 285°C, isothermal for further 2 min
Column 2	4% OV-17 on Chromosorb W-HP, 100–120 mesh
Column temperature 2	170°C isothermal for 18 min, then programmed to rise at 8°C/min to 315°C, isothermal for further 6 min
Injection port temperature	245°C
Detector	Thermionic detector, phosphorus-specific Temperature 315°C
Gas flow rates	Nitrogen carrier, 20 ml/min Hydrogen, 11 ml/min Compressed air, 180 ml/min
Attenuation	$10^3 \cdot 128$
Recorder	Hewlett-Packard 7128 A
Injection volume	1–8 µl

##### 6.3.2. Columns 3 and 4

Gas chromatograph	Hewlett-Packard 5750
Column tubes	Glass, 2 mm i. d., 1.9 m long
Column 3	2% SE-30 on Chromosorb W-AW-DMCS, 80–100 mesh
Column temperature 3	190°C 320°C
Injection port temperature	Thermionic detector, phosphorus-specific Temperature 380°
Detector	Helium carrier, 60 ml/min Hydrogen, 38 ml/min Air, 380 ml/min
Gas flow rates	
Column 4	5% QF-1 on Chromosorb W-AW-DMCS, 80–100 mesh
Column temperature 4	200°C 300°C
Injection port temperature	Thermionic detector, phosphorus-specific Temperature 400°C
Detector	Helium carrier, 60 ml/min Hydrogen, 30 ml/min Air, 350 ml/min
Gas flow rates	

Attenuation  $10^3 \cdot 64$   
 Recorder Hewlett-Packard 7128 A  
 Injection volume 1 and 5 µl

The retention times obtained on the different columns are given in Tables 1 and 2.

Table 1. Absolute retention times

Compound	Retention time		Compound	Retention time	
	Column 1 (OV-1)	Column 2 (OV-17)		min	s
Dichlorvos	2	12	Dichlorvos	1	34
Naled	2	41	Mevinphos	3	37
Thionazin	3	56	Thionazin	4	34
Mevinphos	4	53	Naled	5	12
Phorate	5	2	Phorate	5	12
Thiometon	5	12	Thiometon	5	40
Diazinon	6	18	Diazinon	6	18
Disulfoton	6	27	Disulfoton	6	37
Dichlofenthion	7	15	Dichlofenthion	7	15
Parathion-methyl	7	24	Fenchlorphos	8	21
Fenchlorphos	7	52	Parathion-methyl	8	40
Fenitrothion	8	2	Malathion	9	17
Fenthion	8	30	Paraoxon	9	17
Malathion	8	30	Fenitrothion	9	27
Parathion	8	40	Chlorthion	9	55
Paraoxon	8	49	Fenthion	9	55
Chlorthion	8	49	Bromophos	10	5
Bromophos	9	8	Chlorfenvinphos	10	33
Bromophos-ethyl	10	5	Bromophos-ethyl	10	43
Disulfoton sulphone	10	33	Disulfoton sulphone	12	8
Ethion	12	8	Ethion	13	23
Fensulfothion	13	42	Fensulfothion	14	10
Phenkaptone	14	10	Phenkaptone	15	35
Azinphos-ethyl	15	26	Azinphos-ethyl	18	26

Table 2. Absolute retention times

Compound	Retention time		Compound	Retention time	
	Column 3 (SE-30)			Column 4 (QF-1)	
	min	s		min	s
Dichlorvos	19		Dichlorvos	33	
Trichlorfon	19		Trichlorfon	40	
Phorate	23		Omethoate	43	
Dimefox	24		Dimefox	45	
Omethoate	24		Phorate	1	5
Thionazin	28		Thionazin	1	18
Mevinphos	36		Mevinphos	1	25
Sulfotep	45		Sulfotep	1	33
Dioxathion	50		Diazinon	1	49
Demeton-S-methyl	55		Disulfoton	1	50
Dimethoate	57		Dioxathion	1	54
Disulfoton	1	4	Demeton-S-methyl	2	6
Malaoxon	1	6	Fenchlorphos	2	29
Diazinon	1	11	Chlorpyrifos	3	2
Parathion-methyl	1	30	Bromophos	3	11
Fenchlorphos	1	40	Dimethoate	3	16
Paraoxon	1	41	Fenthion	3	42
Fenitrothion	1	44	Malathion	3	59
Malathion	1	51	Parathion-methyl	4	8
Fenthion	1	56	Paraoxon	4	20
Chlorpyrifos	1	58	Malaoxon	4	25
Parathion	2		Fenitrothion	4	46
Amidithion	2	1	Amidithion	5	2
Chlorthion	2	3	Parathion	5	19
Bromophos	2	15	Chlorfenvinphos	6	13
Chlorfenvinphos	2	36	Methidathion	6	37
Methidathion	2	43	Chlorthion	6	49
Ethion	4	44	Ethion	7	8
Carbophenothon	5	17	Phosphamidon	7	24
Fensulfothion	5	19	Carbophenothon	7	31
Phosphamidon	9	51	Fensulfothion	18	40

## 7. Evaluation

### 7.1. Method

Identification is achieved by determining the retention time and comparing it with that of compounds from appropriately diluted standard solutions. Quantitation is performed by measuring the peak area of the sample solution and comparing it with the peak areas of appropriately diluted standard solutions. Equal volumes of the sample solution and the standard solutions should be injected.

### 7.2. Recoveries and lowest determined concentration

The tested combinations of compounds and crops are listed in two tables, viz. in Table 3 for sweep co-distillation operating conditions I, and in Table 4 for sweep co-distillation operating conditions II. The figures given in the tables represent the residue levels in mg/kg tested in recovery experiments. In about 90% of the cases, the recoveries ranged from 80 to 100%; in the other cases, they were somewhat lower. The routine limit of determination in most of the cases marked thus + in the tables was approx. 0.1 mg/kg. It was approx. 0.05 mg/kg for diazinon, dimefox, mevinphos, parathion, parathion-methyl, phenkapton and thionazin, and approx. 0.01 mg/kg for disulfoton, phorate and sulfotep.

Table 3. Tested combinations of compounds and crops, using operating conditions I in sweep co-distillation

Crop	Azinphos-ethyl	Bromophos	Bromophos-ethyl	Chlorthiophos	Chlorthion	Diazinon	Dichlofenthion	Dichlorvos	Disulfoton	Disulfoton sulphone	Ethion	Fenchlorphos
Compounds												
Apples						+ 0.3				+ 0.5		
Beans	+ 0.6					+ 0.3		+ 0.2		+ 0.5		
Carrots	+ 0.4		+ 0.4			+ 0.05						
Celeriac	+ 0.6		+ 0.4			+ 0.3						
Cherries	+ 0.6					+ 0.3						
Cucumbers	+ 0.6					+ 0.3						
Head cabbage	+ 0.4		+ 0.4	+ 0.2				- 0.2	- 0.2		- 0.1	+ 0.4
Kohlrabi	+ 0.2			+ 0.2		- 0.2		+ 0.2	+ 0.2			
Lettuce	+ 0.6					+ 0.3		+ 0.2		+ 0.5		
Radishes	+ 0.6					+ 0.3				+ 0.5		
Red cabbage		+ 0.4	+ 0.2	+ 0.2	+ 0.1	+ 0.2	+ 0.2	+ 0.2	+ 0.2		+ 0.1	+ 0.2
Spinach	+ 0.4	+ 0.6			+ 0.3	+ 0.2						+ 0.4
Tomatoes		+ 0.6			+ 0.3					+ 0.5		

The figures give the concentrations (mg/kg) at which the recovery experiments were performed.

+ Signifies that the compound, at the given concentration (expressed in mg/kg), was recovered at a rate of more than 80%.

- Signifies that at the given concentration the compound was recovered at a rate of less than 80%.

Where no data are given for a compound alongside a substrate, this indicates that determination of the compound in the particular analytical material has not yet been tried.

Table 3. (contd.)

Crop	Fenitrothion	Fensulfothion	Fenthion	Malathion	Mevinphos	Naled	Parathion	Parathion-methyl	Paraoxon	Phenkapton	Phorate	Thiometon	Thionazin
Compounds													
Apples				+ 0.4			+ 0.5			+ 0.5			
Beans				+ 0.5	+ 0.3	+ 0.2				+ 0.5			
Carrots					+ 0.05	+ 0.1			+ 0.15				
Celeriac					+ 0.3				+ 0.15				
Cherries			- 0.2		+ 0.3				+ 0.15				
Cucumbers				+ 0.5	+ 0.3								
Head cabbage	+ 0.4	+ 0.2			+ 0.2			+ 0.15			- 0.2		
Kohlrabi	+ 0.2					+ 0.2		- 0.15				- 0.3	+ 0.2
Lettuce				+ 0.5		+ 0.2			+ 0.5				
Radishes					+ 0.3			+ 0.2	+ 0.5				
Red cabbage	- 0.4	+ 0.2			- 0.3	+ 0.2		+ 0.15			+ 0.2		+ 0.2
Spinach		+ 0.2				+ 0.2				+ 0.25	+ 0.2		
Tomatoes				+ 0.4				+ 0.5					

Table 4. Tested combinations of compounds and crops, using operating conditions II in sweep co-distillation (for explanations see footnotes to Table 3)

Crop	Compounds	Amidithion 0.25	Bromophos 0.25	Carbopheno- thion 0.25	Chlorfenvinphos 0.5	Chlorpyrifos 0.25	Chlorthion 0.25	Demeton-S- methyl 0.125	Diazinon 0.125
Apples		-				+	+		+
Asparagus			+		+				
Beans				+					
Cauliflower				+			+	+	+
Cherries					+			+	
Cucumbers				+	+			+	
Grapes					+			+	
Kohlrabi		-	+						
Lettuce		-	+	+					
Pears		-	+	+				-	
Peppers						-			
Pineapples			+	+		+			
Potatoes				-	+		+		
Savoy cabbage					+		+		
Strawberries					+		+		
Tomatoes	+		+	+	+				+

Table 4. (contd.)

Crop	Compounds	Dichlorvos 0.025	Dimefox 0.075	Dimethoate 0.125	Dioxathion 0.5	Disulfoton 0.05	Ethion 0.125	Fenchlorphos 0.125
Apples					-	-		+
Asparagus			+					
Beans								
Cauliflower								
Cherries								
Cucumbers			+					
Grapes								
Kohlrabi		-			+			+
Lettuce						+		
Pears		+				+		-
Peppers		-				-		+
Pineapples								
Potatoes								
Savoy cabbage				-				
Strawberries								
Tomatoes		+	-				-	

Table 4. (contd.)

Crop	Compounds	Fenitrothion 0.25	Fenthion 0.25	Malaoxon 1.5	Malathion 0.125	Methidathion 0.125	Mevimphos 0.125	Omethoate 0.75
Apples		+		+			-	-
Asparagus			+					
Beans			+					+
Cauliflower			+			-		
Cherries			+				+	
Cucumbers			+				+	
Grapes	+		+				+	
Kohlrabi			+				+	
Lettuce			+				+	
Pears			+		+		+	
Peppers								
Pineapples			+				-	+
Potatoes								
Savoy cabbage								
Strawberries								
Tomatoes		+	-				+	

Table 4. (contd.)

Crop	Compounds	Parathion 0.2	Parathion-methyl 0.25	Phorate 0.05	Phosphamidon 0.625	Sulfotep 0.025	Thionazin 0.025	Trichlorfon 0.25
Apples	-							
Asparagus								
Beans								
Cauliflower					+			
Cherries					+			
Cucumbers					+			
Grapes					+			
Kohlrabi	+		+					
Lettuce				+				
Pears	-			+				
Peppers	+		+	+				
Pineapples				+				
Potatoes				+				
Savoy cabbage				+				
Strawberries				+				
Tomatoes				+				

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, of an identified compound is calculated from the following equation:

$$R = \frac{F_A \cdot V_{End} \cdot V_{R2} \cdot V_{Ex} \cdot W_{St}}{F_{St} \cdot V_i \cdot V_{R3} \cdot V_{R1} \cdot G}$$

where

G = sample weight (in g)

$V_{Ex}$  = volume of extract derived from step 6.1.1 or of filtrate from extract derived from step 6.1.2 (in ml)

$V_{R1}$  = portion of volume  $V_{Ex}$  used for further processing (in ml)

$V_{R2}$  = volume of concentrated extract solution derived from step 6.1.1 or 6.1.2 (in ml)

$V_{R3}$  = portion of volume  $V_{R2}$  used for cleanup in step 6.2 (in ml)

$V_{End}$  = terminal volume of sample solution from 6.2 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_{St}$  = amount of compound injected with standard solution (in ng)

$F_A$  = peak area obtained from  $V_i$  (in  $\text{mm}^2$ )

$F_{St}$  = peak area obtained from  $W_{St}$  (in  $\text{mm}^2$ )

## 8. Important points

No data

## 9. References

J. Pflugmacher and W. Ebing, Reinigung Pestizidrückstände enthaltender Rohextrakte mit einer automatisch arbeitenden Apparatur nach dem Prinzip der kombinierten Spül- und Codestillation (Sweep Co-Distillation), Fresenius Z. Anal. Chem. 263, 120–127 (1973).

R. W. Storherr and R. R. Watts, A sweep co-distillation cleanup method for organophosphate pesticides, J. Assoc. Off. Agric. Chem. 48, 1154–1158 (1965).

## 10. Authors

Federal Biological Research Centre for Agriculture and Forestry, Department for Ecological Chemistry, Division for Pesticide Research, Berlin, W. Ebing and J. Pflugmacher

Federal State Control Laboratory of Food and Environmental Chemistry, Offenburg, M. Eichner



# **Triazine Herbicides and Desalkyl Metabolites**

**S 14**

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Soil

Gas-chromatographic determination after cleanup by sweep co-distillation

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(German version published 1979)

## **1. Introduction**

The method is applicable to soil samples for the determination of residues of seven triazine herbicides and several of their desalkyl metabolites. The extracts are cleaned up by sweep co-distillation as described in Cleanup Method 2 in this Manual.

## **2. Outline of method**

The analytical material is extracted with methanol. The evaporation residue of the extract is cleaned up by sweep co-distillation in an appropriate apparatus, and the distillate is analyzed by gas chromatography using a chlorine- or sulphur-sensitive microcoulometric detector or a nitrogen-specific thermionic detector or an electrolytic conductivity detector or a flame photometric detector.

## **3. Apparatus**

Beater-cross mill

Blendor or mixer for soil

Soxhlet apparatus

Rotary vacuum evaporator, 40°C bath temperature

Ultrasonic bath

Sweep co-distillation apparatus

Gas chromatograph equipped with microcoulometric detector or thermionic detector or electrolytic conductivity detector or flame photometric detector

## **4. Reagents**

Cyclohexane, high purity, dist.

Ethanol, absolute

n-Hexane, for chromatography

Methanol, dist.  
 Toluene, high purity, dist.  
 Ethanol + n-hexane mixture 1 : 1 v/v  
 Toluene + cyclohexane + ethanol mixture 4 : 1 : 1 v/v/v  
 Standard solutions of triazine herbicides and desalkyl metabolites: 10 µg/ml ethanol-hexane mixture  
 Air, synthetic  
 Helium  
 Hydrogen, re-purified  
 Nitrogen, re-purified  
 Oxygen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 21 f.

## 6. Procedure

### 6.1. Extraction

Extract 50 g of the analytical sample (G) with 100 ml methanol in a Soxhlet apparatus for 2 h. Rotary-evaporate the extract to dryness, and dissolve in 4 ml of the toluene-cyclohexane-ethanol mixture ( $V_{\text{Ex}}$ ). If the evaporation residue does not dissolve completely, use an ultrasonic bath.

### 6.2. Cleanup

Cleanup is performed by sweep co-distillation as described in Section 5.3 of Cleanup Method 2, using 2 ml ( $V_{\text{RI}}$ ) of the extract solution derived from step 6.1.

Evaporate the distillate to dryness on a rotary evaporator or with a gentle stream of purified air. Dissolve the residue in 2.0 ml ethanol-hexane mixture ( $V_{\text{End}}$ ).

### 6.3. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the sample solution derived from step 6.2 into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Varian 1700 or 200
Column packing	3% Carbowax 20 M on Chromosorb G, 80–100 mesh

#### 6.3.1. Column 1

Column tube	Glass, 3 mm i. d., 1 m long
Column temperature	200 °C isothermal for 8 min, then programmed to rise at 8 °C/min to 240 °C
Injection port temperature	240 °C

Detector	Microcoulometer, chlorine- or sulphur-sensitive
Gas flow rates	Pyrolysis furnace temperature 800 °C Nitrogen carrier, 70 ml/min Oxygen reaction gas, 100 ml/min

### 6.3.2. Column 2

Column tube	Glass, 2 mm i. d., 2 m long
Column temperature	220 °C isothermal for 16 min, then programmed to rise at 16 °C/min to 240 °C
Injection port temperature	240 °C
Detector	Coulson conductivity cell, nitrogen-sensitive Furnace temperature 780–800 °C Nickel wire catalyst Strontium hydroxide acid-absorber
Gas flow rates	Helium carrier, 35 ml/min Hydrogen, 40 ml/min

### 6.3.3. Column 3

Column tube	Glass, 2 mm i. d., 1 m long
Column temperature	200 °C isothermal for 13 min, then programmed to rise at 10 °C/min to 240 °C
Injection port temperature	240 °C
Detector	Flame photometric detector, sulphur-sensitive Temperature 180 °C
Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 80 ml/min Oxygen, 30 ml/min

### 6.3.4. Column 4

Column tube	Glass, 2 mm i. d., 1 m long
Column temperature	210 °C isothermal for 13 min, then programmed to rise at 10 °C/min to 240 °C
Injection port temperature	240 °C
Detector	Thermionic detector, nitrogen-sensitive, with Rb <sub>2</sub> SO <sub>4</sub> tip Temperature 240 °C
Gas flow rates	Nitrogen carrier, 30 ml/min Hydrogen, 35 ml/min Air, 235 ml/min

The retention times obtained on the different columns are given in Table 1.

Table 1. Retention times of triazine herbicides and their desalkyl metabolites

Parent compound, metabolite	Retention time							
	Column 1		Column 2		Column 3		Column 4	
	min	s	min	s	min	s	min	s
Atrazine	11	55	9	36			7	31
Desethyl-atrazine	15	12	18	18			16	28
Secbumeton			8	14			6	24
Simazine	13	12	12	29			9	58
bis-(Desethyl)-simazine			31	24			24	5
Simeton			8	27			6	24
Terbumeton			5	48			4	15
Des-tert-butyl-terbumeton			14	12			11	51
Des-tert-butyl-desethyl-terbumeton			21	2			19	19
Terbutylazine	10	55	8	8			6	7
Des-tert-butyl-terbutylazine	16	46	21	12			19	28
Terbutrynl			13	14	9	18		
Des-tert-butyl-terbutrynl			24	56	17	57		
Des-tert-butyl-desethyl-terbutrynl			34	8	21	4		

## 7. Evaluation

### 7.1. Method

Identification is achieved by determining the retention times and comparing them with those of compounds from appropriately diluted standard solutions. Quantitation is performed by measuring the peak area of the sample solution and comparing it with the peak areas of appropriately diluted standard solutions. Equal volumes of the sample solution and the standard solutions should be injected.

### 7.2. Recoveries and lowest determined concentration

The recoveries obtained in fortification experiments in which the triazine herbicides and their metabolites were added at levels of 0.2 to 1.0 mg/kg, are given in Table 2. The routine limit of determination was approx. 0.05 mg/kg.

Table 2. Results of recovery experiments

Parent compound, metabolite	Added mg/kg	Recovery %
Atrazine	0.2–1.0	91–112
Desethyl-atrazine	0.2–1.0	85–107
Secbumeton	0.5	87
Simazine	0.5	99–103
bis-(Desethyl)-simazine	0.5	72
Simeton	0.5	93
Terbumeton	0.2	100
Des-tert-butyl-terbumeton	0.2–0.5	78–105
Des-tert-butyl-desethyl-terbumeton	0.2	99
Terbutylazine	0.5	91
Des-tert-butyl-terbutylazine	0.5	91
Terbutryn	0.5	105
Des-tert-butyl-terbutryn	0.5	86–102
Des-tert-butyl-desethyl-terbutryn	0.5	105

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, of an identified compound is calculated from the following equation:

$$R = \frac{F_A \cdot V_{End} \cdot V_{Ex} \cdot W_{St}}{F_{St} \cdot V_i \cdot V_{RI} \cdot G}$$

where

G = sample weight (in g)

V<sub>Ex</sub> = volume of concentrated extract derived from step 6.1 (in ml)

V<sub>RI</sub> = portion of volume V<sub>Ex</sub> used for cleanup in step 6.2 (in ml)

V<sub>End</sub> = terminal volume of sample solution from 6.2 (in ml)

V<sub>i</sub> = portion of volume V<sub>End</sub> injected into gas chromatograph (in µl)

W<sub>St</sub> = amount of compound injected with standard solution (in ng)

F<sub>A</sub> = peak area obtained from V<sub>i</sub> (in mm<sup>2</sup>)

F<sub>St</sub> = peak area obtained from W<sub>St</sub> (in mm<sup>2</sup>)

## 8. Important points

It is recommended to use an automatic sampler and to employ peak integration for the gas-chromatographic determination. The conditions 6.3.1 and 6.3.3 as well as the conditions 6.3.2 and 6.3.4 can be used optionally.

Further data on gas chromatography will be found in Method S 7.

## 9. References

- J. Pflugmacher and W. Ebing*, Reinigung Pestizidrückstände enthaltender Rohextrakte mit einer automatisch arbeitenden Apparatur nach dem Prinzip der kombinierten Spül- und Codestillation (Sweep Co-Distillation), Fresenius Z. Anal. Chem. 263, 120–127 (1973).
- K. Ramsteiner and B. Karlhuber*, An improved modification of the sweep co-distillation cleanup system for triazine herbicide residue analysis in soil. Internal Report SPR 122/72, Ciba-Geigy AG.
- R. W. Storherr and R. R. Watts*, A sweep co-distillation cleanup method for organophosphate pesticides, J. Assoc. Off. Agric. Chem. 48, 1154–1158 (1965).

## 10. Authors

Ciba-Geigy AG, Agricultural Division, Basle, Switzerland, *B. Karlhuber* and *K. Ramsteiner*

# Dithiocarbamate and Thiuram Disulphide Fungicides

S 15

Apples, barley, carrots, celeriac (leaves and bulbs), cereal straw, cherries, cucumbers, currants (black and red), grapes, leeks, lettuce, peaches, pears, potatoes, rape (green matter), red beet, small radishes, strawberries, sugar beet (foliage and edible root), wheat

Photometric determination

Soil

(German version published 1979)

## 1. Introduction

Salts of aliphatic dithiocarbamates (e.g. mancozeb, maneb, propineb and zineb) and thiuram disulphides (e.g. thiram) are frequently used as fungicides. Assignment of their residues in plant material to a specific compound is very difficult to achieve, and usually is not attempted. The maximum residue limits are established also for the whole group of these compounds; they relate to carbon disulphide ( $\text{CS}_2$ ).

## 2. Outline of method

Heating dithiocarbamates and thiuram disulphides with a solution of stannous chloride and hydrochloric acid yields carbon disulphide which is distilled, purified and collected in an ethanolic solution of cupric acetate and diethanolamine. Two yellow cupric-N,N-bis(2-hydroxyethyl) dithiocarbamate complexes with the molar ratio  $\text{Cu} : \text{CS}_2 = 1 : 1$  and  $1 : 2$ , respectively (Cullen, 1964), are formed, which are measured jointly by spectrophotometry.

## 3. Apparatus

Heating mantle, electrically operated, at least 450 watt; or powerful gas burner fitted with Babo funnel

Cylindrical flask, 2-l, ground-in socket diameter of approx. 10 cm; fitted with adapter for 3 NS 29 ground joints

Decomposition and distillation apparatus consisting of cylindrical flask with adapter, dropping funnel, gas inlet tube, ascending Liebig condenser and at least 3 absorption tubes, connected by spherical socket joints; see Figure

Water jet pump

Flowmeter

Volumetric flasks, 250-ml, 50-ml and 25-ml, with ground joints

Spectrophotometer for measuring at 435 nm, with 1-cm glass cells

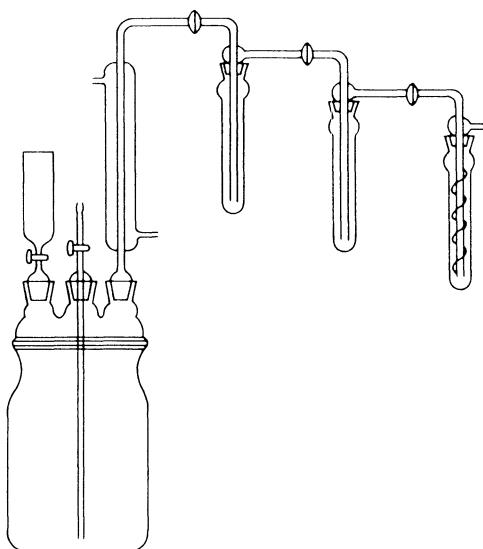


Figure. Decomposition and distillation apparatus.

#### 4. Reagents

Ethanol, p. a.

Carbon disulphide, high purity, colourless

Carbon disulphide stock solution: Weigh a stoppered 50-ml volumetric flask (with ground joint) containing 40 ml ethanol. Add approx. 1.0 ml carbon disulphide (equivalent to approx. 1.25 g) using a pipette, close flask at once and re-weigh to obtain exact weight of carbon disulphide by difference. Dilute to mark with ethanol and mix well. Prepare fresh each time

Carbon disulphide working standard solution: Dilute 5.0 ml of carbon disulphide stock solution with ethanol to 50.0 ml. Make up 5.0 ml of this dilution with ethanol to 250 ml. 1 ml of this working standard solution is equivalent to approx. 50 µg CS<sub>2</sub>. Prepare fresh each time

Hydrochloric acid, p. a., conc.

Sodium hydroxide solution, 10 g/100 ml NaOH p. a.

Lead acetate solution, 30 g/100 ml Pb(CH<sub>3</sub>COO)<sub>2</sub> · 3 H<sub>2</sub>O

Colour reagent: Successively introduce 100 ml ethanol, 30.0 ml cupric acetate solution and 25.0 g diethanolamine into a volumetric flask, and dilute with ethanol to 250 ml

Cupric acetate solution: Dissolve exactly 400 mg cupric acetate monohydrate, Cu(CH<sub>3</sub>COO)<sub>2</sub> · H<sub>2</sub>O, at most with gentle warming, in 250 ml ethanol. Dilute 25 ml of this solution to 100 ml with ethanol

Stannous chloride solution, 40 g/100 ml SnCl<sub>2</sub> · 2 H<sub>2</sub>O in conc. hydrochloric acid

Stannous chloride-hydrochloric acid solution: Mix 20 ml stannous chloride solution, 20 ml conc. hydrochloric acid and 200 ml water

Diethanolamine, p. a.

## 5. Sampling and sample preparation

For products consisting of small units (e.g. legume vegetables, grains, small fruits), mix the laboratory sample well, and weigh the portion required for analysis.

For products consisting of large units (e.g. large fruits and vegetables), cut wedge-shaped portions that include outer surfaces from each unit. Mix and weigh portion required for analysis. For vegetables like lettuce, detach individual leaves and mix. Deep-freeze soft and juicy crops (e.g. tomatoes, peaches) beforehand. Always mix opposite quarters.

After sample is prepared, proceed with analysis without undue delay.

## 6. Procedure

### 6.1. Preparation of apparatus

To the first absorption tube on the decomposition and distillation apparatus add 10 ml lead acetate solution. Fill the second one with 10 ml sodium hydroxide solution. To the third absorption tube (best fitted with a Widmer helix) add 15 ml colour reagent. To check for complete absorption of the evolved carbon disulphide, a fourth absorption tube containing colour reagent can be attached; its contents must remain colourless during the procedure.

Turn on the Liebig condenser cooling system, and regulate the water jet pump connected to the last absorption tube so that the rate of air flow through the apparatus indicated on the flowmeter is approx. 300 ml/min.

### 6.2. Decomposition and distillation

Add 200–500 g of the analytical sample (G) to the flask. Close the apparatus. Then add 240 ml stannous chloride-hydrochloric acid solution from a dropping funnel. If the amount of liquid is not enough for a sample of bulky crop material (e.g. lettuce) to be fully immersed, add appropriately more stannous chloride-hydrochloric acid solution. Then immediately heat flask contents rapidly to boiling. Continue boiling for a total period of 60 min. At end of boiling period, switch off water jet pump and disconnect the third absorption tube.

### 6.3. Photometric measurement

Transfer the yellow-coloured contents of the third absorption tube to a 25-ml volumetric flask and rinse with ethanol. Dilute to the mark with ethanol and mix. Measure absorbance at 435 nm in a 1-cm lightpath cell on the spectrophotometer against a blank solution made up of 15 ml colour reagent and 10 ml ethanol.

## 7. Evaluation

### 7.1. Method

Evaluation is performed by preparing a calibration curve as follows. Add 15 ml colour reagent to each of a series of ten 25-ml volumetric flasks. Then add, from a graduated pipette or a burette, 1, 2, 3, 4 and 5 ml of the carbon disulphide standard solution (equivalent to approx. 50, 100, 150, 200 and 250 µg CS<sub>2</sub>) to each of two volumetric flasks. Dilute to the mark with ethanol, and let the 10 mixtures stand for 60 min. Perform the photometric measurement as described in step 6.3.

On plotting µg CS<sub>2</sub> per mixture on the abscissa (x) versus absorbance value on the ordinate (y), a calibration curve is obtained that is linear in the given range. However, its extension does not pass through the zero point but intersects the abscissa at approx. 20 µg CS<sub>2</sub>.

It is recommended to carry the measured values through a correlation analysis, to determine the equation of the regression line and to plot this line as a calibration curve. The simultaneously computed coefficient of correlation should not be smaller than r = 0.998. For 120 µg CS<sub>2</sub>, an absorbance of 0.308 was measured, for example. The mean equation of the regression line determined in 10 laboratories was  $y = -0.056 + 0.00303 \cdot x$ .

Below approx. 50 µg CS<sub>2</sub> (absorbance of approx. 0.10), the calibration straightline slopes towards the zero point. Therefore, a quantitative evaluation should not be performed at absorbances of less than 0.10 but instead the analysis should be repeated using a larger amount of sample.

### 7.2. Recoveries and lowest determined concentration

The recoveries obtained from untreated control samples fortified with the different compounds in experiments run in five laboratories are given in Table 1. The values obtained for carbon disulphide were converted to the added compounds. The conversion factors given in Table 2 are based on the theoretical molar masses of the compounds.

Sample blanks occur only with few crops. High blanks were observed for rape seed, rutabaga, cauliflower and Savoy cabbage. Due to the slope of the calibration line towards the zero point (see 7.1), less than 50 µg CS<sub>2</sub> should not be quantitatively evaluated. Using 500 g analytical sample, the lower limit for a reliable determination is calculated to be 0.1 mg/kg.

Table 1. Recoveries obtained in fortification experiments run with combinations of the listed compounds and commodities (values from 5 laboratories)

 $\bar{x}$  = mean recovery [%]

s = standard deviation

n = number of individual values

Compound	Commodity	Added mg/kg	$\bar{x}$	s	n
Maneb	Apples	1.0	81	2	3
	Potatoes	1.0	91	6	4
	Carrots	2.0	81	3	3
	Celeriac leaves	1.0	80	6	3
	Celeriac bulbs	1.0	88	3	3
	Wheat (grains)	2.0	88	2	3
	Wheat (green matter)	3.0	88	4	3
	Wheat straw	4.0	80	8	3
	Sugar beet, leaves	2.0	74	6	3
	Sugar beet, edible roots	1.0	83	3	3
Mancozeb	Soil	7.0	87	3	3
	Barley (ears)	4.0	82	5	3
	Barley (grains)	2.0	82	4	3
	Barley (green matter)	3.0	85	5	3
	Barley straw	4.0	76	6	3
	Potatoes	2.0	91	2	3
	Carrots	2.0	83	2	3
	Sugar beet, leaves	2.0	83	5	3
	Sugar beet, edible roots	1.0	80	3	3
Propineb	Apples	2.0	94	2	3
	Currants, red	2.0	92	2	3
	Currants, black	2.0	95	2	3
	Potatoes	2.0	94	2	3
	Cherries	2.0	95	3	3
	Peaches	2.0	87	3	3
	Rape (green matter)	5.0	89	6	3
	Celeriac leaves	2.0	87	6	3
	Celeriac bulbs	2.0	89	2	3
Thiram	Apples	3.0	95	2	3
	Strawberries	2.0	92	4	3
	Potatoes	2.0	81	3	3
	Small radishes	2.0	98	2	3
	Grapes	4.0	99	6	5
		8.0	103	2	4

Table 1. (contd.)

Compound	Commodity	Added mg/kg	$\bar{x}$	s	n
Zineb	Apples	2.0	97	4	2
	Pears	0.8	94	4	7
	Cucumbers	2.0	86	2	2
		5.0	90	4	2
		10.0	81	4	2
	Lettuce	1.8	93	3	4
		2.0	90	4	2
		2.4	97	4	3
		3.6	93	2	4
		4.0	95	4	7
Leeks		4.8	97	4	7
		5.0	86	1	2
		8.0	99	3	7
		9.6	101	1	2
		10.0	78	1	2
		12.8	100	1	8
	Red beet	4.0	94	4	3
		2.0	94	4	2
		5.0	90	3	2
		10.0	82	4	2
Celeriac bulbs		2.0	92	6	2
		5.0	88	3	2
		10.0	81	1	2
Grapes		2.0	93	2	3

Table 2. Factors for conversion of found amounts of carbon disulphide to the respective compounds (see also 7.2)

Mancozeb	1.776
Maneb	1.742
Propineb	1.903
Thiram	1.578
Zineb	1.810

### 7.3. Calculation of residues

The residue R, expressed in mg/kg carbon disulphide, is calculated from the following equation:

$$R = \frac{W_{AR}}{G}$$

where

G = sample weight (in g)

$W_{AR}$  = amount of  $CS_2$  read from calibration curve (in  $\mu g$ )

## 8. Important points

The analytical material should on no account be finely comminuted let alone homogenized otherwise this may result in high and uncontrollable losses of compound.

It is important that the flask contents are rapidly heated to boiling, especially when ethylene bisdithiocarbamates are present. If the flask contents are heated too slowly to boiling, cyclic intermediates presumably form from which carbon disulphide does not cleave quantitatively.

The lead acetate solution can be used for a further analysis provided it has not turned brown or black. The sodium hydroxide solution must be renewed as soon as it becomes yellowish or cloudy or no longer shows an alkaline reaction. Neither of the absorption tubes must be cleaned with ethanol because residues of ethanol may bind carbon disulphide as xanthogenate.

As two different complexes with differing spectra may form from carbon disulphide, diethanolamine and cupric acetate (Cullen, 1964), the levels of the measured absorbance values and the course of the calibration curve are influenced very largely by the copper concentration present. Therefore, special attention must be devoted to the preparation of the colour reagent. The carbon disulphide stock solution and working standard solution also should be prepared as given and not according to other literature data (Keppel, 1969, 1971).

In experiments run to determine the recovery, it proved expedient, especially with the very sparingly soluble dithiocarbamates, to fortify the untreated plant material with freshly prepared triturations of the compounds using talc.

If the colour reagent becomes discoloured during distillation, this is probably due to use of an insufficient amount of stannous chloride. In this case, the analysis should be performed again with twice the amount of stannous chloride-hydrochloric acid solution.

If it is required to measure less than 50 µg carbon disulphide (see 7.2), the UV absorption of xanthogenate can be used for measurement. In this case, 10 ml concentrated sulphuric acid should be added to the second absorption tube, and 4 ml methanolic potassium hydroxide solution (0.5 mol/l) should be added to the third tube. On completion of decomposition and distillation in step 6.2, the contents of the third absorption tube should be transferred to a 5-ml volumetric flask, rinsed with methanol, and diluted to the mark with methanol. To prepare the calibration curve, 0.2 to 1 ml of a solution of 10 µg carbon disulphide/ml methanol should be added to 4 ml of the methanolic potassium hydroxide solution and diluted to 5 ml with methanol. In all cases, the solutions, after being mixed well, are measured in a spectrophotometer at 302 nm in a 2-cm lightpath quartz cell versus a blank solution made up of 4 ml methanolic potassium hydroxide solution and 1 ml methanol.

By this procedure, 0.01 mg/kg carbon disulphide was determined quantitatively in an analytical sample of 200 g cereal grains.

## 9. References

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## 10. Authors

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# **Organophosphorus Pesticides with Thioether Groups**

**S 16**

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Apples, carrots, cherries, cucumbers, curly kale, head cabbage, leeks, lettuce, onions, potatoes, red cabbage, spinach, tomatoes

Gas-chromatographic determination

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(German version published 1979)

## **1. Introduction**

The method is applicable to samples of various crops for the determination of residues of several organophosphorus pesticides containing thioether groups in their molecules. For each parent compound, the sulphoxide and sulphone formed as metabolites are also detected, and the total residue is determined as sulphone.

The compounds determinable by the method, together with details of their physical and chemical properties, are given in Table 1.

## **2. Outline of method**

The residues are extracted from plant material with acetonitrile. The co-extracted water is separated by addition of dichloromethane. The organic phase is dried and evaporated. The residue is dissolved in acetone, and aqueous potassium permanganate solution is added. By this treatment, thioethers and sulphoxides are converted to the analogous sulphones which are partitioned into dichloromethane. The oxidation results in simultaneous removal of most of the interfering plant constituents. It is only in a few cases that oxidation must be preceded by cleanup on a chromatographic column for quantitative determination.

After evaporation of the dichloromethane phase, the residue is dissolved in acetone and the solution is analyzed by gas chromatography using a phosphorus-specific alkali flame ionization detector.

## **3. Apparatus**

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Buchner porcelain funnel, 9 cm dia.

Beakers, 400-ml and 50-ml

Round-bottomed flasks, 1-l and 250-ml, with ground joints

Separatory funnels, 500-ml and 250-ml, with ground stoppers and Teflon stopcocks

Chromatographic tube, 25 mm i. d., 25 cm long, with sintered glass disk and stopcock

Rotary vacuum evaporator, 30°C bath temperature

Volumetric flasks, 10-ml

Table 1. Organophosphorus pesticides with thioether groups and their properties

Common name	Empirical formula	Molar mass	Boiling point	Vapour pressure	Solubility in water
Carbophenothion	C <sub>11</sub> H <sub>16</sub> ClO <sub>2</sub> PS <sub>3</sub>	342.88	82°C at 0.013 mbar	4 · 10 <sup>-7</sup> mbar at 20 °C	0.04 g/1 at 20 °C
Demeton	C <sub>8</sub> H <sub>19</sub> O <sub>3</sub> PS <sub>2</sub>	258.35	92–93 °C at 0.2 mbar	3.4 · 10 <sup>-4</sup> mbar at 20 °C	0.06 g/1 (demeton-O); 2 g/1 (demeton-S)
Demeton-S-methyl	C <sub>6</sub> H <sub>15</sub> O <sub>3</sub> PS <sub>2</sub>	230.29	74°C at 0.06 mbar	1.45 · 10 <sup>-3</sup> mbar at 30 °C	3.3 g/1
Disulfoton	C <sub>8</sub> H <sub>19</sub> O <sub>2</sub> PS <sub>3</sub>	274.41	128°C at 1.33 mbar	2.4 · 10 <sup>-4</sup> mbar at 20 °C	0.025 g/1 at 20 °C
Fenamiphos	C <sub>13</sub> H <sub>22</sub> NO <sub>3</sub> PS	303.36	[m.p. 49 °C]	1.33 · 10 <sup>-6</sup> mbar at 30 °C	0.7 g/1 at 20 °C
Fensulfothion	C <sub>11</sub> H <sub>17</sub> O <sub>4</sub> PS <sub>2</sub>	308.36	138–141 °C at 0.013 mbar	10 <sup>-6</sup> mbar at 30 °C	1.6 g/1 at 20 °C
Fenthion	C <sub>10</sub> H <sub>15</sub> O <sub>3</sub> PS <sub>2</sub>	278.34	87 °C at 0.013 mbar	4 · 10 <sup>-5</sup> mbar at 20 °C	0.055 g/1 at 20 °C
Phorate	C <sub>7</sub> H <sub>17</sub> O <sub>2</sub> PS <sub>3</sub>	260.39	118–120 °C at 1.1 mbar	1.1 · 10 <sup>-3</sup> mbar at 20 °C	0.05 g/1 at 25 °C

Gas chromatograph equipped with phosphorus-specific alkali flame ionization detector (AFID)  
Microsyringe, 10- $\mu$ l

## 4. Reagents

Acetone, technically pure, dist.

Acetonitrile, p. a., dist.

Dichloromethane, technically pure, dist.

Petroleum ether, technically pure, boiling range 40 – 60 °C, distilled on sodium

Eluting mixture 1: petroleum ether + dichloromethane 6:4 v/v

Eluting mixture 2: dichloromethane + acetone 9:1 v/v

Pesticide and metabolite standard solutions: 1  $\mu$ g/ml acetone

Potassium permanganate solutions: a) 0.1 mol/l and b) 0.5 mol/l KMnO<sub>4</sub> p. a.

Sodium sulphate, p. a., anhydrous, heated at 600 °C

Activated charcoal, p. a. (Merck No. 2186)

Filter aid, e. g. Celite 545 (Roth)

Silica gel for column chromatography, 0.05 – 0.200 mm (Merck No. 7734)

Cottonwool, degreased

Air, synthetic

Hydrogen, re-purified

Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Homogenize 100 g analytical sample (G) with 170 ml acetonitrile for approx. 3 min in a blender. Suction-filter the homogenate through a filter paper covered with 10 g acetonitrile-moistened Celite in a Buchner funnel. Rinse the filter cake with 30 ml acetonitrile, and continue to press with a glass stopper until it is completely dry. Transfer the filtrate with a 50-ml washing of dichloromethane to a 500-ml separatory funnel, and add another 150 ml dichloromethane. Swirl cautiously, let the lower organic phase drain into a 1-l round-bottomed flask, and re-extract the aqueous phase with 200 ml dichloromethane. Dry the combined organic phases on 10 to 20 g sodium sulphate for approx. 15 min, and filter through a dichloromethane-moistened fluted filter paper into a 1-l round-bottomed flask. Rinse flask and filter paper with a total of 30 ml dichloromethane. Rotary-evaporate the filtrate to dryness.

## 6.2. Cleanup by column chromatography

This cleanup step is necessary only in cases where it is required to quantify residues of carbophenothion and residues of demeton in material with a high content of chlorophyll (e.g. lettuce, spinach); see Section 8.

Slurry 5 g silica gel with 20 ml eluting mixture 1 into a column. Then mix 15 g silica gel with 1 g activated charcoal in a beaker, stir with 30 ml eluting mixture 1, and pour carefully through a small funnel onto the silica gel layer in the column, with the column stopcock open. Use run-out solvent to rinse beaker and funnel. Then top the column with 5 g sodium sulphate.

Dissolve the residue remaining after evaporation in step 6.1 in 10 ml eluting mixture 1, and quantitatively transfer to the column with a washing of the same solvent mixture. Elute with 100 ml eluting mixture 1, and discard the eluate. Then elute with 150 ml eluting mixture 2, and evaporate this eluate to dryness on a rotary evaporator.

## 6.3. Oxidation

Dissolve the evaporation residue derived from 6.1 or 6.2 in 5 ml acetone. To the solution add 25 ml potassium permanganate solution a, with swirling, and let stand for 15 min at room temperature. In the case of sample material with a high content of chlorophyll (e.g. lettuce), add 50 ml potassium permanganate solution a. For quantitative determination of carbophenothion and demeton, oxidize the extract, after cleanup by column chromatography, for 2 min with 25 ml potassium permanganate solution b (see Section 8).

Then transfer the mixture to a 250-ml separatory funnel, and rinse the flask with 25 ml water. Extract twice with 100-ml portions of dichloromethane, and rinse the flask each time with the dichloromethane portions.

Drain the almost colourless organic phases through a cottonwool pad covered with 10 g sodium sulphate in a glass funnel, into a 250-ml round-bottomed flask, and rinse with approx. 10 ml dichloromethane. Evaporate the solution just to dryness on a rotary evaporator. Transfer the residue with acetone to a 10-ml volumetric flask, and make up to the mark with acetone ( $V_{\text{End}}$ ).

## 6.4. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the solution derived from step 6.3 into the gas chromatograph.

### *Operating conditions*

Gas chromatograph	Varian 1400
Column	Glass, 2 mm i.d., 1 m long; packed with 10% DC-200 + 1.5% QF-1 on Gas Chrom Q, 80–100 mesh
Column temperature	215 °C [A] or programmed to rise from 180 to 245 °C at 6 °C/min [B]
Injection port temperature	220 °C
Detector	Alkali flame ionization detector (AFID) for Varian 1400 (Rb <sub>2</sub> SO <sub>4</sub> ) Temperature 280 °C

Gas flow rates	Nitrogen carrier, 37 ml/min
	Hydrogen, 40 ml/min
	Air, 250 ml/min
Attenuation	$10^{-12} \cdot 64$
Recorder	2 mV; chart speed 60 cm/h
Injection volume	2–2.5 µl
Retention times for	[A] [B]
demeton-O sulphone	1 min 35 s 3 min 50 s
demeton-S-methyl sulphone	1 min 50 s 4 min 10 s
phorate sulphone	2 min 4 min 15 s
demeton-S sulphone	2 min 25 s 5 min
disulfoton sulphone	3 min 5 s 5 min 50 s
fenthion sulphone	4 min 40 s 7 min
fensulfothion sulphone	5 min 10 s 7 min 30 s
fenamiphos sulphone	8 min 55 s 10 min 10 s
carbophenothion sulphone	9 min 30 s 10 min 5 s

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak area of the sample solution and comparing it with the peak areas of appropriately diluted reference solutions. When symmetrical, very narrow peaks (width at half height of less than 3 mm) occur, it is better to perform quantitation by the peak height method.

To prepare a reference solution, oxidize 5 ml of a parent compound standard solution (as in step 6.3) for 15 min with 25 ml potassium permanganate solution a, and make up the terminal volume, as for the sample solution, to 10 ml. Prepare the reference solutions for carbophenothion and demeton also by this procedure. 1 µl of reference solution is then equivalent to 0.5 ng of parent compound. Inject equal volumes of the sample solution and appropriate dilutions of the reference solution.

### 7.2. Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified with the compounds at levels of 0.01 to 1.0 mg/kg generally ranged from 80 to 100%. It was only for fensulfothion that the recoveries usually were somewhat lower. The recovery rates obtained for the different compounds and crops are given in Tables 2 and 3.

Table 4 gives the recoveries from untreated control samples fortified with sulfoxides and sulphones each at a level of 0.1 mg/kg.

The deviation of individual values is evident from the following percent data obtained from sets of five recovery experiments run on untreated control samples of potatoes fortified with each of the given compounds at a level of 0.1 mg/kg:

Compound	Mean recovery %	Standard deviation
Carbophenothion	81	4.1
Demeton	89	3.5
Demeton-S-methyl	97	4.4
Disulfoton	97	2.9
Fenamiphos	90	3.0
Fensulfothion	72	3.8
Fenthion	87	3.6
Phorate	91	3.7

The routine limit of determination was less than 0.01 mg/kg.

### 7.3. Calculation of residues

The sum total of the residue R, expressed in mg/kg, of parent compound (thioether), sulphoxide (parent compound for fensulfothion) and sulphone of an organophosphorus pesticide is calculated, relative to the parent compound, from the following equation:

$$R = \frac{F_A \cdot V_{End} \cdot W_{St}}{F_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

V<sub>End</sub> = terminal volume of oxidized extract solution from 6.3 (in ml)

V<sub>i</sub> = portion of volume V<sub>End</sub> injected into gas chromatograph (in µl)

W<sub>St</sub> = amount of compound injected as sulphone with the reference solution (in ng)

F<sub>A</sub> = peak area obtained from V<sub>i</sub> (in mm<sup>2</sup>)

F<sub>St</sub> = peak area obtained from W<sub>St</sub> (in mm<sup>2</sup>)

In accordance with the Federal German Regulation on Maximum Pesticide Residue Limits, the maximum residue limit for the sum total of demeton, disulfoton and their oxidation products is calculated as disulfoton. For the corresponding conversion to disulfoton, the levels found for demeton-O and demeton-S are each multiplied by the factor 1.062.

## 8. Important points

The usual 15-minute oxidation with 25–50 ml potassium permanganate solution does not run quantitatively for carbophenothion in a plant extract processed only by the procedure described in step 6.1 (recoveries of around 20%). The same applies to demeton in the presence of plant material with a high chlorophyll content like lettuce or spinach (recoveries of around 50%).

Although the sulphones of these two compounds can be detected and identified in the gas chromatogram also under these conditions, it is necessary for quantitative determination that

Table 2. Recoveries in % from fortification experiments on different compounds and crops, with quantification of residues as sulphones (without column chromatographic cleanup). Means from at least 3 recovery experiments (1.0 and 0.1 mg/kg) or from 2 recovery experiments (0.05 and 0.1 mg/kg)

	Added mg/kg	Demeton	Demeton-S-methyl	Disulfoton	Fenamiphos	Fensulfothion	Fenthion	Phorate
Apples	1.0	84	100	83	92	71	78	
	0.1	105	104	95	99	76	97	
	0.05	103	95	102	102	86	100	
	0.01	104	102	100	102	87	101	
Curly kale	1.0		89	93	94	78	92	
	0.1		84	86	89	73	87	
Cucumbers	1.0	81	98	93	98	79	90	
	0.1	77	100	98	92	90	102	
Potatoes	0.1	89	97	97	90	72	87	91
Cherries	1.0	90	90	95	96	74	92	
	0.1	93	102	91	92	76	85	
Lettuce	1.0		101	94	93	81	90	
	0.1		89	91	88	84	95	
Carrots	1.0	91	109	83	87	63	78	93
	0.1	94	98	84	97	55	74	89
	0.05		95	106	96	79	103	
	0.01	99	100	104	97	75	100	
Leeks	1.0	81	92	74	82	56	73	
	0.1	83	102	73	84	59	71	
	0.05			70	82	50	69	
	0.01		86	76	91	52	66	
Red cabbage	1.0	88	101	94	91	75	83	
	0.1	90	94	88	87	68	79	
Spinach	1.0		94	82	92	69	87	87
	0.1		104	85	92	68	83	83
	0.05		93	86	94	61	81	
	0.01		100	87	93	64	84	
Tomatoes	1.0	85	89	93	96	77	92	
	0.1	81	102	98	100	81	95	
Head cabbage	1.0	89	95	80	84	70	90	
	0.1	93	93	84	84	60	76	
	0.05	92	103	91	95	61	79	
	0.01	96	102	82	94	58	79	
Onions	1.0	93	88	85	98	64	79	
	0.1	91	100	83	94	69	83	

Table 3. Recoveries in % from fortification experiments, with column chromatographic cleanup as described in step 6.2 and quantification of residues as sulphones. Means from at least 2 recovery experiments

	Added mg/kg	Carbo- phenothion	Demeton
Carrots	1.0	80	85
	0.1	82	87
Potatoes	0.1	81	91
Spinach	1.0	78	87
	0.1	75	89

Table 4. Recoveries in % from untreated control samples of carrots and spinach fortified with sulfoxides and sulphones of given compounds each at a level of 0.1 mg/kg; residues quantified as sulphones. Means from 2 recovery experiments

	Sulfoxides		Sulphones	
	Carrots	Spinach	Carrots	Spinach
<b>Without column chromatographic cleanup:</b>				
Demeton-O			83	20
Demeton-S	82	75	98	91
Demeton-S-methyl	88	78	103	95
Disulfoton	80	77	96	90
Fenamiphos	77	79	92	97
Fensulfothion	55	68	90	83
Fenthion	95	93	93	90
Phorate			100	100
<b>With column chromatographic cleanup:</b>				
Carbophenothion			75	82
Demeton-O			83	88
Demeton-S	79	75	91	87

oxidation is preceded by an additional cleanup on a chromatographic column (see Sections 6.2 and 6.3).

In those cases where the compounds are esters of thiophosphoric acid, it is possible that during metabolism the P=O analogs are formed, which then appear as P=O sulphones in the gas chromatogram although with less response than the P=S sulphones. However, cleanup and oxidation of the P=O analogs were not specially tested. Under the operating conditions given in Section 6.4, the following retention times were obtained for P=O sulphones:

	[A]	[B]
P=O demeton-O sulphone	1 min 25 s	3 min 45 s
P=O phorate sulphone	1 min 30 s	3 min 35 s
P=O fenthion sulphone	4 min	7 min 5 s
P=O fensulfothion sulphone	4 min 20 s	7 min 15 s
P=O carbophenothion sulphone	9 min 20 s	9 min 55 s

When samples are analyzed for conformity with the Federal German Regulation on Maximum Pesticide Residue Limits, account must be taken of the fact that in that regulation the sulphoxides and sulphones are included in the maximum tolerances only for fenaminophos, demeton-S-methyl, demeton and disulfoton.

In those cases where it is required for the thioether, the sulphoxide and the sulphone of a compound to be determined singly or together in an analytical sample, then the solution derived from step 6.1 also can be directly injected into the gas chromatograph. In this case, it is best to use a column oven temperature programmed to rise from 150–245 °C at a rate of 8 °C/min. As the extract is not subjected here to the oxidation procedure in step 6.3, there is the likelihood of additional peaks appearing at low pesticide concentrations (less than 0.05 mg/kg) with some sample materials. The retention times were:

	Thioether	Sulphoxide	Sulphone
Demeton-S-methyl	3 min 5 s	40 s	6 min 50 s
Demeton-O	3 min 20 s		6 min 20 s
Phorate	3 min 55 s		6 min 50 s
Demeton-S	4 min 5 s	1 min	7 min 50 s
Disulfoton	4 min 55 s	1 min 20 s	8 min 40 s
Fenthion	6 min 35 s	10 min 20 s	10 min 5 s
Fenamiphos	8 min 40 s	12 min 30 s	12 min 20 s
Carbophenothion	10 min 5 s		12 min 30 s
Fensulfothion	—	10 min	10 min 30 s

## 9. References

H. P. Burchfield and E. E. Storrs, Analysis for organophosphorus insecticides and metabolites, J. Chromatogr. Sci. 13, 202–211 (1975).

H. Frehse, Zur Rückstandsanalyse von Organophosphor-Pflanzenschutzmitteln II, Pflanzenschutz-Nachr. 24, 269–282 (1971).

*J. Hild and H.-P. Thier*, Aufarbeitung von Pflanzenmaterial zur schnellen Rückstandsanalyse lipoid- und wasserlöslicher Organophosphor-Pestizide, Dtsch. Lebensm. Rundsch. 73, 330–332 (1977).

*J. H. van der Merve and W. B. Taylor*, Gaschromatographische Bestimmung der Rückstände von Demeton-S-methyl und seinen Metaboliten in Pflanzenmaterial, Pflanzenschutz-Nachr. 24, 263–268 (1971).

*J. S. Thornton and C. A. Anderson*, Determination of residues of Di-Syston and metabolites by thermionic emission flame gas chromatography, J. Agric. Food Chem. 16, 895–898 (1968).

## 10. Authors

Institute of Food Chemistry, University of Münster, *J. Hild and H.-P. Thier*

Apples, applesauce, beans, Brussels sprouts, carrots, cauliflower, Chinese cabbage, curly kale, head cabbage, kohlrabi, lettuce, red cabbage, spinach

Gas-chromatographic determination after cleanup by gel permeation chromatography

(German version published 1979)

## 1. Introduction

The method permits determination of residues of 25 organophosphorus insecticides and some of their metabolites. The extracts from the plant material are cleaned up by gel permeation chromatography on Sephadex LH-20 as described in Cleanup Method 3 in this Manual.

## 2. Outline of method

The plant material is extracted with acetone. The extract is diluted with water, shaken with dichloromethane, and the organic phase is evaporated. The extract is cleaned up by gel permeation chromatography on Sephadex LH-20 using ethanol as solvent, and analyzed by gas chromatography with a phosphorus-specific thermionic detector.

## 3. Apparatus

Homogenizer, e.g. Ultra-Turrax, or high-speed blender fitted with leak-proof glass jar and explosion-proof motor

Buchner porcelain funnel, 12 cm dia.

Filtration flask, 500-ml

Fluted filter paper, 24 cm dia. (Schleicher & Schüll, No. 1450 1/2)

Round filter paper, 12 cm dia. (Schleicher & Schüll, No. 602 eh)

Separatory funnel, 1-l

Kuderna-Danish evaporative concentrator flask, see Figure

Rotary vacuum evaporator

Volumetric flask, 1-ml

Gas chromatograph equipped with phosphorus-specific thermionic detector

Microsyringe, 10- $\mu$ l

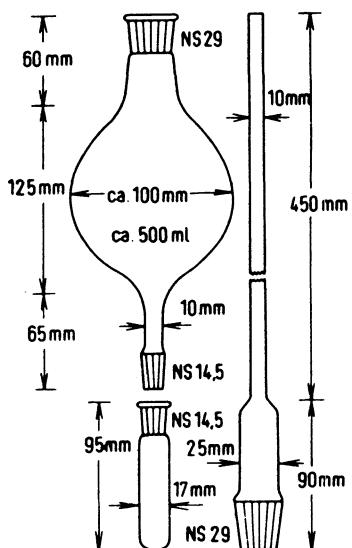


Figure. Kuderna-Danish evaporative concentrator flask.

#### 4. Reagents

Acetone, p. a.

Dichloromethane, p. a.

Ethanol, absolute, not denatured

Insecticide and metabolite standard solutions: 0.5 to 4 µg/ml ethanol

Sodium chloride solution, saturated

Sodium sulphate, p. a., anhydrous

Filter aid, e. g. Celite 545 (Roth)

Compressed air

Hydrogen, re-purified

Nitrogen, re-purified

#### 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

Homogenize 100 g of the sample material (G) with 200 ml acetone for 3 min. Mix the homogenate with 5 g Celite, and suction-filter through an acetone-moistened round filter paper in a Buchner porcelain funnel. Rinse homogenizer jar and filter cake with two 25-ml portions of acetone. Measure the volume of the filtrate ( $V_{\text{Ex}}$ ).

Transfer one fifth ( $V_{\text{RI}}$ ) of the filtrate volume to a separatory funnel, and dilute with 250 ml distilled water and 35 ml saturated sodium chloride solution. Add 60 ml dichloromethane, and shake vigorously for 2 min. Collect the dichloromethane phase, then extract the aqueous phase once more with 50 ml dichloromethane and discard. Wash the combined dichloromethane phases with a mixture of 250 ml distilled water and 35 ml saturated sodium chloride solution, and dry on 10 g sodium sulphate. Filter the dried extract, and rinse the filter cake three times with 10-ml portions of dichloromethane. Then concentrate the filtrate in a Kuderna-Danish evaporative concentrator flask attached to a rotary evaporator to 1–2 ml, add 5 ml ethanol, and concentrate again. Repeat this procedure two or three times, transfer the extract to a 1-ml volumetric flask, and adjust to the 1-ml mark under a stream of nitrogen.

### 6.2. Cleanup

Clean up the whole of the solution derived from 6.1 by gel permeation chromatography on Sephadex LH-20 with ethanol as described in Cleanup Method 3.

Concentrate the appropriate fraction of the column eluate to give a volume of 1 ml ( $V_{\text{End}}$ ) cleaned-up extract solution.

### 6.3. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the solution derived from step 6.2 into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph	Hewlett-Packard 7620A
Column	Glass, 1.9 mm i. d., 2.06 m long; packed with 4% OV-17 on Chromosorb W-HP, 100–120 mesh
Column temperature	Programmed to rise at 4°C/min from 200 to 230°C
Injection port temperature	240°C
Detector	Thermionic detector, Hewlett-Packard 15150
Temperature	300°C
Gas flow rates	Nitrogen carrier, 70 ml/min Hydrogen, 43 ml/min Air, 370 ml/min
Attenuation	$10^3$
Recorder	Hewlett-Packard 7127A; chart speed 6.35 mm/min
Injection volume	2.7 µl

## 7. Evaluation

### 7.1. Method

Identification is achieved by determining the retention time and comparing it with that of compounds from appropriately diluted standard solutions. Quantitation is performed by measuring the peak area of the sample solution and comparing it with the peak areas of appropriately diluted standard solutions. Equal volumes of the sample solution and the standard solutions should be injected.

### 7.2. Recoveries and lowest determined concentration

In recovery experiments run on 113 combinations of insecticides/metabolites and analytical materials, the added compounds were recovered at levels ranging from 80 to 100%. The recovery rates for a further 10 combinations ranged from 70 to 80%. The recovery level was 62% for dichlorvos in curly kale and for ethion in lettuce, and only 49% for fenitrothion in spinach. See Table 1.

The routine limit of determination in the cases marked thus + in Table 1 averaged between 0.1 and 0.05 mg/kg.

Table 1. Tested combinations of compounds and analytical materials (see also Section 7.2)

Analytical material	Azinphos-ethyl	Bromophos	Bromophos-ethyl	Chlорfenvin-phos	Chlorthion	Diazinon	Diazoxon	Dibrom (Naled)	Dichlofenthion	Dichlorvos	Dimethoate	Disulfoton
Apples												
Applesauce			+ 0.1									
Beans, green	+ 0.4	+ 0.1	+ 0.4		+ 0.1	+ 0.05						+ 0.05
Brussels sprouts			+ 0.4									+ 0.1
Carrots			+ 0.1					+ 0.3	- 0.2	+ 0.3	+ 0.1	
Cauliflower			+ 0.4	+ 0.2	+ 0.2		+ 0.2	+ 0.1	+ 0.2	- 0.3	- 0.05	
Chinese cabbage		+ 0.1	+ 0.5									+ 0.1
Curly kale				+ 0.2					- 0.1	+ 0.4		
Head cabbage		+ 0.4	+ 0.1		+ 0.2	+ 0.05		+ 0.1		+ 0.4	+ 0.1	
Kohlrabi	+ 0.4		+ 0.4					- 0.1				- 0.1
Lettuce		+ 0.1	+ 0.3									+ 0.05
Red cabbage			+ 0.4	+ 0.2	+ 0.1	+ 0.2			+ 0.1			+ 0.05
Spinach			+ 0.1	- 0.4	+ 0.2	+ 0.1		+ 0.2		+ 0.3	+ 0.1	

The figures give the concentrations (in mg/kg) at which the recovery experiments were performed.

- + Signifies that the compound, at the given concentration (expressed in mg/kg), was recovered at a rate of more than 80%.
  - Signifies that at the given concentration the compound was recovered at a rate of less than 80%.
- Where no figures are given for a compound alongside a substrate, this indicates that determination of the compound in the particular analytical material has not yet been tried.

Table 1. (contd.)

Analytical material	Disulfoton sulphone	Ethion	Fenchlorphos	Fenitrothion	Fensulfothion	Fenthion	Malaoxon	Malathion	Mevinphos	Omethoate	Paraoxon	Paraoxon-methyl
Apples	+ 0.1						+ 0.4	+ 0.5				
Applesauce							+ 0.4					
Beans, green	+ 0.05	+ 0.1		+ 0.2							+ 0.1	
Brussels sprouts			+ 0.1									
Carrots	+ 0.1	+ 0.1								+ 0.3		+ 0.1
Cauliflower		+ 0.1								- 0.1		
Chinese cabbage			+ 0.1									
Curly kale												
Head cabbage	+ 0.1		+ 0.2		- 0.4	+ 0.1	+ 0.4	+ 0.4	+ 0.1	+ 0.3	+ 0.1	+ 0.1
Kohlrabi	+ 0.1	+ 0.1	+ 0.2	+ 0.2		+ 0.1						+ 0.1
Lettuce			- 0.1									
Red cabbage			- 0.1	+ 0.2								
Spinach	+ 0.05	+ 0.1		- 0.2	+ 0.4	- 0.2	+ 0.4	+ 0.3		+ 0.3		+ 0.1

Table 1. (contd.)

Analytical material	Parathion	Parathion-methyl	Phenkapton	Phorate	Thionazin	Vamidothion	Vamidothion sulphone
Apples	+ 0.1		+ 0.1			+ 0.3	+ 0.3
Applesauce	+ 0.1		+ 0.1		+ 0.1	+ 0.3	
Beans, green		+ 0.01					
Brussels sprouts							
Carrots	+ 0.1	+ 0.1					
Cauliflower		+ 0.05					
Chinese cabbage			+ 0.05				
Curly kale				+ 0.2			
Head cabbage	+ 0.1	+ 0.1	+ 0.2				
Kohlrabi	+ 0.1	+ 0.05	+ 0.1	+ 0.2			
Lettuce			+ 0.1				
Red cabbage					+ 0.1		
Spinach			+ 0.1				

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, of an identified compound is calculated from the following equation:

$$R = \frac{F_A \cdot V_{End} \cdot V_{Ex} \cdot W_{St}}{F_{St} \cdot V_i \cdot V_{RI} \cdot G}$$

where

G      = sample weight (in g)

$V_{Ex}$     = volume of filtrate after extraction (in ml)

$V_{RI}$     = portion of volume  $V_{Ex}$  used for further processing (in ml)

$V_{End}$    = terminal volume of sample solution from 6.2 (in ml)

$V_i$       = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu$ l)

$W_{St}$     = amount of compound injected with standard solution (in ng)

$F_A$       = peak area obtained from  $V_i$  (in  $\text{mm}^2$ )

$F_{St}$     = peak area obtained from  $W_{St}$  (in  $\text{mm}^2$ )

## 8. Important points

It is recommended to use an automatic sampler and to employ peak integration for the gas-chromatographic determination.

## 9. References

*W. Ebing*, Gaschromatographischer Rückstandsnachweis von 47 phosphorhaltigen Insektizid-Wirkstoffen nach einem Einheitsverfahren, Pflanzenschutzberichte 38, 1–22 (1968).

*J. Pflugmacher* and *W. Ebing*, Reinigung von Phosphorsäureinsektizidrückständen in Gemüseextrakten durch Gelchromatographie an Sephadex LH-20, J. Chromatogr. 93, 457–463 (1974).

## 10. Authors

Federal Biological Research Centre for Agriculture and Forestry, Department for Ecological Chemistry, Division for Pesticide Research, Berlin, *W. Ebing* and *J. Pflugmacher*

# Bromine-Containing Fumigants (as total inorganic bromide)

S 18

Cereals (grains), dried fruit, dried mushrooms, dried vegetables, fresh fruit, fresh mushrooms, fresh vegetables, lettuce, milled cereal products, nuts, onions, parsley, sage

Gas-chromatographic determination

(German version published 1982)

## 1. Introduction

Bromine-containing fumigants, especially methyl bromide, are used in crop protection for soil disinfection, and also post-harvest for the protection of stored raw agricultural commodities. Inorganic bromide is formed as a degradation product of these fumigants. It may enter plants by being absorbed from treated soil by the roots, or be retained in fumigated goods.

The described method permits the determination of the total content of inorganic bromide in the material to be analyzed, including its natural bromide content. Any residual amounts of the fumigant itself are not determined.

## 2. Outline of method

The analytical material is suspended in an aqueous solution of ethylene oxide acidified with sulphuric acid whereupon inorganic bromide is extracted simultaneously and converted to 2-bromoethanol. This derivative is partitioned into ethyl acetate and determined, without further cleanup, by electron capture gas chromatography.

## 3. Apparatus

High-speed blender fitted with glass jar  
Erlenmeyer flask, 200-ml, with ground joint  
Test tube, 20-ml, with ground joint  
Gas chromatograph equipped with electron capture detector  
Microsyringe, 10- $\mu$ l

## 4. Reagents

Ethyl acetate for residue analysis: Before use, check each newly opened bottle of this solvent by injecting 5  $\mu$ l into the gas chromatograph under the conditions described in Section 6.3. If interfering peaks are observed, purify by distillation over a fractionating column

Bromide standard solution, 50 µg/ml bromide: Dissolve 149 mg potassium bromide p. a. in 100 ml water, and dilute 5.0 ml of this solution with water to a volume of 100 ml

2-Bromoethanol standard solution, 1 µg/ml ethyl acetate

Sulphuric acid, p. a., 3 mol/l

Ethylene oxide solution, 4 g/100 ml: Prepare by pouring 96.0 ml ice-cold water into a 100-ml volumetric flask, and adding ethylene oxide dropwise to the mark from the completely inverted, ice-cooled pressurized can. Shake several times to mix. Prepare fresh daily. Use fume hood

Ammonium sulphate, p. a. (e. g. Merck No. 1217)

Sodium sulphate, p. a., anhydrous, heated 5 h at 500 °C

Ethylene oxide, pure (99.8%), in pressurized can (e. g. Fluka No. 03901) fitted with valve

2-Bromoethanol (e. g. Merck No. 820175)

Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction and simultaneous derivatization

For cereals, dried fruit, dried vegetables or dried mushrooms, weigh 1.00 g (G) of the finely ground powder into an Erlenmeyer flask, slurry in 10 ml ethylene oxide solution and add 1.0 ml sulphuric acid. For fresh fruit and vegetables, homogenize the analytical material in a blender, weigh 5.0 g (G) of the homogenate into an Erlenmeyer flask, and add 5 ml ethylene oxide solution and 1 ml sulphuric acid. In both cases, stopper the flask and let stand for 30 min at room temperature.

### 6.2. Partition

To the reaction mixture prepared in step 6.1 add 50 ml ethyl acetate ( $V_{End}$ ) and 4 g ammonium sulphate. Stopper the flask, shake vigorously for 1 min, and then let stand for 20 min with occasional shaking. Decant approx. 10 ml of the upper organic phase into a test tube. Add 0.5 g sodium sulphate, stopper the test tube and shake vigorously.

### 6.3. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the solution derived from 6.2 into the gas chromatograph.

#### *Operating conditions*

Gas chromatograph

Perkin-Elmer F-11

Column

Glass, 3 mm i. d., 1.5 m long; packed with 10% Carbowax 20 M on Chromosorb W-HP, 100–120 mesh

Column temperature

130 °C

Injection port temperature	200 °C
Detector	$^{63}\text{Ni}$ electron capture detector Temperature 250 °C
Gas flow rate	Nitrogen carrier, 40 ml/min
Attenuation	1 · 8
Recorder	1 mV; chart speed 10 mm/min
Injection volume	5 $\mu\text{l}$
Retention time for 2-bromoethanol	3 min 15 s

Adjust the attenuation setting for 5  $\mu\text{l}$  of the 2-bromoethanol standard solution (equivalent to 5 ng 2-bromoethanol) to yield a peak height of at least 50% full-scale deflection.

Check for complete derivatization by injecting into the gas chromatograph 5  $\mu\text{l}$  of a reference solution prepared by adding 10 ml ethylene oxide solution and 1 ml sulphuric acid to 1 ml of the bromide standard solution and subjecting it to the procedure described in steps 6.1 and 6.2. The resultant peak must be about 1.5 times higher than the peak obtained for 5 ng 2-bromoethanol.

Inject approx. 5  $\mu\text{l}$  of a reagent blank solution prepared by simply mixing 10 ml ethylene oxide solution and 1 ml sulphuric acid and carrying the mixture through steps 6.1 and 6.2. In this case, the chromatogram should not show a peak with the same retention time as 2-bromoethanol. Occasionally a tailing peak with a longer retention time appears, which is due to ethylene glycol and does not interfere with the determination.

## 7. Evaluation

### 7.1. Method

Quantitation is performed by measuring the peak height or peak area of the sample solution and comparing it with the peak height or peak area of 2-bromoethanol from the appropriate standard solution. Inject equal volumes of sample solution and standard solution. If the peak of the sample solution is substantially higher than that of the standard solution, prepare a suitable dilution in ethyl acetate and inject again.

### 7.2. Recoveries and lowest determined concentration

In experiments with 1 ml of an aqueous solution containing 50  $\mu\text{g}$  bromide, a mean recovery of 99% ( $s = 6\%$ ;  $n = 6$ ) was obtained. During analyses of various commodities treated with methyl bromide, the values obtained were compared with the results of a series of spectrophotometric determinations of the total bromide content [G. Hunter and A. G. Goldspink, Analyst 79, 467–472 (1954)]:

Commodity	Spectro-photometry mg/kg	Gas chroma- tography mg/kg	s mg/kg	n
Tomato powder	73	72	4	6
Mushroom powder	216	233	5	7
Maize flour	50	49	3	7
Buckwheat flakes	305	340		
Wheat	27	28		
Wheat	58	56		
Carrots, dehydrated	24	28		
Lettuce, lyophilized	260	280		

In experiments in which foods of vegetable origin were fortified with inorganic bromide, the following recoveries were obtained (making allowance for the natural content):

Food	Added mg/kg	Found mg/kg	Recovery %
Wheat flour	—	3	—
Wheat flour	10	14	110
Wheat flour	25	30	108
Wheat flour	50	50	94
Leeks	—	9	—
Leeks	50	53	88
Tomato flakes	—	10	—
Tomato flakes	50	55	92
Tomato flakes	100	98	89
Dehydrated mushrooms	—	5	—
Dehydrated mushrooms	50	52	91
Dehydrated mushrooms	100	95	90
Dehydrated mushrooms	250	270	106
Dehydrated mushrooms	500	535	108

The routine limit of determination was approx. 1 mg/kg for fresh plant material and approx. 5 mg/kg for dried material.

### 7.3. Calculation of residues

The residue R, expressed in mg/kg total bromide, is calculated from the following equation:

$$R = \frac{F_A \cdot V_{End} \cdot W_{St}}{F_{St} \cdot V_i \cdot G} \cdot 0.639$$

where

G      = sample weight (in g)

V<sub>End</sub>    = volume of ethyl acetate used for partition in step 6.2 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into gas chromatograph (in  $\mu\text{l}$ )

$W_{St}$  = amount of 2-bromoethanol injected with standard solution (in ng)

$F_A$  = peak area (in  $\text{mm}^2$ ) or peak height (in mm) obtained from  $V_i$

$F_{St}$  = peak area (in  $\text{mm}^2$ ) or peak height (in mm) obtained from  $W_{St}$

0.639 = factor for conversion of 2-bromoethanol to bromide

## 8. Important points

Since the yield of 2-bromoethanol decreases sharply with increased sample size, it should not be attempted to take more than the prescribed quantities.

For the sake of economy, the method can be miniaturized by reducing the sample weight and the amount of reagents by a factor 10. Weigh 100 mg of the dried material into a 10-ml test tube. Add 1.0 ml ethylene oxide solution and 0.1 ml sulphuric acid. Stopper and allow to react for 30 min. Then add 0.4 g ammonium sulphate and 5.0 ml ethyl acetate, shake for 1 min, and let stand for 20 min with occasional shaking. If emulsions form, centrifuge for 15 min at 3000 r.p.m. Decant the organic phase into a clean vial and dry with a little anhydrous sodium sulphate.

The *GDCh-Arbeitsgruppe "Pestizide"* optimized the analytical method in two collaborative studies and tested it successfully for its reproducibility. In 12 laboratories, the bromide concentration was found to be 42.0 mg/kg ( $s = 2.1 \text{ mg/kg}$ ) in a sample of maize flour and 26.1 mg/kg ( $s = 3.2 \text{ mg/kg}$ ) in a sample of carrot flakes.

## 9. References

- Arbeitsgruppe "Pestizide"*, 8. Mitteilung: Überprüfung einer gaschromatographischen Analysenmethode für Bromidrückstände, Lebensmittelchem. gerichtl. Chem. 35, 49 (1981).  
*T. Stijve*, Improved method for the gas chromatographic determination of inorganic bromide residues in foodstuffs fumigated with methyl bromide, Dtsch. Lebensm. Rundsch. 73, 321–325 (1977).  
*T. Stijve*, Gas chromatographic determination of inorganic bromide residues – a simplified procedure, Dtsch. Lebensm. Rundsch. 77, 99–101 (1981).

## 10. Authors

Nestec Ltd., Central Laboratory for Quality Assurance, Vevey, Switzerland, *T. Stijve*  
 Working Group on Pesticides, Food and Forensic Chemistry Division of the Gesellschaft  
 Deutscher Chemiker (German Chemical Society), *H.-P. Thier*



# **Organochlorine, Organophosphorus, Nitrogen-Containing and Other Pesticides** S 19

Crops and Foods as listed in Table 1

Gas-chromatographic determination after cleanup by gel permeation chromatography and silica gel minicolumn chromatography

(German version published 1982)

## **1. Introduction**

The method permits determination of the residues of some 85 lipid- and water-soluble pesticides and several metabolites. The broad applicability of the method results mainly from standardization of the extraction conditions for samples of differing water content, from the ability to process the extract without loss of water-soluble compounds, from the use of gel permeation chromatography for cleanup, and from the use of several selective detectors for gas chromatography. Cleanup by gel permeation chromatography is performed by the procedure described in Cleanup Method 6 in this Manual.

## **2. Outline of method**

Plant material is extracted with acetone. Water is added beforehand in an amount that takes full account of the natural water content of the sample so that during extraction the acetone : water ratio remains constant at 2 : 1 v/v. The extract is saturated with sodium chloride and diluted with dichloromethane, resulting in separation of excess water. The evaporation residue of the organic phase or a fat solution is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel, using a mixture of cyclohexane and ethyl acetate as eluant and an automated gel permeation chromatograph. The residue-containing fraction is concentrated, and analyzed directly by gas chromatography using a phosphorus- or nitrogen-selective detector. For analysis by electron capture and in some cases also for nitrogen-selective detection, a supplemental cleanup on a small silica gel column is necessary. But in this cleanup step, the other compounds are also separated in several fractions thus providing additional leads for identification.

Table 1. Crops and foods on which method was tested

Apples	Meat and meat products
Bananas	Melons
Beans	Milk and dairy products
Beer	Must
Carrots	Nuts
Cauliflower	Oilseed
Cereals	Onions
Cherries	Peaches
Citrus fruit	Peanuts
Cocoa products	Pears
Coffee, raw	Peppers, sweet
Cucumbers	Pineapples
Curly kale	Plums
Currents, red	Potatoes
Dried egg products	Spices (herbs)*)
Fats (vegetable and animal)	Strawberries
Grapes	Sugar beet (edible root)
Head cabbage	Tea and tea-like products*)
Hop cones	Tobacco
Kohlrabi	Tomatoes
Lettuce	Wine

\* bay leaf, borage, caraway, cassia, camomile, chillies, cinnamon, clove, curry, estragon (tarragon), fennel, garlic, ginger, lemon balm, mace, mallow, mugwort, nutmeg, origanum, peppermint, pepper seeds, pimento, star aniseed, summer savory

### 3. Apparatus

High-speed blender fitted with leak-proof glass or stainless steel jar and explosion-proof motor  
Buchner porcelain funnel, 13.5 cm dia.

Filtration flask, 1-l

Filter paper, 13.5 cm dia., fast flow rate (Schleicher & Schüll), extracted exhaustively with dichloromethane

Separatory funnel, 500-ml, with ground stopper and Teflon stopcock

Round-bottomed flasks, 500-ml and 250-ml, with ground joints

Round-bottomed flask, 100-ml, with ground joint and 9-cm long neck

Chromatographic tube, 7 mm i.d., 23 cm long, with extended outlet

Graduated cylinder, 250-ml

Rotary vacuum evaporator, 30–40°C bath temperature

Test tubes, capacity of 12 to 15 ml, with ground stoppers and graduation marks at 2.5, 5.0 and 10.0 ml

Gas chromatographs fitted with electron capture detector, thermionic detector and flame photometric detector

Microsyringe, 10-µl

*Important:* All glassware must be rinsed with acetone and dried before use

## 4. Reagents

Acetone, for residue analysis  
Dichloromethane, for residue analysis  
Ethyl acetate, for residue analysis  
n-Hexane, for residue analysis  
Isooctane, for residue analysis  
Toluene, for residue analysis  
Water, distilled from glass apparatus  
Eluant 1: hexane + toluene mixture 65:35 v/v  
Eluant 2: toluene  
Eluant 3: toluene + acetone mixture 95:5 v/v  
Eluant 4: toluene + acetone mixture 8:2 v/v  
Eluant 5: acetone  
Pesticide standard solutions: 0.01–10 µg/ml (depending on detector sensitivity), in a suitable solvent  
Sodium chloride, p.a.  
Sodium sulphate, p.a., heated at 550°C for at least 2 h  
Filter aid, e.g. Celite 545 (Roth)  
Silica gel, deactivated with 1.5% water: Heat Silica gel 60, 70–230 mesh (Merck No. 7734), for at least 5 h at 130°C, allow to cool in a desiccator, and store in a tightly stoppered container in the desiccator. To 98.5 g dried silica gel in a 300-ml Erlenmeyer flask (with ground joint), add 1.5 ml water dropwise from a burette, with continuous swirling. Immediately stopper flask with ground stopper, shake vigorously for 5 min until all lumps have disappeared, next shake for 2 h on a mechanical shaker, and then store in a tightly stoppered container  
Glass wool, extracted exhaustively with dichloromethane  
Cottonwool, extracted exhaustively with dichloromethane  
Air, synthetic, re-purified  
Argon + methane mixture 9:1 v/v  
Helium, re-purified  
Hydrogen, re-purified  
Nitrogen, re-purified

## 5. Sampling and sample preparation

The analytical sample is taken and prepared as described on p. 17 ff.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Plant material and other foods with a water content exceeding 70 g/100 g

Homogenize 100 g (G) of the comminuted sample having a water content of x g/100 g (see Table 2) with (100 – x) g water and 200 ml acetone for 3 min in the blender. Add 10 g Celite and again homogenize for 10 s.

Table 2. Average water content of crops and foods, on which calculation of the amount of acetone and water to be added in steps 6.1.1 and 6.1.2 can be based

Average water content in g/100 g	Crops and foods
5	Peanuts, cocoa powder, nuts
10	Cereals, spices (herbs), coffee (raw), oilseed, tea, tea-like products
75	Bananas, horse radish
80	Peas, currants (black), potatoes, parsley, grapes
85	Pineapples, apples, pears, currants (red), cherries, milk, oranges, plums, chives
90	Beer, cauliflower, beans (green), broccoli, strawberries, grapefruit, curly kale, kohlrabi, melons, carrots, mustard, peppers, peaches, mushrooms, red beet, red cabbage, spinach, wine, head cabbage, lemons, sugar beet (edible root), onions
95	Witloof chicory, endives, cucumbers, radish, rhubarb, lettuce, celeriac, asparagus, tomatoes

#### 6.1.2. Plant material with low water content

Weigh out 10 to 50 g (G) of the dry or dried analytical material having a water content of x g/100 g (for example, 25–50 g for dried fruit and dried vegetables; 10–20 g for spices and tea; 50 g for cereal grains). Then add sufficient water to adjust the total water present to 100 g. The amount of water (W) to be added is calculated from the equation  $W = 100 - (G \cdot x)/100$ . Blend and let stand for 10 to 20 min. Next add 200 ml acetone and homogenize for 3 min. Then add 10 g Celite and homogenize again for 10 s.

#### 6.1.3. Vegetable and animal fats

The solution of fats is subjected directly to cleanup by gel permeation chromatography in step 6.3, by the procedure described in Cleanup Method 6. To achieve a lower limit of determination, a 20 to 30-g amount of fat can also be cleaned up firstly by the procedure described in Cleanup Method 5.

## 6.2. Partition

Filter the homogenate derived from step 6.1.1 or 6.1.2 through a fast flow-rate filter paper in a Buchner funnel, with low water jet pump suction, until more than 200 ml filtrate is collected. Therefore, do not allow the filter cake to pull dry.

Measure out 200 ml of filtrate ( $V_{R1}$ ) in a graduated cylinder, and transfer to a 500-ml separatory funnel. Add 20 g sodium chloride, and shake vigorously for 3 min. Next add 100 ml

dichloromethane, shake for 2 min, and then let stand for approx. 10 min to allow the phases to separate. Discard the lower aqueous phase. To the organic phase add approx. 25 g sodium sulphate, let stand for approx. 30 min with occasional swirling, and then filter through a cotton-wool plug layered with 3 cm sodium sulphate in a funnel. Collect the filtrate in a 500-ml round-bottomed flask, and rinse separatory funnel and filter twice with 20-ml portions of ethyl acetate. Concentrate the solution to 2 ml on a rotary evaporator. Remove the last traces of solvent with a gentle stream of air.

### **6.3. Cleanup by gel permeation chromatography**

Dissolve the whole of the evaporation residue derived from step 6.2, and clean up by gel permeation chromatography on Bio Beads S-X3, as described in Cleanup Method 6.

Concentrate the appropriate fraction of the column eluate, and make up with ethyl acetate to give a volume of 5.0 ml ( $V_{R4}$ ). This addition of ethyl acetate must not be omitted on any account in order to ensure complete dissolution of the residue.

### **6.4. Chromatography on a silica gel minicolumn**

#### **6.4.1. Preparation of column**

Pack the chromatographic tube in the following order: glass wool plug, 1.0 g deactivated silica gel, 5 to 10-mm layer of sodium sulphate, glass wool plug. Before use, rinse the column with 5 ml hexane and discard the eluate. As soon as the hexane has drained to the top of the silica gel, add the sample solution.

#### **6.4.2. Checking separation efficiency of silica gel**

To the column pre-washed in step 6.4.1, add 1.0 ml of a solution containing 0.05 µg HCB, 0.10 µg lindane, 0.20 µg heptachlor epoxide, 0.25 µg α-endosulfan, 0.25 µg dieldrin and 1.25 µg endosulfan sulphate/ml hexane. Provided the activity of the silica gel is correctly adjusted, the added compounds should be present (after elution as described in step 6.4.3 and electron capture gas-chromatographic analysis as described in step 6.5.2) in the following fractions:

Eluate 1: HCB (100%), lindane (100%), heptachlor epoxide (partial amount), α-endosulfan (partial amount)

Eluate 2: Heptachlor epoxide (residual amount), α-endosulfan (residual amount), endosulfan sulphate (95–100%), dieldrin (100%).

#### **6.4.3. Fractionation of sample extract**

Pipet 2.5 ml ( $V_{R5}$ ) of the solution derived from 6.3 into a long-neck round-bottomed flask, and add 5 ml isoctane. Carefully evaporate to 1 ml (on no account to dryness) on a rotary evaporator (rotate slowly, immerse flask only a little). If the solution still smells of ethyl acetate, again add isoctane and repeat evaporation.

Pipet the solution remaining after evaporation onto the silica gel column pre-washed in step 6.4.1, and rinse with approx. 1 ml hexane. Then rinse the flask with 2 ml eluant 1, and as soon as the hexane has drained to the top of the column packing add the rinsing to the column. From

this point, collect the eluate in a receiver consisting of a graduated test tube. Then elute with a further 6 ml of eluant 1, and fill the receiver with eluant 1 to a volume of 10 ml ( $V_{End}$ ). The resultant solution represents eluate 1.

Rinse the flask used for evaporation once again with 2 ml toluene (eluant 2). Then add this rinsing to the column. Collect the solution in a second graduated test tube, and elute with 6 ml toluene. Fill the receiver with toluene to a volume of 10 ml ( $V_{End}$ ) to give eluate 2. Continue chromatography by the same procedure performed consecutively with eluant 3, eluant 4 and acetone (eluant 5). Each time, rinse the flask with 2 ml, elute with a further 6 ml and make up the eluate to a volume of 10 ml ( $V_{End}$ ) to give eluates 3, 4 and 5.

The distribution of the compounds among the different eluates is shown in Table 3.

Table 3. Distribution of compounds in eluates of silica gel column chromatography (step 6.4.3)

Compound	Eluate 1	Eluate 2	Eluate 3	Eluate 4	Eluate 5
<i>Organochlorine compounds</i>					
Aldrin	+++ +	-	-	-	-
Camphechlor	+++ +	-	-	-	-
$\alpha$ -Chlordane	+++ +	-	-	-	-
$\gamma$ -Chlordane	+++ +	-	-	-	-
Chlorfenson	(+)	++ +	-	-	-
$o,p'$ -DDD	+++ +	-	-	-	-
$p,p'$ -DDD	+++ +	-	-	-	-
$o,p'$ -DDE	+++ +	-	-	-	-
$p,p'$ -DDE	+++ +	-	-	-	-
$o,p'$ -DDT	+++ +	-	-	-	-
$p,p'$ -DDT	+++ +	-	-	-	-
Dicofol	+	+++	-	-	-
Dieldrin	-	+++ +	-	-	-
$\alpha$ -Endosulfan	+	+++	-	-	-
$\beta$ -Endosulfan	-	+++ +	-	-	-
Endosulfan sulphate	-	+++ +	-	-	-
Endrin	-	+++ +	-	-	-
Fenson	-	+++ +	-	-	-
Heptachlor	+++ +	-	-	-	-
Heptachlor epoxide	+++	++	-	-	-
Hexachlorobenzene	+++ +	-	-	-	-
$\alpha$ -Hexachlorocyclohexane	+++ +	-	-	-	-
$\beta$ -Hexachlorocyclohexane	+++ +	-	-	-	-
$\gamma$ -Hexachlorocyclohexane	+++ +	-	-	-	-
$\delta$ -Hexachlorocyclohexane	+++ +	-	-	-	-
Isodrin	+++ +	-	-	-	-
Methoxychlor	-	+++ +	-	-	-
Oxychlordane	+++ +	(+)	-	-	-
Polychlorinated biphenyls	+++ +	-	-	-	-
Quintozone	+++ +	-	-	-	-
Tecnazene	+++ +	-	-	-	-
Tetradifon	-	+++ +	-	-	-
Tetrasul	+++ +	-	-	-	-

Table 3. (contd.)

Compound	Eluate 1	Eluate 2	Eluate 3	Eluate 4	Eluate 5
<i>Organophosphorus compounds</i>					
Azinphos-ethyl	-	-	+++ +	-	-
Bromophos	+++	+	-	-	-
Bromophos-ethyl	+++ +	(+)	-	-	-
Carbophenothion	-	++	-	-	-
Chlorfenvinphos	-	-	+++	++	-
Chlorpyrifos	+	+++	-	-	-
Diazinon	-	-	+++ +	-	-
Dicrotophos	-	-	-	-	+++ +
Dimefox	-	-	-	-	+++ +
Dimethoate	-	-	-	+++	++
Disulfoton sulphone	-	-	+++ +	-	-
Disulfoton sulfoxide	-	-	-	-	+++ +
Ditalimfos	-	-	+++	-	-
Ethion	-	+++ +	-	-	-
Fenchlorphos	+++	+	-	-	-
Fenitrothion	-	+++	-	-	-
Formothion	-	-	+++	-	-
Iodofenphos	+++	+	-	-	-
Malaoxon	-	-	-	++ +	-
Malathion	-	-	++ +	-	-
Methamidophos	-	-	-	-	+++ +
Methidathion	-	-	++ +	-	-
Mevinphos	-	-	-	+++ +	-
Omethoate	-	-	-	-	+++ +
Paraoxon	-	-	-	++ +	-
Parathion	-	+++	(+)	-	-
Parathion-methyl	-	+++	(+)	-	-
Phosalone	-	-	+++	-	-
Pyrazophos	-	-	+++ +	-	-
Sulfotep	-	+++	+	-	-
Thionazin (zinophos)	-	+	++ +	-	-
Triazophos	-	+++	(+)	-	-
<i>Other compounds</i>					
Anilazine	-	-	+++ +	-	-
Binapacryl	-	+++ +	-	-	-
Bitertanol	-	-	-	++ +	-
Captafol	-	-	+++ +	-	-
Captan	-	-	+++ +	-	-
Dichlofluanid	-	++	++	-	-
Dinocap	-	+++ +	-	-	-
Fluotrimazole	-	-	++ +	+	-
Fuberidazole <sup>a)</sup>	-	-	-	+++ +	(+)
Imazalil <sup>a)</sup>	-	-	-	-	+++ <sup>b)</sup>
Metribuzin	-	-	++	-	-
Pentachloroaniline	+++ +	-	-	-	-
Piperonyl butoxide	--	-	++ +	(+)	-

Table 3. (contd.)

Compound	Eluate 1	Eluate 2	Eluate 3	Eluate 4	Eluate 5
Propoxur	—	—	+++	+	—
Pyrethrins	—	—	++++	(+)	—
Rabenazole <sup>a)</sup>	—	—	+++	—	—
Resmethrin	—	+++	—	—	—
Triadimefon	—	—	++	++	—
Triadimenol	—	—	—	+++	+
Trifluralin	+++	—	—	—	—
Vinclozolin	—	++	(+)	—	—

a) with complete exclusion of light

b) complete elution with further 8 ml acetone

(+) less than 10%

+ approx. 10–30%

++ approx. 30–60%

+++ approx. 60–90%

++ + more than 90%

## 6.5. Gas-chromatographic determination

Inject an aliquot ( $V_i$ ) of the eluates 1 to 5 derived from step 6.4.3 into the gas chromatograph. For analyses with the thermionic detector and the flame photometric detector, an aliquot ( $V_i$ ) of the solution derived from step 6.3 ( $V_{R4}$ ) also can be injected into the gas chromatograph in many cases.

### *Operating conditions*

The determinations were performed with different gas chromatographs, columns and detectors.

#### 6.5.1. Gas chromatograph 1

Gas chromatograph	Hewlett-Packard 5750 G
Column	Glass, 4 mm i.d., 1.2 m long; packed with 1.5% OV-17 + 1.95% QF-1 on Chromosorb W-HP, 100–120 mesh
Column temperature	180°C
Injection port temperature	230°C
Detector	Alkali flame ionization detector (N-FID) Temperature 330°C
Gas flow rates	Helium carrier, 60 ml/min Hydrogen, 30 ml/min Air, 200 ml/min
Attenuation	10 · 32
Recorder	1 mV; chart speed 60 cm/h
Injection volume	5 µl

### 6.5.2. Gas chromatograph 2

Gas chromatograph	Hewlett-Packard 5710 G
Column	Glass, 4 mm i. d., 1.8 m long; packed with 3% OV-61 + 7.5% QF-1 + 3% XE-60 on Chromosorb W-HP, 100–120 mesh
Column temperature	225 °C
Injection port temperature	250 °C
Detector	$^{63}\text{Ni}$ electron capture detector, pulse modulated Temperature 300 °C
Carrier gas flow rate	Argon-methane, 40 ml/min
Attenuation	256
Recorder	1 mV; chart speed 75 cm/h
Injection volume	5 µl

### 6.5.3. Gas chromatograph 3

Gas chromatograph	Hewlett-Packard 5750
Column	Glass, 4 mm i. d., 1.8 m long; packed with 6.5% DC-200 + 0.01% Versamid 900 on Gas Chrom Q, 80–100 mesh
Column temperature	210 °C
Injection port temperature	235 °C
Detector	Tracor flame photometric detector (P mode; 526-nm filter) Temperature 190 °C
Gas flow rates	Helium carrier, 55 ml/min Hydrogen, 110 ml/min Air, 150 ml/min
Attenuation	$4 \cdot 10^{-3}$
Recorder	1 mV; chart speed 75 cm/h
Injection volume	5 µl

### 6.5.4. Comments

The retention times under the described operating conditions were 4 min (6.5.2) for aldrin, and 5 min 30 s (6.5.1) and 3 min 36 s (6.5.3) for parathion. The retention times for the analyzed compounds relative to aldrin = 1.00 and to parathion = 1.00 are given in Table 4. Camphechlor, pyrethrins and polychlorinated biphenyls give several peaks on all columns; the positions of these peaks must each be determined by recording a reference chromatogram.

For identification and quantitation, the analysis must be performed on at least two different columns. Suitable additional columns are, for example: 1.8 m long, packed with 4% SE-30 on Chromosorb W-HP, 100–120 mesh (6.5.1); 1.8 m long, packed with 1% OV-1 on Chromosorb G-AW-DMCS, 80–100 mesh (6.5.2); 1.8 m long, packed with 1.5% OV-17 + 1.95% QF-1 on Chromosorb W-HP, 100–120 mesh (6.5.3).

Anilazine, captafol and dinocap were found to be difficult to determine using the column described in Section 6.5.2. For their analysis, it is better to use the above-mentioned additional column of 1.8 m length packed with 1% OV-1 on Chromosorb G-AW-DMCS, 80–100 mesh.

Using this column, the retention times relative to aldrin = 1.00 were 1.31 for anilazine, 3.61 for captafol and 4.65, 4.98, 5.56 and 6.02 for dinocap.

Piperonyl butoxide (pip.) and resmethrin (resm.) were analyzed with a combined GC/MS system by mass fragmentography at m/e 176 and m/e 123, respectively. The operating conditions were as follows:

System	Finnigan GC/MS 4500/3200
Column	Glass, 2 mm i. d., 1.2 m long; packed with 3% OV-1 on Chromosorb W-DMCS, 60–80 mesh
Column temperature	230°C (pip.) and 240°C (resm.)
Injection port temperature	250°C
Detector	Mass spectrometer Separator temperature 250°C Inlet temperature 220°C
Carrier gas flow rate	Helium, 20 ml/min
Attenuation	$1 \cdot 10^8$ at 500 mV (pip.) and at 1000 mV (resm.)
Recorder	1 mV; chart speed 1 cm/min
Injection volume	5 µl

## 7. Evaluation

### 7.1. Method

Identification is achieved by determination of the retention times relative to aldrin and to parathion (Table 4). Further indications of the identity of the assumed compounds are provided by the selective response of the different detectors and by the occurrence of the compounds in the different eluates collected from the silica gel column (Table 3).

Quantitation is performed by measuring the peak area and comparing it with the peak areas from solutions of the found compound having known concentration. Equal volumes of the cleaned-up extract solution and the standard solutions should be injected. When using an ECD, it is especially important to ascertain that the detector is indeed operating within its linear range and then to inject into the gas chromatograph weights of sample residue and reference standard which fall within that range.

Table 4. Examples of relative retention times (RRT), relative to aldrin or to parathion, under the gas-chromatographic conditions in steps 6.5.1 to 6.5.3  
 6.5.1 (Column packed with 1.5% OV-17 + 1.95% QF-1)

Compound	RRT Parathion	Compound	RRT Parathion
Mevinphos	0.18	Parathion	1.00
Thionazin (zinophos)	0.25	Bromophos	1.05
Propoxur	0.29	Chlorfenvinphos	1.20
Diazinon	0.37	Triadimenol	1.27
Dimethoate	0.55	Bromophos-ethyl	1.32
Fenchlorphos	0.68	Methidathion	1.60
Chlorpyrifos	0.73	Imazalil	1.99
Parathion-methyl	0.76	Disulfoton sulphone	2.28
Formothion	0.78	Ethion	2.93
Metribuzin	0.81	Fluotrimazole	2.99
Fuberidazole	0.87	Carbophenothion	3.39
Malathion	0.87	Triazophos	4.76
Paraoxon	0.88	Phosalone	6.84
Triadimefon	0.89	Azinphos-ethyl	10.3
Fenitrothion	0.94	Pyrazophos	12.1
Rabenbazole	1.00	Bitertanol	13.1

Table 4. (contd.)  
 6.5.2 (Column packed with 3% OV-61 + 7.5% QF-1 + 3% XE-60)

Compound	RRT Aldrin	Compound	RRT Aldrin
Trifluralin	0.31	o,p'-DDE	1.64
Tecnazene	0.37	$\alpha$ -Chlordane	1.74
Hexachlorobenzene	0.46	$\alpha$ -Endosulfan	1.80
$\alpha$ -Hexachlorocyclohexane	0.52	Fenson	1.92
Quintozene	0.61	p,p'-DDE	1.96
$\gamma$ -Hexachlorocyclohexane	0.65	Captan	2.12
$\beta$ -Hexachlorocyclohexane	0.73	Dieldrin	2.13
Heptachlor	0.82	Iodofenphos	2.18
Pentachloroaniline	0.85	o,p'-DDD	2.29
$\delta$ -Hexachlorocyclohexane	0.91	Chlorfenson	2.61
Fenchlorphos	0.92	Endrin	2.62
Vinclozolin	0.93	o,p'-DDT	2.76
Aldrin	1.00	Tetrasul	2.84
Chlorpyrifos	1.12	Binapacryl	2.89
Fenitrothion	1.26	p,p'-DDD	2.94
Isodrin	1.30	$\beta$ -Endosulfan	3.06
Dicofol	1.32	Ethion	3.37
Oxychlordane	1.33	Carbophenothion	3.43
Dichlofluanid	1.34	p,p'-DDT	3.50
Bromophos	1.35	Endosulfan sulphate	4.58
Heptachlor epoxide	1.44	Methoxychlor	5.96
Bromophos-ethyl	1.60	Tetradifon	7.31
$\gamma$ -Chlordane	1.61		

Table 4. (contd.)

6.5.3 (Column packed with 6.5% DC-200 + 0.01% Versamid 900)

Compound	RRT Parathion	Compound	RRT Parathion
Dimefox	0.16	Malathion	0.90
Methamidophos	0.22	Chlorpyrifos	0.99
Mevinphos	0.26	Parathion	1.00
Disulfoton sulphoxide	0.27	Bromophos	1.11
Omethoate	0.38	Chlorfenvinphos	1.23
Thionazin (Zinophos)	0.39	Methidathion	1.38
Dicrotophos	0.41	Disulfoton sulphone	1.39
Sulfotep	0.45	Bromophos-ethyl	1.40
Dimethoate	0.52	Ditalimfos	1.48
Diazinon	0.59	Iodofenphos	1.63
Formothion	0.62	Ethion	2.11
Malaoxon	0.74	Triazophos	2.20
Parathion-methyl	0.78	Carbophenothion	2.45
Paraoxon	0.82	Phosalone	4.48
Fenchlorphos	0.88	Azinphos-ethyl	5.33
Fenitrothion	0.89	Pyrazophos	5.47

## 7.2. Recoveries

Recoveries from untreated control samples of a wide variety of analytical material of plant and animal origin fortified with the analyzed compounds at levels of 0.01 to 10 mg/kg generally ranged from 80 to 100%. See Table 5.

Table 5. Combinations of compounds and crops/foods for which the recovery rate exceeded 70% in recovery experiments

Compound	Added mg/kg	Crops and foods
<i>Organochlorine compounds</i>		
Aldrin	0.01	Apples, pears, potatoes, lettuce, citrus fruit
	0.1	Oilseed*), tobacco, tea and tea-like products
Camphechlor	0.3	Apples, kohlrabi, carrots, peaches
$\alpha$ -Chlordane	0.01	Pears, lettuce
$\gamma$ -Chlordane	0.01	Pears, lettuce
Chlorfenson	1.0	Apples, cucumbers, carrots
$\text{o},\text{p}'$ -DDD	0.05	Apples, milk and dairy products, tomatoes, grapes
	0.1	Cocoa products*)
	0.2	Dried egg products*), meat and meat products
	1.0	Fats (vegetable and animal)*), tobacco

Table 5. (contd.)

Compound	Added mg/kg	Crops and foods
p,p'-DDD	0.05	Apples, milk and dairy products, tomatoes, grapes
	0.1	Cocoa products*)
	0.2	Dried egg products*), meat and meat products
	1.0	Fats (vegetable and animal)*), tobacco
o,p'-DDE	0.02	Apples, milk and dairy products, grapes, citrus fruit
	0.1	Dried egg products*), meat and meat products, cocoa products*)
	0.5	Fats (vegetable and animal)*), tobacco
p,p'-DDE	0.02	Apples, milk and dairy products, grapes, citrus fruit
	0.07	Bananas, cucumbers
	0.1	Dried egg products*), meat and meat products, cocoa products*), nuts*)
	0.5	Fats (vegetable and animal)*), tobacco
o,p'-DDT	0.05	Milk and dairy products
	0.1	Apples, pears, cocoa products*), peppers, grapes
	0.2	Dried egg products*), meat and meat products
	1.0	Fats (vegetable and animal)*)
	2.0	Tobacco
p,p'-DDT	0.05	Milk and dairy products
	0.1	Apples, pears, cocoa products*), nuts*), peppers, grapes
	0.2	Dried egg products*), meat and meat products
	1.0	Fats (vegetable and animal)*)
	2.0	Tobacco
Dicofol	1.0	Citrus fruit
Dieldrin	0.01	Apples, pears, potatoes, lettuce, citrus fruit
	0.1	Fats (vegetable and animal)*), tobacco
$\alpha$ -Endosulfan	0.1	Beans, cereals, cucumbers
	0.5	Tea and tea-like products
$\beta$ -Endosulfan	0.1	Beans, cereals, cucumbers
	0.5	Tea and tea-like products
Endosulfan sulphate	0.2	Beans, cereals, cucumbers
	1.0	Tea and tea-like products
Endrin	0.01	Apples, potatoes, carrots
Fenson	0.2	Apples, strawberries, cherries
$\alpha$ -HCH	0.01	Apples, pears, cocoa products*), nuts*)
	0.02	Bananas, lettuce
	0.1	Spices, tea and tea-like products

Table 5. (contd.)

Compound	Added mg/kg	Crops and foods
$\beta$ -HCH	0.01 0.02 0.1	Apples, pears Cocoa products *) Spices, oilseed *)
$\gamma$ -HCH (lindane)	0.005 0.01 0.02 0.5	Milk and dairy products Apples, cucumbers, carrots, lettuce Cocoa products *), nuts *) Spices, tea and tea-like products, grapes
$\delta$ -HCH	0.01	Apples, cocoa products *)
Heptachlor	0.01 0.05	Apples Fats *)
Heptachlor epoxide	0.01	Apples
Hexachlorobenzene	0.01 0.1	Apples, meat and meat products, spices, cocoa products *) Dried egg products *)
Isodrin	0.01	Apples
Methoxychlor	5.0	Apples, spices, carrots, head cabbage
Oxychlordane	0.01	Pears, dried egg products *), lettuce
Polychlorinated biphenyls	2.0	Fats *)
Quintozene	0.01 0.03	Cereals Bananas, lettuce, grapes
Tecnazene	0.3	Strawberries, peppers, lettuce
Tetradifon	0.5	Apples, carrots
Tetrasul	1.0	Apples, carrots
<i>Organophosphorus compounds</i>		
Azinphos-ethyl	0.04 2.5	Apples Tobacco
Bromophos	0.5	Spices, lettuce, citrus fruit
Bromophos-ethyl	0.5	Apples, grapes
Carbophenothion	0.1	Citrus fruit
Chlorfenvinphos	0.2	Carrots
Chlorpyrifos	0.1	Potatoes, lettuce, citrus fruit
Diazinon	0.2	Apples, carrots, lettuce
Dicrotophos	2.0	Tobacco
Dimefox	0.01	Tobacco
Dimethoate	0.2	Apples, cauliflower, beans, curly kale, cucumbers, cherries, carrots, plums, tomatoes, grapes, sugar beet

Table 5. (contd.)

Compound	Added mg/kg	Crops and foods
Disulfoton sulphone	0.1	Cereals
Disulfoton sulphoxide	0.1	Cereals
Ditalimfos	1.5	Bananas, cucumbers
Ethion	0.1 0.5	Apples Citrus fruit
Fenchlorphos	0.2 1.0	Apples, cucumbers, carrots Fats*)
Fenitrothion	0.05 0.1	Cereals Apples
Formothion	0.2	Citrus fruit
Iodofenphos	0.1	Milk and dairy products
Malaoxon	0.2	Cereals
Malathion	0.2	Beans, cereals, cherries, lettuce
Methamidophos	0.2	Bananas
Methidathion	0.2 5.0	Apples, cucumbers Hop cones
Mevinphos	0.1	Apples, cucumbers, carrots
Omethoate	0.2 0.4 10.0	Cauliflower, beans, curly kale, cucumbers, red currants, cherries, carrots, plums, tomatoes, grapes, sugar beet Onions Tobacco
Paraoxon	0.5	Tobacco
Parathion	0.05	Apples, cucumbers
Parathion-methyl	0.1	Apples, lettuce
Phosalone	1.0	Cherries, citrus fruit
Pyrazophos	0.2	Apples, cereals, cucumbers, grapes
Sulfotep	0.1	Apples, cereals, potatoes, grapes
Thionazin (zinophos)	0.1	Sugar beet
Triazophos	0.1	Apples, cauliflower, beans, strawberries, potatoes, sugar beet, onions
<i>Other compounds</i>		
Anilazine	0.1	Cereals
Binapacryl	0.3	Apples
Bitertanol	0.5	Apples, bananas, pears, beans, peanuts*, cereals, red currants, cherries, plums

Table 5. (contd.)

Compound	Added mg/kg	Crops and foods
Captafol	0.3	Cereals
Captan	0.1	Cereals
Dichlofluanid	0.2	Apples, red currants
	0.5	Apples, cucumbers, red currants
Dinocap	1.0	Apples, lettuce
Fluotrimazole	0.5	Apples, cereals, cucumbers, melons, grapes
Fuberidazole	0.1	Cereals
Imazalil	0.2	Cereals
Metribuzin	0.5	Apples
Pentachloroaniline	0.05	Cereals
Piperonyl butoxide	1.0	Apples, lettuce, head cabbage
Propoxur	0.2	Potatoes
	0.5	Strawberries
Pyrethrins	0.5	Apples, lettuce, head cabbage
Rabenazole	0.5	Cereals
Resmethrin	0.5	Lettuce, head cabbage
Triadimefon	0.5	Pineapples, apples, bananas, beer, strawberries, cereals, red currants, coffee (raw), must, peppers, wine, grapes, sugar beet
	5.0	Hop cones
Triadimenol	1.0	Pineapples, apples, bananas, beer, strawberries, cereals, red currants, coffee (raw), must, peppers, wine, grapes, sugar beet
Trifluralin	0.05	Cereals
Vinclozolin	0.1	Lettuce

\*) with cleanup by method described on p.71 ff (Cleanup Method 5)

### 7.3. Calculation of residues

The residue R, expressed in mg/kg, of an identified compound is calculated from the following equation:

$$R = \frac{F_A \cdot W_{st}}{F_{st} \cdot V_i \cdot G} \cdot f$$

in which

$$f = \frac{V_{\text{End}} \cdot V_{\text{Ex}} \cdot V_{R2} \cdot V_{R4}}{V_{R1} \cdot V_{R3} \cdot V_{R5}}$$

where

- $G$  = sample weight (in g)
- $V_{\text{Ex}}$  = volume of acetone and water added in extraction step 6.1.1 or 6.1.2 plus water contained in sample in ml, less an empirical volume shrinkage of 5 ml
- $V_{R1}$  = portion of volume  $V_{\text{Ex}}$  used for partition in step 6.2 (in ml)
- $V_{R2}$  = volume of solution of evaporation residue prepared for gel permeation chromatography by method described in Cleanup Method 6, step 5.3 (in ml)
- $V_{R3}$  = portion of volume  $V_{R2}$  injected for gel permeation chromatography (volume of sample loop) (in ml)
- $V_{R4}$  = volume of solution obtained after gel permeation chromatography by procedure described in Cleanup Method 6, step 5.3 (in ml)
- $V_{R5}$  = portion of volume  $V_{R4}$  used for chromatography in step 6.4.3 (in ml)
- $V_{\text{End}}$  = terminal volume of eluate solution obtained from step 6.4.3 (in ml)
- $V_i$  = portion of volume  $V_{\text{End}}$  injected into gas chromatograph (in  $\mu\text{l}$ )
- $W_{\text{St}}$  = amount of compound injected with standard solution (in ng)
- $F_A$  = peak area obtained from  $V_i$  (in  $\text{mm}^2$ )
- $F_{\text{St}}$  = peak area obtained from  $W_{\text{St}}$  (in  $\text{mm}^2$ )

Provided the given volumes are strictly adhered to, a value of 88.5 is obtained for  $f$ .

## 8. Important points

The *GDCh-Arbeitsgruppe "Pestizide"* tested the described analytical method in two collaborative studies conducted by 8 laboratories and 5 laboratories, respectively. In these tests, samples of cucumbers and banana flakes were each fortified with 6 compounds (hexachlorobenzene, quintozene, dicloran, triadimefon, ditalimfos and triamiphos;  $\alpha$ -hexachlorocyclohexane, p,p'-DDE, dieldrin, chlorpyrifos, pirimiphos-methyl and dimethoate). After processing, recoveries of the compounds from the eluates collected from the silica gel column averaged 80 to 100%.

## 9. References

*W. Specht and M. Tillkes, Gaschromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach Clean-up über Gel-Chromatographie und Mini-Kieselgelsäulen-Chromatographie.*

1. Mitt.: Organochlor-Pflanzenbehandlungsmittel in Tabak und Tabakerzeugnissen, Beitr. Tabakforsch. Intern. 10, 73–79 (1979).
2. Mitt.: Bestimmung der Fungizide Bitertanol, Fluotrimazol, Fuberidazol, Imazalil, Triadimefon und Triadimenol in Pflanzen und Boden, Pflanzenschutz-Nachr. 33, 61–85 (1980).
3. Mitt.: Methode zur Aufarbeitung von Lebensmitteln und Futtermitteln pflanzlicher und tierischer Herkunft für die Multirückstandsbestimmung lipoïd- und wasserlöslicher Pflanzenbehandlungsmittel, Fresenius Z. Anal. Chem. 301, 300–307 (1980).

## 10. Authors

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# **Phthalimide Fungicides (Captafol, Captan, Folpet)**

**S 20**

Apples, barley (green matter, grains and straw), grapes,  
lettuce, pears, tomatoes  
Soil

High-performance  
liquid chromato-  
graphic determination

(German version published 1984)

## **1. Introduction**

The chemical and physical properties of the compounds named in the heading above are given in the methods for the individual compounds contained in this Manual.

## **2. Outline of method**

The compounds are extracted with acetone. The extract is diluted with water and saturated sodium chloride solution, and shaken with n-hexane. The hexane phase is evaporated to dryness. The compounds are separated from interfering co-extractives on a gel permeation column. Following separation by high-performance liquid chromatography, the compounds are determined quantitatively with a photoconductivity detector.

## **3. Apparatus**

High-speed blender fitted with leak-proof glass jar and explosion-proof motor  
Meat mincer  
Beater-cross mill (e. g. Retsch)  
Wide neck bottle, 500-ml  
Homogenizer, e. g. Ultra-Turrax (Janke & Kunkel)  
Laboratory mechanical shaker  
Buchner porcelain funnel, 9 cm dia.  
Filter paper, 9 cm dia. (e. g. Schleicher & Schüll)  
Filtration flask  
Fluted filter paper  
Funnel  
Graduated cylinder, 250-ml  
Beaker, 1-l  
Round-bottomed flasks, 500-ml, 100-ml and 25-ml  
Separatory funnel, 1-l  
Rotary vacuum evaporator, 40°C bath temperature

Chromatographic tube, 2.5 cm i. d., 60 cm long

Glass syringe, 5-ml or 10-ml

Automated gel permeation chromatograph, e.g. Autoprep 1001 (Analytical Biochemistry Laboratories)

High performance liquid chromatograph equipped with photoconductivity detector (Tracor) Microsyringe, 100- $\mu$ l

## 4. Reagents

Acetone, high purity

Cyclohexane, p. a.

Ethyl acetate, p. a.

n-Hexane, p. a.

Methanol, p. a.

Propanol-(2) (isopropanol), p. a.

2,2,4-Trimethylpentane (isooctane), p. a.

Eluting mixture: cyclohexane + ethyl acetate 1:1 v/v

Mobile phase: isooctane + isopropanol + methanol mixture 85:5:10 v/v/v; conditioned for several hours by recirculation through ion exchange cartridge of detector

Captafol, captan and folpet standard solutions: 100  $\mu$ g/ml mobile phase

Sodium chloride solution, saturated

Sodium sulphate, p. a., anhydrous

Bio-Beads S-X3, 200–400 mesh (Bio-Rad)

Cottonwool, chemically pure

Dry ice

## 5. Sampling and sample preparation

The analytical sample is taken as described on p. 17 ff and p. 21 f. Green plants, straw and fruits are chopped. Chopped straw and grains are ground in a beater-cross mill in the presence of dry ice. The portion required for analysis is weighed from the prepared sample.

## 6. Procedure

### 6.1. Extraction

#### 6.1.1. Cereals

Weigh 40 g green plant material, 40 g straw or 100 g grains (G) into a wide neck bottle. To the green plant material add an equal amount by weight of sodium sulphate. Add 300 ml acetone ( $V_{Ex}$ ) and homogenize for 2 min with an Ultra-Turrax. Tightly stopper the bottle and shake for 1 h on a mechanical shaker. Filter a 150-ml aliquot ( $V_{Vi}$ ) through a fluted filter paper into a graduated cylinder.

### 6.1.2. Fruit, vegetables, soil

Weigh 50 g (G) of the homogenized fruit sample, vegetable sample or soil sample into a wide neck bottle. Add 100 ml acetone, tightly stopper the bottle, and shake for 1 h on a mechanical shaker. Suction-filter the solution through a filter paper in a Buchner porcelain funnel. Wash the bottle and the filter cake each with 20 ml acetone. Combine the filtrate and washings.

## 6.2. Partition

Transfer the acetone extract derived from step 6.1.1 or 6.1.2 with 20 ml sodium chloride solution, 500 ml water and 75 ml n-hexane to a separatory funnel. Shake vigorously, let the phases separate, drain the aqueous phase into a beaker, and collect the hexane phase in a 500-ml round-bottomed flask. Repeat the partition twice with 75-ml portions of n-hexane. Return the combined hexane phases to the separatory funnel, and wash with 100 ml water. Discard the water phase. Drain the hexane through a cottonwool pad into a 500-ml round-bottomed flask, and rotary-evaporate to dryness.

### 6.3. Cleanup by gel permeation chromatography

Allow 50 g Bio-Beads S-X3 to swell in the eluting mixture, and then place, free from air bubbles, in the column of the gel permeation chromatograph. Compress the column packing to a bed length of approx. 30 cm. Pump eluting mixture through this column at a constant flow rate of 2.5 ml/min and at an operating pressure of 0.2 bar.

Prior to cleanup of the sample, determine the elution volumes of the three phthalimide fungicides.

To accomplish this, inject 5 ml of the eluting mixture (containing known amounts of the three fungicides) into the gel permeation chromatograph. Then elute the compounds with 230 ml of the same mixture, collecting twenty-three 10-ml fractions. Collect the gel column eluate for 4 min in each of the 23 numbered sample collection tubes at a constant flow rate of 2.5 ml/min. Evaporate each fraction to dryness, and analyze by injecting into the high performance liquid chromatograph, as described in step 6.4, to determine the elution volume. Under the described conditions, captafol, captan and folpet were eluted in the 120 to 190-ml fractions.

Dissolve the evaporation residue of the acetone-hexane partition from step 6.2 in 10 ml eluting mixture ( $V_{R1}$ ). Inject a 5-ml aliquot ( $V_{R2}$ ) into the gel permeation chromatograph and clean up according to the conditions given above. Collect the fraction in which the compounds are eluted (in this case approx. 120–190 ml) and evaporate to dryness on a rotary evaporator. See Important points in Section 8.

### 6.4. High-performance liquid chromatographic determination

Dissolve the residue cleaned up by gel permeation chromatography in step 6.3 in conditioned mobile phase, and dilute to an appropriate volume ( $V_{End}$ ), at least 5 ml. Inject aliquots of this solution ( $V_i$ ) into the high performance liquid chromatograph.

*Operating conditions*

Chromatograph	High performance liquid chromatograph
Pump	Orlita
Injector	Valco injection valve fitted with 50- $\mu$ l sample loop
Columns	Two stainless steel columns, each 4.6 mm i.d. and 25 cm long, connected in series; packed with Zorbax CN, 6 $\mu$ m (Du Pont)
Mobile phase	Isooctane-isopropanol-methanol, conditioned by recirculation through ion exchange cartridge of detector
Column pressure	150 bar
Flow rate	1.6 ml/min
Detector	Photoconductivity detector (Tracor 965), halogen-specific, operated with an Hg lamp at 254 nm
Attenuation	1 · 10
Injection volume	50 $\mu$ l
Temperature	approx. 20°C
Retention times for	
captafol	9 min 48 s
captan	9 min 6 s
folpet	7 min 48 s

**7. Evaluation****7.1. Method**

Prepare a calibration curve as follows. Dilute aliquots of the captafol, captan and folpet standard solutions with appropriate amounts of conditioned mobile phase to produce captafol, captan and folpet working solutions of 0.2, 0.5, 1.0 and 2.0  $\mu$ g/ml. Introduce 50- $\mu$ l aliquots, equivalent to 10, 25, 50 and 100 ng captafol, captan and folpet, onto the column through the sample loop. Plot measured peak heights vs. ng compound to obtain a linear curve.

## 7.2. Recoveries and lowest determined concentration

The following table provides examples of recoveries obtained in experiments on untreated control samples fortified with the three compounds.

Crop	Added mg/kg	Captafol	% Recovery Captan	Folpet
Grapes	0.04	92	113	105
	0.2	95	95	105
Apples	0.04	113	90	120
	0.2	90	85	100
Cereal grains	0.02	95	90	95
	0.04	92	75	95
Cereal straw	0.1	94	97	94
	0.5	94	94	84

The routine limit of determination was 0.02 mg/kg for fruit, vegetables and grains, and 0.05 mg/kg for green plant parts and straw.

## 7.3. Calculation of residues

The residue R, expressed in mg/kg captafol, captan or folpet, is calculated from the following equation:

$$R = \frac{V_{Ex} \cdot V_{End} \cdot V_{R1} \cdot W_A}{V_{V1} \cdot V_{R2} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

$V_{Ex}$  = volume of acetone used for extraction of cereal sample in step 6.1.1 (in ml)

$V_{V1}$  = portion of volume  $V_{Ex}$  used for partition in step 6.2 in analysis of cereals (in ml)

$V_{R1}$  = volume of eluting mixture used for dissolution of residue from step 6.2 (in ml)

$V_{R2}$  = portion of volume  $V_{R1}$  used for cleanup by gel permeation chromatography in step 6.3 (in ml)

$V_{End}$  = volume of mobile phase used for dissolution of residue from step 6.3 (in ml)

$V_i$  = portion of volume  $V_{End}$  injected into high performance liquid chromatograph (in  $\mu$ l)

$W_A$  = amount of compound for  $V_i$  read from calibration curve (in ng)

## 8. Important points

If an automated gel permeation chromatograph is not available, cleanup by gel permeation chromatography can also be performed manually with the aid of a pump and a gel column (see Cleanup Method 6). The elution pattern must be determined at all events.

To obtain a stable baseline, the mobile phase must be conditioned for several hours, preferably overnight, by recirculation through the ion exchange cartridge of the detector otherwise interfering peaks may occur. Therefore, the conditioned mobile phase must be used also for preparing the sample solution in step 6.4 and the calibration solutions in step 7.1.

## 9. Reference

*B. Büttler and W. D. Hörmann*, High-pressure liquid chromatographic determination of captan, captafol, and folpet residues in plant material, *J. Agric. Food Chem.* 29, 257–260 (1981).

## 10. Authors

Ciba-Geigy AG, Agricultural Division, Basle, Switzerland, *B. Büttler and W. D. Hörmann*

# Ethylene and Propylene Bisdithiocarbamate Fungicides

S 21

Apples, beans, carrots, cucumbers, lettuce, potatoes,  
tomatoes

Photometric determina-  
tion after gel permea-  
tion chromatographic  
cleanup

(German version published 1984)

## 1. Introduction

The method permits determination of residues of ethylene bisdithiocarbamate fungicides and of propineb on the surface of harvested crops without decomposition of the dithiocarbamates. As these residues are present only on the surface and not within the plants, they need only be removed from the surface.

## 2. Outline of method

The unchopped plant material is shaken with an aqueous solution of ethylenedinitrilotetra-acetic acid (tetrasodium salt). An aliquot of the solution is chromatographed on Sephadex LH-20 with the same salt solution. The separated disodium ethylene and propylene bisdithiocarbamates are determined by measuring the UV absorbance of the eluate at a wavelength of 285 nm.

## 3. Apparatus

Beakers of different sizes, 500-ml to 3000-ml

Centrifuge, e.g. HC 122 Labofuge 1 (Heraeus-Christ)

Centrifuge tubes, 10-ml

Pipette, 10-ml

Graduated cylinder, 200-ml

Chromatographic tube, 1.5 cm i.d., 45 cm long (e.g. Quickfit No. 108 002/160)

Pump, e.g. Duramat (Chemie und Filter)

Sample introduction valve (made of PTFE) fitted with 1-ml sample loop (e.g. Beckman)

UV continuous-flow photometer, e.g. LC 3 (Pye Unicam-Philips)

Potentiometric recorder, e.g. Servogor RE 511 (Metrawatt)

## 4. Reagents

EDTA solution: 0.1 mol/l ethylenedinitrilotetraacetic acid, tetrasodium salt-tetrahydrate (e.g. Merck No. 10964), in bidistilled water

Standard solutions: 1–10 µg *anion* of maneb, nabam, propineb or zineb fungicide/ml EDTA solution

Reference solution: 0.01 ml acetone in 100 ml water

Sephadex LH-20, 40–60 µm sieve fraction (Pharmacia)

## 5. Sampling and sample preparation

The analytical sample is taken and prepared in accordance with the guidelines given on p. 17 ff. However, the plant parts must not be comminuted at any cost otherwise the fungicidal compounds will be decomposed by plant constituents like acids and enzymes. The analysis must be initiated immediately after sampling.

## 6. Procedure

### 6.1. Extraction

Weigh between 100 and 1500 g of the unchopped sample (G) (actual amount depends upon sample unit size) into a beaker of appropriate size. Then wash the sample by pouring 200 to 2000 ml EDTA solution (actual amount depends upon sample size) ( $V_{Ex}$ ) over it, and swirl for 15 min. The actual size of the samples is given by the weight of the representative units.

The analytical sample should consist of the following units:

Apples	6 to 8 units
Beans	30 to 35 units
Carrots	15 to 20 units
Cucumbers	at least 3 units*
Lettuce	4 to 6 units
Potatoes	8 to 10 units
Tomatoes	8 to 10 units

\* For cucumbers, halves can be used, with cut surface facing upwards

The amount of washing that is used must be kept as small as possible but care must be taken that the samples are completely covered with the washing while swirling.

Next withdraw 10 ml of the washing with a pipette, centrifuge at 3000 r. p. m., and proceed immediately to step 6.3.

### 6.2. Assembly of apparatus and determination of elution volumes

Allow 16 g Sephadex LH-20 to swell in EDTA solution for 24 h. Pack the column in the usual manner, from a loading reservoir. Then pump EDTA solution through the column until the level of the packing is constant. Next, the gel bed is settled in position by adjusting and tighten-

ing the plungers. The total volume of the gel bed will be approx. 40 ml. Assemble and connect, in following order, pump, sample introduction valve, gel permeation column, UV continuous-flow photometer and potentiometric recorder.

Chromatograph first 1.0 ml of the reference solution with EDTA solution at a flow rate of 1 ml/min. Measure the UV absorbance of the eluate at 285 nm, and record it using the potentiometric recorder. Then chromatograph 1.0 ml of the standard solutions in the same manner.

The elution volumes, relative to acetone = 1.00, were found to be 1.90 for the ethylene bisdithiocarbamates and 1.68 for propineb. Using the column dimensions given in Section 3 and a gel bed volume of 40 ml, elution volumes were found to be 42 ml for acetone, 80 ml for the ethylene bisdithiocarbamates and 71 ml for propineb.

### 6.3. Cleanup and determination

Add 1.0 ml ( $V_i$ ) of the solution derived from 6.1 onto the column through the sample introduction valve, and chromatograph with EDTA solution at a flow rate of 1 ml/min. Measure the UV absorbance of the eluate at 285 nm, and record it using the potentiometric recorder.

## 7. Evaluation

### 7.1. Method

Evaluation is performed by measuring the peak area of the sample and comparing it with the peak areas obtained for the ethylene and propylene bisdithiocarbamate anions (molar mass of 210.37 and 224.40 g/mol, respectively) from appropriate dilutions of the standard solutions. The spectral decadic absorbance (extinction) measured for the ethylene bisdithiocarbamate anion at, for example, a concentration of 5 µg/ml was 0.49 at 285 nm and 1 cm lightpath.

### 7.2. Recoveries and lowest determined concentration

The recoveries from untreated control samples fortified with the compounds at levels of 0.5 to 1 mg/kg ranged from 80 to 93%; for cucumbers and tomatoes, they ranged from 95 to 100% (see Table).

The routine limit of determination was 0.05 mg/kg. For crops with a large surface area like lettuce, which require a particularly large amount of EDTA solution for washing them, the routine limit of determination may be as high as 0.5 mg/kg.

Table. Mean recovery rates from 3 to 5 fortification experiments

Crop	Compound	Added mg/kg	Recovery %
Apples	Mancozeb	0.5	92
Apples	Maneb	0.5	91
Apples	Zineb	0.5	94
Beans	Zineb	1.0	93
Cucumbers	Maneb	1.0	106
Cucumbers	Maneb	0.5	99
Cucumbers	Maneb	0.25	102
Potatoes	Mancozeb	1.0	82
Potatoes	Maneb	1.0	95
Potatoes	Nabam	1.0	85
Potatoes	Propineb	1.0	84
Potatoes	Zineb	2.0	82
Potatoes	Zineb	1.0	85
Potatoes	Zineb	0.5	81
Lettuce	Mancozeb	1.0	78
Lettuce	Maneb	1.0	88
Lettuce	Metiram	1.0	81
Lettuce	Zineb	1.0	88
Carrots	Maneb	1.0	79
Tomatoes	Maneb	1.0	84
Tomatoes	Zineb	2.0	95
Tomatoes	Zineb	1.0	94
Tomatoes	Zineb	0.5	96

### 7.3. Calculation of residues

The residue R, expressed in mg/kg ethylene or propylene bisdithiocarbamate anion, is calculated from the following equation:

$$R = \frac{F_A \cdot W_{St} \cdot V_{Ex}}{F_{St} \cdot V_i \cdot G}$$

where

G = sample weight (in g)

V<sub>Ex</sub> = volume of EDTA solution used for washing the analytical sample (in ml)

V<sub>i</sub> = portion of volume V<sub>Ex</sub> used for gel permeation chromatography in step 6.3 (in ml)

W<sub>St</sub> = amount of ethylene or propylene bisdithiocarbamate anion added with standard solution (in µg)

F<sub>A</sub> = peak area obtained from V<sub>i</sub> (in mm<sup>2</sup>)

F<sub>St</sub> = peak area obtained from W<sub>St</sub> (in mm<sup>2</sup>)

The mg/kg residue of a certain fungicidal compound or the mg/kg residue of carbon disulphide is calculated by multiplying R by the following factors:

Conversion of ethylene bisdithiocarbamate anion (molar mass of 210.37 g/mol) to

Maneb (molar mass of 265.31 g/mol):	1.26
Zineb (molar mass of 275.75 g/mol):	1.31
Carbon disulphide (molar mass of 76.14 g/mol):	0.72

Conversion of propylene bisdithiocarbamate anion (molar mass of 224.40 g/mol) to

Propineb (molar mass of 289.78 g/mol):	1.29
Carbon disulphide (molar mass of 76.14 g/mol):	0.68

The conversion factors for the mixed fungicides mancozeb (20% Mn, 2.5% Zn) and vondozeb (14.5% Mn, 2.1% Zn) should be determined experimentally because their stoichiometric composition is not known with certainty.

From the found amount of anion, the sum total of the residues of ethylene bisdithiocarbamate fungicides also can be calculated given acceptance of a certain error. The maximal size of the error arising from the conversion may be between + 7% and - 7%. From a list of the errors likely to arise from the use of conversion factors, it is evident that use of the conversion factor anion → maneb (1.26) may result in errors not exceeding + 4% to - 3%, and then only if either only metiram and/or nabam or only zineb is present in the residue. If several fungicides are present, the residue will at all events be smaller; see also Important points in Section 8.

## 8. Important points

Spectrophotometric measurements of solutions of sodium, manganese and zinc salts of ethylenedinitrilotetraacetic acid revealed that the chromatographic peaks are due to the dithiocarbamate anions. The elution volumes also depend only on the anion. Accordingly, the individual ethylene bisdithiocarbamate fungicides have the same elution volumes and their peaks appear at the same position in the chromatogram. On the other hand, the size of the peak areas is dependent upon the percentage of anion in the molecule.

Compared with the recovery rates obtained in the hitherto almost exclusively employed carbon disulphide evolution method of Keppel (e.g. Multiresidue Method S 15), those recorded by the method reported herein are equally high in some cases whilst in others they are also somewhat higher. This is attributed to the fact that in the other method, enzymes, acids, etc. are liberated during the requisite comminution of the analytical material so that carbon disulphide already begins to evolve before the actual analysis is initiated. Decomposition of dithiocarbamates during sample preparation has been reported also by Howard and Yip.

When dithiocarbamate fungicides act for a prolonged period on plant tissue, they form the toxicologically significant metabolites ethylene thiourea and propylene thiourea. These compounds are not determined by the reported method. They are present mainly within the tissues of the crops whereas the parent compounds are found on the outer plant surface only.

## 9. References

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- J. Pflugmacher and W. Ebing*, Eine neue Schnellmethode zur Bestimmung von Alkylen-bis-dithiocarbamat-Fungizid-Rückständen, Z. Lebensm. Unters. Forsch. 170, 349–354 (1980).

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Federal Biological Research Centre for Agriculture and Forestry, Department for Ecological Chemistry, Division for Pesticide Research, Berlin, *J. Pflugmacher and W. Ebing*

# **Indexes**



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# List of Suppliers Referenced in the Text-Matter of the Manual

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*Alpine AG*, Maschinenfabrik, Peter-Dörfler-Str. 13–25, D-8900 Augsburg 22, FRG.

*Analytical Bio Chemistry Laboratories*, 7200 ABC Lane, P. O. Box 1097, Columbia, MO 65205, USA; in Europe: N. Foss Electric GmbH, Waidmannstr. 12 b, D-2000 Hamburg 50, FRG.

*Becker*: see Packard.

*Beckman Instruments Inc.*, 2500 Harbor Blvd., Fullerton, CA 92634, USA.

*Bender und Hobein GmbH*, Lindwurmstr. 71–73, Postfach 150229, D-8000 München 15, FRG.

*J. C. Binzer Papierfabrik*, Berleburger Str. 71, D-3559 Hatzfeld 1, FRG.

*Bio-Rad Laboratories*, 2200 Wright Ave., Richmond, CA 94804, USA; Dachauer Str. 511 + 364, Postfach 500167, D-8000 München 50, FRG.

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*Carlo-Erba Strumentazione*, Strada Rivoltana, I-20090 Rodano (Milano), Italy.

*Chemie-Mineralien KG*, Löningstr. 35, D-2800 Bremen, FRG.

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*Chromatronics Inc.*, 2300 Leghorn St., Mt. View, CA 94043, USA.

*Dohrmann Div.*, Xertex Corp., 3240 Scott Blvd., Santa Clara, CA 95042, USA.

*E. I. du Pont de Nemours & Co., Inc.*, 1007 Market St., Wilmington, DE 19898, USA; Dieselstr. 18, D-6350 Bad Nauheim 1, FRG.

*EGA-Chemie*; now: Aldrich-Chemie, D-7924 Steinheim, FRG.

*Günther Ehle*, Glasinstrumentenfabrik, Holzheimer Str. 56 a, D-6250 Limburg, FRG.

*Fluka AG*, CH-9470 Buchs, Switzerland.

*F & M Scientific*: see Hewlett-Packard.

*Finnigan MAT*, 355 River Oaks Pkwy., San Jose, CA 95134, USA; Paradise, Hemel Hempstead, Herts. HP2 4TG, U. K.

*Heraeus-Christ GmbH*, Postfach 1220, D-3360 Osterode, FRG.

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*Janke & Kunkel GmbH & Co. KG*, Neumagenstr. 16, D-7813 Staufen, FRG.

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*Dr. H. Knauer*, Wissenschaftl. Geräte KG, Heuchelheimer Str. 9, D-6380 Bad Homburg, FRG.

*Kontes Glass Co.*, Spruce St., P. O. Box 729, Vineland, NJ 08360, USA.

*Lehmann & Voss & Co.*, Alsterufer 19, D-2000 Hamburg 36, FRG.

*Macherey-Nagel*, Neumann-Neander-Str. 6–8, Postfach 307, D-5160 Düren, FRG.

*Mallinckrodt Inc.*, 675 McDonnell Blvd., P. O. Box 5840, St. Louis, MO 63134, USA.

*Melpar*: see Tracor.

*E. Merck*, Frankfurter Str. 250, Postfach 4119, D-6100 Darmstadt, FRG.

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*Metrawatt*, GmbH, Thomas-Mann-Str. 16-20, Postfach 1333, D-8500 Nürnberg, FRG.  
*Micro Tek* Instrument Co.: see Tracor.  
*Nopco* Chemical Co., 350 Mt. Kemble Ave., Morristown, NJ 07960, USA.  
ICN *Nutritional Biochemicals*, 26201 Miles Road, Cleveland, OH 44128, USA.  
*Orlita* GmbH & Co. KG, Dosiertechnik, Max-Eyth-Str. 10, D-6300 Gießen, FRG.  
*Packard* Instrument B. V., P. O. Box 519, NL-2600 AM Delft , The Netherlands.  
*Perkin-Elmer* & Co. GmbH, Bodenseewerk, Postfach 1120, D-7770 Überlingen, FRG.  
*Pharmacia* Fine Chemicals AB, Box 175, S-751 04 Uppsala, Sweden.  
*Philips*, Scientific and Analytical Equipment Div., Lelyweg 1, NL-7602 EA Almelo, The Netherlands.  
*Pye Unicam* Ltd., York St., Cambridge CB1 2PX, Great Britain.  
*Quickfit* Laborglas GmbH, Hüttenstr. 8, D-6200 Wiesbaden-Schierstein, FRG.  
*W. Reiss & Co.*, Krähenweg 5, D-2000 Hamburg 61, FRG.  
*Retsch* GmbH & Co. KG, Rheinische Str. 36, Postfach 1554, D-5657 Haan 1, FRG.  
*Riedel-de Haen* AG, Wunstorfer Str. 40, D-3016 Seelze 1, FRG.  
*Carl Roth*, Chem. Fabrik, Schoemperlenstr. 1-5, Postfach 211162, D-7500 Karlsruhe 21, FRG.  
*Schleicher & Schüll*, GmbH, Postfach 4, D-3354 Dassel, FRG.  
*Schoeffel* Instrument Corp., 24 Booker St., Westwood, NJ 07675, USA.  
*Schott & Gen.*, Jenaer Glaswerke, Hattenbergstr. 10, Postfach 2480, D-6500 Mainz, FRG.  
*Serva* Feinbiochemica GmbH & Co., Carl-Benz-Str. 7, Postfach 105260, D-6900 Heidelberg 1, FRG.  
*Spectra-Physics*, 3333 North First Street, San Jose, CA 95134, USA; Siemensstr. 20, D-6100 Darmstadt-Kranichstein, FRG.  
*Tracor* Instruments, 6500 Tracor La., Bldg. 27-7, Austin, TX 78725, USA.  
*Varian* Instrument Group, 611 Hansen Way, Palo Alto, CA 94303, USA.  
*Weisser*: supplied by Hans Otto, Feldstr. 26-28, D-2000 Hamburg 6, FRG.  
*Woelm*: ICN Biomedicals GmbH, Max-Woelm-Straße, Postfach 369, D-3440 Eschwege, FRG.

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