

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

Chemistry and specifications of pesticides

Second Report of the WHO Expert Committee
on Vector Biology and Control

World Health Organization
Technical Report Series
620



World Health Organization Geneva 1978

ISBN 92 4 120620 9

© World Health Organization 1978

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. For rights of reproduction or translation of WHO publications, in part or *in toto*, application should be made to the Office of Publications, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

PRINTED IN SWITZERLAND

CONTENTS

	Page
1. Pesticides for use in public health	5
1.1 New pesticides and formulations	6
1.2 Malathion	7
1.3 Specifications for insecticides	10
1.4 New insecticides	10
1.5 Rodenticides	13
1.6 Molluscicides	13
1.7 Impurities in pesticide formulations	14
2. Methods, standards, and sampling	14
2.1 Methods for general use in specifications	14
2.2 Standards for use in analytical procedures	14
2.3 Gas-liquid and high-performance liquid chromatography	16
2.4 Sampling and analysis of environmental materials	17
2.5 Identification methods	17
3. Collaboration with other organizations	18
3.1 Food and Agriculture Organization	18
3.2 United Nations Children's Fund	19
3.3 United Nations Industrial Development Organization	19
3.4 Collaborative International Pesticide Analytical Council	19
3.5 International Group of National Associations of Pesticide Manufacturers	19
4. Recommendations	20
Annex 1. Recommended changes in specifications for insecticides	21
Annex 2. Recommended changes in methods used in specifications	35

WHO EXPERT COMMITTEE ON VECTOR BIOLOGY AND CONTROL

Geneva, 29 November – 5 December 1977

Members *

- Dr W. R. Bontoyan, Supervisory Chemist, Quality Assurance, Office of Pesticide Programmes, Beltsville, MD, USA
- Mr J. Henriët, Department of Agriculture, National Phytopharmacological Research Establishment, Gembloux, Belgium (*Rapporteur*)
- Dr M. A. Klisenko, Head, Department of Chemistry, All-Union Scientific Research Institute of Hygiene and Toxicology of Pesticides, Polymers and Plastics, Kiev, USSR
- Dr P. J. Madati, Chief Chemist, Government Chemical Laboratory, Dar es Salaam, United Republic of Tanzania
- Dr J. W. Miles, Chief, Pesticides Branch, Vector Biology and Control Division, Center for Disease Control, Atlanta, GA, USA (*Chairman*)
- Dr G. H. Sanai, Professor of Industrial Hygiene and Toxicology, Department of Occupational and Environmental Health, University of Teheran, Iran (*Vice-Chairman*)

Representatives of other organizations

Food and Agriculture Organization of the United Nations

- Dr A. V. Adam, Pesticides Officer, Plant Protection Service, FAO, Rome, Italy

United Nations Children's Fund

- Mr M. W. Irmer, Supply Division, UNICEF, Geneva, Switzerland
- Dr H. M. Probst, Supply Division, UNICEF, Geneva, Switzerland

United Nations Industrial Development Organization

- Dr K. Szabo, Senior Industrial Development Officer, Chemical Industries Section, Industrial Operations Division UNIDO, Vienna, Austria

Secretariat

- Dr W. N. Aldridge, Medical Research Council Toxicology Unit, Carshalton, England (*Temporary Adviser*)
- Mr F. Barlow, Division of Chemical Control, Ministry of Overseas Development, Centre for Overseas Pest Research, London, England (*Temporary Adviser*)
- Mr H. H. Povlsen, Director, Chemical Control Laboratory, Lyngby, Denmark (*Temporary Adviser*)
- Dr A. R. Stiles, Chief, Pesticide Development and Safe Use, Division of Vector Biology and Control, WHO, Geneva, Switzerland (*Secretary*)

* Unable to attend: Dr W. Weinmann, Federal Biological Establishment for Agriculture and Forestry, Brunswick, Federal Republic of Germany; Dr Widodo Koesmijati, Pharmacist, Central Public Health Laboratory, National Institute of Medical Research, Jakarta, Indonesia.

CHEMISTRY AND SPECIFICATIONS OF PESTICIDES

Second Report of the WHO Expert Committee on Vector Biology and Control

The WHO Expert Committee on Vector Biology and Control¹ met in Geneva from 29 November to 5 December 1977 to study pesticide specifications and their use in public health, to establish new or revised specifications for the WHO manual *Specifications for pesticides used in public health*, and to provide guidance for improving the specifications of new types of formulations and active ingredients.

1. PESTICIDES FOR USE IN PUBLIC HEALTH

Chemical pesticides continue to be the mainstay of most public health vector control programmes. Although WHO is trying to develop biological control agents and environmental modifications for use in vector control, there is at present and during the immediate future an urgent need for safe and effective pesticides and thus for valid specifications for these pesticides.

The Committee therefore addressed itself to the task of revising the specifications and analytical methods for the pesticides currently being used in public health and of establishing specifications and analytical methods for newer pesticides that could prove safe and effective in vector control. In these deliberations account was taken of the recommendations made by the WHO Expert Committee on Insecticides in its nineteenth report² and in the report of the Scientific Group on the Chemical and Biochemical Methodology for the Assessment of Hazards of Pesticides for Man.³

¹ The Committee was previously known as the WHO Expert Committee on Insecticides.

² WHO Technical Report Series, No. 475, 1971.

³ WHO Technical Report Series, No. 560, 1975.

1.1 New pesticides and formulations

During the past 17 years WHO has conducted a research programme to develop pesticides for vector control. The programme was originally directed to finding residual insecticides for use in malaria control in those areas where DDT did not give control of the anopheline vectors. This is still an important aim of the programme today, but the research work has grown in complexity with the inclusion of research on other vectors and with the need for more information on the safety of pesticides and on the effects of these substances on the environment. As it happens, this intensification of research on new materials has occurred at a time when fewer and fewer candidate insecticides are available. Thus, in view of the problems faced by the malaria control programmes in many countries owing to the development of physiological resistance in anopheline mosquitos, it is essential that any new compounds that can be applied residually be continually examined.

The Committee was informed that the compounds bromophos, jodfenphos, chlorphoxim, and pirimiphos-methyl have been examined closely since the nineteenth meeting and merit consideration for the establishment of specifications. The Committee reviewed the chemical evaluations of the powder formulations of these materials carried out by a WHO research unit in Nigeria. The investigations included the determination of suspensibilities of the powders after accelerated storage treatment and after storage under local ambient conditions. The duration of local storage varied with each compound, but the measurements showed a correlation between accelerated and local storage and thus allowed the Committee to establish valid specifications for these materials.

The Committee was also informed of the intensive research being carried out to develop new insecticides for use in controlling larvae of the *Simulium damnosum* complex in the rivers of west Africa as part of the Onchocerciasis Control Programme in the Volta River Basin area.

Chlorpyrifos and fenthion are used extensively for the control of *Culex pipiens fatigans*, the main vector of Bancroftian filariasis, a disease widespread in both urban and rural areas of Asia and Africa.

Temephos, particularly as 1% on sand, is used extensively against *Aedes aegypti*, which breeds in containers of clean and potable water. This mosquito is the vector of yellow and dengue haemorrhagic fevers in south-east Asia and the western Pacific. The latter disease has acquired considerable importance in recent years.

1.2 Malathion

1.2.1 Poisoning incident

The Committee was given details of a poisoning incident that occurred in 1976 during the application of malathion water-dispersible powder during malaria control operations in Pakistan. Among 7500 field workers, cases of poisoning occurred in probably more than 2500, of whom five died. Staff of WHO and the Center for Disease Control, Atlanta, GA, USA, attributed the cause to organophosphorus poisoning, and subsequent chemical and toxicological investigations showed the presence of other organophosphorus compounds as impurities in some batches of the malathion powder. These impurities made the malathion exceptionally toxic. For almost 20 years it has been known from laboratory studies that a number of organophosphorus compounds can potentiate the toxicity of malathion in mammals by inhibiting the normal detoxification mechanism. This incident of poisoning in malaria control workers was a tragic demonstration that the phenomenon could be of critical importance for man under certain circumstances of field use of malathion.

1.2.2 Some contributory factors

The incident is believed to have been partly caused by failure to use elementary precautions during the handling and spraying of the formulation, and it thus shows that despite clear handling directions and package labels poor handling practices will occasionally occur in large-scale vector control programmes in developing countries. It is therefore necessary to improve and refine specifications continually so that all the factors that might cause problems during field use will have been determined, and limits will have been imposed in the specifications, before procurement. It was pointed out that odour was a limiting factor governing the acceptability of malathion water-dispersible powders in some malaria control programmes.

The recent increase in the use of malathion in malaria control programmes has coincided with an increase in the number of manufacturers of the insecticide—a result of the expiry of patents. This change from a single source of supply to several sources reinforces the need to pay careful attention to the specification requirements. Similar considerations apply to other insecticides. One example mentioned was fenitrothion, which is being increasingly used in public health programmes and is now made and formulated by several manufacturers.

In view of the possible increase in toxicity of a formulation after shipment or field storage, the Committee observed that there is a need for field methods of identifying active material and impurities in pesticide formulations. These methods should be simple, easy to use in developing countries, and if possible of a quantitative nature.

1.2.3 *Laboratory investigations*

The Committee reviewed the research that had been carried out as a result of the poisoning incident and agreed that there was a need to change to a new and specific method of analysis for specification purposes. It was considered worth while to summarize the steps undertaken in this research to show the basis for the new requirements and analytical methods.

After the poisoning incident and in collaboration with the various national malaria control programmes, WHO obtained samples of malathion water-dispersible powders from current malaria programmes for investigation of toxicity and presence of impurities. This urgent research was carried out by three laboratories in collaboration with WHO. The acute oral toxicity to rats was determined and the content of malathion and impurities was measured by gas-liquid chromatography. Forty-nine samples representing different manufacturers, dates of manufacture, and periods of field storage were collected and analysed, and the results allowed a correlation to be made between the presence of certain impurities and the degree of mammalian toxicity. From these investigations and from the literature^{1, 2} it was concluded that the presence of an impurity (or perhaps more than one) was causing potentiation of the toxicity of the malathion itself.

1.2.4 *Effect of impurities*

From this investigation, isomalathion was implicated as the main cause of the increased toxicity because, although it occurs in technical malathion only in very low concentrations, it is formed in larger amounts in some of the powder formulations. Other investigations and information from manufacturers implicated certain of the inert diluents and perhaps even the surfactants as the agents causing isomerization of the malathion to isomalathion after manufacture and during shipment and

¹ PELLEGRINI, G. & SANTI, R. *Journal of agricultural and food chemistry*, **20**: 944-950 (1972).

² UMETSU, N. ET AL. *Journal of agricultural and food chemistry*, **25**: 946-953 (1977).

field storage. As part of the research, several of the impurities found in and derived from malathion (including isomalathion) were prepared, and mixtures in various proportions with highly purified malathion were fed to rats. Pure malathion has an acute oral toxicity to rats of 10–13 g/kg of body weight and three impurities in addition to isomalathion were shown to produce potentiation. Isomalathion itself, however, was proved to be the dominant potentiator. The other components, which were present only in minor amounts and were not formed to any appreciable extent during storage of the powders, contributed only slightly to potentiation.

The Committee was informed of the very ready cooperation of the malathion manufacturers in solving this problem and in providing samples and methods for use in developing the new specifications. This cooperation and close working relationship resulted in improved formulations. A collaborating laboratory had developed gas-liquid chromatographic methods for malathion and for the isomerization product isomalathion, and these methods were tested collaboratively in nine laboratories including those of the manufacturers. An accelerated storage test at 55°C for 6 days was developed, and this was considered to be equivalent to field storage conditions in which isomalathion was likely to be generated.

Tests on samples of powder formulations of malathion from field programmes showed that all those that have been proved safe for indoor residual spraying have an acute oral LD₅₀ to rats of at least 2 g/kg of body weight. The isomalathion content of those samples never exceeded 2% of the nominal malathion content. Thus impurities, including isomalathion itself, present in small amounts in good-quality malathion water-dispersible powder do not cause significant changes in the mammalian toxicity of the formulation, even though there is a large difference between the toxicities of such impurities and that of highly purified malathion. The samples with significantly increased mammalian toxicity had, as a rule, an isomalathion content 2–5 times greater than usual. An acceptable level of this contaminant, required for setting a limit to its presence in water-dispersible powder after accelerated storage, was then considered. The Committee was aware of uncertainties in the accuracy and precision of the analytical method for isomalathion. There is also uncertainty in the correlation of isomalathion formation during the accelerated storage treatment with that occurring under various field-storage conditions. However, it considered that a limit could be set that would be acceptable from an analytical point of view and that would ensure that the malathion passing this requirement in the labora-

tory would not cause problems in the field. The Committee considered that the limit for isomalathion should be 1.5–1.6% of the nominal malathion content. However, in view of the range of results obtained in a limited collaborative study and in view also of the possibility that the recommended accelerated storage test may be too severe as compared to field conditions, the Committee considered that a practical limit for isomalathion of 1.8% of the nominal malathion content should be the highest acceptable value (see Annex 1, section 6).

The Committee recommended that additional information be obtained from laboratory and field studies so that the isomalathion limit can be reviewed at a future date.

The Committee also recommended that research be continued on the analytical methods for determining isomalathion and on the development of the accelerated storage test so that the results obtained with it correlate closely with the field stability actually found for malathion water-dispersible powders formulated according to the new specifications.

1.3 Specifications for insecticides

The Committee reviewed the specifications for insecticides contained in the WHO manual *Specifications for pesticides used in public health*¹ and recommended a number of changes. It also considered the suitability for publication of several interim specifications.² A full account of the Committee's deliberations on this subject is given in Annex 1.

1.4 New insecticides

1.4.1 Synthetic pyrethroids

In its nineteenth report³ the WHO Expert Committee on Insecticides noted the development of synthetic pyrethroids, which were then looked upon as replacements for the natural product for use in aerosols for space treatment. Since that time the situation has been completely changed by the discovery of pyrethroids that are both highly insecticidal and much more stable on exposure to light and air. They can be used for the control of agricultural and public health pests in situations where natural pyrethrum and the earlier synthetic pyrethroids would never have been considered.

¹ *Specifications for pesticides used in public health*, 4th edition, Geneva, World Health Organization, 1973.

² Unpublished WHO document WHO/VBC/73.462.

³ WHO Technical Report Series, No. 475, 1971, p. 18.

Their exceptional activity against insects is important because they are relatively expensive to manufacture and will therefore need to be used at dosage rates much lower than those used with other insecticides. This high activity may cause some difficulties in preparing formulations, in drawing up specifications, and in developing the necessary analytical methods.

The pyrethroids now being developed have both optical and geometrical isomers, the ratios of which vary with the source of the technical material. The stereoisomers vary in insecticidal activity and toxicity to mammals. Thus specifications will be required to define the ratio of these isomers, particularly the *cis-trans* isomers, and to describe analytical methods that can distinguish between them. Gas-liquid chromatography is the method of choice; it is already employed by manufacturers and users. There is no difficulty in obtaining stable standards, in contrast to the situation with natural pyrethrum. High-performance liquid chromatography is also likely to be used.

The new compounds are already available as the usual formulations of solutions, emulsion concentrates, and water-dispersible powders. To achieve low dosage rates, the formulations must contain low concentrations of active ingredient, and they must be more highly diluted before application. The low-concentration formulations may require changes in the accelerated storage tests and in the emulsion and suspension stability tests.

New members of this group of substances have properties that make them of great potential value in vector control. The Committee considered that, when field trials have shown which compounds and formulations are the most effective, support should be given to collaborative testing of analytical methods and the development of specifications.

1.4.2 *Insect growth regulators*

It is well known that some insect vectors have become so resistant to the standard insecticides that they cannot be controlled efficiently. The same insecticide can also be toxic to nontarget organisms and persist in the environment. There has therefore been great interest in a new strategy in which synthetic chemicals are used to interfere with the growth of the pre-adult stages of the insects. The chemicals may mimic the natural juvenile hormone so that their presence keeps the insects in the larval stages and prevents metamorphosis or, alternatively, they can inhibit the synthesis of chitin between larval stages or when the pupa is formed. They should be more specific than most insecticides because their sites of action are unique to the insect. Moreover, they are readily

decomposed. An isoprenoid hormone mimic and a chitin-inhibiting substituted urea have already been extensively tested for the control of mosquito larvae and have shown considerable promise.

Methods of analysis have already been published that would probably be suitable for inclusion in specifications. Gas-liquid chromatography is satisfactory for esters such as the isoprene-derived hormone mimics but not for the benzoylurea derivatives that are used to inhibit chitin formation. The latter have, however, been determined by high-performance liquid chromatography.

Hormone mimics cannot always be applied at exactly the time when the larvae are most susceptible, and since they are unstable in water it is often essential to use them in slow-release formulations such as granules or microcapsules.

Good control of mosquito larvae has been obtained with isoprenoid and substituted-urea compounds, which are safer to use and less polluting than more generally toxic insecticides. The Committee recommended that, if further trials confirm the suitability of these compounds for vector control, methods of analysis and specifications be developed.

1.4.3 *Controlled release formulations*

The main use of granules is now in agriculture, although they were originally developed as a way of applying mosquito larvicides and molluscicides. They are convenient and safe to handle, they reduce drift hazards, and they can be placed on the target more accurately than can conventional formulations. Most granules are based on inorganic materials such as clays, but organic polymers have also been used and all the new insecticides could certainly be prepared in this form. The choice of carrier, solvents, and binders provides control over wetting and breakdown and subsequent release of the active ingredient. Alternatively the granule may remain whole, with diffusion governing the release. Both these systems have been used with larvicides.

Another procedure is microencapsulation. The capsules containing insecticides are always much smaller than granules and can be prepared and stored as suspensions in water and diluted for spraying. As with water-dispersible powder formulations of solid insecticides, one great advantage of capsules is that the sizes of the individual units of pesticide are determined during manufacture and do not depend on mixing and application procedures. Some capsule walls may be designed to disintegrate and release the whole contents very quickly, but the greatest benefits from this formulation are obtained if the wall remains and is used to control the rate at which the chemical leaves. This control

depends mainly on choice of polymer, degree of cross-linking, capsule size, and wall thickness. Other factors include the nature of the core (i.e., whether it comprises technical grade material, a solution, or a suspension) and the effect of diffusion of water or air inwards. Chlorpyrifos and pirimiphos-methyl are being developed as encapsulated formulations, and if these formulations prove effective specifications will be prepared. The properties of capsules should ideally be tailored for a particular use. In the case of *Simulium* larvae, for example, important features would be density, a size range that would allow the capsules to be ingested by the larvae, and a wall material that remains intact while the capsules are suspended in the river but disintegrates once they are ingested by the larvae. Mosquito larvicides or the hormone mimics should be released from the capsule at a constant rate all the time they are immersed.

1.5 Rodenticides

Because most of the rodenticides used in public health are the same active materials and formulations as those used in agriculture, a joint FAO/WHO informal consultation of rodenticide experts was convened at FAO headquarters in Rome from 8 to 12 December 1975. The report of the consultation¹ outlined the major chemical and physical characteristics of effective rodenticides and described analytical methods needed to determine them.

The Committee recommended that newly available technical details, including some analytical methods, be inserted in the report, the data being obtained from national laboratories and the industry itself, and that, if resources were available, the revised version, for use in both public health and agriculture, be published separately from the manual on pesticides in collaboration with FAO.

1.6 Molluscicides

The Committee did not recommend any changes in the established specifications and analytical methods for molluscicides. No new molluscicides for public health use had been developed. It was understood that development of slow-release formulations of existing molluscicides is under way and that some new molluscicides are being tested. When these products are sufficiently developed, interim specifications will be prepared.

¹ Unpublished WHO document VBC/76.3.

1.7 Impurities in pesticide formulations

It has been known for some time that impurities in organophosphorus pesticides can cause major changes in their biological properties.¹ Other groups of pesticides can also contain impurities of very high biological activity. It is the Committee's view that the determination of impurities by sensitive and specific analytical techniques may be of great importance in some instances. The research carried out in connection with the development of improved specifications for malathion has brought to light that some simple thiophosphate esters may be present in commercial malathion and perhaps in other organophosphorus pesticides. The Committee was informed about work on these compounds now being carried out and recommended that such studies be strengthened by the further development of analytical techniques that would help in evaluating the biological effects of these compounds and in assessing their potential hazard to man.

The Committee understood that the general problem of impurities in pesticides and their toxicological significance is under review and recommended that it should be considered in detail by the WHO Expert Committee on the Safe Use of Pesticides in 1978.

2. METHODS, STANDARDS, AND SAMPLING

2.1 Methods for general use in specifications

The Committee reviewed the methods of work described in the WHO manual *Specifications for pesticides used in public health*² and recommended a number of changes. These are recorded in Annex 2.

2.2 Standards for use in analytical procedures

A basic element in most procedures for the specific analysis of pesticides is the use of a standard. The Committee was informed that for the assistance of national laboratories WHO provides standard materials (such as *p,p'*-DDT, dichlorvos, malathion, and the alpha and gamma isomers of HCH) in small lots without charge. At present

¹ ALDRIDGE, W. N. & REINER, E. *Enzyme inhibitors as substrates*. Amsterdam, North Holland, 1972 (Frontiers of biology, vol. 26), pp. 30-33.

² *Specifications for pesticides used in public health*, 4th edition, Geneva, World Health Organization, 1973.

these few standards are obtained through the generosity of the various pesticide manufacturers.

The Committee supported this effort but noted that with the need for better standards and a wider variety of them (as exemplified by the new procedure for malathion, which requires high-purity malathion and isomalathion) this aspect of the use of WHO specifications may become difficult to manage from the viewpoint of source of supply and storage of stocks of known purity. In addition the highly pure standards may not have the reproducibility required to maintain a constant level of standardization over a period of time.

This is now a significant problem for control laboratories supplying the large variety of materials needed for the quality control of pesticide formulations used in agriculture. Because of the limited number of pesticides used in vector control, the problem is not yet so great for public health programmes. It is likely to become so quite rapidly, however, with the development of more specific methods and with the increasing need for methods of identifying and quantifying impurities that may endanger the user or contaminate the environment.

In reviewing this subject the Committee considered several categories of standards used in pesticide analysis. Primary analytical pesticide standards used for calibrating secondary standards have a purity of more than 99%, as determined accurately with sophisticated instruments and techniques. Secondary pesticide standards are usually in the 90-98% purity range and are generally used for calibration of the analytical methods for pesticides in technical-grade and formulated products. Technical pesticide standards are materials without a definite chemical composition but with a definite range of elemental composition (e.g., chlordane and campheclor); they are used in some qualitative analytical procedures for the assay of technical-grade and formulated products. Standards for pesticide degradation products, by-products, and metabolites usually have purities of 90% or more, as determined by the use of sophisticated instruments and techniques. Internal standards are usually common laboratory reagent-grade organic chemicals for calibrating and quantifying the analytical procedures when using gas-liquid and high-performance liquid chromatography.

The Committee recommended that, when an analytical procedure for a pesticide is developed, the standard be specified and the method of determining the purity be defined before the acceptance of the procedure for inclusion in a specification. In making this recommendation the Committee was aware of the difficulties of applying it to established pesticide specifications; it therefore urged that all ways and means be

explored to improve the descriptions of existing standards and the definition of their purities. The Committee noted that WHO had designated some national laboratories as centres for standardizing various materials in the past and suggested that this possibility be further explored.

On being informed that FAO had been requested by various national laboratories to assist in obtaining pesticide standards, the Committee recommended that FAO and WHO collaborate in developing a better reference service.

2.3 Gas-liquid and high-performance liquid chromatography

The Committee noted the rapid expansion in the use of gas-liquid chromatography for all types of pesticide analysis in the past few years. The reproducibility of the results obtained by different laboratories with this technique was confirmed by the collaborative trials carried out on malathion and the contaminant isomalathion (see section 1.2.4). It was further noted that the Association of Official Analytical Chemists, Washington, DC, USA, has been studying the use of gas-liquid chromatography and has issued the report of the Committee on Gas Chromatography of Pesticide Formulations,¹ which gives general guidelines for the use of this technique. In addition, several collaborative analytical trials have been completed and published in the journal of the Association. On the grounds that this technique is now becoming more common and that reliable results can be easily obtained with relatively modest equipment, the Committee considered that its use can be recommended for those pesticides for which no simpler technique of specific analysis is available or for which there is a special need for detection of minor components. The Committee recognized that in many laboratories, especially in developing countries, such equipment is not available and that there is need to develop simple but specific analytical methods. This aspect of the problem of pesticide analysis is discussed in section 2.5.

The development of high-pressure liquid chromatography was noted to be progressing rapidly, and the Committee considered that the method should be acceptable for routine analysis in the future, with the drawback that equipment may be lacking in laboratories in developing countries. However, the Committee considered that it would be of much value where specificity is essential and where thermally labile materials are being investigated. The Committee noted that this analytical procedure has been included in the draft rodenticide specifications as an alternative to the ultraviolet analysis of many anticoagulant compounds.

¹ *Journal of the Association of Analytical Chemists*, 59 : 420 (1976).

2.4 Sampling and analysis of environmental materials

The sampling and analysis of pesticides after their application in the environment is of increasing importance. This had been noted by a WHO Scientific Group in 1974, and the Committee concurred with the suggestions and recommendations made by that group.¹

This subject was considered by the Committee since the analytical techniques described in specifications should take into account the ecotoxicology and environmental chemistry of pesticides. The stability of a pesticide and the presence of impurities in the manufactured material and its formulations may both affect the fate and distribution of the pesticide after application. The Committee noted with interest the research work being carried out by WHO on developing methods for the chemical monitoring of the environment during the *Simulium* larviciding operations of the Onchocerciasis Control Programme in the Volta River Basin area. The techniques used require careful extraction and preservation of the samples from the field, with subsequent analysis in the laboratory. While this is satisfactory for research purposes, it would be useful to develop simpler methods that could be applied in the field in developing countries, where routine checking may be required for more than one pesticide.

Unlike the sampling of formulations, the sampling of environmental biological materials is far more intricate. It is also vulnerable to many factors, such as packaging, location and quantities taken, lapse of time, availability of sampling, and laboratory facilities. An approach to solving some of these problems would be the development of directions on sampling, extraction, packaging, and analytical procedures for use in the field. It was noted that techniques are being developed for temephos and chlorphoxim for the Onchocerciasis Control Programme. The Committee recommended that this type of research be carried out with pesticides applied in other vector control programmes and that the description of the procedures, including those for chlorphoxim and temephos, be issued to field personnel.

2.5 Identification methods

Specific methods for identifying a pesticide and any contaminants and impurities it may contain must be included in the specification for that pesticide. In addition, specific methods for pesticides and perhaps

¹ WHO Technical Report Series, No. 560, 1975 (*Chemical and biochemical methodology for the assessment of hazards of pesticides for man*).

impurities, contaminants, and degradation products are needed for direct use in the field. A simple and rapid method would be of immense value in controlling the pesticide during transport and use, especially in those countries where no control or registration schemes exist and where there is a high possibility of misuse. Analytical methods that could be applied in the field would also be useful when faced with problems such as possible toxicity or lack of efficacy, and it becomes necessary to determine the identity of the products being used as soon as possible.

The Committee therefore strongly recommended that WHO encourage the development of simple chemical and biological methods of identifying the pesticides and their major decomposition products in pure, technical, or formulated materials and in biological materials. Such analytical methods should preferably be of a kind that can be easily converted to semi- or fully quantitative methods for use in the laboratory. Since these methods are also of much interest in agriculture, the Committee urged that their development be carried out in close collaboration with FAO.

In making this recommendation, the Committee supported the similar recommendation made by the WHO Scientific Group on Chemical and Biochemical Methodology for the Assessment of Hazards of Pesticides for Man¹ and suggested, as a step in its implementation, that consideration be given to the preparation of a review or bibliography of already existing methods, for the information of field personnel. The development of a field kit for the determination of cholinesterase values was commended.

3. COLLABORATION WITH OTHER ORGANIZATIONS

3.1 Food and Agriculture Organization

The Committee noted with satisfaction the long-standing collaboration between WHO and FAO on the chemistry and specifications of pesticides. A tangible result was the proposal to collaborate on a joint manual on rodenticides in public health and agriculture (see section 1.5). The Committee felt that there was a need for further coordination of all relevant programme activities with the object of increasing the uniformity of specifications and publishing them as joint standards

¹ WHO Technical Report Series, No. 560, 1975, p. 23.

where appropriate. It was agreed that, in addition to assisting Member States to ensure an adequate quality of pesticides, the availability of such joint FAO/WHO specifications would also contribute substantially to the international harmonization of pesticide registration requirements, which both organizations are promoting.

3.2 United Nations Children's Fund

The Committee was informed that UNICEF uses the specifications established by WHO for purchasing pesticides for various national programmes. It noted with satisfaction the close technical cooperation existing between WHO and UNICEF in the use of these specifications and urged that this be continued.

3.3 United Nations Industrial Development Organization

The Committee was informed that UNIDO is interested in promoting the manufacture of insecticides and formulations in developing countries and that the specifications governing such manufacture should be of a kind that can be applied under the conditions existing in those countries. The Committee considered that the established specifications were valid for such manufacture—particularly the manufacture of DDT water-dispersible powder other than the 75% water-dispersible powder for overseas shipment—and that UNIDO should encourage local manufacturers to use the WHO specifications for those materials that are destined for use in public health.

3.4 Collaborative International Pesticide Analytical Council

The Committee noted the collaboration between WHO and CIPAC in the development of standardized chemical and physicochemical analytical methods for pesticides and urged its continuation. Such collaboration would prevent the emergence of a multiplicity of analytical methods and so avoid future difficulties in establishing specifications for new materials.

3.5 International Group of National Associations of Pesticide Manufacturers

The Committee noted the assistance provided by the technical committee of the International Group of National Associations of Pesticide Manufacturers (GIFAP) in preparing the requirements and analytical

sections of the draft rodenticide specifications and in preparing and improving the specifications and analytical methods for nonproprietary pesticides in general. It was considered that this cooperation should be encouraged.

4. RECOMMENDATIONS

The Committee recommended the establishment of specifications for new insecticides and the revision of existing specifications and methods (see Annexes 1 and 2). During its deliberations, the Committee made recommendations and suggestions of a more general nature, and these are given in the following paragraphs.

1. The development of specification requirements for new types of formulations should be carried out in parallel with field tests on efficacy and safety.

2. Research should be conducted on the presence of impurities in technical organophosphorus insecticides and the possible formation of such impurities in formulations. The implications of these impurities for the efficacy and safety of the insecticides in the field should be fully evaluated, particular attention being paid to the simple esters in the organophosphorus class of compounds.

3. Close collaboration should be maintained with FAO in developing an information service in pesticide standards.

4. If resources are available, the established specifications and methods should be published in a fifth edition of *Specifications for pesticides used in public health*, and specifications for rodenticides should be published separately in collaboration with FAO.

ACKNOWLEDGEMENTS

The Committee acknowledged the special contributions to its deliberations made by the following WHO staff members : Dr J. F. Copplestone, Pesticide Development and Safe Use ; Miss C. M. Moreau, Pesticide Development and Safe Use ; Dr D. Muir, Malaria and Other Parasitic Diseases ; Dr C. Pant, Ecology and Control of Vectors ; Dr G. Quélenec, Pesticide Development and Safe Use ; Dr M. Vandekar, Pesticide Development and Safe Use, and Dr J. de Zulueta, Malaria Adviser, Pakistan.

Annex 1

RECOMMENDED CHANGES IN SPECIFICATIONS¹ FOR INSECTICIDES

1. DDT

Technical DDT (Specifications WHO/SIT/1.R4), DDT water-dispersible powders (Specifications WHO/SIF/1.R4 and WHO/SIF/26.R1), DDT emulsion concentrate (Specification WHO/SIF/4.R4), and DDT dusting powder (Specification WHO/SIF/16.R3)

The Committee reviewed a report on methods of analysis of technical and formulated DDT and methoxychlor, including the results of a collaborative study on the analysis of *p,p'*-DDT and DDT water-dispersible powders.

The Committee recommended that, in the specifications for technical DDT and all DDT formulations, the Caldwell technique² be adopted for use in the determination of chlorine after the sodium reduction step in the analysis for total chlorine. The Committee further recommended that the correction factor *f* be omitted from the calculations in the determination of organic chlorine in all specifications for technical DDT and DDT formulations. It was, however, recommended that a blank determination be made using the exact procedure, in order to obtain a correction value for all reagents used. This determination should become a part of the procedure for obtaining organic chlorine content.

The Committee took note of the ambiguity that could result from having two methods of determining DDT content and recommended that the hydrolysable chlorine method be deleted from all specifications for DDT formulations.

Observing that the determination of inorganic chlorine is unnecessary in procedures in which the sample is dissolved in nonpolar organic solvents, the Committee recommended that it be omitted from all specifications for DDT formulations.

¹ *Specifications for pesticides used in public health*, 4th edition, Geneva, World Health Organization, 1973.

² CALDWELL, J. R. *Industrial and engineering chemistry, analytical edition*, 1: 38 (1935).

2. Methoxychlor

Technical methoxychlor (Specification WHO/SIT/4.R2) and methoxychlor emulsion concentrate (WHO/SIF/11.R2)

The Committee recommended that, for consistency, some of the changes that had been recommended in the methods of analysis for DDT be adopted for methoxychlor as well. The changes recommended were the adoption, in the specifications for methoxychlor and methoxychlor emulsion concentrate, of the Caldwell technique for determination of chlorine after the sodium reduction step in the analysis for total chlorine; the omission of the *f* factor from the calculations in the determination of organic chlorine; the provision for a blank determination to obtain a correction value for all reagents used; the deletion of the hydrolysable chlorine method for methoxychlor emulsion concentrate; and the omission of the determination of inorganic chlorine in the specification for methoxychlor emulsion concentrate.

3. HCH

Technical and refined HCH (Specification WHO/SIT/2.R4), HCH water-dispersible powder (Specification WHO/SIF/2.R4), HCH emulsion concentrate (Specification WHO/SIF/5.R4), and HCH dust (Specification WHO/SIF/17.R3)

The Committee recommended the deletion, in footnotes to the specifications, of hydrolysable chlorine as an alternative analytical method for analysis of HCH content.

4. Pyrethrum

Pyrethrum (Specification WHO/SIT/7.R1)

The method of analysis was reviewed. It is complicated and time-consuming, and it needs to be performed by an experienced chemist. However, it is the product of many years of development, and there are no obvious ways in which it can be simplified. It is the method still used by the producers.

Gas-liquid chromatography is a much easier procedure. It is also more specific for the active components and has been used to measure their relative amounts. However, these components are unstable and difficult to prepare and therefore cannot be used as standards. The

total amounts of the mixed esters "pyrethrin I" and "pyrethrin II" must still be measured by reference to a pyrethrum extract whose concentration has itself been recently determined by the method of the specification. The Committee therefore concluded that no changes could be recommended in the specification and that the analytical method should be retained.

5. Diazinon

Technical diazinon (Specification WHO/SIT/9.R3) and diazinon water-dispersible powder (Specification WHO/SIF/9.R3)

The Committee was informed that technical diazinon containing an acid scavenger for stabilizing purposes was no longer available and therefore the minimum purity of the technical material could be raised to 95% by weight to reflect current manufacturing practice. It was also agreed that, in the description of the technical material, the words "a dark liquid" should be replaced by the words "a yellow to brown liquid" and that, in the requirements, the maximum content of solid material insoluble in acetone should be reduced from 0.5% to 0.15% by weight while the maximum water content should be reduced from 0.1% to 0.06% by weight.

For diazinon water-dispersible powder, the Committee agreed to delete, in the description, the words "yellow to light brown" and to delete, in the requirements, the section entitled "1.2.4 Acidity", because the powder formulation is not acidic in reaction.

6. Malathion

Technical malathion (Specification WHO/SIT/10.R3), malathion water-dispersible powder (Specification WHO/SIF/10.R3), malathion emulsion concentrate (Specification WHO/SIF/14.R3), and malathion dusting powder (Specification WHO/SIF/22.R2)

The Committee took note of the difficulties encountered with the nonaqueous copper colorimetric method of analysis of malathion, namely, the high results obtained on poor quality products and the

tedious and time-consuming nature of the method.¹ The Committee reviewed the results of a collaborative study² of the new gas-liquid chromatography (GLC) method conducted on malathion (technical, emulsion concentrate, water-dispersible powder, and dust). It also reviewed the results of a collaborative study³ of GLC methods for malathion and isomalathion in technical material and water-dispersible powder.

The Committee recommended that :

(1) the new GLC method² with minor modifications^{3, 4} be adopted as the official method of analysis in specifications for technical malathion and malathion formulations, in place of the colorimetric method.

(2) the minimum content of malathion in technical malathion be changed from 95% to 92% to reflect the improved accuracy of the method.

(3) a limit on isomalathion content be incorporated in the specification for malathion water-dispersible powder—namely, that the isomalathion content of the powder shall not exceed 1.8% of the nominal malathion content after storage of the powder for 6 days at $55 \pm 1^\circ\text{C}$ when tested by the method described in the appendix to this annex.

The Committee noted that the adoption of the highly specific GLC method of analysis makes the specific gravity requirement for technical malathion superfluous and therefore recommended its deletion.

The Committee was informed of the problem posed by the strong odour of malathion, which causes its rejection in some malaria control programmes. However, no reliable quantitative test for odour is available for incorporation in the specifications, the method of controlling odour is still that given in the footnote of the current malathion specifications. It was recommended that the same footnote be included in the new specifications. Some of the recent powders have shown much improved odour characteristics, and this may be related to the improvement of the formulations to ensure more stable malathion in the field.

¹ STILES, A. R. ET AL. *Journal of the Association of Official Analytical Chemists*, **60** : 1148-1153 (1977).

² WAYNE, R. S. Collaborative study of the determination of malathion in formulations and technical materials by gas-liquid chromatography. *Journal of the Association of Official Analytical Chemists*, in press.

³ Unpublished working document PDS/EC24/77.23.

⁴ Unpublished working document PDS/EC24/77.13.

7. Trichlorfon

Technical trichlorfon (Specification WHO/SIT/13.R1)

The Committee agreed to change the description of the material from "white to pale yellow crystalline powder" to "white crystalline material". It was also agreed to replace "setting point" by "melting point", with a minimum limit of 77°C, to raise the minimum purity from 95.0% to 97.0%, to reduce the maximum acidity from 0.7% to 0.3%, and to reduce the maximum water content from 1.0% to 0.4%, all these values being in agreement with the FAO specifications.

8. Fenthion

Technical fenthion (Specification WHO/SIT/15) and fenthion emulsion concentrate (Specification WHO/SIF/28.R1)

The Committee was informed that current manufacturing methods give technical fenthion of higher purity than previously and agreed that the minimum purity value be raised from 90.0% to 94.0% by weight. It was also agreed to limit the water content to 0.2% instead of 0.5%, taking into account that technical fenthion is susceptible to hydrolytic decomposition, and to delete the clause "solid material insoluble in acetone".

The Committee agreed to raise the maximum acidity content from 0.3% to 0.6% for fenthion emulsion concentrate.

9. Dichlorvos

Technical dichlorvos (Specification WHO/SIT/16)

The Committee reviewed the specifications and considered suggestions from three manufacturers. It agreed, in the light of improved manufacturing practices, that the minimum content of active ingredient be raised from 93.0% to 95.0% by weight, that the maximum acidity content (as H₂SO₄) be raised from 0.1% to 0.2% by weight, and that the maximum water content be raised from 0.02% to 0.05% by weight. In addition the specification should contain a footnote stating that "dichlorvos is hygroscopic and should be sampled in such a way as to ensure minimum entry of water vapour". In view of the fact that no samples have shown any solid material insoluble in acetone, it was agreed that this clause could be deleted.

10. Fenitrothion

Technical fenitrothion (Specification WHO/SIT/17), fenitrothion water-dispersible powder (Specification WHO/SIF/29), and fenitrothion emulsion concentrate (Specification WHO/IS/3.0043-2)

Several changes in the specifications suggested by manufacturers were considered by the Committee. It was recommended that :

(1) The description of technical fenitrothion be changed from "tan coloured" to "yellow to brown".

(2) The maximum water content be reduced to 0.1% by weight in technical fenitrothion.

(3) No change be made in the present titrimetric method of analysis for fenitrothion content. A new colorimetric method proposed by the industry was not adopted by the Committee because it had not been collaboratively tested.

(4) The requirement governing solid material insoluble in acetone and the requirement governing specific gravity be omitted from the specification for technical fenitrothion.

(5) The requirement governing alkalinity be reduced from 0.5% to 0.2% in the specification for fenitrothion water-dispersible powder.

The Committee reviewed a report on a gas-liquid chromatography method for the analysis of technical fenitrothion and fenitrothion water-dispersible powder. Although excellent precision was obtained by the method, the Committee did not recommend its adoption because there was no assurance that a reference standard would be available.

The Committee recommended that an interim specification for fenitrothion emulsion concentrate be established and published in a new edition of the WHO manual *Specifications for pesticides used in public health*. It also recommended that development and collaborative testing be carried out on the gas-liquid chromatography method of analysis.

11. Propoxur

Technical propoxur (Specification WHO/SIT/18) and propoxur water-dispersible powder (Specification WHO/SIF/30)

The Committee agreed, in the description of the technical material, to replace the words "light yellow" by the words "white to grey".

It was also agreed to raise the minimum purity from 95.0% to 97.0% by weight, to raise the minimum melting point from 87.0°C to 88.5°C, and to reduce the maximum water content from 0.5% to 0.2% by weight. Reviewing the analytical methods, the Committee did not think there should be any changes until a collaborative trial has proved the suitability of new methods. The Committee was aware that the technical material might contain isopropoxyphenol as an impurity but felt that the changes being recommended in minimum purity and melting point would help to ensure a high-quality product. If a new and more specific analytical method were developed it should include a procedure to determine the phenol impurity.

The Committee reviewed the clause "1.2.4 Acidity or alkalinity" in the specification for water-dispersible powder and agreed to limit the alkalinity to a maximum of 0.2% instead of 0.3%. It also recommended the inclusion of the following footnote: "Maximum acidity applies only to products not containing an acid stabilizer".

12. Temephos

Technical temephos (Specification WHO/SIT/19), temephos emulsion concentrate (Specification WHO/SIF/31), and temephos 20% emulsion concentrate for Simulium control (WHO/IS/3.0786)

The Committee reviewed reports on a new thin-layer chromatography method for the clean-up step in the analysis of temephos in technical and formulated products. Although not collaboratively tested it was considered to be an improvement on the present column chromatographic clean-up step and was recommended for inclusion in the specification for technical temephos and temephos formulations. A gas-liquid chromatographic method was reviewed but this was considered to be in the development stage and not ready for use in specifications. It was noted that a high-performance liquid chromatographic method is being developed and may be useful for future specifications.

The Committee reviewed the interim specification for temephos emulsion concentrate for *Simulium* control, which has been used for the past two years in purchasing supplies for the Onchocerciasis Control Programme in the Volta River Basin area. The emulsion type and stability and the procedures for their measurement were considered sufficiently defined to justify the establishment of the specifications, but further work is necessary to obtain a better correlation between the laboratory measurement of these characteristics and the efficacy of the formulation in the field.

The Committee noted that the 1% formulation of temephos on sand granules is widely used in the control of mosquito larvae and considered that interim specifications should be developed.

13. Interim specifications for newer insecticides

Bromophos

The Committee recommended the publication of the specification for technical bromophos, WHO/IS/1.0658-3, after the following changes have been made.

(1) The material should be described as "a white or light-cream-coloured powder".

(2) The method used to determine the acidity should be WHO/M/3.¹

(3) The summary of the method should read: "The sample is dissolved in glacial acetic acid and oxidized with standard solution containing a mixture of potassium bromide and potassium bromate. The excess of bromate is determined by titration with standard sodium thiosulfate solution."

(4) Potassium bromide solution should be deleted from the special reagents.

(5) The procedure should be revised to read: "Weigh accurately about 0.065 g of the sample into a 300 ml flask. Add ... the sample. Add 20.0 ml of 0.1 mol/l standard bromide-bromate solution. Stopper the flask and allow the solution to stand in the dark at room temperature for 15 minutes, shaking it occasionally. Add 20 ml ... reagents."

(6) Section 2.2 on acidity should be deleted.

The Committee recommended the publication of the specification for bromophos water-dispersible powder formulation, WHO/IS/2.0658-3, subject to the following changes.

(1) The acidity should be no greater than 0.3% when tested by method WHO/M/3.

(2) The summary of the method should read: "The sample is suspended in glacial acetic acid and oxidized with standard bromide-

¹ Methods indicated by reference numbers in this report are fully described in: *Specifications for pesticides used in public health*, 4th edition, Geneva, World Health Organization, 1973.

bromate solution. The excess bromate is determined by titration with standard sodium thiosulfate solution.”

(3) Potassium bromide solution should be deleted from the reagents.

(4) The procedure should read: “ Weigh accurately a sample containing about 0.065 g of bromophos into a 300 ml flask. Add ... completely. Add 20.0 ml of 0.1 mol/l standard bromide-bromate solution. Stopper the flask and allow the solution to stand in the dark at room temperature for 15 minutes, shaking it occasionally. Add ... reagents.”

The Committee recommended the publication of the specification for bromophos emulsion concentrate, WHO/IS/3.0658-3, subject to the following changes.

(1) The acidity should be greater than 0.3% when determined by method WHO/M/3, and paragraph 2.4 on free acid determination should be deleted.

(2) In the analytical procedure a revised clean-up step using a silica-gel column should be included.

In recommending establishment of these specifications for bromophos, the Committee recognized that the analytical method was not specific and that research should be carried out to develop a more specific method, which should then be collaboratively tested.

Jodfenphos

The Committee reviewed the interim specifications for technical jodfenphos, WHO/IS/1.1211-2, and jodfenphos water-dispersible powder, WHO/IS/2.1211-2, and considered certain changes suggested by the manufacturer. The Committee recommended the acceptance of these specifications for publication subject to the following changes.

(1) The content of technical jodfenphos should be 93% minimum instead of 94%, its melting point should be omitted, and the maximum weight of solid material insoluble in acetone should be 0.5% instead of 0.3%.

(2) The cautionary notice on packages of both technical jodfenphos and the water-dispersible powder should state that the contents may be hazardous if swallowed. It should also warn the user not to inhale the spray mist and to wash hands and exposed skin thoroughly after work.

The Committee recommended that a gas-liquid chromatography method for analysis of the active ingredient be tested, with the goal of eventually substituting it for the nonspecific method given in the new specifications.

Chlorpyrifos

Interim specifications for technical chlorpyrifos (WHO/IS/1.0971-2), and chlorpyrifos emulsion concentrate (WHO/IS/3.0971-2), were reviewed. The Committee recommended their adoption for publication but considered that only the gas-liquid chromatographic method of analysis should be included.

Other interim specifications

The interim specifications for technical chlorphoxim (WHO/IS/1.1197-2), chlorphoxim water-dispersible powder (WHO/IS/2.1197-2), chlorphoxim emulsion concentrate for *Simulium* control (WHO/IS/4.1197-1), technical pirimiphos-methyl (WHO/IS/1.1424-1), pirimiphos-methyl water-dispersible powder (WHO/IS/2.1424-1), and pirimiphos-methyl emulsion concentrate (WHO/IS/3.1424-1) were reviewed, but the amount of information available was not considered sufficient to enable the Committee to recommend their acceptance for publication. The Committee considered that these specifications should remain as interim specifications and be used in such a form for any procurement of supplies. When further experience has been gained they may be reconsidered.

The Committee reviewed the interim specifications for trichlorfon water-soluble powder (WHO/IS/4.0800), fenthion water-dispersible powder (WHO/IS/2.0002-1), dichlorvos emulsion concentrate (WHO/IS/3.0014-2), and chlorpyrifos-methyl (WHO/IS/1.1155-1 and 3.1155-1) but, on being informed that the use of these formulations for public health purposes has not increased, recommended that they be maintained as interim specifications for the time being. It was noted that work was proceeding on new encapsulated formulations of chlorpyrifos-methyl and that if such formulations could be developed the appropriate interim specifications would be prepared.

The interim specifications for dicapthon, Landrin, and Mobam were recommended to be dropped because these insecticides are no longer being manufactured and developed.

14. Specifications no longer needed

Technical dieldrin (WHO/SIT/6.R3), dieldrin water-dispersible powders (WHO/SIF/3.R3), and dieldrin emulsion concentrates (WHO/SIF/6.R4)

The Committee recognized that dieldrin-based formulations continue to be used in public health, as in campaigns against the tsetse fly. However, it was considered that the danger of environmental contamination was such that these specifications should be omitted from the new edition of *Specifications for pesticides used in public health*.

**ANALYSIS FOR ISOMALATHION IN MALATHION
WATER-DISPERSIBLE POWDER****1. Summary of method**

Chloroform is added to a sample of malathion water-dispersible powder to dissolve the active ingredient as well as any isomalathion present. An internal standard is added to the solution and an aliquot is injected into a gas-liquid chromatograph. The ratio of the response of the isomalathion and the internal standard is determined. This is compared to the response of a standard containing a known quantity of isomalathion to give the percentage of isomalathion in the sample.

2. Special apparatus ¹

Gas-liquid chromatograph. The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame ionization detector, an electrometer having a sensitivity of at least 10^{-11} amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1 mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to assay precision.

Chromatography column. The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.

Column-packing material. Chromosorb W HP (100/120 mesh) treated with 7.5% OV-210.

Glass wool, silane-treated.

3. Special reagents

*Isomalathion standard.*² A solution of a known quantity of isomalathion (approximately 20 mg/g) dissolved in malathion purified by recrystallization with tetraethyl thiodisuccinate added as a check on resolution.

Internal standard. 1,3-Diphenoxybenzene.

4. Preparation of standard solutions

Internal standard solution. Prepare a 4 g/l solution of the internal standard in reagent-grade chloroform. This solution is stable for 4 weeks if kept tightly sealed

¹ Advice on sources of supply of special apparatus is obtainable from Pesticide Development and Safe Use, World Health Organization, 1211 Geneva 27, Switzerland.

² Reference samples may be obtained from Pesticide Development and Safe Use, World Health Organization, 1211 Geneva 27, Switzerland.

and under refrigeration. Allow the solution to warm to room temperature before use.

Isomalathion standard solution. Weigh accurately about 1.5 g of the isomalathion standard in a 25 ml volumetric flask. Add 2.0 ml of internal standard solution to the flask and make up to volume with chloroform. Multiply the weight (in grams) of the isomalathion standard by the concentration of isomalathion in the standard (in mg/g) to determine the weight (in mg) of isomalathion in the standard.

5. Preparation and conditioning of chromatography column

Pass dry nitrogen through the column to remove moisture. Fill the column with a solution of 50 g dimethyldichlorosilane per litre of toluene and allow to stand for 5 minutes. Empty the column, rinse with toluene, and then rinse several times with methanol until the rinsings are neutral to litmus.

Attach an 8-cm funnel to the exit end of the prebent glass tube. While tapping the tube with a small wooden rod, add the prepared packing material in small quantities until the exit end of the tube is filled to about 1.5 cm from the end of the tube. Move the funnel to the entrance end of the column. Insert a small wad of silane-treated glass wool in the exit end of the column and attach a vacuum pump of moderate power to that end. Continue to add packing material slowly, while tapping, until the tube is filled to within about 2 cm of the entrance end. Insert a small wad of glass wool in the entrance end, compressing the glass wool only enough to hold the packing in place.

The column should be conditioned for at least 15 hours (overnight) at 250°C. This step should be conducted with the exit end of the column unconnected to the detector but with the carrier gas flowing at the recommended rate.

Connect the exit end of the column to the detector and set the controls to provide the conditions described below. Allow the instrument to come into equilibrium. Inject 3 µl aliquots of the isomalathion standard solution into the chromatograph until constant response is obtained. This criterion is met when at least three consecutive injections give response ratios (see section 9 for definition) that agree to within 2%.

6. Gas-liquid chromatography conditions

Typical operating parameters for an automated gas-liquid chromatograph are given below.

Temperatures

Oven	175°C ¹
Injection port	190°C
Flame detector	280°C

Gas flow rates

Hydrogen	30 ml/min
Air	300 ml/min
Carrier gas (helium or nitrogen)	30 ml/min

¹ When a sample is run, the temperature of the oven must be programmed to rise to 240°C following the emergence of tetraethyl thiodisuccinate in order to remove another minor component, tetraethyl dithiodisuccinate. After several minutes at 240°C, the oven may be returned to 175°C to begin the analysis of the next sample.

7. Resolution check

The efficiency of the gas chromatography column must be sufficient to partially resolve the diastereoisomers of tetraethyl thiodisuccinate. The resolution may be considered satisfactory when the distance measured from the top of the tetraethyl thiodisuccinate peaks to the valley between the peaks is at least 10% of the height of the peaks. This measurement should be made on a chromatogram of the isomalathion standard solution.

8. Preparation and analysis of sample

Weigh accurately an amount of sample equivalent to about 1.5 g of malathion in a 30-ml screw-cap bottle. Add 2.0 ml of the internal standard solution and about 25 ml of chloroform. Shake to dissolve the malathion, allow the phases to separate, and filter a portion of the supernatant solution and hold it for gas chromatography analysis.

Inject duplicate aliquots of 3 μ l of the isomalathion standard solution. Calculate the response ratios by dividing the area of the isomalathion peak by the area of the internal standard peak. Response ratios should agree to within 2%. If the precision limit is not met, inject two more aliquots of the solution. Failure to meet the precision requirements with the second pair of injections indicates that the apparatus is not functioning properly, and this problem must be resolved before proceeding with the analyses. Average the duplicate response ratios obtained with the isomalathion standard solution.

Inject duplicate aliquots (3 μ l each) of the sample solution. Calculate the response ratios, which, as for the standard solution, should agree to within 2%. Average the duplicate response ratios obtained with the sample solution.

9. Calculation

For each sample injection

$$\text{let } R = \text{response ratio} = \frac{\text{area of isomalathion peak}}{\text{area of internal standard peak}}$$

Then isomalathion content (in g/100 g of sample)

$$= \frac{R_2 \times W_1 \times 100}{R_1 \times W_2 \times 100}$$

where: R_1 = average response ratio for standard isomalathion solution

R_2 = average response ratio for sample solution

W_1 = weight of isomalathion taken (mg)

W_2 = weight of sample taken (g).

Annex 2

RECOMMENDED CHANGES IN METHODS USED IN SPECIFICATIONS¹

1. Visual suspensibility test for 75% DDT water-dispersible powders (Method WHO/M/2.R1)

The Committee was informed that people using this test find it particularly difficult to evaluate the small volume (4 ml) of sediment in the bottom of the graduated cylinder. The Committee recommended that advice on calibrating the 4 ml volume should be included in the description of the method. Noting that it could be useful to extend this test for a rapid evaluation of the suspensibility of other water-dispersible powders, the Committee supported the recommendation made at the nineteenth meeting of the Expert Committee on Insecticides that further work be carried out to establish the suitability of the method for other materials by adapting the initial concentration and the volume of sediment.

2. Emulsion stability test (Method WHO/M/13)

The specifications for emulsion concentrates require that the emulsions for this test be prepared by diluting 5 ml of concentrate to 100 ml with water. The repeatability and reproducibility of the test with this dilution are satisfactory, but the description of the method refers only to "the required volume". The Committee felt that it should be made clear that the test is not suitable for emulsions that are more dilute than 5 ml/100 ml because the volumes of creaming or separation cannot be measured with any accuracy and the formation of such dilute emulsions is not reproducible.

3. Revised Stepanow (total organic chlorine) method (Method WHO/M/16)

Having recommended modifications in the procedure for determining organic chlorine in the specifications for DDT and methoxychlor, the Committee considered that similar changes should be made in the

¹ *Specifications for pesticides used in public health*, 4th edition, Geneva, World Health Organization, 1973.

method WHO/M/16. It further recommended the deletion of the determination of inorganic chlorine in the analysis of HCH water-dispersible powders, HCH dusting powders, and HCH emulsion concentrates. Deletion of the *f* factor was also recommended.

4. Standard water for suspensibility and emulsifiability tests

The Committee noted that it is required to test the suspensibility of water-dispersible powders and the emulsion stability of emulsion concentrates both in distilled water and in standard hard water (342 mg/l calculated as calcium carbonate). The requirement for a test in distilled water, which was added by the WHO Expert Committee on Insecticides in 1965 mainly for controlling the quality of the surfactants in powders used with rainwater, has been criticized on the grounds that distilled water does not exist in nature and that the test is too severe. Taking account of subsequent work on 200 g/kg temephos emulsion concentrate formulation for use in *Simulium* control, in which a 10 : 1 dilution of the standard hard water was used to reflect field conditions, the Committee recommended that the requirement for use of distilled water be changed to a requirement for use of a standard soft water made by diluting 1 part of the standard hard water with 9 parts of distilled water.

5. Methods no longer needed

The Committee recommended that the specifications for rodenticides should be published separately (see section 1.5 of the report). The toxicity test for rodenticides (Method WHO/M/14) is therefore no longer needed and should be deleted. It is also recommended that gas-liquid chromatography should be used for the determination of malathion instead of the colorimetric method. The preparation of the potassium salt of *O,O*-dimethyl phosphorodithioic acid (Method WHO/M/20) is therefore not required and should be deleted. The Committee recommended deletion of the hydrolysable chlorine method for determining HCH content (Method WHO/M/17), because it is not now recommended as an alternative to the analysis of HCH and its formulations.