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**CHEMICAL AND BIOCHEMICAL  
METHODOLOGY FOR THE  
ASSESSMENT OF HAZARDS OF  
PESTICIDES FOR MAN**

**Report of a WHO Scientific Group**

WORLD HEALTH ORGANIZATION

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**WHO SCIENTIFIC GROUP ON CHEMICAL AND BIOCHEMICAL  
METHODOLOGY FOR THE ASSESSMENT OF HAZARDS  
OF PESTICIDES FOR MAN**

*Geneva, 17-23 September 1974*

**Members :**

Dr U. Ahlborg, Department of Toxicology, Swedish Medical Research Council, Karolinska Institute, Stockholm, Sweden

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, Principal Chemist, Government Chemical Laboratory, Dar-es-Salaam, Tanzania (*Vice-Chairman*)

Dr J. W. Miles, Chief, Pesticides Branch, Vector Biology and Control Division, Bureau of Tropical Diseases, Centre for Disease Control, Public Health Service, Atlanta, Ga., USA (*Chairman*)

Dr E. Reiner, Head, Department of Biochemistry, Institute for Medical Research, Zagreb, Yugoslavia (*Rapporteur*)

Dr G. H. Sanai, Professor Industrial Hygiene and Toxicology, Department of Occupational and Environmental Health, School of Public Health, University of Teheran, Teheran, Iran

Mr H. R. Wolfe, Chief, Field Studies Section, Environmental Protection Agency, Wenatchee, Wash., USA

Dr S. H. Zaidi, Director, Industrial Toxicology Research Centre, Lucknow, India

**Secretariat :**

Mr J. Henriët, State Phytopharmaceutical Station, Gembloux, Belgium (*Temporary Adviser*)

Professor R. Lauwerys, Industrial and Medical Toxicology Unit, University of Louvain, Brussels, Belgium (*Temporary Adviser*)

Dr A. R. Stiles, Scientist, Vector Biology and Control, WHO, Geneva, Switzerland (*Secretary*)

# CHEMICAL AND BIOCHEMICAL METHODOLOGY FOR THE ASSESSMENT OF HAZARDS OF PESTICIDES FOR MAN

## Report of a WHO Scientific Group

A Scientific Group on the Chemical and Biochemical Methodology for the Assessment of Hazards of Pesticides for Man was held in Geneva from 17 to 23 September 1974. Dr L. Bernard, Assistant Director General, opened the meeting and welcomed the participants on behalf of the Director General. He stressed the increasing concern for safe use of pesticides in both public health and agriculture and noted the need for improved chemical and biochemical technology in these areas.

### INTRODUCTION

#### Use of pesticides in public health vector control

During the past 15 years the World Health Organization has conducted a systematic search for pesticides that could be used to control disease vectors. This research work has grown in complexity owing to the inclusion of additional vectors and to the increased need for information on safety in the variety of conditions imposed by vector control programmes. There is a growing awareness of the great effect some pesticides have on nontarget organisms and this has very serious implications on the use of pesticides in public health work and in agriculture.

The Organization has conducted a Programme for Evaluating and Testing New Insecticides to find materials that are both effective against the target species and readily degradable and thus safe to use. During the development of these new compounds considerable work has been done on determining possible hazards when these compounds are used with ordinary and thus acceptable precautionary measures (see pages 6-7).

The Scientific Group addressed its deliberations in part to the recommendations made by the WHO Expert Committee on Insecticides in its nineteenth and twentieth reports (1, 2). The rapidly changing techniques of analysis and their application to the effects on nontarget organisms were taken into consideration and were discussed in relation to the various vector control programmes and their needs for chemicals as the control agents.

It was recognized that the chlorinated hydrocarbons, especially DDT, have been, and in many cases will remain, the mainstay of many control programmes, although the development of resistance to these has required the use of alternative compounds in some places. Concern for the short- and long-term effects on the environment of persistent compounds used outdoors has also stimulated the use of alternative chemicals.

The needs for chemicals and the manner of their application was briefly reviewed in relation to a range of vectors. The residual application of DDT and compounds such as malathion and propoxur for malaria control represents the largest use of chemicals in public health at present. However, the rapidly increasing need for control of mosquitos in urban areas for control of arbovirus diseases, etc., has resulted in a great increase in the use of larvicides. In view of their extensive use in flowing river water for *Simulium* control, and the use of molluscicides in irrigation schemes much more information is required on the effect of these compounds on nontarget organisms. This in turn means that more information must be obtained on the fate of the chemical after application.

The shortage of food supplies in the world, in particular in certain developing countries, has caused much concern and has led to large-scale schemes for vector control in fertile areas that have become depopulated because of the prevalence of disease. One such programme was discussed in some detail, the 7-country Onchocerciasis Control Programme that is being implemented in West Africa by means of the application of a larvicide to river water to control the breeding sites of the blackfly vector. The large scale of such programmes and their speed of implementation served to stress the importance of developing analytical techniques that would allow progress to be made in an orderly and safe manner.

#### **Hazards to man from exposure to pesticides**

In considering the risks associated with the use of pesticides in vector control programmes, it is essential to bear in mind the benefits to health that may result from effective vector control. Thus, it would be unjustified to limit the use of an effective pesticide because of possible hazards associated with ingestion of residues in food and water or with contamination of the home. The occurrence and severity of toxic effects are related to the dose, i.e., the extent of the exposure. For pesticides this is greatest in the short-term for formulators, mixers, and those applying the chemical. The phased introduction of a pesticide, which is essential for determining its ability to control the vector, also provides an opportunity to seek the earliest evidence of toxic effects among those most heavily exposed.

There has now been enough experience in agriculture and public health work to make a realistic assessment of toxic hazards from the pesticides in

use today for vector control. Acceptable insecticides, for example, the pyrethrins, DDT, and compounds with anticholinesterase activity, all exert their effects by an essentially reversible action on the nervous system. These compounds, with the exception of a few organophosphorus compounds not used in vector control, do not produce structural damage to the nervous system in mammals, so that there is no basis for undue concern about the effects of exposure to low doses. Some effective molluscicides are compounds that interfere with oxidative phosphorylation, a toxic effect involving biochemical disturbances but not causing structural damage. It is improbable that any pesticide dependent for its action on interference with genetic material, for example some chemosterilants, will ever be accepted for use under conditions that might lead to human exposure.

For more than 12 years, pesticides being evaluated for use in public health have undergone a thorough toxicological scrutiny, their safety being assessed concurrently with their efficacy for a particular use. Toxicological assessment begins during the early stages of the WHO Evaluation Programme and many otherwise suitable compounds are rejected at this time for toxicological reasons. Since skin contamination is the most important source of exposure, the dermal toxicity of a compound is frequently the limiting factor in preventing its further development. If a pesticide is to be used under conditions where drinking water might contain residues for a long period of time, special studies are carried out to assess its hazard to the general population. On the basis of data obtained from these toxicological studies, the WHO Expert Committee on Insecticides (2, 3, 4,) has examined the possible hazards of using pesticides in public health practice. In discussing the specific needs for chemical and biochemical methods for the estimation of hazards, the Scientific Group took note of the recommendations of these Committees.

The hazards of any new type of insecticide can be assessed only on the basis of some knowledge of its action and metabolism in mammals. The introduction of a new material into a vector control programme might demand special studies, the nature of which will become clear during the introductory phases. Special attention must be given to the possible hazards of those most heavily exposed.

#### **Importance of chemical and biochemical methods in public health programmes**

The increasing use of pesticides in various public health programmes necessitates increased use and perfection of analytical methods as a means of determining the magnitude of contamination of nontarget materials as well as of controlling the dosage on the pest. Chemical methods usually

measure the amount of the pesticide and/or its degradation or metabolic products while biochemical methods measure the effects caused by the pesticide or its metabolic products after absorption. The Group reviewed methods in both these categories in relation to the use of pesticides in public health vector control programmes and in other programmes that may result in human exposure (except those in which the presence of pesticide residues in food results from normal agriculture practice).

Chemical and biochemical methods play an important role in the early stages of development of a compound for vector control use. At this stage a wide range of methods, mostly laboratory methods, are used to evaluate the efficiency of the candidate pesticide and to assess the potential hazards to nontarget organisms. When a compound passes all the trial stages, including field trials, and is introduced into operational use, there is sometimes a need for simpler methods to monitor regularly the amounts of the compound in the environment or in workers applying the pesticide. Thus, during the introductory trials of a residual insecticide to be applied indoors, the requirement for laboratory methods to monitor exposure is considered. If there is shown to be a need for monitoring, a true field method should be evaluated in a large-scale field trial.

Some analytical methods can be used under field conditions but this depends on the particular equipment, reagents, etc., involved. It is essential continually to reassess methods and to search for modifications that are easily adaptable to field use because of the numerous and sometimes insurmountable problems associated with transport of samples to a laboratory (rapid degradation, bulk of sample, lack of transport, etc.). Such problems are often encountered in developing countries where pesticides are being utilized more and more. In some of these countries the control of pesticide use, from the viewpoint of legislation, administration, and facilities for chemical/biochemical analysis, is very inadequate at present.

The Group considered that the development of field methods would be especially valuable, as many of them could be of great value in developing countries as laboratory methods; there is a need to simplify the instrumentation as well as the procedures themselves. The development of simpler methods would permit the existing multipurpose government laboratories to carry out rapid and reliable analyses. It was considered desirable that these simplified methods for field use and for monitoring should be scaled-down versions of the standard laboratory methods.

## DETERMINATION OF EXPOSURE AND ABSORPTION OF PESTICIDES IN MAN

### Measurement of inhalation and dermal exposure

Measurement of inhalation or dermal exposure of pesticides can be made by direct methods. Such methods are not intended to reflect absorption; however, they do indicate the amount of pesticide that is potentially available for absorption. This is accomplished by measuring the amount of pesticide that may impinge on exposed skin areas or the amount calculated to be available for inhalation exposure.

The Group considered that for research purposes, there is a need to evaluate the inhalation exposure of workers applying relatively toxic compounds by use of operator-carried ultra-low-volume (ULV) machines. This is especially important during indoor applications where exposure may be relatively high. Inhalation exposure during indoor spraying has usually been calculated by using the values for ambient air concentration obtained using conventional air sampling equipment. However, these estimates are open to question as the distribution of the pesticide in the air under such conditions is not uniform. The direct measurement of exposure using a respirator filter pad (5) allows the worker himself to provide the air flow during respiration. This technique could be used, suitably modified if necessary, to estimate inhalation exposure more accurately under field conditions when new relatively toxic compounds are being tested.

In this technique, inhalation exposure is estimated from the contamination of the special respirator filter pads. These are covered with inverted plastic funnels, modified to a specific aperture size, to reproduce as nearly as possible the pattern of air flow through the nostrils. The funnels also prevent direct impingement of droplets or particles on to the pads except for those carried through the aperture by respiratory action. Even though the respirator pads tend to retain some vapour as well as particulate material, the technique is, of course, less efficient where highly volatile compounds are involved. Chemical analysis of the respirator pads can be used to calculate the potential inhalation exposure of workers who do not wear respirators.

There may be situations where there is need to compare inhalation with dermal exposure. In such cases the direct methods for measurement of dermal exposure (5) may be used. The technique involves attaching absorbent pads made of alpha-cellulose or filter paper at different locations on the worker's skin or clothing and collecting washings from the hands using a suitable solvent such as ethanol. The amount of pesticide found on the dermal pads or in the washings indicates the amount of pesticide accumu-

lated on exposed skin areas during a specific period of work. The amount of pesticide that comes into contact with unprotected skin areas is then calculated in milligrams per hour by calculating the total surface area of the unclothed body parts according to published values (6) for skin surface area.

When determinations are carried out on both respiratory and dermal exposure as outlined above, the percentage of the toxic dose to which the operator is exposed can be estimated using the formula :

$$\frac{\text{dermal exposure (mg/h)} + [\text{respiratory exposure (mg/h)}] \times 10}{[\text{dermal LD}_{50} \text{ (mg/kg)}] \times 70} \times 100$$

Where highly volatile compounds are used, the respirator and dermal pad methods are not considered useful as an accurate direct measurement of exposure. If estimation of inhalation exposure is required and impinger-type air samplers are used to estimate levels of pesticide in air near the indoor application activity, the volume of air inspired by a worker during a timed period needs to be accurately determined for the particular work situation. The published values for tidal air volume at different levels of physical activity are difficult to relate to spraying operations in the field.

#### **Chemical analysis of biological materials for pesticides and metabolites**

In some circumstances, the degree of exposure of man to a pesticide may be evaluated by measuring the concentration of the parent compound and/or its biotransformation products in biological materials such as blood, urine, adipose tissue, etc. When such methods are feasible, they may offer advantages over environmental monitoring : (1) the total amount of the chemical absorbed by all the routes of exposure can be estimated for a particular time interval that usually depends on the biological half-life of the compound ; (2) variations in rates of absorption of different compounds may be determined ; (3) less stringent storage conditions are required for the biological samples than with biochemical (enzyme) methods of analysis ; and (4) the direct methods can even provide a means of detecting excessive exposure at an early stage, i.e., before the occurrence of clinical signs of intoxication or before any biochemical responses are measurable.

The selection of specific methods for analysis of biological materials for early detection of excessive exposure to a pesticide depends upon several factors :

1. Basic information must be available concerning the distribution, biotransformation, rate of excretion, etc., of the pesticide in the human body. For example, with urine samples the rate of urinary excretion of the metab-

olite during and after exposure should be determined in order to select the best time for sample collection. In fact, under field conditions it is usually impracticable to collect urine at well defined intervals and the analysis must usually be performed on spot samples. Furthermore, it has sometimes been found useful, when measuring the level of a urinary metabolite in a spot sample, to correct for the degree of urine dilution either by expressing the results in relation to the level of urinary creatinine or by reporting the results with reference to a particular specific gravity. The latter can be easily determined under field conditions. It should also be stressed that direct collection of urine samples by a technician is preferable to collection by the worker himself.

2. The relationship between the degree of human total exposure and the concentration of the pesticide or its metabolite(s) in the biological materials analysed must be known, mainly for exposures below the maximum tolerated ; hence a meaningful biological threshold limit value can be proposed above which removal from further exposure is indicated.

3. Field methods are only of value for pesticides and/or their metabolite(s) that may be estimated by a quick, and relatively simple analytical technique.

4. The compound analysed should be sufficiently stable in the biological medium to allow the sample to be stored for a certain period of time. It is very important to investigate the effects of methods of sample collection and storage conditions. For example, methods of controlling possible absorption on storage-container walls and the effects of different preservatives on loss of metabolite(s) due to pH changes or bacterial action during storage must be evaluated. No general rules can be formulated for the conservation of biological samples as the method will depend on the metabolite to be analysed and the technique to be used for its determination.

#### **Specific applications**

The Group discussed the analysis of several compounds, in particular from the viewpoint of public health work.

##### *Chlorinated hydrocarbons*

*DDT*. Gas-liquid chromatographic methods utilizing an electron-capture detector are available for the analysis of *DDT* and its metabolites in human blood or tissues. Body fat is the most important storage tissue for most of the chlorinated hydrocarbons and several comparisons have been made

in humans between the levels of chlorinated hydrocarbons in fat and in blood. These studies show that there is a relationship between the levels of DDT and DDE in the adipose lipids and in serum and that the concentration of DDT in fat is very roughly about 300 times that in serum. Thus the available data indicate that plasma levels of DDT and DDE can be used as indicators of exposure to DDT. The relationship between the plasma levels of DDT and DDE can indicate whether the exposure was recent or chronic: in cases of recent exposure DDT predominates, while in cases of chronic exposure the level of DDE usually considerably exceeds that of DDT.

A newly developed method (7) that uses heptachlorepoxyde as an internal standard has a sensitivity of 1 ng/ml for *p,p'*-DDE and 3 ng/ml for *p,p'*-DDT. (The sample size needed is 1 ml of plasma). These levels are below those of the general population in most countries.

DDT in man is partly metabolized to DDA and the excretion of DDA in urine can be estimated and used as an indirect measurement of acute exposure; for this purpose colorimetric and gas-liquid chromatographic methods are available (8, 9).

*Lindane.* From human and animal data it is indicated that plasma levels of lindane reflect the extent of exposure. Lindane is a compound with a comparatively rapid turnover in man and analysis of blood levels would therefore only indicate fairly recent exposure. The method mentioned above for DDT also detects lindane, with a sensitivity of 0.3 ng/ml of plasma.

*Other chlorinated hydrocarbons.* Thin-layer chromatographic techniques are available for the analysis of several chlorinated hydrocarbons (10) but the sensitivity is limited to 100 ng/g of sample with blood or tissue samples. This is a serious limitation in relation to determination of exposure in man. For most chlorinated hydrocarbons, gas-liquid chromatographic methods are available. However, there is a lack of simple and sensitive methods suitable for field work or routine laboratory work.

#### *Organophosphorus compounds*

Most of the organophosphorus compounds have a very rapid turnover in man and thus the analysis of blood or tissue for a certain pesticide or its metabolites is of little value. However, exposure to various organophosphorus compounds produces an increase in the amount of ether-extractable organic phosphorus excreted (11).

*Parathion, parathion-methyl, and EPN.*<sup>a</sup> The determination of urinary 4-nitrophenol excretion has been demonstrated to be a reliable index of parathion, parathion-methyl, and EPN exposure (12-17) and is even more sensitive as an absorption index than determination of blood cholinesterases. This metabolite can also be readily measured by a colorimetric, TLC, or gas-liquid chromatography procedure (13, 18, 19).

*Abate.*<sup>b</sup> The thiodiphenol, a urinary metabolite of the pesticide Abate, is amenable to analysis.

*Fenitrothion.* 4-nitro-3-methylphenol is a urinary metabolite of fenitrothion that can be easily measured.

*Dicaphon.*<sup>c</sup> The urinary concentration of 2-chloro-4-nitrophenol is a potential indicator of exposure to this compound.

*Fenchlorphos.* The main hydrolysis degradation product of fenchlorphos, 2,4,5-trichlorophenol, is amenable to analysis in the urine of workers exposed to this pesticide.

*Dursban.*<sup>d</sup> The urinary metabolite, 3,5,6-trichloro-2-pyridinol, is a potential compound for evaluating exposure to Dursban.

#### *Carbamates*

*Carbaryl.* The estimation of free and conjugated 1-naphthenol excreted in the urine has been used to measure exposure of workers to carbaryl. This metabolite is measured colorimetrically or by a gas-liquid chromatographic technique (20).

*Propoxur.* The main urinary metabolite of propoxur, 2-isopropoxyphenol, can be measured satisfactorily by a colorimetric or gas-liquid chromatographic method (21).

#### *Others*

*Pentachlorophenol.* This compound can be measured by a gas-liquid chromatographic technique using a flame-ionization or an electron-capture detector (22, 23).

<sup>a</sup> *O*-ethyl *O*-(4-nitrophenyl) phenyl phosphonothioate.

<sup>b</sup> *O,O'*-(thiodi-4,1-phenylene)-*O,O',O'*-tetramethyl phosphorothioate.

<sup>c</sup> *O*-(2-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate.

<sup>d</sup> *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate.

*DNOC*. In man, a significant proportion of absorbed *DNOC* appears in the urine as 4-amino-2-methyl-6-nitrophenol. The measurement of this metabolite may constitute the basis for a test of exposure.

### **Biochemical analysis**

Biochemical assays can be used to evaluate the degree of absorption of a pesticide in man and thus are indirect methods of assessing the history of exposure to a compound. If the biochemical assay is performed on the target organ or enzyme, it will serve to establish the degree of hazard produced by a given pesticide. In the ideal case, the assay will reveal the absorption of a toxic compound before a severe lesion has been produced. The selection of an assay method to meet such complex requirements can be made only when the mechanism of action of the pesticide is known. As this is seldom the case, more basic research is needed on the mode of action of pesticides.

Organophosphorus compounds and carbamates are inhibitors of many hydrolytic enzymes, but their toxic effects are primarily due to inhibition of tissue cholinesterases. The assay of human blood cholinesterase, the most sensitive index of absorption, is easily performed and this assay has therefore been widely and successfully used for monitoring persons exposed to anticholinesterase pesticides.

The WHO Expert Committee on Insecticides has critically evaluated the various methods for determining cholinesterase activity: those methods suitable for field or simple laboratory conditions have been described in detail, indicating the advantages and disadvantages of each method (2,4). There are today two field methods available, one is the Acholest method (24-26) for human plasma cholinesterase and the other is a colorimetric method (27) for human whole blood cholinesterase. In both methods, the time of assay is relatively long and it has been observed that with some anticholinesterases, spontaneous reactivation of the inhibited enzyme occurs during assay. This has revealed the need for a more suitable method that could be used in both the laboratory and the field. The Scientific Group discussed the development of an adequate field method and some features of this method are reviewed in the following section.

Chlorinated hydrocarbons are known to induce liver microsomal enzymes. This biochemical effect can be indirectly tested by means of a drug metabolism test, for example, the determination of the plasma half-life of a suitable drug.

A moderate increase in activity of the microsomal enzymes of the liver has been demonstrated in men subject to occupational exposure to

lindane (28) and in other men exposed to DDT (29). The reported changes have been found not to affect the subjects clinically but may be regarded as early biological responses to exposure even to rather low levels of organochlorine compounds.

The use of drug metabolism tests as indicators of the liver microsomal enzyme activity seems to be of limited value as a general approach in monitoring exposure but would be of considerable interest for studies on limited groups of people to determine the effects of intensive exposure to certain organochlorine compounds.

### **Methods for determining the activity of cholinesterases in human blood**

#### *Sampling and storage of blood*

Amounts of blood obtained from finger pricks are sufficient for determining cholinesterase activity. It is preferable to assay the enzyme immediately after sampling as storage can result in changes in enzyme activity. As this is not always possible, more studies are required on adequate procedures for storage.

#### *Enzyme assay*

Many methods are available for determining the activity of acetylcholinesterase and cholinesterase (30). All these methods are applicable to cholinesterase in human blood when measured under laboratory conditions. However, if assays are performed in poorly equipped laboratories or under field conditions there is a need for simple and reliable procedures. The Expert Committee on Insecticides (2) suggested that the spectrophotometric method of Ellman et al. (31) would be suitable as a field method after appropriate modification.

Under laboratory conditions, this method is simple to perform, gives reliable results, and is considered as a reference method. By converting it into a field method it becomes possible directly to compare enzyme activities measured in the laboratory and in the field. The time of assay is short, 1-2 minutes, and thus spontaneous reactivation during the assay is negligible for all anticholinesterases that are currently in use as pesticides. It is equally suitable for determination of erythrocyte acetylcholinesterase and serum or plasma cholinesterase. The choice of enzyme to be measured will depend on which is more inhibited by a given compound. Thus using the same method for both enzymes makes it easier to organize an assay.

When the enzyme assay is performed without constant temperature conditions, it is necessary to use temperature conversion tables to compare activities determined at different temperatures (32).

The Group was informed that work is underway to convert this method into a field method, the field kit containing suitably packed and bottled reagents and a reliable, simple to operate spectrophotometer.

#### **Drug metabolism test for microsomal enzyme induction**

Liver microsomal enzyme activity is measurable by two main procedures, determination of the half-life of either phenylbutazone or phenazone in plasma. The method using phenazone is to be preferred because there is a smaller risk of adverse reactions, it is very soluble in water, it has low protein-binding ability, the plasma samples may be stored for several days in the refrigerator, and the sampling time is shorter.

The level of phenazone in plasma is measured by spectrophotometric (33) or gas-liquid chromatographic (34) methods.

### **DETERMINATION OF PESTICIDES AND DEGRADATION PRODUCTS IN CERTAIN ENVIRONMENT MATERIALS**

Analysis of samples for pesticide residues in a variety of media including human tissues can yield valuable data on the distribution, persistence, and possible harmful effects of a given pest control programme on man and his environment. The mode of application and type of pesticide applied should be studied prior to launching a programme of environmental monitoring. For example, in a malaria control programme where spraying is confined to the inside walls of houses, it could be assumed that the residual pesticide would be confined to dwellings and that sampling of other areas would be useless. In such a programme, the monitoring of blood or urine of the spraymen and general population would be more valuable. On the other hand, in operations where pesticides are sprayed over land areas, as in the case of ULV application, or are applied to water systems, as in larviciding or mollusciciding operations, monitoring of the water, mud, and fish is recommended.

#### **Sampling and storage**

Recent advances in man's knowledge of the hazards of pesticides as environmental pollutants have made all users aware of the responsibility to minimize the impact of their programmes on the environment. With the

possible exception of indoor spraying, all large-scale vector control programmes should be accompanied by a programme of chemical monitoring to determine the concentration and distribution of a given pesticide in the environment. Sampling techniques will vary depending upon the type of pesticide used and the mode of application. The more important elements of the environment to be sampled and analysed include air, water, soil, mud, and fish. The type of sample taken for analysis will depend upon the programme. For example, samples of air and static bodies of water would be appropriate for programmes involving ULV pesticide applications, whereas samples of water, mud, and fish should be taken to determine the extent of contamination caused by a larviciding programme.

In view of the low levels of pesticides, or their degradation products, likely to be found in environmental samples, it is probable that the samples will have to be analysed in a well equipped analytical laboratory rather than in the field. In many cases this will cause serious problems owing to the long distances over which the samples will have to be transported. This is further complicated by the fact that it may not be practical to freeze or, in some cases, even to cool the samples. This is not so important in the case of chlorinated hydrocarbons because of their stability. On the other hand, problems may be encountered in storage and shipment of the less stable organophosphorus compounds and carbamates. In cases where degradation of the parent compound cannot be prevented, there is always the possibility of analysis of the degradation products. Such data would be useful, although it would be more desirable to have a quantitative measurement of the original compound.

In some cases, special techniques have been developed that make it possible to transport environmental samples without decomposition. A procedure for the shipment of water samples contaminated with malathion from ULV operations has been reported (35). In this case, hydrolysis is prevented by the addition of an acid buffer.

A technique for field extraction of water samples contaminated with Abate from larviciding operations has been developed (36). In this method decomposition is averted by shipment to a central laboratory in an organic solvent. Methods for analysis of fish and mud for Abate have been developed. However, preservation techniques for field use at present allow storage for only about two weeks (Miles et al., unpublished data). Methods for storage and transport of environmental samples contaminated with the *Simulium* larvicides Dursban-methyl, fenitrothion, phoxim, and chlorphoxim have not been reported.

## Specific applications

### Control of larvae

The use of chemicals for the control of larval forms of insects has increased in recent years. Larvicidal compounds have been applied to static and moving water for the control of *Culex* and *Aedes* larvae, as well as *Simulium* larvae. Attention has been drawn to the latter in view of the Onchocerciasis Control Programme that is now in progress in Africa. Chemical monitoring of the environment will be required as larvicidal chemicals are introduced into the rivers and streams.

A number of methods for analysis of Abate in water have been reported. The most recent method (36) includes field extraction and gas-liquid chromatographic determination. The method is sensitive to 0.03 µg/litre of water and appears to be quite satisfactory for monitoring purposes. Methods for analysis of fish have been developed that use a colorimetric technique to measure the hydrolysis product of Abate (37) or a gas-chromatographic technique to measure the Abate directly (38). In the latter method the oxidation product, Abate sulfoxide, is reduced to Abate and then measured by GLC. Another method now being developed determines both Abate and Abate sulfoxide directly by GLC (Miles et al., unpublished data).

Methods for analysis of Abate in mud have been developed (37-39, and Miles et al., unpublished). Two of these methods (38 and Miles et al., unpublished) are based on gas-liquid chromatography and are suitable for use in monitoring environmental samples.

Methods for the determination of Dursban in water, fish, and mud have been published and these should be satisfactory for the determination of Dursban-methyl in the same substrates. No data are available for the storage and transport of these samples.

No procedures for determination of phoxim and chlorphoxim in water, fish, or mud have been reported. Research on extraction, clean-up, and analysis of these compounds is needed. Data on storage of samples containing residues of phoxim and chlorphoxim are also needed.

Methods for determination of fenthion in animal tissues have been reported; however, research will be needed to adapt these to fish tissues. Work on analysis of water and mud samples is also needed. No work on the stability of fenthion residues in storage has been reported.

### Snail control

Snail control trials and programmes have been carried out based on the use of niclosamide and trifenmorph. Besides these, well-known com-

compounds, such as copper(3+) sulfate, triphenyltin acetate and Zectran<sup>a</sup> are still in use in some situations. A new compound, yurimin,<sup>b</sup> is under study and new formulations, such as slow-release rubber mixtures with organic tin compounds, are being considered. Application dosages are of the order of magnitude of 0.5 mg/litre.

Methods for determining these compounds at this level are available. They are based on the development of a colour reaction and are applicable both in the field and in the laboratory.

#### *Niclosamide*

*Field method.* Colorimetric methods based on the reaction of niclosamide with safranin-TH at pH 9.0–9.5 have been published (40, 41). The limit of detection using a comparator is 0.2 mg/litre.

*Laboratory method.* Two colorimetric methods, based on the reduction of the nitro-group to an amino-group to give a diamine have been developed (42). In one the diamine is diazotated and coupled with 1-naphthalenylethylene diamine to produce a blue colour and in the other, the diamine is reacted with Ehrlich reagent to give a yellow colour. A similar method has been developed in which the diamine is coupled with 1-naphthol in alkaline media (43). The limit of detection is 0.02 mg/litre.

The above methods have been used for the analysis of treated water. If niclosamide is to be determined in other environmental materials, more accurate methods should be sought.

#### *Trifenmorph*

A colorimetric method based on extraction with an organic solvent and reaction with sulfuric acid to produce yellow coloration has been developed (44).

*Field method.* Measurement of the yellow coloration with a comparator is possible with a limit of detection of 0.1 mg/litre.

*Laboratory method.* Measurement of the yellow coloration with a spectrophotometer gives a limit of detection of 0.01 mg/litre.

These methods have been used for the determination of trifenmorph in water, soil, and plants. A gas-liquid chromatography method has been developed for the determination of trifenmorph as well as the metabolite triphenylcarbinol in crops and soils (45). This involves conversion to the trichloroethyl ether derivative and gives a sensitivity of 0.01 µg/g.

<sup>a</sup> 4-(dimethylamino)-3,5-dimethylphenyl methylcarbamate.

<sup>b</sup> 2,6-dibromo-4-[(4-nitrophenyl)azo] phenol.

### *Zectran*<sup>a</sup>

A colorimetric method of analysis for Zectran has been developed based on extraction of the compound with methanol and saponification with potassium hydroxide to produce the coloured phenylate ion (46).

### **Control of rodents**

The normal use of recommended (2) rodenticides presents little hazard to the general population, but there is a possibility of accidental exposure of children through consumption of treated baits, or of the general population through consumption of accidentally contaminated foodstuffs. There is also a danger of exposure of operators handling equipment or preparing baits.

Some rodenticides such as sodium fluoracetate, calcium cyanide, strychnine and thallium sulfate are extremely toxic for humans and should be used only by trained operators with a full knowledge of the hazards. Other rodenticides, such as the anticoagulants, present little hazard to the general population.

Analytical methods are available for most rodenticides and are adequate for analysis in cases of accidental poisoning. The Group did not consider that monitoring on a larger scale is necessary.

## **ANALYTICAL METHODS**

### **Spectrophotometric**

Spectrophotometers capable of operation in the visible range of the spectrum and filter photometers are now considered to be standard items of equipment for most laboratories. Some instruments are battery operated and may be used in the simplest field laboratory. Although photometric methods lack the specificity of GLC methods, they can be used for monitoring pesticide residues or metabolites in many cases. Some examples are the determination of 4-nitrophenol in the urine of persons exposed to parathion or the determination of 2-isopropoxyphenol in the urine of persons exposed to propoxur. Spectrophotometric methods for analysis of residues of many pesticides in water or other environmental samples are available and may be used for monitoring if more sophisticated equipment is not available. Compounds for which spectrophotometric methods have been

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<sup>a</sup> 4-(dimethylamino)-3,5-dimethylphenyl methylcarbamate.

developed include malathion, parathion, Abate, propoxur, fenitrothion, and many others.

#### **Thin-layer chromatography**

The development of thin-layer chromatography (TLC) has provided the chemist with a useful tool for separation and semi-quantitative analysis of many pesticides and their metabolites. Reagents are available that, when sprayed on a developed chromatogram, make possible the detection of less than 1 microgram of many compounds. Equipment for the preparation and development of TLC plates is simple and can be used in field laboratories. Environmental samples may be extracted and spotted on to thin-layer plates with a minimum of clean-up. Development of chromatograms is simple and, with the proper selection of reagents, identification of the spots is not difficult.

The Group noted that commercially prepared thin-layer chromatographic plates are available and that these could be used to carry out TLC analytical procedures in the field.

#### **Gas-liquid chromatography**

At the present stage of development, gas-liquid chromatography instruments cannot be considered for use in the field but it was the opinion of the Group that this technique should be considered for routine use by laboratories in developing countries. Methods have been developed for the majority of pesticides in use. It is obvious that the use of identical analytical methods in field and research laboratories offers several advantages. The technique is usually more specific than the colorimetric technique and gives the possibility of simultaneous determination of several compounds. The use of internal standards allows manipulation of the samples with less meticulous attention than is required for other methods, since the parameter measured is a ratio of the compound to the internal standard and not their absolute values. The use of specific detectors, such as flame-thermionic detectors for phosphorus compounds, allows the clean-up procedures to be considerably simplified.

It was noted that gas-liquid chromatography can be used for the analysis of other compounds, such as drugs and toxicants, that might need to be carried out in the same laboratory as pesticide analysis in a developing country. It was also noted that a simple change of the detector to a catharometer would allow analysis of high concentrations of compounds such as are found in formulations. The use of this technique was considered necessary to analyse technical products and formulations for contaminants or impurities.

The Scientific Group recognized, however, that several practical problems have to be solved before this technique can be used widely in developing countries. The lack of pure reagents and suitable column packings also presents a problem. However, it should be stressed that the lack of availability of a specified column does not necessarily exclude the determination being made with other available columns.

A gas-liquid chromatograph combined with a mass-spectrometer functioning as the detector permits direct identification of compounds. This technique is called "mass fragmentography". The spectrometer can be focused on certain typical mass numbers for a given compound and these are then monitored and recorded (47, 48). Mass fragmentography is one of the more sensitive detection systems and can be applied to virtually any compound that can be determined by gas-liquid chromatography and that has a suitable fragmentation pattern. Both magnetic and quadrupole mass spectrometers have been used. Mass fragmentography certainly cannot be used in routine laboratory work but it appears that it will be used on an increasing scale in the future for checking other procedures and in the development of new and simpler methods for chemical analysis.

## RECOMMENDATIONS

### Research items

#### Environment samples

In recent years there have been more programmes in which pesticides are applied directly to static and flowing water to control snails and the larval stages of insects. The Group noted the increasing need for measurement of the fate and distribution of these pesticides in the aquatic environment and recommended that work on the development of analytical methodology applicable under these conditions be continued and broadened. The following specific needs were noted :

1. Analytical methods for small quantities of insecticides and their degradation products in mud, water, fish, and other associated materials.
2. Methods for sampling and preservation of samples of mud, fish, and other biological material to prevent deterioration of the chemical compounds before analysis can be undertaken in an analytical laboratory.
3. Analytical methods for new types of materials, such as insect growth inhibitors, organo-tin compounds, and plant products (e.g., *Phytolacca*

*dodecandra*) used as molluscicides, etc., as they are developed or are used in programmes.

#### **Mechanism of action of pesticides**

The Group recommends that WHO should promote research on the metabolism and degradation of pesticides with special reference to new insecticides, in order to elucidate the relationships between absorption, metabolism, and excretion of the parent compound and its degradation products in mammals.

#### **Cholinesterase determination**

The Group noted with commendation the progress being made in the development of a method and kit suitable for use in the field for measurement of cholinesterase in blood samples. It was noted that the method under development yields results equivalent to the laboratory method and thus the field and laboratory results would be directly comparable. In reviewing this work the Group considered that more research is needed on the methods of storage of the blood samples to allow preservation without deterioration of the enzymes.

#### **Simple analytical techniques**

It was recommended that WHO should encourage the development of simple reliable methods of analysis of pesticides for use in developing countries, or in the field, that would allow accurate identification of the compounds and estimation of the amounts present. These methods could be based on currently available food residue or other methods but with less expensive or less complicated apparatus. Simple detector devices for determining the presence of pesticides in air and water should also be considered.

#### **Fields trials and new application techniques**

Whenever trials of new pesticides are undertaken, or when new methods of application such as ULV are initiated, a programme to monitor the blood or urine of exposed persons should be established. Existing techniques for assessment of exposure to pesticides by inhalation or dermal absorption should also be used. In the case of experimental work with ULV application indoors, existing techniques should be modified to measure exposure accurately under those conditions.

## Information

The Group noted that information on reliable methods and equipment suitable for use by laboratories in developing countries was often difficult to find. It was recommended that WHO should promote a better exchange of information on pesticide analytical methodology and in particular, on those methods that are suitable for use in developing countries and in the field where facilities are limited. Such information could include techniques using GLC, TLC, and spectrophotometric equipment when these were suitable for use under such conditions.

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