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Journal of Chromatography B, 769 (2002) 191–219

JOURNAL OF CHROMATOGRAPHY B

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Review

# Biological monitoring of pesticide exposure: a review of analytical methods

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Received 30 July 2001; received in revised form 15 January 2002; accepted 15 January 2002

**Abstract**

A wide range of studies concerned with analytical methods for biological monitoring of exposure to pesticides is reviewed. All phases of analytical procedures are assessed, including sampling and storage, sample preparation and analysis, and validation of methods. Most of the studies aimed at measuring metabolites or unchanged compounds in urine and/or blood as biological indicators of exposure or dose. Biological indicators of effect, such as cholinesterase, are also evaluated. The principal groups of pesticides are considered: organophosphorus pesticides, carbamate pesticides, organochlorine pesticides, pyrethroid pesticides, herbicides, fungicides and other compounds. Choice of the method for biological monitoring of exposure depends on the study population: a detection limit of 1 μg/l or less is required for the general population; higher values are adequate for occupationally exposed subjects. Interpretation of results is also discussed. Since biological indices of exposure are only available for a few compounds, biological reference values, established for the general population, may be used for comparison with levels of professionally exposed subjects. © 2002 Published by Elsevier Science B.V.

*Keywords:* Reviews; Biological monitoring; Pesticides

## Contents

1. Introduction ..... 192
2. Analytical methods ..... 193
3. Insecticides ..... 195
   3.1. Organophosphorus pesticides (OPs) ..... 195
      3.1.1. Serum and erythrocyte cholinesterase activity ..... 195
      3.1.2. Unchanged compounds ..... 195

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1570-0232/02/$ – see front matter 
PII: S1570-0232(02)00044-2

3.1.2.1. Acephate and methamidophos ... 195
3.1.3. Metabolites ... 196
3.1.3.1. Alkylphosphates ... 196
3.1.3.2. 3,5,6-Trichloro-2-pyridinol (TCP) ... 197
3.1.3.3. *p*-Nitrophenol (PNP) ... 199
3.1.3.4. 3-Methyl-4-nitrophenol (MNP) ... 199
3.1.3.5. Malathion α-monocarboxylic acid (MCA) and malathion dicarboxylic acid (DCA) ... 200
3.2. Carbamate insecticides ... 200
3.2.1. Serum cholinesterase and erythrocyte activity ... 200
3.2.2. Unchanged compounds ... 200
3.2.3. Metabolites ... 201
3.2.3.1. Benomyl metabolites ... 201
3.2.3.2. 1-Naphthol (1NAP) and carbaryl ... 201
3.2.3.3. 2-Isopropoxyphenol (IPP) ... 202
3.2.3.4. Carbofuranphenol (CFP, 2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran) ... 202
3.3. Organochlorine compounds (OCs) ... 203
3.3.1. Unchanged compounds and metabolites ... 203
3.3.1.1. Chlorobenzylate and dicofol ... 203
3.4. Pyrethroids (PYRs) ... 203
3.4.1. Unchanged compounds ... 203
3.4.2. Metabolites ... 204
3.4.2.1. 3-Phenoxybenzoic acid (3-PBA), 3-(4-hydroxy)-phenoxybenzoic acid (4OH-3PBA), 3-(2,2-dichlorovinyl)-2.2-dimethyl cyclopropane acid (DCVA), 3-(2.2-dibromovinyl)-2.2-dimethyl cyclopropane acid (DBVA), 2-(4-chlorophenyl)-3-methyl-1 butanoic acid (CPBA), 4-fluoro-3-phenoxybenzoic acid (F-PBA) ... 204
4. Herbicides ... 205
4.1. Unchanged compounds and metabolites ... 205
4.1.1. 2,4-Dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), pichloram, mecoprop, dichlorprop ... 205
4.1.2. Glyphosate ... 208
4.1.3. 2,6-Diethylaniline (DEA) and 2-(1-hydroxyethyl)-6-ethylanyline (HEEA) ... 208
4.1.4. Diquat and paraquat ... 208
5. Fungicides ... 208
5.1. Dithiocarbamate pesticides (DTC) ... 208
5.1.1. Unchanged compounds and metabolites ... 208
5.1.1.1. Carbon disulfide ($CS_2$) ... 208
5.1.1.2. 2-Thiazolidinethione-4-carboxylic acid (TTCA) ... 209
5.1.1.3. Ethylenethiourea (imidazolidin-2-thione, ETU) ... 211
6. Other pesticides ... 212
6.1. Unchanged compounds and metabolites ... 212
6.1.1. Tetrahydrophthalimide (THPI) (captan metabolite) ... 212
6.1.2. 4-Chloro-*o*-toluidine (CT) (chlordimeform metabolite) ... 212
6.1.2.1. Pentachlorophenol (PCP) ... 213
6.1.2.2. Dinitro-*o*-cresol (DNOC) ... 214
6.1.2.3. Chlorotriazine ... 214
7. Sampling and storage ... 215
7.1. Blood ... 215
7.2. Urine ... 215
8. Interpretation of the results ... 216
References ... 216

## 1. Introduction

Pesticides are chemicals manufactured specifically to be toxic to living species and are released deliberately into the environment. More than one thousand active ingredients are produced and used worldwide by millions of industrial, agricultural and public health workers. A large segment of the human population may therefore be exposed to these chemicals in the general environment or work place. Since

pesticides may be not entirely specific for their target organisms, their ubiquitous presence in the environment may endanger other living species, including man.

Knowledge of exposure levels is a first step in the risk-evaluation process, and can be acquired by measuring the dose entering the body. This is usually done by biological monitoring. In cases where exposure fluctuates in time, and/or the skin is a significant route of absorption, biological monitoring has proven to be reliable for obtaining information on absorbed dose.

Biological indicators currently available for monitoring pesticide exposure in man can be divided into three main groups: indicators of dose or exposure, indicators of effect and indicators of effective dose.

A 'biological indicator of dose' means the measurement of chemical agents (or their metabolites) either in tissues, secreta, excreta, exhaled air, or any combination of them in order to evaluate exposure and health risk and compare them with an appropriate reference [1].

In some cases it is possible to measure early changes caused by exposure. If these changes are 'non adverse' and reversible, and if a dose–effect relationship is known, these changes can be used as biological indicators of effect.

In other cases it is possible to measure the binding product of the chemical or its metabolites to specific cell receptors. These indicators are the so-called 'biological indicators of effective dose'.

Despite the importance of this problem, very few published studies have been concerned with biological monitoring of pesticide exposure. Here we review original papers on biological monitoring published since 1973 and listed in Medline excluding reviews and guidance documents. Interestingly, the number of studies aimed at measuring biological indicators of dose, biological indicators of effect, and biological indicators of effective dose varies over time: at first most regarded biological indicators of exposure, whereas more recently a significant proportion of studies have regarded indicators of effect or active dose (Fig. 1).

Unfortunately, biological monitoring of pesticide exposure is not usually carried out in routine field activities. The reasons for this are, briefly, as follows:

1. Analytical methods currently available are often very complicated (laborious preparation of samples, chromatographic analysis with mass spectrometry detection) and require highly specialized laboratories.
2. Pure standards for measuring metabolites are not commercially available.
3. There are few completely validated methods suggested by reference organisations.
4. In field studies on pesticide exposure it is difficult collect representative samples and to define a correct sampling time.
5. Permissible exposure limits and biological exposure indexes are only available for a limited group of compounds. This lack is partially compensated by the efforts which have gone into defining reference values (i.e. concentrations of indicator(s) in the general 'unexposed' population). Unfortunately, these values only enable definition of the degree of exposure, but do not provide the information necessary for estimating the risk to health.

Based on the above considerations, we review the publications on biological monitoring, looking at analytical methods and practical aspects, such as sampling times, methods for storage and conservation of samples, special containers, etc. The availability of reference values and biological exposure indexes for various compounds is also mentioned.

## 2. Analytical methods

Biological indicators can usually be measured by different analytical methods. The choice of method should be based on the objective. Limits of detection (LODs) of analyses, for example, vary widely. For monitoring the general population, LOD must be 1 μg/l or less; higher LODs may be adequate for monitoring occupationally exposed workers, and LODs of the order of 100 μg/l may be adequate for acute poisoning cases. Accuracy and precision of analytical methods should be established first. For complex analytical methods, an admissible relative standard variation (RSD) is usually more than 10%; for methods involving a simple preparation procedure, the RSD is of the order of 5–10%.

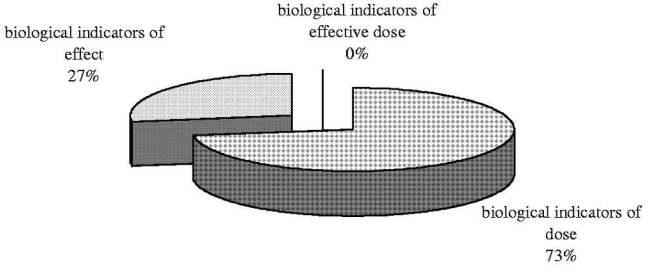


**Fig. 1a Literature data from 1972 to 1982**

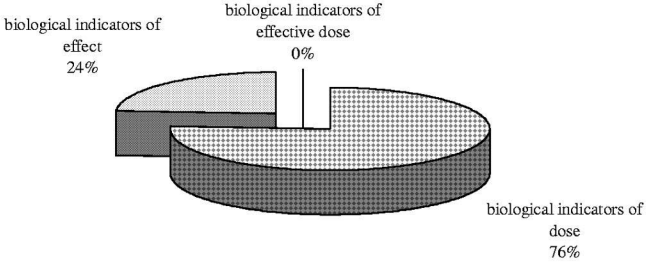


**Fig. 1b Literature data from 1983 to 1992**

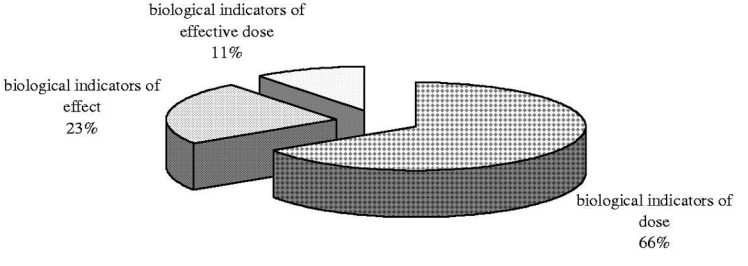


**Fig. 1c Literature data from 1993 to 2000**

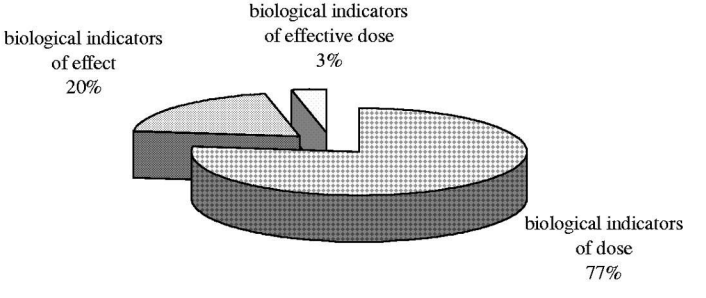


**Fig. 1d Literature data from 1972 to 2000**

Fig. 1. Literature from (a) 1972–1982, (b) 1983–1992, (c) 1993–2000 and (d) 1972–2000.

## 3. Insecticides

### 3.1. Organophosphorus pesticides (OPs)

#### 3.1.1. Serum and erythrocyte cholinesterase activity

OPs act through inhibition of acetylcholinesterase activity in the central nervous system. This enzyme is essential for acetylcholine hydrolysis and transmission of nerve impulses and its activity is closely correlated with that measured in red blood cells (AChE) and to a lesser extent with pseudocholinesterase or plasma cholinesterase (BuChe) activity. This is why the determination of AChE or BuChe activity can be used for biological monitoring of exposure to pesticides acting through cholinesterase inhibition.

Methods for the detection of cholinesterase activity can be divided into four groups: electrometric [2] and colorimetric [3], which are used in field surveillance and research in developed countries; titrimetric [4] and tintometric [5], mainly used in developing countries. Titrimetry is extremely accurate and precise but is rarely used because of its high cost and complexity. Electrometric method is less sensitive than colorimetric method and less accurate than tirimetric methods. Tintometric method is mainly used in the field.

The colorimetric method developed by Ellman et al. [3] measures cholinesterase activity on substrates such as acetylcholine and butyrylthiocholine. Thiocholine released through hydrolysis reacts with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) to form a yellow anion (5-thio-2-nitrobenzoate), which is measured by spectrophotometric analysis at 405 nm. This method (available in two kits, for laboratory and field use) has been evaluated by various authors. The laboratory kit showed a RSD of 3.8 (precision between series) in detecting erythrocyte cholinesterase [6]. The mean RSD between series of the field kit was 4.1% for erythrocyte cholinesterase and 5.6% for blood cholinesterase [7]. In the standard operating procedure developed by Deutsche Forschungsgemeinschaft [8], RSD values of 5.8 and 10.6% (within-series imprecision) are reported for AChE and BuChE respectively, and 14.0% (between-day imprecision) for BuChE; LOD was 235 U/l.

For the laboratory kit, a reduction of 15% in erythrocyte cholinesterase and 7.5% in plasma cholinesterase activities between two subsequent samples was considered to be a sign of significant inhibition, if the RSD values of the two analyses were 3.5 and 2.5% [9]. For the field kit, a 22% reduction in cholinesterase activity, with respect to pre-exposure values, indicated that over-exposure to OPs has taken place [10].

Inter- and intra-individual variations in cholinesterase activity are very high. The results should therefore preferably be compared with the baseline of each subject, if possible the median of three samples obtained in a pre-exposure period. In evaluating the results, confusing factors must be taken into account. Plasma cholinesterase activity may be depressed by chronic hepatitis, cirrhosis and other liver disease as well as by use and abuse of drugs. There are no differences in cholinesterase activity associated with race, but plasma cholinesterase activity in North American black races has been reported to be lower than in whites of the same sex [3].

With regard to interpretation of results, a reduction to 70% of the individual AChE baseline (30% inhibition) has been suggested as an indication of risk of over-exposure. This level is adopted by ACGIH [11] and DFG [12] as a biological limit. Since BuChE is more sensitive, but less specific, 50% inhibition level has been suggested as a biological limit [13].

#### 3.1.2. Unchanged compounds

Unmetabolised OPs may be measured in blood and/or urine, or in gastric content, to confirm exposure in cases of poisoning. In fatalities, unchanged compounds may be measured in central nervous system and other tissues. Available analytical methods and their main characteristics are summarised in Table 1.

*3.1.2.1. Acephate and methamidophos*. Acephate is metabolised relatively little in the human body, 73–77% of the absorbed dose being excreted unchanged in urine. Most is excreted within 12 h of exposure [14]. Methamidophos may also be found in urine samples of subjects exposed to acephate [14].

Table 1
Analytical procedures for the determination of intact organophosphorous pesticides in biological samples

| | [125] | [126] | [127] | [128] | [129] | [130] | [131] |
|---|---|---|---|---|---|---|---|
| Analytes | Azinphos-ethyl | Malathion | Malathion | Fenitrothion[b] | Fenthion | Acephate and methamidophos | Dimethoate, dichlorvos, ronnel, dichlofenthion, carbophenothion, parathion, EPN, leptophos |
| Sample | Blood, urine, gastric lavage liquid | Blood | Blood, stomach contents, liver | Blood, gastric lavage liquid | Blood, urine, post-mortem samples | Blood and brain of exposed animals | Blood and fatty tissue of exposed animals |
| Sample preparation | Extraction with benzene | HS-SPME | Extraction with solvents | Extraction with solvents after absorption on Extrelut | SPE $C_8$ | Treatment with acetonitrile and ethyl acetate | Extraction with solvents and purification on silica gel |
| Apparatus | GLC–FPD or thermoionic detector | GC–MS-EI SIM $m/z$ 173 | GC–MS-EI SIM $m/z$ 173 | GC–FID GC–FPD and GC–MS | RP HPLC–DAD and GC–MS EI SCAN[a] | GC–MS EI SIM $m/z$ 94 and 136 for acephate; $m/z$ 94 for methamidophos | GLC–FPD |
| LOD | 1 μg/l | 1 μg/g | 1 μg/g | – | – | – | – |
| Recovery (%) | 92–102 | 86.4±6.3 | – | – | 71±12.5 (blood) 46±8 (liver) | 75–80 | – |
| RSD (%) | – | 4 at 25 μg/g | – | – | – | – | – |

[a] After derivatization with diazomethane.
[b] Urine samples were analyzed with the same procedure for the determination of 3-methyl-4-nitrophenol, aminofenitrothion, aminofenitroxon, acetylaminofenitroxon and S-methylfenitrothion.

Gas chromatography (GC) with capillary columns and alkaline flame detection (NPD) is the only method fully described in the literature [15] but GC–MS could improve the detection limit [Aprea, unpublished). Table 2 shows conditions of analysis.

Acephate in urine may be used as an indicator of exposure: available data is insufficient to establish exposure limits.

### *3.1.3. Metabolites*

*3.1.3.1. Alkylphosphates.* Dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP) and diethyldithiophosphate (DEDTP) are metabolic products of various OP compounds. They are formed by the hydrolysis of the ester bond in the OP molecule. Dimethyl OPs produce dimethyl metabolites and diethyl OPs produce diethyl metabolites [16]. Alkyphosphates are excreted in urine as sodium or potassium salts. Excretion is usually quite rapid (80–90% of the total dose within 48 h) [16].

The most commonly used analytical methods are GC with photometric detection (HRGC–FPD) [17–20] or mass detection (HRGC–MS) [21]. Sample preparation often requires azeotropic distillation of urine with acetonitrile [17,19,20]. Extraction with solvents [21–23] or ion-exchange resins [18,24] may be used to isolate the metabolites from urine.

Before carrying out GC analysis, alkylphosphates must be converted into volatile compounds with derivatizing agents such as diazomethane [25], diazopentane [23], triazenes [26] and pentafluorbenzylbromide (PFB-Br) [17,19–21]. The latter has the advantage of yielding a single reaction product for DMTP and DETP, whereas the others form two isomers for each metabolite.

Three methods are particularly reliable and practical. They involve PFB-Br [17,20,21] and were

Table 2
Analytical procedures for the determination of acephate in urine

| | Aprea (unpublished) | [15] |
|---|---|---|
| Analytes | Acephate, methamidophos | Acephate, methamidophos |
| Sample volume | 10 ml | 10 ml |
| Analyte isolation | Extraction with dichloromethane on ChemElut column | Extraction with dichloromethane on ChemElut column |
| Apparatus | GC–MS SIM ($m/z$ 94 for methamidophos, $m/z$ 94 and 136 for acephate, $m/z$ 156 for I.S.) | GC–NPD |
| Column | MDN-5S 30 m×0.25 mm×0.25 μm | SE52 25 m×0.23 mm |
| Column temperature | 50–300 °C | 120–200 °C |
| Injection temperature | 280 °C | 250 °C |
| Detector temperature | 280 °C | 250 °C |
| Injection volume | 1 μl | 0.5 μl |
| Injection type | Splitless | Not reported |
| I.S. | Omethoate | – |
| LOD (μg/l) | 6 | 30 |
| Recovery (%) | 85 | Not reported |
| RSD (%) | 8 for methamidophos 10 for acephate | Not reported |

derived from a previous more complex method [19]. In the Nutley and Cocker method [20] and the Hardt and Angerer method [21], the six alkylphosphate derivatives are obtained simultaneously at 40–50 °C in acetonitrile without further sample purification steps. In the method of Aprea et al. [17], derivatization of compounds containing sulfur (DMTP, DMDTP, DETP, DEDTP) is obtained with acetone at room temperature; for DMP and DEP derivatization is with acetonitrile at 90 °C. The pentafluorobenzyl esters of alkylphosphates are then purified with CN bound phase SPE columns. Purification of the derivatized sample before GC is advisable when many samples have to be analysed, because excess PFB-Br (corrosive) decomposes at high temperature giving rise to halogen acid that would damage column and detector [17]. Table 3 shows conditions of analysis.

The method proposed by Aprea [17] has been used for monitoring exposure in occupationally exposed subjects [7,27–31] and the general population [32,33]. Alkylphosphates in urine are more sensitive indicators of exposure than acetylcholinesterase inhibition. Unfortunately, biological limits of exposure have not yet been established, and it is complicated to interpret the results in terms of risk for human health.

*3.1.3.2. 3,5,6-Trichloro-2-pyridinol (TCP).* TCP is a product of esterase cleavage of chlorpyrifos and chlorpyrifos-methyl [34,35]. It constitutes 96% of total urinary chlorpyrifos metabolites in rats; 12% is free and the rest conjugated, mainly with glucuronic acid [36]. After oral and dermal administration of chlorpyrifos to volunteers, the biological half-life was found to be 27 h [37]. In man, about 70% of the oral dose, and less than 3% of the dermal dose, were excreted in urine as TCP [37].

Reversed-phase HPLC with spectrophotometric detection [38,39] and GC are the main techniques used for determining TCP in urine. In the GC methods, summarized in Table 4, derivatization of samples is important to obtain more stable and more volatile compounds. The two techniques have been compared analysing 42 urine samples from the general population in two different laboratories. Comparison did not show any statistically significant differences: linear regression analysis showed a statistically significant adjustment to data ($P<0.01$),

Table 3
Analytical procedures for the determination of alkylphosphates in urine

| | | [17] | [20] | [21] |
|---|---|---|---|---|
| Apparatus | | GC–FPD | GC–FPD | GC–MS SIM (*m*/*z* 306 for DMP, 334 for DEP, 322 for DMTP, 350 for DETP, 338 for DMDTP, 366 for DEDTP and 335 for I.S.) |
| Column | | SPB20 (25 m×0.32 mm×0.4 μm) | BP 10 (25 m×0.33 mm×0.5 μm) | DB 5MS (60 m×0.25 mm×0.25 μm) |
| Column temperatures | | 70–180 °C | 140–280 °C | 90–250 °C |
| Injection temperature | | 250 °C | 280 °C | 260 °C |
| Detector temperature | | 300 °C | 300 °C | 300 °C |
| Injection volume | | 1 μl | 1 μl | 1 μl |
| Injection type | | Splitless | Splitless | Splitless |
| I.S. | | Sulfotep | Dibutylphosphate | Dibutylphosphate |
| LOD (n*M*) | DMP | 18 | 50 | 5 μg/l |
| | DEP | 16 | 10 | 1 μg/l |
| | DMTP | 12 | 20 | 1 μg/l |
| | DETP | 10 | 10 | 1 μg/l |
| | DMDTP | 10 | 10 | 1 μg/l |
| | DEDTP | 9 | 10 | 1 μg/l |
| Recovery (%) | DMP | 100 | 73 | 88–108 |
| | DEP | 88 | 82 | 100–114 |
| | DMTP | 86 | 79 | 71–89 |
| | DETP | 99 | 88 | 72–88 |
| | DMDTP | 90 | 83 | 75–85 |
| | DEDTP | 101 | 89 | 68–83 |
| RSD (%) | DMP | 11.4[a] (1.9[b]) | 11.6[c] (14.2[d]) | 12.2[c] (13.4–17.0[d]) |
| | DEP | 7.9[a] (2.5[b]) | 6.5[c] (13.3[d]) | 9.7[c] (7.9–10.0[d]) |
| | DMTP | 9.1[a] (4.5[b]) | 8.7[c] (22.6[d]) | 8.8[c] (9.1–9.4[d]) |
| | DETP | 8.8[a] (3.0[b]) | 4.4[c] (6.3[d]) | 15.5[c] (9.5–13.7[d]) |
| | DMDTP | 11.9[a] (4.8[b]) | 4.5[c] (6.4[d]) | 9.2[c] (10.8–12.2[d]) |
| | DEDTP | 9.8[a] (4.0[b]) | 3.8[c] (7.4[d]) | 11.0[c] (9.7–11.8[d]) |

[a] Whole analysis.
[b] GC analysis only.
[c] Within-series.
[d] Between-day.

and correlation and angular coefficients were close to one [40].

The LODs of the HPLC methods [38,39] (about 100 μg/l) are too high for monitoring low levels of exposure in workers or the general population. The GC methods with MS–MS detection [41,42] are too complicated for routine analyses, being more suitable for the simultaneous determination of different substances.

In a study carried out in the USA (National Health and Nutrition Examination Survey III, NHANES III), 82% of 1000 urine samples from the general population over a 7-year period (1988–1994), had TCP concentration above the LOD of 1 μg/l (mean value 3.1 μg/g creat.) [42]. These results were similar to those of a subsequent study based on 42 'unexposed' subjects in Italy [40].

Urinary TCP, like other OP metabolites, may be

Table 4
Analytical procedures for the determination of 3,5,6-trichloro-2-pyridinol (TCP) in urine

| | [37] | [132] | [40] | | [41] | [42] |
|---|---|---|---|---|---|---|
| Sample volume | 10 ml | 2 ml | 10 ml | | 5 ml | 10 ml |
| Hydrolysis | Hot $H_2SO_4$ | Hot HCl | Hot HCl | | Hot HCl | β-Glucuronidase |
| Analyte isolation | $C_{18}$Sep-Pak | Extraction with toluene | Extraction with toluene | | Extraction with ether | Extraction with 1-chlorobutane–ether |
| Derivatization | BSA[a] (1 h room temperature) | BSA[a] (1 h room temperature) | BSA[a] (1 h room temperature) | | 1 MTBSTFA[a] (1 h 60 °C) | 1-chloro-3-iodopropane (6 h 70 °C) |
| Purification | – | – | – | – | – | SPE silica |
| Apparatus | GC–ECD | GC–ECD | GC–ECD | GC–MS SIM (*m*/*z* 254 and 256 for TCP, *m*/*z* 181 and 183 for I.S.) | GC–MS–MS in NCI | GC–MS–MS in PCI |
| Column | 3% OV-1 on GC Q 100–120 mesh | RLS-200 15 m×0.32 mm | CP-Sil5 25 m×0.32 mm×0.4 μm | PONA 50 m×0.32 mm×0.5 μm | DB-5 30 m×0.25 mm×0.25 μm | DB-5 30 m×0.25 mm×0.25 μm |
| Program of temperature or elution | 145 °C | 120–220 °C | 90–250 °C | 90–250 °C | 175–275 °C | 80–260 °C |
| Injection temperature | 220 °C | 150 °C | 225 °C | 225 °C | 280 °C | Not reported |
| Detector temperature | 350 °C | 300 °C | 250 °C | 250 °C | 280 °C | Not reported |
| Injection volume | 4 μl | 1 μl | 1 μl | 1 μl | Not reported | Not reported |
| Injection type | Not reported | Splitless | Splitless | Splitless | Splitless | Not reported |
| I.S. | – | Heptachloroepoxide | γ-Hexachlorocyclohexane | γ-Hexachlorocyclohexane and 2,4,6-TCPh | 3,4,5-Trichloropyridinol | $^{13}$C TCP (isotopic dilution) |
| LOD (μg/l) | 10.0 | 5.0 | 1.2 | 1.5 | 0.5 | 1.0 |
| Recovery (%) | – | 71.8 | – | – | 80.6–89.9 | – |
| RSD (%) | – | 7 | 8.2 at 5.4 μg/l | – | <3 day-to-day | 24 between series |

[a]BSA, N,O-bis(trimethylsilyl)acetamide; MTBSTFA, *N*-(*tert.*-butyldimethylsilyl)-N-methyltrifluoroacetamide; 2,4,6-TCPh, 2,4,6-trichlorophenol.

used as an indicator of exposure to chlorpyrifos and chlorpyrifos-methyl, although available data is still insufficient to define biological exposure limits [43].

*3.1.3.3. p-Nitrophenol (PNP).* PNP is a metabolic product of esterase cleavage of parathion, parathion-methyl and EPN.

PNP can be determined in urine of occupationally exposed subjects by GC with ECD detection. Sample preparation involves acid hydrolysis, extraction with ethyl ether, derivatization with diazoethane, and purification on silica gel columns. LOD is 20 μg/l with a recovery of 85–98% [44]. This method has also been used in a general population study [45].

A more complicated technique has recently been used to detect PNP in the general population [46], namely GC with MS–MS detection in positive chemical ionization (PCI), after derivatization with 1-chloro-3-iodopropane. Sample preparation involves hydrolysis with β-glucuronidase, several phases of extraction with solvents, and purification on SPE (silica). Quantification in isotopic dilution involves using $^{13}$C PNP as internal standard. The LOD was 1 μg/l, RSD between series was 24%, and mean accuracy was 1 μg/l [42].

In 2.4% of the urine samples analysed by NHANES II, PNP was above the LOD of 10 μg/l [45] and in 41% of samples analysed by NHANES III [46] it was >1 μg/l. The highest values measured in the two studies were 143 μg/l and 44 μg/g creat.

*3.1.3.4. 3-Methyl-4-nitrophenol (MNP).* MNP is a metabolite produced by esterase cleavage of fenitrothion.

GC–ECD has been suggested for the measurement of MNP in occupationally exposed subjects. Sample preparation involves extraction with ethyl ether, derivatization with diazoethane and purification on silica gel columns. LOD is 50 μg/l with a recovery of 88–98% [44].

*3.1.3.5. Malathion α-monocarboxylic acid (MCA) and malathion dicarboxylic acid (DCA).* The mono- and dicarboxylic phosphoric acids, derived from hydrolysis of diethylsuccinic ester in the lateral chain, are the main urinary metabolites of malathion [47].

GC with FPD detection has been suggested for their determination. Sample preparation involves extraction of acidified urine with ethyl ether–acetonitrile, derivatization with diazomethane and purification on a silica-gel column. This method [47] has a LOD of 30 μg/l and has been used in a study on the general population [45].

Another method, used by some authors [48], involves conversion of MCA and DCA into alkylphosphates (DMTP and DMDTP), by alkaline hydrolysis. The compounds are then derivatized with pentafluorbenzylbromide, as previously described.

### 3.2. Carbamate insecticides

#### 3.2.1. Serum cholinesterase and erythrocyte activity

Like OP, carbamate insecticides are inhibitors of ChE. The inhibition is labile, of short duration and reversible, unlike that induced by OP compounds. Blood samples must therefore be obtained and analysed as soon as possible after exposure. The analytical methods and sampling procedures have already been discussed.

#### 3.2.2. Unchanged compounds

Measurement of unmodified carbamate insecticides in blood and/or urine has often been performed to confirm exposure in acute poisoning cases [49–51]. In fatal cases, unmodified compounds may be measured in various organs [49]. The analytical methods are summarised in Table 5.

In cases of occupational exposure, unmodified compound is rarely measured since the metabolic

Table 5
Analytical procedures for the determination of intact carbamate pesticides in biological samples

| | [49] | [133] | [50] |
|---|---|---|---|
| Analytes | Carbaryl | Methomyl | Furathiocarb |
| Sample | Blood, urine, liver, stomach (postmortem) | Blood | Blood, stomach contents |
| Sample preparation | Extraction with *n*-butyl chloride–methanol on Extrelut column for blood and urine<br>Protein precipitation with acetonitrile for viscera | Extraction with benzene and ether | Extraction with ethylacetate |
| Apparatus | RP HPLC–UV (214 nm) | GC–FPD<br>LC–MS–MS to confirm | GC–NPD for quantitation in blood<br>TLC and GC–MS to confirm the pesticide in stomach contents |
| LOD | – | – | – |
| Recovery (%) | 99 for blood<br>95 for viscera | 50 | 93 for blood |
| RSD (%) | 2.7 for blood | – | – |

pathway of these substances is very complex and yields polar compounds, readily soluble in water. Carbofuran measured in urine of occupationally exposed farmers showed that about 7% of the total absorbed dose (dermal and inhalation) is excreted in urine in 24 h [52]. The analytical method is summarised in Table 6.

### 3.2.3. Metabolites

*3.2.3.1. Benomyl metabolites*. The main benomyl metabolites are carbendazim (methyl 2-benzimidazole carbamate) (II), and methyl 5-hydroxy-2-benzimidazolecarbamate (III), which have been detected in experimental animals but never in biological fluids of exposed workers or the general population.

*3.2.3.2. 1-Naphthol (1NAP) and carbaryl*. 1NAP is the main metabolite of carbaryl in humans, accounting for more than 85% of its metabolites in urine [53]. 1NAP is also a metabolite of naphthalene and napropamide. It has been studied in exposed workers [54]. The analytical methods are described in Table 6.

Table 6
Analytical procedures for the determination of carbaryl, 1-naphthol (1NAP) and carbofuran in biological samples

| | [42] | [134] | [135] | [52] |
|---|---|---|---|---|
| Analytes | 1NAP | Carbaryl, 1NAP[a] | 1NAP | Carbofuran |
| Sample volume | 10 ml urine | 0.5–1.0 ml plasma or urine | 5 ml urine | 50 ml urine |
| Hydrolysis | β-Glucuronidase | β-Glucuronidase | Acid reflux for 90 min[b] | – |
| Analyte isolation | Extraction with 1-chlorobuthane–ether | Extraction with hexane–ethylacetate | Extraction with benzene | Extraction with dichloromethane |
| Derivatization | 1-Chloro-3-iodopropane | – | Chloroacetic anhydride in pyridine | – |
| Purification | SPE (silica) | – | Silica gel | SPE ($C_{18}$) |
| Apparatus | HRGC MS–MS in PCI ($CH_4$) | HPLC UV (210 nm) | GC ECD | HPLC–fluorescence detector ($\lambda_{ex}$ 280 nm, $\lambda_{em}$ 315 nm) |
| Column | DB-5 30 m×0.25 mm 0.25 μm | ODS 5 μm RP | Glass column packed with 1.5% OV-17 and 1.95% QF1 on Chromosorb W | Partisil PXS 10/15 ODS 3 |
| Programme of temperature or elution | 80–260 °C | Acetonitrile–water | 185 °C | Acetonitrile–water |
| Injection temperature | Not reported | – | 210 °C | – |
| Detector temperature | Not reported | – | 210 °C | – |
| Injection volume | Not reported | – | 5–10 μl | 25 μl |
| Injection type | Not reported | – | Not reported | – |
| I.S. | $^{13}C$ 1NAP (isotopic dilution) | Mesurol | – | – |
| LOD (μg/l) | 1 | 5 for carbaryl 10 for 1NAP | 20 | 100 |
| Recovery (%) | – | 90 for carbaryl 60 for 1NAP | Mean 92% at 20–1000 μg/l | 101±7 |
| RSD (%) | 20 between series (8.1 μg/l) | <10% Intra- and inter-assay | Not reported | Not reported |

[a] Modification of HPLC elution phase enables the determination of carbaryl metabolites, hydroxylated in positions 4 and 5.
[b] Hydrolysis converts carbaryl to 1NAP.

The procedure of Hill and Coll. [42] was used to analyse urine samples of subjects participating in the NHANES III study. 1NAP was detected in 86% of subjects. The highest concentration found was nearly 1400 μg/g creat.; 95% of the results were less than 36 μg/g creat. [46].

*3.2.3.3. 2-Isopropoxyphenol (IPP).* About 83% of propoxur absorbed is metabolised to IPP [55,56] which is excreted quite rapidly. After oral administration of propoxur, 24.7% of the total dose is excreted in urine within 8–10 h [57]. After intraperitoneal administration in rats, 75% of the dose was recovered in urine as IPP (probably conjugated with glucuronic acid) within 24 h.

Analytical procedures are summarised in Table 7. The GC–ECD technique [58] is simplest but is not sensitive enough for biological monitoring of occupational exposure. The LOD of 50 μg/l only enables detection in cases of heavy exposure. Two analytical methods recently published show substantial difference in duration, that of Leenheers et al. [59] being the more practicable, though its detection limit is high at 6 μg/l and does not permit measurement of the metabolite in subjects exposed to low levels of propoxur.

IPP has been measured in the general population by various authors [46,60]: 6.8% of urine samples in NHANES III, were positive (LOD 1 μg/l). Concentrations never exceeded 10 μg/l (9.6 μg/g creat.), and 95% of the samples were below 1.7 μg/l (1.6 μg/g creat.) [46].

Table 7
Analytical procedures for the determination of 2-isopropoxyphenol (IPP) in urine

| | [58] | [42] | [59] |
|---|---|---|---|
| Sample volume | – | 10 ml | 4 ml |
| Hydrolysis | Hot HCl | β-Glucuronidase | Hot HCl |
| Analyte isolation | Extraction with dichloromethane | Extraction with 1-chlorobuthane–ether | Extraction with *n*-hexane |
| Derivatization | 2,4-dinitro-1-fluoro-benzene | 1-Chloro-3-iodopropane | – |
| Purification | TLC (silica) | SPE (silica) | Washing at pH 11 |
| Apparatus | GC–ECD | GC–MS–MS PCI ($CH_4$) | GC–MS EI |
| Column | Packed with DC-200 10% on Chromosorb WHMD 80–100 mesh | DB-5 30 m×0.25 mm×0.25 μm | OV-1701 25 m×0.25 mm×0.2 μm |
| Programme of temperature or elution | 230 °C | 80–260 °C | 40–250 °C |
| Injection temperature | 240 °C | Not reported | 200 °C |
| Detector temperature | 260 °C | Not reported | 280 °C |
| Injection volume | Not reported | Not reported | 1 μl |
| Injection type | Not reported | Not reported | Splitless |
| I.S. | – | $^{13}C$ IPP (isotopic dilution) | Trimethylphenol |
| LOD (μg/l) | 50 | 1.0 | 6.0 |
| Recovery (%) | 75 | – | >95 |
| RSD (%) | – | 17% between series (4.2 μg/l) | 7% (16 μg/l) |

*3.2.3.4. Carbofuranphenol (CFP, 2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran).* CFP is a metabolite of several pesticides (e.g. carbofuran, benfuracarb, carbosulfan and furathiocarb). Biological monitoring of this metabolite was performed in urine samples of NHANES III. Positive samples were 1.5% of the total with concentrations never exceeding 8.5 μg/g creat. [46]. The analytical method [42] has already been described for other metabolites: LOD was 1 μg/l, RSD between series 13% at a concentration of 4.6 μg/l and accuracy +2 μg/l at an expected value of 27 μg/l.

### 3.3. Organochlorine compounds (OCs)

OCs are a broad class of pesticides that were widely used as insecticides in the 1950s and 1960s. Their use was subsequently discontinued in many countries due to persistent contamination of the environment. They can be divided into three groups: benzene hexachloride isomers (e.g. lindane), cyclodienes (aldrin, dieldrin, endrin, chlordane, heptachlor, endosulfan) and DDT and analogues (methoxychlor, dicofol, chlorobenzylate).

Biological monitoring of OC exposure can be carried out by determination of intact compounds or their metabolites in blood and urine. Because of their persistence in the environment, most OC pesticides are ubiquitous pollutants and can usually be detected in biological samples from the general population.

#### 3.3.1. Unchanged compounds and metabolites

After absorption, aldrin is rapidly converted to dieldrin. Exposure to both compounds has been assessed by measuring dieldrin concentrations in blood, serum, fatty tissue and milk.

Technical chlordane is a mixture of α- and γ-chlordane, nonachlor and heptachlor. Biological monitoring of human exposure has been based on measurement of concentrations of chlordane and related compounds (oxychlordane, nonachlor, heptachlor-epoxide) in blood, fatty tissue and milk.

Endrin is rapidly metabolised to 12-hydroxyendrin, and excreted as sulphate and glucuronide conjugate. Intact pesticide is usually undetectable in blood, fatty tissue and milk of occupationally exposed workers and the general population.

Exposure to heptachlor has been monitored by measuring its main metabolite, heptachlor epoxide, in blood, fatty tissue and milk of exposed subjects and the general population.

Technical grade hexachlorocyclohexane (HCH) consists of 65–70% α-HCH, 7–10% β-HCH, 14–15% γ-HCH and about 10% of other isomers and compounds. Lindane contains >90% γ-HCH. Exposure to lindane and HCH isomers has been monitored through concentrations of intact compounds in blood, fatty tissue and milk.

After absorption, DDT is largely transformed to DDE, and several intermediate metabolites have been measured in body tissues.

Analytical methods for monitoring OC compounds in biological samples are shown in Table 8.

*3.3.1.1. Chlorobenzylate and dicofol*. The determination of unchanged chlorobenzylate and its metabolites (*p,p′*-dichlorobenzylic acid and *p,p′*-dichlorobenzyldrol) requires oxidation to *p,p′*-dichlorobenzophenone, which is then analysed by CG. The same analytical procedures are suitable for measuring occupational exposure to dicofol because *p,p′*-dichlorobenzylic acid is the major urinary metabolite of this substance [61]. Methods are summarised in Table 9.

### 3.4. Pyrethroids (PYRs)

#### 3.4.1. Unchanged compounds

Occupational exposure to PYRs may be assessed by measuring intact compounds or their metabolites in urine. Because of their rapid metabolisation, determination in blood is only appropriate for recent high exposure. In a recent study [62], cypermethrin, cyfluthrin and permethrin were determined in plasma samples of 30 pest control operators. Pyrethroid concentrations were <5 μg/l (LOD) in all cases. For the analysis, 1 ml plasma underwent precipitation of protein, liquid–liquid extraction and GC–ECD detection using bifenthrin as internal standard [62].

Determination of unmodified compounds in urine involves simpler analytical procedures than those necessary for metabolites: deltamethrin and fenvalerate may be monitored by HPLC (LOD 4.0 and 0.2 μg/l respectively) [63] or GC–ECD (LOD 0.2 μg/l) [64,65].

Because of their rapid elimination, unmodified

Table 8
Analytical procedures for the determination of organochlorine compounds and metabolites in biological samples

| | [136] | [137] | [138] | [139] | [140] | [141] | [142] |
|---|---|---|---|---|---|---|---|
| Analytes | α- and β-Endosulfan | α, β,γ HCB[a], *p,p*-DDE, *p,p*-DDD, *p,p*-DDT, *o,p*-DDT, A[a], endosulfan, | α, β,γ HCH[a], *p,p*-DDE, *p,p*-DDT, *o,p*-DDT | HCB[a], β,γ HCH[a], OC[a], HE, TN[a], *p,p*-DDE, DDT, D[a], E[a] | HCB[a], γ HCH[a], HC[a], A[a], D[a], E[a], *p,p*-DDE, *p,p*-DDD, *p,p*-DDT, *o,p*-DDT, *p,p*-DDE, *o,p*-DDD | β HCB, γ HCH, HE, DDE, D, E, *o,p*-TDE, *p,p*-TDE, *p,p*-DDT, *o,p*-DDT, OC | DDT, DDE, β,γ HCH[a] |
| Sample | Postmortem samples | Urine | Serum | Serum | Fatty tissue | Fatty tissue, liver | Fatty tissue |
| Sample volume or mass | 5–20 g | 200 ml | 1 ml | 4 ml | 2–5 g | – | – |
| Analyte isolation | Addition of sea-sand and extraction with acetonitrile | Extraction with *n*-hexane | Addition of silica gel and extraction with *n*-hexane–acetone | Extraction of denatured serum with *n*-hexane–ether | Extraction with methanol | Extraction with petroleum ether | Extraction with petroleum ether |
| Purification | Extraction with benzene and purification on alumina | Liquid–liquid partition with acetonitrile | Deactivated alumina | Florisil chromatography and separation of three fractions | SPE ($C_{18}$) | Activated florisil | Treatment with $H_2SO_4$ |
| Apparatus | GC–ECD | GLC–ECD | GLC–ECD | GLC–ECD | GLC–ECD | GLC–ECD | GLC–ECD |
| Column | Packed 3.8% UCCW-982 on Chromosorb W | Packed 1.5% OV-17 and 1.95% OV-210 on Chrome WHP | Packed 1.5% OV-17 and 1.95% QF-1 on Chromosorb W-AW-DMCS | Packed columns | SE 54 50 m, 0.32 mm, 0.2 μm | Silicon 30 m, 0.25 mm, 0.25 μm | Packed 1.5% SP 2250 and 1.95% SP 2401 on Supelcoport |
| Programme of temperature | 200 °C | 190 °C | 200 °C | Not reported | 70–220 °C | 50–280 °C | 200 °C |
| Injection temperature | 210 °C | 250 °C | 230 °C | Not reported | Not reported | 250 °C | 250 °C |
| Detector temperature | 210 °C | 250 °C | 300 °C | Not reported | 300 °C | 350 °C | 350 °C |
| Injection volume | Not reported | Not reported | 2–5 μl | Not reported | – | 1 μl | 1 μl |
| Injection type | Not reported | Not reported | Not reported | Not reported | On column | Split | – |
| I.S. | Aldrin | – | – | DCB, Mirex, TN | chlorothalonil | – | – |
| LOD (μg/l) | – | 1–2 | <1 | <1 | <1 ng/l | Not reported | 0.01 mg/kg |
| Recovery (%) | 83.6±3.4 α-ES 95.8±7.7 β-ES | >90 | 80–99 | 50.4–121.6 in range 1–10.7 μg/l | 62–66 at 20 pg/ml | Not reported | 91–99 |
| RSD (%) | – | – | ≤15 | – | – | Not reported | <10 |

[a]HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; OC, oxychlordane; HE, heptachlor epoxide; TN, *trans*-nonachlor; HC, heptachlor; A, aldrin; D, dieldrin; E, endrin.

compounds are less sensitive indicators of exposure than metabolites, although certainly more specific.

### *3.4.2. Metabolites*

*3.4.2.1. 3-Phenoxybenzoic acid (3-PBA), 3-(4-hydroxy)-phenoxybenzoic acid (4OH-3PBA), 3-(2,2-dichlorovinyl)-2.2-dimethyl cyclopropane acid (DCVA), 3-(2.2-dibromovinyl)-2.2-dimethyl cyclopropane acid (DBVA), 2-(4-chlorophenyl)-3-methyl-1 butanoic acid (CPBA), 4-fluoro-3-phenoxybenzoic acid (F-PBA).* Hydrolysis of the ester bond of permethrin, cypermethrin, deltamethrin, cyfluthrin and fenvalerate, produces acid metabolites and 3-phenoxy benzyl (4-fluoro-3-phenoxybenzyl for cyfluthrin) alcohol. The acid metabolites are: *cis–trans*-DCVA (permethrin, cypermethrin and cyfluthrin), *cis–trans*-DBVA (deltamethrin) and CPBA (fenvalerate). Phenoxybenzoic compounds (3-PBA and 4OH-3PBA), derived from the alcohol group, are metabolites of permethrin, cypermethrin, deltamethrin and fenvalerate, [63–66]; F-PBA is a metabolite

Table 9
Analytical procedures for the determination of chlorobenzylate metabolites in urine

| | [143] | [144] |
|---|---|---|
| Analytes | *p,p′*-Dichlorobenzophenone | *p,p′*-Dichlorobenzophenone |
| Sample volume | 5 ml | 10 ml |
| Oxidation | $K_2CO_3$–$H_2SO_4$ (90 °C for 1 h) | $CrO_3$–$CH_3COOH$ (20 min)[a] |
| Analyte isolation | Extraction with *n*-hexane | Extraction with *n*-hexane |
| Derivatization | – | – |
| Purification | – | – |
| Apparatus | GC–ECD | GC–ECD |
| Column | Glass column packed with 1.5% OV-17 and 1.95% OV-210 on Gas Chrom Q | Glass column packed with 4% SP2100 and 2% 2401 on Supelcoport |
| Programme of temperature or elution | 210 °C | 190 °C |
| Injection temperature | 250 °C | 210 °C |
| Detector temperature | 300 °C | 300 °C |
| Injection volume | Not reported | Not reported |
| Injection type | Not reported | Not reported |
| I.S. | – | – |
| LOD (μg/l) | 2 | 10 |
| Recovery (%) | 97 | 91 |
| RSD (%) | 9 | 7 |

[a] This technique cannot be confused with the human urinary metabolite of DDT in man (4,4′-dichlorodibenzoacetic acid, DDA) because DDA is not oxidized to *p,p′*-dichlorobenzophenone using this system.

of cyfluthrin [67]. These metabolites are excreted as conjugates in a very short time: after oral and dermal administration they are detectable in urine for no more than 5 days (biological half-life between 8 and 27 h) [67–69].

Available analytical methods for PYR metabolites are summarized in Table 10. The method of Aprea et al. [70] has the advantage of enabling formation of the pentafluorobenzyl ester of 3-PBA at room temperature with a small quantity of reagent. It has been used to monitor 3-PBA in a worker exposed to fenvalerate. This metabolite is suitable for biological monitoring of exposure to the many pyrethroid insecticides which have an ester bond with 3-phenoxybenzyl alcohol and a hydrogen of the benzyl group substituted with a CN group.

The method of Chester et al. [71], is less time-consuming, but produces a greater number of interfering compounds, which the method of Aprea et al. is not able to eliminate through purification [70]. Besides, the volume of urine required by this method (50 ml), makes it unpracticable. The analytical procedure of Yao et al. [72] for DBVA has been used to assess exposure to deltamethrin in cotton field pest control operators. Metabolite concentration of 9–33 μg/l were found in urine samples collected from the first day of spraying to 2 days after the end of exposure [64,66].

The standard operating procedure of Deutsche Forschungsgemeinschaft [73] enables determination of all metabolites, with better LODs and good precision and recovery.

Biological limits are not available for pyrethroid insecticides.

## 4. Herbicides

### *4.1. Unchanged compounds and metabolites*

#### *4.1.1. 2,4-Dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), pichloram, mecoprop, dichlorprop*

These compounds are poorly metabolised and are excreted largely unchanged in urine [74]. Excretion is slow with a maximum on the first and second days after oral administration of 2,4-D; 40% of MCPA is excreted within 24 h and 80% within 5 days of administration. For dermal absorption, maximum

Table 10
Analytical procedures for the determination of pyrethroid metabolites in urine

| | [70] | [145] | [69] | [68] | [71] | [72] | [146] | [73] |
|---|---|---|---|---|---|---|---|---|
| Analytes | 3-PBA | 3-PBA | 3-PBA; 4OH-3PBA; DCVA | DCVA | 3-PBA; 4OH-3PBA; DCVA | DBVA | CPBA | *cis*, *trans*-DCVA, *cis*-DBVA, 3-PBA, F-PBA |
| Sample volume | 5 ml | 5 ml | 5 ml | 5 ml | 50 ml | 2 ml | 25 ml | 5–10 ml |
| Hydrolysis | $H_2SO_4$ 100 °C | $H_2SO_4$ 100 °C | $H_2SO_4$ 100 °C | HCl reflux | HCl reflux | β-G–S[a] | NaOH 100 °C | $H_2SO_4$ 90 °C |
| Analyte isolation | Extraction with dichloromethane | SEP-PAK $C_{18}$ cartridges | Extraction with ether | – | Extraction with dichloromethane | Extraction with *n*-hexane | – | $C_{18}$ cartridges or extraction with *n*-hexane |
| Derivatization | PFB-Br (2 μl) overnight room temperature | PFB-Br (2 μl) 20 min | PFPA–PFPOH–TAA[a] (60 min at 90 °C) | Methanol–$H_2SO_4$ reflux 1.5 h | PFB-Br (30 μl) 90 min room temperature | – | – | methanol–$H_2SO_4$ 75 °C, 1 h |
| Purification | SPE (silica) | – | – | – | Activated Florisil | – | SPE (silica) and SPE ($C_{18}$) | Extraction with *n*-hexane–saturated NaCl solution |
| Apparatus | HRGC–ECD | HRGC–ECD | HRGC–MS (EI) | HRGC–ECD | HRGC–MS (EI) | HPLC (UV 254 nm) | HPLC | HRGC–MS SIM (*m*/*z* 187 for DCVA, 231 for DBVA, 246 for F-PBA, 228 for 3-PBA and 197 for I.S.) |
| Column | SPB 608 30 m×0.25 mm ×0.4 μm | Not reported | SGE BP10 25 m×0.32 mm×0.5 μm | Not reported | Methyl silicone 28 m×0.25 mm | $C_{18}$ Ultrasphere (4.6 mm×15 cm ×5 μm) | Whatman $C_{18}$ Partisphere (12.5 cm) | 5% Phenylmethyl polysiloxane (60 m× 0.25 mm×0.25 μm) |
| Programme of temperature or elution | 50–250 °C | Not reported | 75–260 °C | Not reported | 120–280 °C | Methanol–water | Methanol–tetra-hydrofuran–acetic acid 1% | 90–270 °C |
| Injection temperature | 300 °C | Not reported | 200 °C | Not reported | 275 °C | – | – | 250 °C |
| Detector temperature | 350 °C | Not reported | 250 °C | Not reported | Not reported | – | – | 300 °C |
| Injection volume | 1 μl | Not reported | 1 μl | Not reported | Not reported | – | – | 1 μl |
| Injection type | Splitless | Not reported | Not reported | Not reported | Not reported | – | – | Not reported |
| I.S. | 2-PBA[a] | Mecoprop | 4-PBA[a]; 4OH-4PBA[a] | – | Not reported | – | – | 2-PBA[a] |
| LOD (μg/l) | 0.5 | 2.0 | 0.5 | 5–10 | 1–2 | 10 | – | 0.1–0.5 |
| Recovery (%) | 91 | 75–95 | – | – | – | 95 | – | 77.5–106.8 |
| RSD (%) | 9.58[b]–10.34[c]–3.18[d] | 10 | 5 | – | Not reported | 3 | – | 1.6–2.7[e]/6.2–8.7[f] |

[a] β-G–S, β-glucuronidase–sulfatase; PFPA–PFPOH–TAA, pentafluoropropionic anhydride–1H-1H-pentafluoropropanol–trifluoroacetic acid; 2-PBA, 2-phenoxybenzoic acid; 4-PBA, 4-phenoxybenzoic acid; 4OH-4PBA, 3-(4-hydroxy)-phenoxybenzoic acid.
[b] Whole analysis.
[c] Whole analysis on different urine samples.
[d] Chromatographic analysis only (concentration 24.96 μg/l).
[e] Within-series.
[f] Between-day.

urinary excretion is detected on the first and second days after application of 2,4-D [55,56] and 48 h after application of MCPA [75,76].

Methods for analysis in urine are summarised in Table 11. A LOD of 15 μg/l makes the HPLC method useful only for occupational exposure. The GC method (LOD 1 μg/l) is sensitive enough to monitor the general population. At concentrations

Table 11
Analytical procedures for the determination of phenoxyacetic herbicides in urine

| | [77] | | [42] | [80] | [147] | [79] |
|---|---|---|---|---|---|---|
| Analytes | 2,4-D; MCPA | | 2,4-D | 2,4-D; 2,4,5-T | 2,4-D; PIC[e]; DCP[e] | 2,4-D; MCPA; MCP[a]; DCP[a] |
| Sample volume | 20 ml | | 10 ml | 10 ml | 50 ml | 25 ml |
| Hydrolysis | – | | β-Glucuronidase | $H_2SO_4$ | NaOH | – |
| Analyte isolation | Extraction with dichloromethane | | Extraction with 1-chlorobutane–ether | Extraction with benzene | Extraction with ether | $C_{18}$ cartridges |
| Derivatization | – | PFBBr (2 μl overnight room temperature) | 1-chloro-3-iodo-propane | diazoethane | $BF_3$ in methanol | Methanol–$H_2SO_4$ 5 min room temperature |
| Purification | SPE (silica) | SPE (silica) | SPE (silica) | Silica gel | – | Extraction with hexane |
| Apparatus | HPLC–DAD (230 nm) | HRGC–ECD | HRGC MS–MS PCI | HRGC MS–MS PCI for 2,4-D and MS–MS NCI for 2,4,5-T | GLC | GC–MS (*m/z* 199 for 2,4-D, 214 for MCPA, 228 for MCP, 162 for DCP, 242 for I.S.) |
| Column | $LC_8$ (25 cm×4.6 mm ×5 μm) | CP Sil 8 50 m×0.32 mm 0.4 μm | DB-5 30 m×0.25 mm× 0.25 μm | DB-5 30 m×0.25 mm 0.25 μm | Packed with 5% Dexsil 300 GC on chromosorb W | DB-WAX 30 m×0.25 mm×0.25 μm |
| Programme of temperature or elution | Acetonitrile–phosphate buffer pH 3.2 | 50–270 °C | 80–260 °C | 70–220 °C | 160 °C for 2,4-D and DCP, 200 °C for PIC | 35–220 °C |
| Injection temperature | – | 250 °C | Not reported | 250 °C | Not reported | 210 °C |
| Detector temperature | – | 300 °C | Not reported | 250 °C | Not reported | Not reported |
| Injection volume | 10 μl | 1 μl | Not reported | 1–2 μl | Not reported | 1 μl |
| Injection type | – | Splitless | Not reported | splitless | Not reported | Splitless |
| I.S. | 4-CPA[e] | 4-CPA[a] and 2,3-D[e] | $^{13}C_6$2,4-D (isotopic dilution) | 2,3-D[a] (for 2,4-D) ($^{13}$C)PCP[e] (for 2,4,5-T isotopic dilution) | – | CMBA[e] |
| LOD (μg/l) | 15 for 2,4-D and MCPA | 1 for 2,4-D and MCPA | 1 | 1 for 2,4-D and 2,4,5-T | Not reported | 10 for all analytes |
| Recovery (%) | 81.4 for 2,4-D 85.3 for MCPA | 87.1 for 2,4-D 94.3 for MCPA | Accuracy –6% | 94 for 2,4-D 99 for 2,4,5-T | 76–90 | 90–95 |
| RSD (%) | 6.2[a] (1.9[b]) for 2,4-D 6.8[a] (1.7[b]) for MCPA | 8.0[a] (2.5[b]) for 2,4-D 5.5[a] (2.1[b]) for MCPA | 8.7 | 9.7 for 2,4-D 22.2 for 2,4,5-T | Not reported | 2.4–4.2[c]/6.9–11.3[d] |

[a] Whole analysis.
[b] Chromatographic analysis only.
[c] Within-series.
[d] Between-day at concentration <17.4 μg/l.
[e] PIC, picloram; MCP, mecoprop; DCP, dichlorprop; 4-CPA, 4-chlorophenoxyacetic acid; 2,3-D, 2,3-dichlorophenoxyacetic acid; ($^{13}$C)PCP, $^{13}$C-pentachlorophenol; CMBA, 4-chloro-2-methylbutyric acid.

above 15 μg/l, the results of the two methods coincide [77]. The HPLC procedure [77] has been widely tested in occupationally exposed subjects engaged in weed control spraying with 2,4-D and MCPA on wheat [78]. The GC–ECD method was used for 2,4-D and MCPA in a group of 100 children aged 6–7 years: 2,4-D was detectable in 20% of samples, and the maximum detected value was 2.5 μg/l, MCPA was never detectable [77].

The standard operating procedure of Deutsche Forschungsgemeinschaft [79] for 2,4-D, MCPA, mecoprop and dichlorprop have LODs (10 μg/l) too

high for monitoring the general population. The GC method with MS–MS detection [42,80] is less practicable for routine analyses.

In the USA, 2,4-D was only detected in 0.3% of urine samples in the NHANES II study were LOD was 30 μg/l. Urinary 2,4,5-T concentrations did not reach the LOD of 10 μg/l [45].

The procedure of Hill et al. [42] for 2,4-D in urine was used in NHANES III: it was detected in 12% of samples and never exceeded the concentration of 15 μg/g creat.; 95% of the results were below 1.5 μg/g creat. [46].

Biological exposure limits are not available for 2,4-D and MCPA but some authors [75,76] suggest that urinary MCPA levels up to 0.5 μg/ml may be observed under good working conditions.

#### *4.1.2. Glyphosate*

Exposure to glyphosate may be monitored through the unchanged compound and aminomethylphosphonic acid in urine [81,82]. Studies in monkeys showed that 89% of the dose absorbed through the skin is excreted in urine within 5 days [83]. The analytical procedure is summarised in Table 12.

#### *4.1.3. 2,6-Diethylaniline (DEA) and 2-(1-hydroxyethyl)-6-ethylanyline (HEEA)*

Urinary DEA and HEEA are metabolic products of alachlor, after alkaline hydrolysis. Studies in monkeys have shown their relative proportions to be 8:2 and a similar relationship is reported in urine of exposed human subjects [83].

Analytical methods for the two compounds in urine are summarised in Table 12. The HPLC method with electrochemical detection [84] has been modified by other authors [85,86], using SPE in the analyte isolation phase. The method of Driskell et al. [87] is for alachlor mercapturate without prehydrolysis.

Alachlor metabolites may also be measured in urine with a commercial ELISA kit, originally developed for analysis of alachlor in water [85,86]. The method is based on inhibition of the reaction between enzyme-labeled alachlor and immobilised polyclonal antialachlor antibodies, by free alachlor in the test sample. Since the kit was standardised using parent alachlor, the concentration of alachlor metabolites is reported as μg of alachlor equivalents per ml urine. The LOD for urine metabolites was empirically set at 1 μg/l. The ELISA and HPLC methods give statistically significantly different results but a linear association ($r=0.89$) was observed. The relationship was: ELISA=4.12HPLC+9.25. The basis of this systematic bias is unknown [85].

#### *4.1.4. Diquat and paraquat*

Determination of the unchanged quaternary ammonium compounds in blood and urine has been used to monitor human exposure [88]. The two herbicides may be measured by HPLC [89], GC [90], second derivative spectroscopy [91] and spectrometry [92]. Table 13 shows the analytical procedures. The procedure suggested by Fuke [91] seems to be less sensitive than HPLC, but is very fast (10 min for serum and 5 min for urine samples), and can therefore be used for emergency analyses.

## 5. Fungicides

### *5.1. Dithiocarbamate pesticides (DTC)*

These substances can be divided into thiurams (thiram, methiram, disulfiram), dimethyldithiocarbamates (ferbam, ziram) and alkylenbisdithiocarbamates (ethylene and propylene). The metabolic pathway of DTC is very complex, producing a great number of metabolites. One is carbon disulfide, which is further partially metabolised to 2-thiazolidinethione-4-carboxylic acid [93]. These compounds have both been determined in urine of occupationally exposed and unexposed subjects, though they are not specific indicators of exposure to DTC. Ethylenethiourea, on the contrary, is the specific metabolite of ethylenebisdithiocarbamates (EBDC) (mancozeb, zineb, maneb, etc.) and is the more promising indicator of exposure for biological monitoring.

#### *5.1.1. Unchanged compounds and metabolites*

*5.1.1.1. Carbon disulfide ($CS_2$).* Table 14 describes the analytical procedure employed in the measurement of $CS_2$ in blood [94,95]. The same method has

Table 12
Analytical procedures for the determination of various compounds in urine

| | [82] | [84] | [87] |
|---|---|---|---|
| Analytes | GLY[a], AMPA[a] | DEA, HEEA | DEA |
| Sample volume | 1 ml | 25 ml | 2 ml |
| Hydrolysis | – | Methanol and NaOH (30 min at 150 °C)[b] | Methanol and NaOH (30 min at 150 °C)[b] |
| Analyte isolation | SPE SAX | Extraction with dichloromethane | SPE $C_{18}$ |
| Derivatization | TFE[a] and TFAA[a] (1 h at 100 °C) | – | – |
| Purification | – | Addition of isooctane and re-extraction with HCl | – |
| Apparatus | GC–ECD and GLC–MS | RP HPLC–Electrochemical detector | LC–MS–MS PCI ($m/z$ 150 molecular ion and $m/z$ 105 daughter ion) |
| Column | 25 m×0.25 mm | Zorbax $C_8$ (15 cm×4.6 mm) | ODS-3 (4.6 mm×25 cm) |
| Programme of temperature or elution | 50–280 °C | Na acetate–methanol, pH 4.8 | Water–methanol–0.1% acetic acid |
| Injection temperature | Not reported | – | – |
| Detector temperature | 330 °C | – | – |
| Injection volume | Not reported | 50 μl | 50 μl |
| Injection type | Not reported | – | – |
| I.S. | – | – | – |
| LOD (μg/l) | 1 for GLY and 0.5 for AMPA | 5 total (alachlor equivalent) | – |
| Recovery (%) | 94 from water | 85–86 for DEA 74–87 for HEEA | 75 |
| RSD (%) | – | 8.1–14.3 for DEA 5.5–11.6 for HEEA | – |

[a] GLY, glyphosate; AMPA, aminomethylphosphonic acid; TFE, trifluoroethanol; TFAA, trifluoroacetic anhydride.
[b] Base-pressure hydrolysis of alachlor metabolites to DEA and HEEA.

subsequently been used in the analysis of the metabolite in urine [96].

*5.1.1.2. 2-Thiazolidinethione-4-carboxylic acid (TTCA).* $CS_2$ is metabolised to TTCA by addition to the cysteinyl-SH group of glutathione and subsequent ring condensation [97]. Consumption of brassica vegetables [98] is a non-negligible source of urinary TTCA. The pesticide captan may also produce TTCA during metabolisation [99]. TTCA is a well known marker for biological monitoring of exposure to $CS_2$ (ACGIH BEI 5 mg/g creat.) [11], and the WHO has also suggested this indicator for monitoring exposure to DTC [100].

HPLC and GC–MS are the analytical techniques. The three procedures developed for assessment of exposure to $CS_2$, alkylenebisdithiocarbamates and captan are compared in Table 14.

Table 13
Analytical procedures for the determination of paraquat and diquat in biological samples

| | [91] | [92] | [89] | [90] |
|---|---|---|---|---|
| Analytes | Diquat, paraquat | Diquat, paraquat[a] | Diquat, paraquat | Diquat, paraquat |
| Sample | Serum, urine | Urine | Serum, urine | Blood, urine |
| Sample volume | 1 ml | 4 ml | 50 μl | 1 ml |
| Sample preparation | Deproteinization with sulfosalicylic acid for serum | – | – | Deproteinization with $HClO_4$–trichloroacetic acid for blood |
| Analyte isolation | – | Ion-pair (bromothymol) extraction with dichloromethane Extraction of organic phase with saturated NaCl solution | Gel filtration chromatography (TSK precomn PW, 3.5 cm 4.6 mm) with $NaClO_4$–$NaH_2PO_4$(pH3) | Precipitation of reinechate[c] complexes of paraquat and diquat |
| Reduction | Dithionite and NaOH to form free radical | Dithionite and NaOH to form free radical | – | $NaBH_3$–$NiCl_2$ to form perhydrogenated products |
| Derivatization | – | – | – | – |
| Purification | – | – | – | – |
| Apparatus | UV–Vis spectrophotometer/ second derivative (396–403 nm for paraquat, 454–464 nm for diquat) | UV–Vis spectrophotometer | HPLC–UV (290 nm) | GLC–HFID |
| Column | – | – | Ion exchange chromatography (TSK gel SP-2 SW 25 cm, 4.6 mm)[b] | Glass column packed with 5% potassium hydroxide + 5% Apiezon L on Chromosorb W AW DMCS |
| Programme of temperature or elution | – | – | $NaH_2PO_4$(pH3)/ acetonitrile | Isothermal (205 °C) |
| Injection temperature | – | – | – | 250 °C |
| Detector temperature | – | – | – | |
| Injection volume | – | – | – | 1 μl |
| Injection type | – | – | – | |
| I.S. | – | – | – | Xanthene |
| LOD (μg/l) | 500 for serum 250 for urine | 30 for paraquat | 100 for paraquat and diquat | 1000 |
| Recovery (%) | – | 70±3.4 for paraquat | 96.7–106.5 for paraquat and diquat in serum and urine | 50–91 for blood 52–97 for urine |
| RSD (%) | <5 within day and day-to-day | – | 0.64–3.13 | 2.7–13 for blood 3.7–16.5 for urine |

[a] The method is suitable for quantitative work with paraquat but not with diquat: it can be used to obtain qualitative information on diquat in urine.
[b] Automated pretreatment apparatus connected to ion-exchange HPLC using a column switching method.
[c] Ammonium tetrathiocyanodiammonochromate.

Table 14
Analytical procedures for the determination of $CS_2$ and TTCA in biological samples

| | [94] | [148] | [149] | [99] | [99] |
|---|---|---|---|---|---|
| Analytes | $CS_2$ | TTCA | TTCA | TTCA | TTCA |
| Sample | Blood | Human urine | Human urine | Rat urine | Human urine |
| Sample volume | 3 ml for free $CS_2$<br>1 ml for total $CS_2$ | 2 ml | 5 ml | 2 ml | 5 ml |
| Hydrolysis | HCL 1% (1 h 100 °C) | – | – | – | – |
| Analyte isolation | Dynamic head space and concentration on cryogenic trap (tenax) | Extraction with ether after addition of HCl | Extraction with ether after addition of HCl | Extraction with ethylacetate after addition of HCl | Extraction with ethylacetate after addition of HCl |
| Derivatization | – | – | Diazoethane (12 h room temperature) | – | Diazomethane (1 h room temperature) |
| Purification | – | – | – | – | – |
| Apparatus | GC–MS SIM ($m/z$ 76 and 78) | HPLC–UV (280 nm) | GC–MS SIM ($m/z$ 191 and 146 for TTCA and $m/z$ 211 and 142 for I.S.) | HPLC–UV (273 nm)[b] | GC–FPD |
| Column | 5% Phenyl-methylsilicone<br>50 m×0.32 mm×0.17 μm | μ-Bonda-Pac C25<br>25 cm×4.3 mm | 35% Phenyl methylpolysiloxane<br>60 m×0.22 mm×0.25 μm | RP-18 Lichrosorb<br>15 cm×4.6 mm×5 μm | OV-1701<br>25 m×0.32 mm×0.29 μm |
| Programme of temperature or elution | 35–100 °C | Acetic acid 2%–methanol after addition of HCl | 100–250 °C | Acetic acid 2% in water | 140–240 °C |
| Injection temperature | – | – | 260 °C | – | 290 °C |
| Detector temperature | – | – | 300 °C | – | 250 °C |
| Injection volume | – | 10 μl | 1 μl | 10 μl | 2 μl |
| Injection type | – | – | Splitless | – | – |
| I.S. | – | – | MCPBA[a] | – | E-DCP-MA |
| LOD | 0.025 μg/l | 1 μ*M* | 0.7 μg/l | 1000 μg/l | 110 μg/l |
| Recovery (%) | – | – | 95 | 97 | 97 |
| RSD (%) | <20 | – | 4.3 within-series<br>3.3–7.8 between day | – | 10.1 |

[a] MCPBA, 4-(4-chloro-2-methylphenoxy)butanoic acid; E-DCP-MA, *N*-acetyl-S-(E-3-chloropropenyl-2-)-L-cysteine.
[b] Identification by GC–MS EI SIM ($m/z$ 132 and 191) after derivatization as for human urine (LOD=234 μg/l).

*5.1.1.3. Ethylenethiourea (imidazolidin-2-thione, ETU).* Besides being an environmental, animal and human metabolite of EBDCs, ETU is an impurity of EBDC formulations [101,102]. It is also used in vulcanisation of rubber. NIOSH classifies ETU as carcinogenic for humans; OSHA classifies it as suspected carcinogenic, as does IARC [103,104]. Urinary excretion of ETU after exposure to EBDCs is relatively slow: it begins 6 h after oral administration in rats and peaks after 24 h; 52 and 86% of the total dose are excreted in urine after 24 and 48 h, respectively. Low levels of ETU are detected in urine within 15 days of administration [105].

Most analytical procedures involve HPLC, only a few GC with FPD detection [105]. The HPLC methods and their reliability characteristics are summarised in Table 15.

HPLC of purified urine extracts [106] is the

Table 15
Analytical procedures for the determination of ETU in urine by HPLC

| | [150] | [151] | [152] | [109] |
|---|---|---|---|---|
| Sample volume (ml) | 10 | 10 | 1 | 18 |
| Sample preparation | – | Evaporation to dryness | – | Addition of $NH_4Cl$ and KF |
| Analyte isolation | Extraction with ethanol–chloroform after absorption on Gas-Chrom S and alumine | Addition of methanol and silica gel, transfer to aluminum oxide column and elution with methanol–dichloromethane | Extraction with dichloromethane | Extraction on Extrelut with dichloromethane |
| Purification | – | – | – | SPE (silica) |
| Column | Zorbax ODS (25 cm×4.6 mm) | Hypersil 5 ODS (25 cm×4.6 mm×5 μm) | Spherisorb ODS (25 cm×4.6 mm×5 μm) | Supelco ABZ (15 cm×4.6 mm×5 μm) |
| Column temperature | 28 °C | 40 °C | 25 °C | Room |
| Mobile phase | Acetonitrile–buffer | Methanol–buffer | Acetonitrile–buffer | Buffer |
| Flow (ml/min) | 1.0 | 1.0 | 0.8 | 0.8 |
| Injection volume (μl) | 30 | 25 | 5 | 10 |
| Detector | Electrochemical | DAD (230 nm) | DAD (235 nm) | DAD (232 nm) |
| I.S. | – | – | – | – |
| LOD (μg/l) | 25 | 0.2[a] | 900 | 1.0 |
| Recovery (%) | 91 (25–100 μg/l) | 87–96 | >61.5 | 91.1±8.9 (6.9 μg/l) |
| RSD (%) | 7–8 (25–100 μg/l) | Not reported | Not reported | 9.8 (6.9 μg/l) |

[a] The same LOD was obtained using TSP-MS (thermospray mass spectrometry) ($m/z=103$) [111].

technique of choice for assessment of occupational [7,107,108] and non-occupational [109,110] exposure. The procedure that involves HPLC–TSP-MS [111] is quite sensitive and selective, but is too expensive for routine analyses.

Biological exposure limits for ETU are not available. Since ETU is detectable in urine of the general population [109,110], results of biological monitoring should be compared with those of adequate reference groups or with baseline levels of the monitored workers.

## 6. Other pesticides

### *6.1. Unchanged compounds and metabolites*

#### *6.1.1. Tetrahydrophthalimide (THPI) (captan metabolite)*

In mammals captan is primarily metabolised to thiophosgene and tetrahydrophthalimide (THPI) which are excreted in urine [99]. Thiophosgene is conjugated with glutathione (GSH) and excreted as 2-thiazolidinethione-4-carboxylic acid (TTCA), after enzyme degradation and ring closure. The analytical procedures for analysis of THPI in urine are summarized in Table 16; methods for TTCA are discussed in the DTC Section.

THPI may be used as a quite sensitive indicator of exposure to captan, but not enough data is available for its use as a biological indicator of dose.

#### *6.1.2. 4-Chloro-o-toluidine (CT) (chlordimeform metabolite)*

Chlordimeform is an acaricide insecticide. Occupational exposure has been assessed by measurement of the unchanged compound and CT in urine (that accounts for 70–90% of chlordimeform excretion products). These two compounds increase rapidly in urine of spray operators with a peak 4–6 h after exposure [112]. The amount excreted gradually increases during 3 days of exposure and begins to

Table 16
Analytical procedures for the determination of various compounds in urine

| | [153] | [154] | [154] | [99] |
|---|---|---|---|---|
| Analytes | CT | THPI | THPI | THPI |
| Sample volume | 5 ml | 5 ml | 0.5 ml | 5–10 ml |
| Hydrolysis | NaOH 1 h at 95 °C | – | – | – |
| Analyte isolation | Extraction with *n*-hexane | Extraction with ethylacetate | Extraction with ethylacetate | Extraction with dichloromethane |
| Derivatization | – | – | – | – |
| Purification | – | – | – | Silica gel and acid–base clean-up |
| Apparatus | RP HPLC–UV (254 nm) | GC–MS PCI (*m/z* 152 for THPI and *m/z* 180 for I.S.) | GC–NPD | GC–electrolytic conductivity detector |
| Column | Nova Pak $C_{18}$ 15 m×3.2 mm×5 μm | Bester QC2/BP20 25 m×0.22 mm×0.25 μm | 25-AQ2/BP20 25 m×0.22 mm×0.25 μm | Not reported |
| Programme of temperature or elution | Acetonitrile–0.1 M $NH_4$ acetate | 100–250 °C | 100–240 °C | Not reported |
| Injection temperature | – | 275 °C | 275 °C | Not reported |
| Detector temperature | – | 250 °C | 300 °C | Not reported |
| Injection volume | 25 μl | 5 μl | 1 μl | Not reported |
| Injection type | – | – | Splitless | Not reported |
| I.S. | – | Phenacetin | Phenacetin | |
| LOD (μg/l) | 20 | 2.7 | 79 | 5.0 |
| Recovery (%) | 83–85 | 54 | – | 78±5 |
| RSD (%) | – | 9.5 at 5.4 μg/l | – | – |

decrease immediately after the end of exposure, going back to the pre-exposure level within 5 days. Total urinary excretion of both compounds is correlated with dermal exposure and can be used as biological indicator of exposure [112]. Table 16 shows the analytical method.

*6.1.2.1. Pentachlorophenol (PCP)*

PCP concentrations in blood and urine have been proposed as indices to monitor occupational exposure. Biological exposure indices (BEI) for PCP have been recommended by ACGIH [11] and biological tolerance values by the DFG [12]. PCP only occurs in urine as a consequence of exposure to the compound. The adsorbed dose is excreted largely unmodified (86%): 74% free, 12% conjugated with glucuronic acid [113].

Several methods are available for assay of PCP in urine (Table 17) and blood. The Renner method [114] has been used to assess PCP and tetrachlorohydroquinone (a major metabolite of PCP) excretion in rats treated subacutely. The procedure proposed by Treble [115] has been used to monitor the general population using 24 h urine samples: 94% of samples were positive, with a maximum concentration of 3.6 μg/l. The Shafik method [44] was used in NHANES II: PCP was detected in 71.6% of samples and the maximum concentration was 2670 μg/l [45]. The procedure of Hill et al. [42] was used in NHANES III: 64% of samples were positive, concentrations never exceeded 29 μg/g creat., and 95% of the detected values were under 5.4 μg/g creat. [46].

Two standard operating procedures have been developed by Deutsche Forschungsgemeinschaft

Table 17
Analytical procedures for the determination of pentachlorophenol (PCP) in urine

| | [44] | [155] | [115] | [42] | [80] | [114] | [116] | [117] |
|---|---|---|---|---|---|---|---|---|
| Analytes | PCP | PCP | PCP | PCP | PCP | PCP, TCH[a] | PCP | PCP |
| Sample volume | 1–5 ml | 2.5 ml | 10 ml | 10 ml | 10 ml | 20 ml | 2 ml | 2 ml |
| Hydrolysis | HCl 1 h reflux | $H_2SO_4$ | $H_2SO_4$ | β-Glucuronidase | $H_2SO_4$ | HCl 1 h 100 °C | HCl 0.5 h 95 °C | $H_2SO_4$ 1 h 80 °C |
| Analyte isolation | Extraction with ether | Extraction with *n*-hexane | Extraction with petroleum ether | Extraction with 1-chlorobutane–ether | Extraction with benzene | Extraction with ether on Extrelut | Extraction with *n*-hexane | Extraction with toluene |
| Derivatization | Diazoethane (15 min) | – | Diazomethane | 1-Cl-3-IP[a] | Diazoethane | – | Acetic anhydride in *n*-hexane– | Diazomethane |
| Purification | Silica gel | – | – | SPE (silica) | Silica gel | – | 0.1 M $K_2CO_3$ | – |
| Apparatus | GC–ECD | GC–MS SIM (*m*/*z* 266) | GC–MS SIM (*m*/*z* 263, 265, 278, 280 for PCP; 288, 290 for I.S.) | HRGC–MS–MS in PCI ($CH_4$) | HRGC–MS–MS PCI ($CH_4$) | GC–ECD for PCP HPLC–UV (303–370 nm) for TCH | HRGC–MS SIM (*m*/*z* 264, 308 for PCP; 274 for I.S.) | GC–ECD |
| Column | Glass, packed with 4% SE-30/6% QF-1 on Chromosorb W | SE-54 25 m×0.30 mm ×0.15 μm | DB-5MS 15 m×0.25 mm ×0.25 μm | DB-5 30 m×0.25 mm ×0.25 μm | DB-5 30 m×0.25 mm ×0.25 μm | 5% Phenyl-methyl silicon (GC) and RP-8 (HPLC) | 94% Methyl silicon 25 m× 0.25 mm×0.25 μm | DB-1 30 m×0.32 mm ×0.25 μm |
| Programme of temperature or elution | 175 °C | 60–240 °C | 120–300 °C | 80–260 °C | 70–220 °C | 38–160 °C (GC) methanol–HCl (HPLC) | 100–290 | 170–220 |
| Injection temperature | 210 °C | 240 °C | Not reported | Not reported | 250 °C | Not reported | 250 °C | 250 °C |
| Detector temperature | 210 °C | 230 °C | 200 °C | Not reported | 250 °C | 260 °C | 280 °C | 300 °C |
| Injection volume | Not reported | 2 μl | Not reported | Not reported | 1–2 μl | Not reported | 2 μl | 1 μl |
| Injection type | Not reported | Not reported | Splitless | Not reported | Splitless | Not reported | Splitless | Split |
| I.S. | – | – | $^{13}C_6$ PCP (isotopic dilution) | $^{13}C$ PCP (isotopic dilution) | $^{13}C$ PCP (isotopic dilution) | Not reported | $^{13}C_6$ PCP (isotopic dilution) | 2,4,6-Tribromophenol |
| LOD (μg/l) | 10 | – | 0.05 | 1 | 1 | Not reported | 1 | 1 |
| Recovery (%) | 92–96 | – | – | Accuracy 1% | 164 | Not reported | 95–115 | 93.2 |
| RSD (%) | – | – | – | 24[c] | 30.4 | Not reported | 2.5[b]–9.3[c] | 7.6[b]/13.1[c] |

[a] TCH, tetrachlorohydroquinone; 1-Cl-3-IP, 1-chloro-3-iodopropane.
[b] Within-series.
[c] Between-day at a concentration of 10.0 μg/l.

[116,117]. These validated methods can be also used for serum/plasma samples after deproteinisation with $NaHSO_4$ [116] or acidification with nitric acid [117]; the LOD is 2 μg/l.

### *6.1.2.2. Dinitro-o-cresol (DNOC)*

The Shafik procedure [44], already described in the PCP section, has been employed to monitor DNOC in urine. LOD was 50 μg/l and recovery 86–96%.

### *6.1.2.3. Chlorotriazine*

The most representative compound in this group is atrazine. Since atrazine metabolism gives rise to bidealkylated (80%), deisopropylated (10%) and deethylated metabolites, intact compound and metabolites can be detected in body fluids of exposed subjects [118,119]. GC–MS [118], GC–NPD [119] and HPLC methods [120] are usually employed. An enzyme-linked immunosorbent assay with a LOD of 0.1 μg/l for atrazine thioether conjugates in human

urine was proposed by Lucas [121]. In urine samples of sprayers, the mercapturic acid conjugate of atrazine was found to be the major urinary metabolite, having concentrations at least ten times higher than those of dealkylated products and the parent compound.

Since other chlorotriazines (simazine, propazine, terbutylazine) follow the same metabolic pathway as atrazine, urinary excretion of bidealkylated, deisopropylated and deethylated metabolites are not compound-specific. The unmodified compound in urine represents only a minor portion of the absorbed dose, however its determination may be useful for qualitative confirmation of exposure.

## 7. Sampling and storage

### *7.1. Blood*

In occupationally exposed subjects, samples for measuring biological indicators should be at the end of exposure. Since a significant number of compounds (e.g. organochlorines) may be present in the general 'unexposed' population, pre-exposure sampling is recommended for comparison with postexposure levels.

### *7.2. Urine*

If analysis is aimed at assessing the total absorbed dose of pesticide, it is advisable to collect a 24-h urine sample (in a single container) or to carry out intermittent sampling throughout the day. An alternative strategy is to obtain spot samples. In this case the right time has to be determined according to the specific kinetics of the biological marker and its urinary excretion speed (Table 18). For monitoring exposure to compounds characterised by slow absorption and excretion, 24–48 h sampling of urine may be needed, starting from the beginning of exposure (azinphos-methyl, chlorpyrifos, phorate, ethylenethiourea, pyrethroids).

Table 18
Relationship between half-life and sampling strategy [156]

| Half-life (h) | Sampling time |
|---|---|
| $<2$ | Too short for sampling |
| 2–10 | End of shift or beginning of next shift |
| 10–100 | End of shift, end of work-week |
| $>100$ | Time of sampling not critical |

Exposure of agricultural workers is mainly dermal, thus absorption is quite slow and steady over time. In such cases, collection of a single sample of urine at the end of the work-shift may not be significant. A 24-h sample or intermittent collection of different samples (during the work-shift, and from the end of the work-shift to the beginning of the work-shift next day) has been performed in different studies [7,27–31,78]. It was found advisable to continue collection of samples from the same subject for a definite period, based on the half-life of the substance. The number of samples to be collected should be determined according to the final use of the data and the required level of statistical reliability, etc. With regard to field exposure, variability may be more accurately assessed by increasing the number of subjects instead of repeatedly monitoring the same subjects.

In any case, but particularly when biological monitoring is not performed on the first day of exposure, a baseline spot sample of urine before the work-shift is advisable.

In acute poisonings, urine sampling (usually 24 h) should be performed for a few days, or at least until urinary levels of metabolites have returned to reference values.

When using spot urine samples, creatinine or specific mass should also be determined, in order to normalise results for concentration and rule out overdiluted or overconcentrated samples. For 24-h samples and 1-day intermittent samples (taken at scheduled times), the total volume of urine excreted should be measured to permit the absolute quantity of metabolites in the sample to be determined.

Urine should be placed in plastic containers shielded with aluminium foil to prevent light-induced breakdown of metabolites. Ethylenethiourea is transformed into ethyleneurea when exposed to light or in the presence of specific activators, such as chlorophyll and organic solvents [122].

If not specifically required by the procedures, use of preservatives or stabilisers should be avoided. Some authors [64] report addition of hydrochloric acid (1 ml in 100 ml urine) for measurement of

Table 19
Recommended biological limits

| Compound | Biological indicator | BEIs[a] | BATs[b] | HBBLs[c] |
|---|---|---|---|---|
| Acetylcholinesterase-inhibiting pesticides | AChE | 70% of individual's baseline | 70% of reference value | 70% of reference value |
| DNOC (dinitro *o*-cresol) | DNOC in blood | – | – | 20 mg/l |
| Lindane (HCH) | HCH in blood<br>HCH in plasma/serum | –<br>– | 0.02<br>0.025 | 0.02 mg/l<br>– |
| Parathion | *p*-Nitrophenol in urine<br>AChE | 0.5 mg/g creat.<br>70% of individual's baseline | 0.5 mg/l<br>70% of reference value | –<br>– |
| Pentachlorophenol (PCP) | PCP (total in urine)<br>PCP (free in plasma) | 2 mg/g creat<br>5 mg/l | –<br>– | –<br>– |
| Arsenic elemental and soluble inorganic compounds | Inorganic arsenic plus methylated metabolites in urine | 35 μg As/l | | |

[a] BEI, biological exposure index [11].
[b] BAT, biological tolerance value [12].
[c] HBBL, health-based biological limit [13].

DBVA. If analysis is not carried out immediately, samples should be frozen and stored frozen. In these cases, it is strongly suggested to consider sample stability. Some authors [123] report that alkylphosphates are quite stable in frozen urine for at least 20 weeks. Other authors [124] report that DMP is stable in urine stored at 4 °C for 6 months. In monitoring urinary TCP, degradation has not been observed in samples within 40 days of storage at −18° [40]. TCP added to urine samples and stored at 0 °C showed a good stability for 12 weeks [41]. A stability study showed that conjugated IPP remains stable for at least 6 months if urine is stored at −20 °C [59]. In experiments carried out to assess stability, concentrations of 3-PBA did not significantly vary during 3 months of storage at −18 °C. In monitoring urinary ETU, a stability study proved that after 350 days of storage at −18 °C no significant degradation took place [109].

## 8. Interpretation of the results

The milestone in the interpretation of biological monitoring data is a good knowledge of dose–response and dose–effect relationships. For most active ingredients this knowledge is lacking, and biological indices of exposure are available for very few compounds (see Table 19). Under these conditions, evaluation of potential health risk is very difficult. However, biological monitoring may be useful to assess absorbed dose which may be done by comparison with pre-exposure levels or reference values of the general population (biological reference values). Since these values are the result of background environmental exposure, in preventive strategies they become target values to aim for to minimise additional risk caused by occupational exposure. Moreover, in studies aimed at assessing the efficacy of personal protection, comparison with reference or pre-exposure values makes it possible to evaluate whether absorption of xenobiotics into the organism is still occurring [7,27–31,78]. This information is particularly useful in cases of exposure to active ingredients, the long-term toxicity of which is known, or strongly suspected.

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