Biological Monitoring of Agricultural Workers Exposed to Pesticides: II. Monitoring of Intact Pesticides and Their Metabolites

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Analytical methods have been developed for the detection of a variety of compounds that are found intact or as metabolites in biological samples from workers exposed to pesticides. Such tests are used primarily in research settings to describe patterns of absorption, metabolism, and excretion, to derive exposure limits for occupational exposure, to evaluate the adequacy of these limits and of work practices in field settings, and to confirm the etiology of poisonings for medicolegal purposes. We review here methods used in studies of occupational pesticide exposure, with particular attention to validation in terms of dose-response relationships, to technical complexity and cost, to the requirements for analytical quality control, and to the utility of these methods for field research purposes. Biological monitoring for intact pesticides or metabolites in agricultural workers is limited to a few chemicals, notably, pentachlorophenol, methyl bromide, and chlordimeform. These programs and their use in regulation and enforcement are described.

Biological tests for intact pesticides and their metabolites are used primarily in research settings to establish patterns of absorption, metabolism, and excretion for specific compounds; to derive exposure limits, including permissible exposure levels (PELs) and field reentry intervals; and to evaluate the adequacy of these limits and of work practices and protective equipment. In the clinical setting, these tests are used infrequently to confirm the cause of acute poisonings for medicolegal purposes. Methods of biological testing do not exist for all currently registered compounds, and descriptions of the analytical procedures and results of biological testing for some compounds are held as trade secret information by the Environmental Protection Agency (EPA).

The California Department of Food and Agriculture (CDFA) now requires manufacturers seeking to register new products for use in agriculture to determine a urinary metabolite that can be used in monitoring before registration is granted, and is requiring the same for certain compounds already in use but of renewed concern, including Difolitan, chlorothalonil, bromoxymil, and oxadiazon. These will largely replace the current air, dermal patch, and leaf biopsy tests for estimations of human exposure in field-work situations.

Analytical methods have been developed for the detection of a variety of compounds found intact or as metabolites in biological samples from workers exposed to pesticides. Many of the compounds detected may be classified into one of several broad categories (alkyl phosphates, phenols, anilines, etc) permitting the use of a few basic techniques to monitor a large number of pesticides. Indeed, it is more convenient to examine the analytical methods for some pesticides by classes of metabolites than parent compounds. In some cases, such as the chlorophenols, the same compound is found both as the intact residue of the pesticide and as the metabolite of other pesticides.

We review here methods that have been used by other investigators studying the effects of occupational pesticide exposure. Whenever possible, the use of a

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particular analysis in field studies has been documented, giving an indication of the flexibility of that method. Certain methods (notably those for detecting metabolites from exposure to organophosphorous insecticides) have been validated in terms of dose-response relationships, allowing the assessment of biological effects from metabolite residues in blood or urine samples. Some have been investigated for requirements needed to assure sampling and analytical quality control. It may be noted that gas chromatography is now being replaced by liquid chromatography for measurement of many water-soluble or solvent-soluble biological molecules, because this eliminates the need to convert the compound into volatile derivatives for gas partitioning.

The most recent compendium of methods for detecting pesticides and metabolites in biological samples is the *Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples*, produced in 1980 by the EPA Health Effects Research laboratory (EPA-600/8-80-038).[1] This volume provides information on sampling, general information on laboratory operation, sample preparation, and analytical instrumentation. It is an excellent resource for those initiating a biological monitoring program for pesticide exposure. This review will also incorporate methods not discussed in the EPA document and will provide a compilation of exposure studies applying the different methods. A further compendium of analytic methods developed and used in Europe, *Field Surveys of Exposure to Pesticides: Standard Protocol*, was also published by the Division of Vector Biology and Control of the World Health Organization in 1982.

Organophosphate Compounds: Alkyl Phosphate and Phenolic Metabolites

The best developed data on biological monitoring of intact pesticides and their metabolites among occupationally exposed persons are for organophosphate insecticides. Exposures to organophosphates are commonly measured in field research by the determination of alkyl phosphate or phenolic metabolites excreted in the urine. Generally, detection of alkyl phosphates serves as a better screening technique for exposure, because the degradation of most organophosphate pesticides produces only six alkyl phosphate metabolites. Detection of urinary phenolic metabolites allows identification of the particular pesticide to which a person has been exposed, but because each organophosphate gives rise to a different phenol, a multiphenol screening technique is probably not feasible.[2]

For several years, the basic method for determination of alkyl phosphates was one developed by Shafik et al in 1973.[3] This method can detect the alkyl phosphates dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate (DETP), dimethyldithiophosphate (DMDTP), and diethyldithiophosphate (DEDTP). Residues of DMP and DEP are directly attributable to pesticide exposure; DMDTP and DEDTP residues are less directly associated with pesticide exposure and rapidly degrade to other alkyl phosphate metabolites, whereas nonpesticide sources may confound the interpretation of DMTP and DETP residues. Although the metabolic residues are known for many organophosphates, it is difficult to monitor exposure to a single compound under field conditions because most applicators and many field workers handle more than one organophosphate at a time, and because many separate compounds share the same metabolic end points. In the case of single exposure under field research conditions, however, there is a strong dose-response relationship between exposure and urinary excretion, making urinary metabolites a useful method for the estimation of total exposure.[4]

The analysis involves solvent extraction of the acidified urine sample, conversion of the alkyl phosphates to volatile derivatives using diazopentane, fractionation, and cleanup of the extract with a silica gel column, then gas chromatographic (GC) analysis. Refinements of this method have been developed by Lores and Bradway[5] and Blair and Roderick.[6] Lores and Bradway used an anion-exchange resin to extract the alkyl phosphates from urine, desorption of the residues from the resin with solvent and acid, then derivatization, column cleanup, and GC analysis as before. Advantages of this method included better removal of analytical interferences, a faster analysis (increasing its suitability as a screening technique), and the ability to quantitate phosphonate metabolites of leptophos and EPN. Blair and Roderick used calcium hydroxide to precipitate inorganic phosphate in urine, which interfered with the determination of DMP.

These methods have enjoyed wide application in studies of occupational pesticide exposure (Table). The alkyl phosphate and phenolic screens have been correlated with the effects of exposure to organophosphorus pesticides in some studies.[7,8] The storage stability of both chlorinated phenols and alkyl phosphates in urine has also been investigated.[9,10] A number of drawbacks have been identified in the use of these methods, however. The preparation of the diazopentane reagent requires the use of N-amyl-N′-nitro-N-nitroso-guanidine, which presents potential carcinogenic and explosive hazards to the analyst. Also, derivatization of DMTP or DETP produces two isomeric products; because it is uncertain if the ratio between these is constant, the detection limit for these metabolites is higher than if one product were formed. In the 1980s, alkyl phosphate methods were developed which involved "extractive alkylation," where pentafluorobenzyl bromide (the alkylating reagent) is added to a solvent extract of the urine sample. Analysis is performed by GC.[11–13]

Because organophosphates are hydrolyzed rapidly (often within hours of absorption), the intact compounds are not measured in blood unless very large quantities have been ingested such as may occur in suicide attempts. Urinary metabolites of organophosphates can be detected for several days after exposure and in association with lesser exposures than those necessary to produce cholinesterase inhibition.[14] Under the relatively controlled conditions of field research, urinary metabo-

TABLE
Methods of Biological Surveillance for Intact Pesticides and Their Residues*

Pesticide	Substance(s) Analyzed	Sample Matrix	Analytical Method	References	
				Monitoring Studies	Laboratory Studies
Organophosphates					
Bromophos	2,5-DC-4-BP	Blood	GC	40	3
Chlorpyrifos	3,5,6,-TCPyr, DEP, DETP	Urine	GC	75–77	3, 5, 34
Diazinon	DEP, DETP	Urine	GC	76, 78, 79	3, 5, 6, 11, 12
Dichlorvos	DMP	Urine	GC	76	5
Dicrotophos	DMP	Blood, urine	GC	77	3, 5
Dimethoate	DMP, DMTP, DMDTP	Urine	GC	78	3
Disulfoton	DEP, DETP, DEDTP	Urine	GC	80	3, 5
EPN	PNP	Urine	GC		34
Ethion	DEP, DETP, DEDTP	Urine	GC	81, 82	12
Guthion	DMP, DMTP, DMDTP	Urine	GC	4, 78	3
Malathion	DMP, DMTP, DMDTP, MCA, DCA	Urine	GC	75, 78	3, 17
Mevinphos	DMP	Urine	GC	75, 78	3
Metasystox-R	DMP, DMTP	Urine	GC	78	3
Methyl Parathion	DMP, DMTP	Urine	GC	78	3
Naled	DMP	Urine	GC	78	3
Parathion	DEP, DETP, PNP	Urine	GC	75, 77, 78, 83–86	3, 5, 6, 36
Phorate	DEP, DETP, DEDTP	Urine	GC	78, 80	3, 5
Phosphamidon	DMP	Urine	GC	78	3
Systox	DEP, DETP	Urine	GC	78	3
Halogenateds and nitrophenols					
DNBP	DNBP	Urine, blood	GC		39
DNOC	DNOC	Urine	GC		35
PCP	PCP		GC, LC	38–40	34, 35, 37, 39, 41, 61
Herbicides					
2,4, D	2,4-D	Urine, blood	GC	68, 87	55, 57–59, 61
2,4,5-T	2,4,5-T	Urine, blood	GC	60, 64	56, 60, 61
TCDD	TCDD	Urine, blood	GC		62
2,4-DB	2,4-DB	Urine	GC		61
2,4-DP	2,4-DP	Urine	GC		61
Dicamba	Dicamba	Urine	GC		55, 61
Picloram	Picloram	Urine	GC		61
Pronamide	Pronamide	Urine	GC		61
Silvex	Silvex	Urine	GC		61
Diuron	3,4-dichloroaniline	Urine	LC		65
Linuron	3,4-dichloroaniline	Urine	LC		65
Propanil	3,4-dichloroaniline	Urine	LC		65
Chlorinated hydrocarbons					
DDT	DDA	Urine	GC	30	28–30
DDT	DDT	Blood	GC	88	25, 89
DDE	DDE	Blood	GC		25
BHC isomers	BHC isomers	Blood	GC		25
Aldrin	Aldrin	Blood	GC		25
Dieldrin	Dieldrin	Blood	GC		25
Heptachlor epoxide	Heptachlor epoxide	Blood	GC		25
Lindane	Lindane, 2,3,5-TCP, 2,4,5-TCP, 2,3,4,5-TCP, 2,3,4,6-TCP	Blood	GC	23, 40	34, 25
Miscellaneous					
Carbaryl	1-Naphthol	Urine, blood	GC, LC	89	16, 17
Captan	THPI	Urine	GC	90	90
Chlorbenzilate	4,4′-DBP	Urine	GC	91	92
Mancozeb	ETU	Urine		71	
Diquat	Diquat	Urine	GC	70	
Paraquat	Paraquat	Urine	GC	70	
Chlordimeform	Chlordimeform, 2-methyl-4-chloroaniline	Urine	GC	66, 67	

* Abbreviations used are: GC, gas chromatography; LC, liquid chromatography; DEP, diethylphosphate; DETP, diethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; DEDTP, diethyldithiophosphate; EPN, phosphonothioic acid, phenyl-, O-ethyl O-(p-nitrophenyl) ester; PNP, p-nitrophenyl; MCA, N-methyl carbamic acid; DCA, N,N-dimethyl carbamic acid; DNOC, 4,6-dinitro-o-cresol; PCP, pentachlorophenol; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; 2,4-DB, 2,4-dichlorophenoxy butanoic acid; 2,4-DP, 2,4-dichlorophenoxy propanoic acid; DDT, dichlorodiphenyltrichloroethane; DDA, dichlorodiphenyl acidic acid; DDE, dichlorodiphenyldichloroethylene; TCP, tetra-chlorophenol; BHC, benzene hexachloride; THPI, tetrahydrophthalimide; ETU, ethylene thiourea.

lites have been used frequently to establish reentry intervals and to evaluate the protective value of clothing and equipment.

Although the measurement of urinary metabolites can be an important and valuable tool, its complexity as a field method should be noted. Sequential urine samples are collected throughout the application and until 24 hours after the end of the sampling day, often with separate containers and determinations of volume and metabolite concentration at each voiding. Thus, the utility of urinary metabolites as a means of biological surveillance is limited by practical considerations, including the difficulty of collecting 24-hour urine samples, the variation in kinetics among compounds, the fact that random sampling will estimate an integrated sum of cumulative exposures over several preceding days, and the expense of gas chromatographic analysis.

Finally, biological screening by colorimetric assays can measure the enzyme neurotoxic esterase. Inhibition of this enzyme by organophosphate pesticides has been associated with peripheral neuropathy in animal models, particularly in the hen.[15] The method has been used in an unpublished field study of agricultural workers exposed to DEF and Folex (C. E. Becker and M. Lotti, January 1982).

Carbamates and Metabolites

Few analytical methods have been adapted to detect carbamate residues in biological samples, in part because most carbamates represent less of a risk for agricultural workers than the organophosphate compounds. Shafik et al[16] detected 1-naphthol, the metabolite from carbaryl, in urine. The method requires solvent extraction and column cleanup following alkali hydrolysis of the residue. In this case, derivatization is required to enhance sensitivity to electron capture detector-gas chromatography (EC-DGC);.[16] Recently, a high-performance liquid chromatography (HPLC) method of determination of carbaryl and 1-naphthol in blood was developed, which requires simple extraction of the hemolyzed sample.[17] A number of biological thresholds for urinary concentrations of 1-naphthol in workers have been estimated, but the relationship between exposure and urinary excretion is not clearly defined.[18,19] Like many other tests for pesticide metabolites, this method has practical application in field studies of application procedures and protective clothing rather than in periodic monitoring of exposed agricultural workers.

Organochlorines

The analytical methods used to detect organochlorine pesticides have been extensively developed and widely used in the EPA's National Human Monitoring Program.[20,21] Most of these compounds are lipid soluble and accumulate in adipose tissue. The pesticides and their metabolites, including dichlorodiphenyltrichloroethane (DDT), chlordane metabolites, dieldrin, aldrin, lindane, and benzene hexachloride (BHC), were detected in serum and adipose tissues from a large proportion of the nonoccupationally exposed population in the United States. Beginning in 1972, DDT and several other organochlorines have been cancelled or severely restricted by the EPA for their effects on other species, and the agricultural uses of most organochlorines are now quite limited.

Although the World Health Organization has developed maximum recommended daily intake for DDT and other organochlorines, occupational exposure thresholds have not been established in most cases because information relating exposure to the chronic health effect(s) of concern is largely lacking. In a few cases, data correlate acute signs and symptoms with environmental exposure or biological (serum, adipose tissue, and urinary) levels, but agricultural exposures to organochlorine compounds in developed countries rarely reach the concentrations required to provoke acute illness.

Monitoring occupational pesticide exposure by measuring levels in adipose tissue is impractical because samples must be taken surgically.[20-23] The determination involves solvent extraction, column cleanup, and GC analysis; the determination in serum is essentially similar.[22-25] Serum levels are rarely used for routine surveillance because serum concentrations for most compounds reflect cumulative exposure over a period of months or years rather than recent exposures. The only recent case of agricultural worker monitoring for organochlorine exposure in California was periodic serum sampling of an applicator exposed to Endrin in a soil fumigation process in the early 1980s.

A recently developed cleanup procedure for chlorinated hydrocarbons in adipose tissue uses automated gel permeation and is reportedly more efficient.[26] Few methods have been developed to measure chlorinated hydrocarbons in urine. DDT is dechlorinated in the human body to dichlorodiphenyldichloroethane (DDD) and is then either metabolized to the water-soluble and water-excretable DDA, or excreted directly as DDD. DDT also degrades to form dichlorodiphenyldichloroethylene (DDE) in the environment, which is ingested and stored. DDA is the major urinary metabolite of DDT in occupationally exposed persons, reflecting recent exposure to DDT and peaking approximately 10 hours after the initial exposure.[27] DDT exposure is assessed by detection of derivatized DDA using GC, and the patterns of DDT absorption, storage, metabolism, and excretion as DDA in the urine have been studied in human subjects.[28-31]

In the EPA National Human Monitoring Program, GC analytic methods detected DDT and its analogs in 99% of serum samples. Approximately 80% of the urine samples contained detectable levels of pentachlorophenol, 2% to 4% contained carbamate pesticide metabolites, and 6% to 12% contained dialkyl phosphate residues.[21] In an analysis of human milk samples from 1,436 women in the US, Savage et al[32] found dieldrin above the limit of detection (1.0 ppb) in more than 80% of the samples collected, chlordane metabolites in 74% of the samples, and heptachlor in 63% of all samples. The mean fat-adjusted residue levels for those samples above

the detection limit were in the 100 ppb range for all three of the residues.

Although body burdens of pesticides have not been assessed in large populations of agricultural workers, rural residents in agricultural areas often have more insecticide exposure than does the general population. Chlorinated hydrocarbon residues in the serum of non-occupationally exposed pregnant women in rural Mississippi and from the umbilical cords of their infants at delivery were comparable to mean levels reported in occupationally exposed chemical company employees, and two to five times higher for both mothers and newborns than the average levels for the total US population.[33]

Of those organochlorine compounds still used in agriculture to a significant extent, lindane, chlorobenzilate, and dicofol are all rapidly excreted in urine. Dicofol may contain as much as 10% DDT, and is under EPA review for potential cancellation for this reason. Hexachlorobenzene, a fungicide, has a much longer biological half-life, and serum levels for this chemical, therefore, would be of little use in monitoring occupational exposure.

Chlorophenols and Nitrophenols (Pesticides and Metabolites)

Detection of chlorophenols and nitrophenols in the blood or urine can be used to assess exposure to intact nitrophenolic herbicides (2,4-dinitrophenol, dinitro-o-cresol, and 2-sec-butyl-4,6-dinitrophenol), pentachlorophenol, other chlorinated phenols, metabolites of lindane, hexachlorobenzene, and organophosphorus insecticides. Most nitrophenol and chlorophenol pesticides are excreted relatively rapidly, and several compounds have been monitored in agricultural workers for field research purposes.

The analytical methods generally involve acid hydrolysis of the phenolic residues, solvent extraction, derivatization with diazomethane, column cleanup with either silica gel or alumina, and GC determination. Some investigators have attempted to develop multiresidue screening methods.[34-40] A possible refinement for determination of chlorinated phenols involves extraction by eluting urine samples through a column of porous polymer resin. The residues are quantitated by GC, using chromatographic techniques allowing the separation of underivatized phenols (eliminating the need for using hazardous diazomethane reagents). The use of HPLC is being investigated as a quantitative tool, also eliminating the need for volatile derivatives.[41]

Dinoseb (dinitro,2-sec-butyl-4,6-dinitrophenol) is a contact herbicide with very high oral and dermal toxicities and minimal skin warning properties; fatal doses have failed to cause significant skin irritation in experimental animals.[42] Worker exposure is, therefore, of considerable concern, and CDFA has studied dermal absorption in field applicators, finding a maximal excretion of intact dinoseb 2 days after the exposure.[43] DNOC (4,6-dinitro-o-cresol) is a related compound with similarly high acute toxicity. Because DNOC is only slowly cleared after absorption, blood levels must be followed to assess occupational exposure. Hayes[42] cites a Hungarian study of chemical plant workers exposed to DNOC who evidenced no symptoms while serum concentrations of DNOC ranged from 1.0 to 8.7 ppm and averaged about 3 ppm. Urine concentrations ranged from 0 to 4.2 ppm and averaged 1 to 2 ppm; he also notes that researchers studying highly exposed workers in the 1950s recommended that workers found to have elevated whole blood or serum concentrations be removed from exposure for at least 6 weeks to prevent further accumulation of DNOC.

Pentachlorophenol (PCP) is widely used as a wood preservative in the lumber industry. It is of concern for the acute hypermetabolic syndrome and the fetotoxic effects associated with PCP itself, and the potential for teratogenic and carcinogenic effects associated with hexachlorodibenzodioxin (HxCDD) and other dioxin and dibenzofuran contaminants of the active ingredient. Although PCP has been commonly stated to be metabolized within 2 to 3 days, based on single oral dosage studies in human volunteers,[44] several studies now suggest that excretion in chronically exposed workers may in fact be much longer than previously supposed. The most recent study followed PCP excretion among workers and continued to detect PCP at 30 to 40 days after exposure had ceased; the subjects returned to potential exposure before nondetectable levels could be reached.[45] This would imply that the timing of specimens for PCP analysis in relation to exposure is less critical than previously assumed, and that single spot specimens as opposed to 24-hour samples may be adequate for some monitoring programs.

The amount of PCP detected by GC analysis after acid hydrolysis of the urine sample is 4 to 6 times greater than that detected without hydrolysis.[46] The increased amount of PCP detected in this way also increases the sensitivity of the method, but may or may not reduce the individual variability of samples; determination after hydrolysis is preferred in order to increase sensitivity, to possibly decrease variability, and to encourage standardization so that levels determined in different laboratories can be compared.

Levels of 3 ppm of total PCP in urine after hydrolysis of the urine sample are probably sufficient for protection from the acute effects of PCP exposure. These criteria are based on a number of surveys of workers who did not manifest symptoms of acute illness.[47-49] There are also a number of case reports of acutely symptomatic workers in which the urinary levels of pentachlorophenol were commonly 10 ppm or greater.[50] The EPA recently concluded an 8-year evaluation of the hazards of wood preservatives with a document that included an estimation for the carcinogenic risk from exposure to PCP contaminated with HxCDD.[51] It is possible to use the average daily dose of PCP which is estimated to yield a carcinogenic risk of 1/100,000—assuming that the PCP is excreted evenly over a period of 24 hours and reflects exposure to HxCDD in the same proportion to PCP as that used in the risk estimations (eg, 1 ppm HxCDD in PCP)—and calculate a urine concentration

of PCP that corresponds to this dose. An unpublished estimation of this level (assuming 1 ppm HxCDD, which is less than that for most current commercially used PCP) is approxiamtely 100 to 150 ppb (J. Rosenberg, July 1984). This level also provides margins of safety against fetotoxic and teratogenic effects of greater than 1,000.

A number of paper manufacturers and other employers have now established biological threshold limits and "action levels" for urine PCP levels to protect against the potential carcinogenic and fetotoxic effects of PCP and its contaminants. Most frequently, these have established levels of 300 to 500 ppb of total chlorophenols as the absolute limit for withdrawal of workers, and 150 ppb as an "action level" to trigger investigation of the work process. The major caveat to be applied in biological monitoring of urinary PCP concentrations is that the urinary concentrations are taken as an index of HxCDD exposure; if the work process allows disproportionate volatilization of PCP before absorption, HxCDD absorption may in fact be significantly greater than estimated. The American Conference of Governmental and Industrial Hygienists is currently considering the adoption of a proposed standard to incorporate the findings summarized here.[51]

Tetrachlorophenol (TCP) has replaced PCP in many lumber industry operations recently. Although the data available on TCP are much fewer than those for PCP, it appears to be contaminated with the same dioxins and dibenzofurans in percentages comparable to those found in PCP.[52] TCP is only slightly less acutely toxic than PCP, as might be predicted from the fact that it has one less chlorine than PCP. It is therefore reasonable to use total chlorophenol levels for the previously cited action levels in monitoring workers exposed to PCP and TCP. TCP is excreted more rapidly than PCP and has a reported half-life of approximately 17 hours, however,[45] suggesting that the timing of urine sampling in relation to TCP exposures may be more crucial than for PCP.

Phenoxy Herbicides

Most phenoxy herbicides are rapidly excreted within a matter of hours or days. Although urinary metabolites of all of the phenoxy acids compounds have been monitored in human exposure studies (Table), the more significant concern in occupational exposure to 2,4,5-T and Silvex has been concomitant contamination with the dioxin TCDD, a highly toxic compound which accumulates in adipose tissue.[53] Use of 2,4,5-T and Silvex was severely restricted by the EPA in 1979, and 2,4-D is now the phenoxy herbicide most widely used in agricultural and forestry. Occupational exposure to 2,4-D can be quite adequately assessed by following urinary concentrations, as more than 95% of the compound absorbed (dermally or by inhalation) is excreted in the urine; the rate of excretion is probably pH-dependent.[54]

Common analytical methods detect the intact phenoxy acids (or their hydrolyzable conjugates) in blood or urine. These analyses consist of acid or alkali hydrolysis of the sample, solvent extraction, conversion of the residue to a volatile derivative, and GC analysis.[55-62] One method for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can be used to detect TCDD residues in fat or blood, and this method has been used to detect TCDD in a single urine sample.[63,64] Recently, a GC multiresidue screen for acid herbicide residues in urine has been developed. This is capable of detecting 2,4-D; 2,4,5-T; 2,4,5-trichlorophenoxy propanoic acid (Silvex); 2,4-dichlorophenoxy propanoic acid (2,4-DP); 2,4-dichlorophenoxy butanoic acid (2,4-DB); dicamba; pronamide; picloram; and pentachlorophenol.[61]

Chlorinated Aniline Metabolites

Several classes of pesticides degrade to chlorinated anilines, including substituted ureas and carbamates. An HPLC method is used to detect urinary 3,4-dichloroaniline, which is a metabolite of linuron, diuron, and propanil. The use of HPLC eliminates the need for column cleanup and derivatization (required for GC).[65]

Chlordimeform is a formamidine that is metabolized to 2-methyl-4-chloroaniline. In 1975, employees in a chlordimeform packaging plant developed symptoms of urinary tract irritation associated with exposure. The two companies distributing chlordimeform products voluntarily ceased production and distribution in 1976, and in the same year chlordimeform was found to cause hemangioendotheliomas in a long-term feeding study in mice.[66,67]

When chlordimeform was reintroduced for cotton cultivation in 1978, monitoring determined that chlordimeform metabolites were measurable in the urine of applicators despite extraordinary measures aimed at eliminating worker exposure. A biological monitoring program for pesticide handlers exposed to chlordimeform involved detection of this metabolite in urine using GC. An earlier study of the health of chlordimeform formulation workers had determined the presence of chlorinated aniline by conversion to and GC determination of total amine in the urine.[67] It has been suggested that for chlorinated phenols and anilines, simply fortifying urine samples does not provide adequate analytical recovery data for conjugated (biologically incorporated) residues.[1,46,65]

In 1982, cotton growers petitioned the CDFA, which had not yet reregistered chlordimeform for use in California, for a Special Local Need Registration based on implementation of an IPM program. A trial application was granted contingent upon an extensive field exposure monitoring program. A total of 180 workers were trained to handle chlordimeform. Subsequently, 130 worked with it and were monitored, and 1,000 urine specimens were submitted for analysis. No chlordimeform metabolites were detected in approximately two thirds of all samples (limit of detection = 0.05 ppm). Of all samples, 97% had less than 0.5 ppm, and average levels ranged from 0.10 to 0.12 ppm. In more specific evaluation of job categories, six mixer/loader/applicators had levels greater than 1 ppm.[68]

Methyl Bromide

Bromides occur naturally in soil and food. Bromide levels of 50 ppm may be considered normal, and persons ingesting large quantities of inorganic bromide medications have maintained blood levels of 700 to 1,000 ppm without apparent harm.[42] Exposures to both inorganic bromide and methyl bromide are measured as the inorganic bromide ion by colorimetric and ion-specific electrode methods. The functional changes produced by methyl bromide are apparently due to the intact molecule, however, rather than to the inorganic bromide ion, and the toxicity associated with bromide ion levels resulting from methyl bromide exposure is markedly greater than for similar levels resulting from inorganic bromide absorption.

Findings reported among workers exposed to methyl bromide have been associated with levels of 4 to 23 ppm (electroencephalographic changes and increases in serum transminase levels), over 50 ppm (mild euphoria), 24 to 550 ppm (nonfatal hospital admissions and outpatient cases), and 250 to 400 ppm (fatal cases), as summarized in Hayes.[42] Alexeeff and Kilgore[69] have also reviewed bromide concentrations related to the severity of symptoms and provide estimates for the half-life of the bromide ion.

Although regular medical surveillance of applicators exposed to methyl bromide is not mandated, the CDFA does require biological monitoring when excessive exposure has been demonstrated or is suspected in a particular operation. The standard protocol requires a baseline bromide level after a period of 30 days without exposure, and subsequent blood levels on a weekly basis for several weeks. If these levels suggest that exposure is well controlled, monitoring may be reduced to once a month. In several cases, symptoms have been observed, together with increases in blood levels, providing a good diagnostic tool and a practical means of identifying problem work practices. Practical methods for the measurement of other fumigants and their metabolites do not exist.

Miscellaneous

Paraquat

Colorimetry and GC of volatile derivatives have been used to measure paraquat.[70] Paraquat is formulated and retailed in the United States by Chevron Chemical Corporation, and analysis of biological samples is provided by the Chevron Environmental Health Center in Richmond in cases of suspected poisoning.

Dithiocarbamates

In general, dithiocarbamates are metabolized and excreted too rapidly to be detected in biological samples. The ethylenebisdithiocarbamate (EBDC) fungicides (nabam, maneb, zineb, metiram, mancozeb, and diamonium EBDC) are metabolized to ethylene thiourea (ETU), however, which can be measured in urine. ETU has been determined to be a teratogen and a carcinogen in laboratory rats with supportive studies in other species, and may be associated with thyroid toxicity as well.[71–73]

Summary

Biological tests for intact pesticides and their metabolites have had little use in the routine biological surveillance of occupationally exposed workers in agriculture. Exceptions to this generalization occur when the continued use of highly toxic compounds is profitable enough to justify the expense and complexity of such biological monitoring programs. These include biological monitoring of workers exposed to pentachlorophenol, to methyl bromide under certain circumstances, and more recently to chlordimeform. Nevertheless, the methods reviewed here for biological determination of pesticides and their metabolites are of increasing importance in field investigations.

Field investigations, as distinguished from routine surveillance, are designed to monitor and assess worker exposure for a number of purposes: in the evaluation of occupational exposure limits and field reentry intervals for specific compounds, in the evaluation of the design of application equipment, and in the evaluation of work practices and protective clothing. Medical supervisors of pesticide applicators in agriculture and agencies charged with the regulation of pesticide use will encounter increasing demands for biological monitoring information in the future. Interpretation of this information is made more difficult by the fact that biological thresholds for most agricultural chemicals (no-effect levels or levels estimated to protect against certain outcomes) have not been established by national agencies for effects other than acute illness.[74] Research is urgently needed on the biological indices of exposure associated with adverse health effects in occupational exposure to pesticides, particularly for neurologic, carcinogenic, and reproductive effects.

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