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Review

Analytical methods for biological monitoring of exposure to pesticides: a review

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Abstract

Synthetic pesticides have been used since in the early to mid twentieth century. In the US alone, over 800 pesticide active ingredients are formulated in about 21 000 different commercial products. Although many public health benefits have been realized by the use of pesticides, their potential impact on the environment and public health is substantial. For risk assessment studies, exposure assessment is an integral component, which has unfortunately, often been weak or missing. In the past several decades, researchers have proposed to fill these missing data gaps using biological monitoring of specific markers related to exposures. In this paper, we present a review of existing analytical methodology for the biological monitoring of exposure to pesticides. We also present a critical assessment of the existing methodology and explore areas in which more research is needed.

Keywords: Reviews; Biological monitoring; Pesticides

Contents

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1. Introduction

Pesticides are broadly defined by the United States' Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as a substance or mixture intended to prevent, destroy, repel, or mitigate any pest including insects, rodents, and weeds [1]. They include not only insecticides but also herbicides, fungicides, disinfectants, and growth regulators. Pesticides have been used in some crude form since early times, but the modern use of synthetic pesticides began in the early to mid twentieth century [1]. Currently, there is a catalogue of over 800 pesticides formulated in 21 000 different products that are registered with the US Environmental Protection Agency (EPA) for use in the United States [2].

Many public health benefits have been realized by the use of synthetic pesticides [1,3]. For instance, the supply of food has become safer and more plentiful and the occurrence of vector borne disease has been dramatically reduced. Despite the obvious benefits of pesticides, their potential impact on the environment and public health is substantial. The most recent US EPA public sales and usage report estimates that over 5.5 billion pounds (1 lb=0.45359 kg) of pesticide active ingredients were applied worldwide in 1997 [2]. In the US, about 75% of the pesticides are used for agricultural purposes with the remaining amount used in residential applications. The EPA estimates that about 85% of US households store and use pesticides for their home [4]. With the widespread use of pesticides, it is virtually impossible to avoid exposure at some level [5].

Although epidemiologic studies have been conducted to determine if any relationship exists between pesticide exposure and disease, many lack integral components of the risk assessment equation. In 1995, noted epidemiologist Roy Shore wrote ''the single greatest weakness of epidemiologic risk assessment is that individual [or population sic] quantitative exposure information is very often limited or missing in occupational and environmental studies'' [6]. In the past several decades, researchers have proposed to fill these missing data gaps using biological monitoring of specific markers related to exposures [7–9].

Biomarkers for monitoring toxicant exposures, including pesticides, are typically divided into three broad categories which are depicted in Fig. 1 [10]. Biomarkers of exposure provide information on the dose of a toxicant which, in turn, can be related to the exposure. Biomarkers of susceptibility indicate the variables that affect an individual's response to a particular toxicant. Biomarkers of effect provide information on an event, usually in the preclinical stage, occurring at a target site after exposure that directly correlates to manifestation of disease. In general, as the biomarker approaches the actual manifestation of disease, data indicating a relationship, or lack thereof, between exposure to a toxicant and development of disease are considered more solid. For this paper, we concentrate on biomarkers of exposure.

Biomarkers of exposure can be further divided into three groups: (1) potential dose or external dose, (2) internal or absorbed dose, and (3) biologically effective dose. Because human exposure to these pesticides is multi-media and multi-route and varies with the usage of pesticides, environmental monitoring of exposure, which determines the potential dose, must account for all media and routes in order to accurately calculate individual exposures. Conversely, biomarkers of internal dose integrate all pathways of exposure by estimating the amount of a pesticide that is absorbed into the body via measurements of the pesticide, its metabolite, or its reaction product in biological media (e.g., urine, blood, saliva, meconium, breast milk, etc.). The biologically effective dose is the amount of a toxicant that has

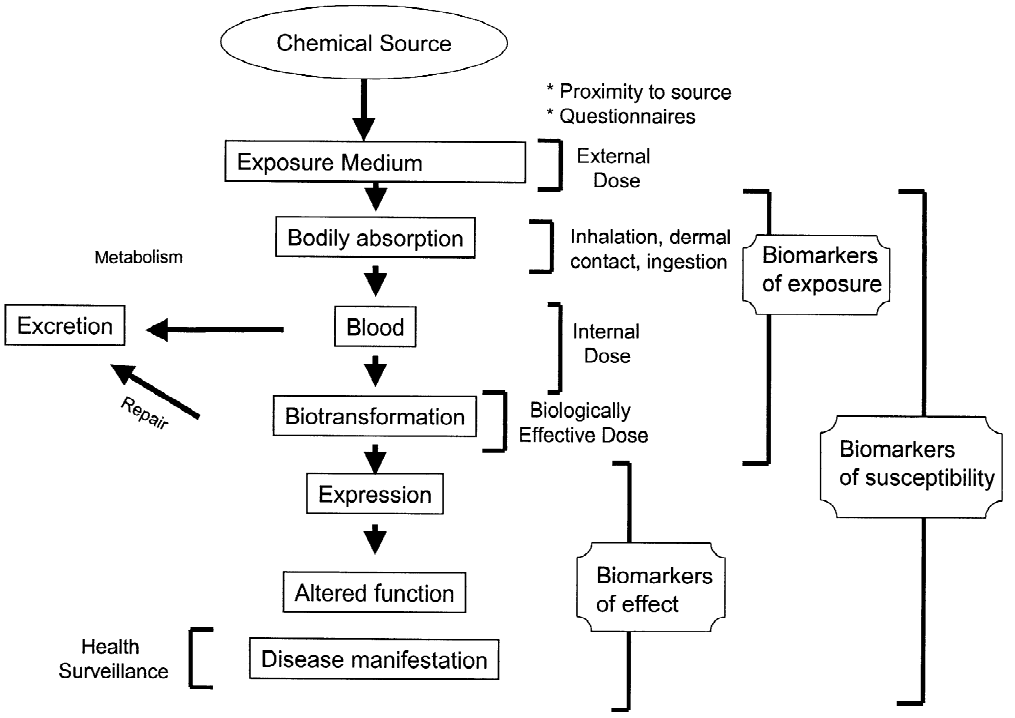

Fig. 1. Schematic representation of the pathway of a toxicant from exposure to induction of health effects.

interacted with a target site and altered a physiological function. An example is a site-specific DNA adduct of a toxicant or inhibited cholinesterase enzymes. These biomarkers may be spontaneously repaired or may lead to the development of disease.

In this paper, we present a review of existing analytical methodology for the biological monitoring of exposure to pesticides. We also present a critical assessment of the existing methodology and explore areas in which more research is needed. In an effort to provide a more concise exploration of existing pesticide biomonitoring methodology that is relevant today, we impose a few limitations on our review. We are only considering methods published no earlier than 1975, because in general, today's affordable technology has surpassed the experimental detail of earlier methods. In addition, as much of the pesticide biomonitoring work involving effective dose measurements is its infancy (adduct measurements) or do not provide sensitive indicators of exposure (as in cholinesterase measurements), we concentrate on internal dose measurements in human matrices.

The methods reviewed, in many instances, are diverse in character. They all use some form of chromatography, but the detection systems range from simple UV absorbance detection to sophisticated mass spectrometric analyses. These methods possess limits of detection (LODs) that span a wide range; some are suitable for only occupational or forensic applications while those with LODs near or lower than the low-μg/l are useful for detecting incidental environmental exposures. In addition, these methods have been used to measure pesticides and/or their metabolites in a variety of matrices including urine, serum, breast milk, saliva, and postpartum meconium.

Pesticides are generally categorized based upon their persistence in the environment. Organochlorine pesticides are considered persistent pesticides. These pesticides have long environmental half-lives and tend to bioaccumulate in humans and other animals and thus biomagnify up to 70 000 times in the food chain [11–17]. Because migratory birds and other animals are at the top of the food chain, they carry these persistent compounds with them wherever they go and are then transferred to the very top of the food chain, humans [18]. Another manner in which

Table 1
Methods for analysis of organochlorine pesticides in human matrices

Method	Analytes[a]	Matrix	Extraction	Analytical system	I.S. type	Recovery[e] (%)	LOD (μg/g)	RSD (%)
Frenzel, 2000 [51]	15	Whole blood	Kieselguhr SPE	GC–MS	None	97	30–40	7
Rohrig, 2000 [35]	1–6	Breast milk	SPME	GC–ECD	None	[b]	Low	[b]
Ward, 2000 [54]	1–10	Serum, breast milk, adipose	SPE	GC–HRMS	Isotope dilution	60–80	0.07–0.26	<20
Najam, 1999 [55]	1–3, 5–12, 13, 17–18	Serum	Solvent extraction, Silica/Florosil cleanup	GC–ECD	Surrogates	39–126	0.15–0.5	7–32
Pauwels, 1999 [36]	[f]	Serum	C_{18} SPE, acid wash	GC–MS GC–ECD	$^{13}C_{12}$ PCB 149	48–140	Low	[b]
Lino, 1998 [37]	[f]	Serum	Florisil SPE	GC–ECD	[f]	>84	1–37 μg/l	<19
Luo, 1997 [38]	1,2	Serum	*n*-Hexane	GC–ECD	[b]	93–106	[b]	[b]
Waliszewski, 1982 [215]	1, 2, 4–6	Adipose	Light petroleum, acid wash	GC–ECD	[b]	91–99	0.01 μg/kg	<10
Brock, 1996 [39]	1–11	Serum	C_{18} SPE, Florosil	GC–ECD	Surrogates	63–80	0.08–0.66 μg/l	0.7–5.9 [c]
Noren, 1996 [56]	$MeSO_2$-DDE	Breast milk	Liquid–gel partitioning, absorption/gel permeation chromatography	GC–HRMS	Surrogates	80–97	0.01–0.05 ng/g lipid	4–14
Gill, 1996 [52]	1–9, 11–13, 15+others	Serum	Solvent extraction/SPE	GC–MS	Surrogates	60–110	[b]	[b]
Guardino, 1996 [40]	1, 2	Blood	C_{18} SPE	GC–ECD; GC–MS confirm	None	[b]	[b]	[b]
Minelli, 1996 [41]	1, 2, 4–6	Serum	Serum–silica suspension, hexane/acetone, alumina cleanup	GC–ECD	[b]	80–99	<1 μg/l	<15
Gallelli, 1995 [216]	[f]	Adipose liver	Light petroleum, Florosil	GC–ECD	[f]	[f]	[f]	[f]
Prapamontol, 1991 [42]	1–2, 4–7, 10–13	Milk	Ethyl acetate/acetone/methanol, C_{18} SPE	GC–ECD	Surrogates	90–110	0.5–2.5 μg/l	≤16
Burse, 1990 [43]	1–3, 5–10, 12	Serum	Hexane/ether, Florosil	GC–ECD	Surrogates	48–122	<1 μg/l	7–23
Saady, 1990 [44]	1–2, 4, 6, 7–10	Serum	C_{18} SPE	GC–ECD	Surrogate	70–75	0.1–0.7 μg/l	4–25
Gomez-Catalan, 1987 [45]	3	Serum	Florosil	GC–ECD	Surrogates	100	0.1	[b]
Stachel, 1989 [46]	1–6, 10	Semen	Liquid extraction	GC–ECD	[b]	72–120	[b]	[b]
Liao, 1988 [53]	1–5, 11	Adipose	solvent, Florosil	GC–MS	Surrogate	–85	5–50 μg/l	[b]
Noren, 1987 [169]	1–5, 8–10	Milk	Lipidex gel, aluminum oxide, silica	GC–HRMS	Surrogates	82–101	[b]	[b]
LeBel, 1983 [47]	[f]	[f]	Acetone/hexane, gel permeation, dichloromethane/cyclohexane	GC–ECD	[b]	>80	[b]	[b]
Bristol, 1982 [48]	3, 5, 7–9, 11	[f]	[f]	GC–ECD[d]	Surrogates	35–99	Low μg/l	3–20
Tessari, 1980 [49]	1–3, 7–10, 13	Breast milk	ACN/hexane, Florosil	GC–ECD	[b]	68–90	0.5–30 μg/l	[b]
Strassman, 1977 [50]	1–13	Breast milk	Solvent, Florosil	GC–ECD[d]	[b]	[b]	10–100 μg/l	[b]
Martinez, 1998 [57,58]	15	Urine	SPE	GC–MS–MS	Dieldrin	>89	0.006–0.018 μg/l	9–13

Nigg, 1991 [59]	17	Urine	Oxidation, solvent	GC (detector not noted)	None	[b]	1	[b]
Angerer, 1981 [60]	14	Urine	Acid hydrolysis, derivatization	GC–ECD	[f]	87–119	5–20 μg/l	4–10
Holler, 1989 [61]	14	Urine	Acid hydrolysis, derivatization	GC–MS–MS	Isotope analogues/surrogates	>50	1	[c]
Hill, 1995 [62]	14	Urine	Enzyme hydrolysis, chlorobutane/ether; derivatization	GC–MS–MS	Stable isotope analogues	[b]	1–2 μg/l	21–24
Mardones, 1999 [63]	14	Urine	Acid hydrolysis, on-line cleanup	MEKC–UV	[b]	58–103	1–12 μg/l	3–7
Mardones, 2000 [64]	14	Urine	SFE	MEKC–UV	[b]	[b]	Low	[b]
Wada, 1999 [65]	14	Urine	Derivatization with fluorophore	HPLC–fluoroscence	[b]	[b]	Low	<14
DFG, 2001 [66]	14	Urine	Acid hydrolysis, steam distillation, C_{18} SPE, diazomethane derivatization	GC–MSD	Surrogate	[b]	0.1–0.5	5–14

I.S.=Internal standard; LOD=limit of detection; RSD=relative standard deviation; SPME=solid-phase microextraction; SPE=solid-phase extraction; SFE=supercritical fluid extraction; GC–ECD=gas chromatography–electron-capture detection; GC–MS=gas chromatography–mass spectrometry, GC–HRMS=gas chromatography–high-resolution mass spectrometry; MEKC–UV=micellar electrokinetic chromatography with UV detection.

[a] 1=*p,p′*-DDT; 2=*p,p′*-DDE; 3=hexachlorobenzene; 4=α-hexachlorocyclohexane; 5=β-hexachlorocyclohexane; 6=γ-hexachlorocyclohexane; 7=heptachlor epoxide; 8=oxychlordane; 9=*trans*-nonachlor; 10=dieldrin; 11=aldrin; 12=endrin; 13=mirex; 14=lindane and/or metabolites (chlorinated phenols); 15=endosulfan and/or metabolites; 16=methylsulfonyl-DDE; 17=*o,p*-DDT; 18=*cis*-nonachlor.

[b] Not given.

[c] Standard error about the mean.

[d] GC–MS used for confirmation of positive samples.

[e] Recovery refers only to the absolute recovery from extraction or isolation of the analyte.

[f] Unable to obtain full article. Details taken from abstract. Missing details may be available in full article.

these persistent compounds are transported transboundary is through a series of evaporation, deposit (condensation), evaporation, deposit steps; this is the so-called ''grasshopper effect'' [19]. By these two means these persistent chemicals are transported thousands of miles from their origin. The contemporary pesticides include organophosphates, carbamates, triazines, chloroacetanilides, synthetic pyrethroids, and others and are considered nonpersistent. These pesticides have much shorter environmental half-lives [20–23] and tend not to bioaccumulate. In fact, most of these pesticides are excreted from humans within 48 h as the parent pesticide, a mercapturate detoxification product, free metabolites, and/or glucuronide- or sulfate-bound metabolites [24–33]. However, because of the heavy agricultural use of these chemicals, humans are continually exposed to many of these nonpersistent chemicals via the food chain and also through residential use.

2. Persistent pesticides

2.1. Organochlorine pesticides

Organochlorine (OC) pesticides were used extensively in the US as insecticides in the mid twentieth century. OC pesticides include the cyclodienes, hexachlorocyclohexane isomers, and DDT and its analogues (e.g., DDE, methoxyclor, dicofol). Nine of the organochlorine pesticides as well as polychlorinated dibenzo-*p*-dioxins, furans, and biphenyls are the subject of the Stockholm Convention on Persistent Organic Pollutants (POPs), which was held in May 2001; this treaty calls for an immediate ban on the production, import, export, and use of most of these POPs as well as disposal guidelines [34]. DDT has a health-related exemption for the control of malaria-carrying mosquitoes. These nine pesticides are aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene. Many of the methods in Table 1 monitor for each of these pesticides, either directly or indirectly. Aldrin is measured as its primary metabolite, dieldrin. Chlordane and heptachlor are generally used together and are monitored as their metabolites, oxychlordane and heptachlor epoxide, as well as their commercial by-product, *trans*-nonachlor. DDT is sometimes measured as DDT but more generally as its biodegraded product and metabolite, DDE. The measurement of toxaphene in biological samples is the most complex because it is a mixture of chlorinated camphenes, some of which have long biological half-lives. In the US only four OC pesticides are still in use (i.e., methoxychlor, dicofol, lindane, and endosulfan); however, these four tend to be much less persistent than those falling under the proposed treaty. Even if the proposed treaty is eventually ratified by 50 countries and thus enters into force, the persistent organochlorine compounds will continue to be monitored in the ecosystems, including humans. The reasons for this is their toxicity (known animal toxicity, known and suspected human toxicity) and the possibilities of human exposure, primarily via the food chain.

Although some OC pesticide metabolites are monitored in urine, they are most commonly measured as the intact pesticide and/or its metabolite in whole blood, serum, plasma, or other lipid-rich matrices. These methods and the specific pesticides measured are outlined in Table 1. Typically, serum or plasma is extracted using a liquid partitioning or solid-phase extraction (SPE) and the extract is analyzed using capillary gas chromatography (GC) with electron-capture detection (ECD) [35–50]. These methods are reliable and use affordable instrumentation. However, GC–ECD analyses have a higher potential for detecting interfering components than do more selective analysis techniques. Other methods for analysis of serum extracts include mass-selective detection (MSD aka MS) [36,51–53] and high-resolution mass spectrometry [54–56] some with isotope dilution quantification [54–63]. These analyses are typically more selective and sensitive than GC–ECD analyses; however, the high cost of instrumentation and isotopically labeled standards and the complex operation and maintenance of these instruments often preclude their routine use in most laboratories.

Methods similar to those employed with serum and plasma are used to measure OCs in other lipid-rich matrices such as adipose tissue and breast milk (Table 1). These methods may involve some modifications in the sample preparation procedures to accommodate the change in matrix properties.

Some OCs are metabolized more readily than others and their polar metabolites are excreted in urine. The most common OCs whose metabolites are

measured in urine are endosulfan and lindane (γ-HCH). Endosulfan and its polar metabolites endosulfan-lactone, endosulfan-ether, and endosulfan-sulfate have been measured in the low-ng/l level in urine using SPE with analysis by GC–tandem mass spectrometry (GC–MS–MS; Table 1) [57,58]. A metabolite of dicofol, 4,4′-dichlorobenzilic acid, was measured in pesticide applicators by GC [59]. Lindane metabolites, primarily chlorinated phenols, have been measured in urine using several methods, all of which employ some deconjugation technique (e.g., acid or enzyme hydrolysis) to liberate glucuronide- and sulfate-bound chlorinated phenols (Table 1) [60–66]. In most instances, the chlorinated phenols are extracted, derivatized, and analyzed using GC–ECD [60] or GC–MS–MS [61,62]. One novel method employs an on-line cleanup or supercritical fluid extraction (SFE) and preconcentration followed by separation and analysis using micellar electrokinetic chromatography with UV detection [63,64].

3. Nonpersistent pesticides

Nonpersistent pesticides are also called contemporary pesticides or current-use pesticides. The development and production of these pesticides escalated after the more persistent pesticides were banned beginning in the mid 1970s. By nature, these pesticides do not persist appreciably in the environment; most decompose within several weeks with exposure to sunlight and water. In addition, these pesticides tend not to bioaccumulate; therefore, they are typically metabolized and excreted from the body in a few days. The contemporary pesticides are structurally diverse and have varied mechanisms of action.

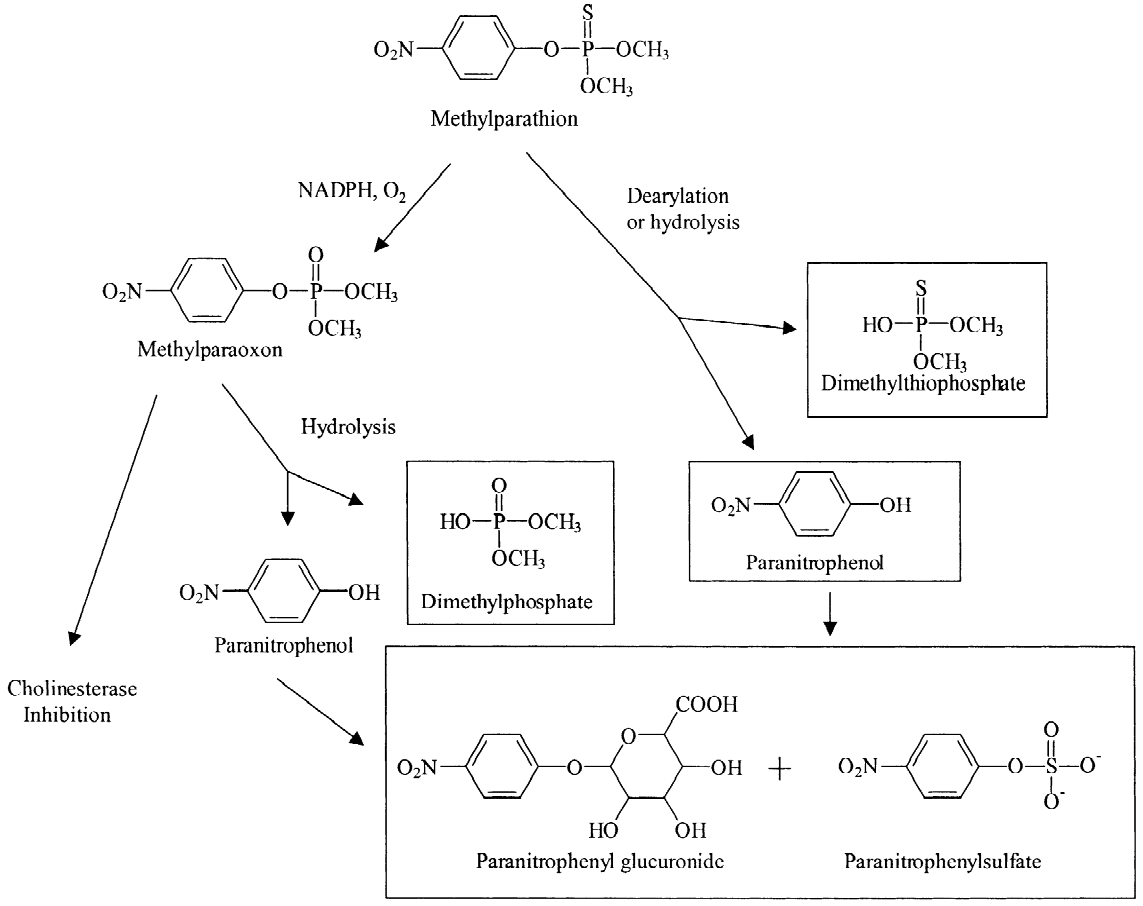

Fig. 2. Metabolic pathway of methyl parathion in the body representative of organophosphate pesticide metabolism.

Table 2
Methods for measuring organophosphate pesticides and their metabolites in biological matrices

Method	Analytes[a]	Matrix	Preparation (isolation; derivatization; cleanup)	Analytical system	I.S. type	Recovery[d] (%)	LOD (μg/l)	RSD (%)
Lores, 1976 [67]	1, 2, 4, 5	Urine	Solvent/ion exchange; diazopentylation	GC–FPD	None	51–97	50–100	[c]
Daughton, 1979 [68]	1, 2, 4–6	Urine	Resin column; benzylation	GC–FPD	None	[c]	<2 pmol	[c]
Reid, 1981 [69]	1–6	Urine	Azeotropic distillation, PFB (two-step); silica	GC–FPD	None	91–102	38–130	<7
Fenske, 1989 [70]	2, 3, 9, 10	Urine	Base hydrolysis, azeotropic distillation, PFB	GC–FPD	None	89 Total malathion equivalent	14–25	[c]
Weisskopf, 1989 [71]	2–6	Urine	CH-SPE; methylation	GC–FPD	None	8–131	2–10	3–13
Nutley[e], 1993 [72]	1–6	Urine	Azeotropic distillation; PFB	GC–FPD	Surrogate	[e]	[e]	[e]
Richardson, 1993 [73]	1–6, 21, 19	Kidney/liver	Homogenized; solvent; tetrabutylation of DAPs	GC–FPD	None	[c]	20–50	[c]
Drevenkar, 1994 [74]	2–6	Urine	Solvent; methylation	GC–FID	Surrogate	[c]	[c]	[c]
Drevenkar, 1983 [75]	1–6	Plasma	Solvent; methylation	GC–MS (ion trap) GC–AFID	Surrogate	36–97	50–150 (w/AFID)	[c]
Aprea, 1996 [76]	1–6	Urine	Azeotropic distillation; Two-step PFB; CN-SPE	GC–FPD	None	86–100	2	<12
Loewenherz, 1997 [77]	1–3	Urine	azeotropic distillation; PFB	GC–FPD	Surrogate	62–80	13–15	[c]
Hardt, 2000 [78]	1–6	Urine	solvent; PFB	GC–MS	Surrogate	68–114	1–5	8–17
Davies, 1997 [79]	1–6	Urine	Freeze dry; benzylation	GC–FPD	None	90–100	[c]	5–15
Moate, 1999 [80]	1–6	Urine	SPE cleanup; azeotropic distillation, PFB (two-step)	GC–FPD	None	85–110	2–10	8–18; 32 for DEP
Whyatt, 2001 [81]	1–6	Meconium	Solvent; chloropropylation	GC–MS–MS	Stable isotope analogues	20–80	0.09–0.5	[c]
Bravo, 2001 [83]	1–6	Urine	Azeotropic distillation, chloropropylation	GC–MS–MS	Stable isotope analogues	60–80	0.1–1.2	8–19
Draper, 1991 [100]	9, 10	Urine	SAX SPE; methylation	GC–MS (ion trap)	Stable isotope analogues	55–60	5–14	7–10
Bradway, 1977 [101]	9, 10	Urine	Solvent extraction; methylation	GC–FPD	None	98–104	2–5	[c]
Meyer, 1998 [110]	18	Post mortem specimens	C_{18} SPE	HPLC GC–MS	Surrogate	46–71	250 100	8–13
Hill, 1995 [62]	7, 8	Urine	Enzyme hydrolysis; solvent; chloropropylation	GC–MS–MS	Stable isotope analogues	[c]	1	13–17
Bartels, 1992 [97]	7	Urine	Acid hydrolyis; solvent; silylation	GC–MS–MS	Surrogate	80–100	0.5	<3
Chang, 1996 [98]	7	Urine	Acid hydrolysis; other details lacking	HPLC	Surrogate	102	2.2 ng/20 μl injected	<10
Maroni, 1990 [111]	11, 12	Urine	Pseudo-solvent extraction[b]	GC–NPD	None	[c]	30	[c]
Marques, 1990 [112]	20	Urine, blood	Solvent	GC–FPD	None	90–100	[c]	[c]
Jitsunari, 1989 [91]	7	Urine	Solvent; silylation	GC–ECD		91	10	[c]
Watts, 1980 [92]	7	Urine	Acid hydrolysis; solvent; silylation	GC–ECD	None	[c]	[c]	[c]

Fenske, 1990 [94]	7	Urine	Acid hydrolysis; solvent; silylation	GC–ECD	None	72	10	[c]
Beeson, 1999 [103]	9, 13	Urine	Enzyme hydrolysis, solvent	HPLC–APCI-MS–MS	Stable isotope analogues	13	0.02	8
Baker, 2000 [102]	9, 13	Urine	Enzyme hydrolysis, solvent	HPLC–APCI-MS–MS	Stable isotope analogues	13	0.02	8
Frenzel, 2000 [51]	12, 22	Whole blood	Hemolysis/deproteination; Kieselguhr	GC–MS	Surrogate	51–97	25–130	5–8
Barr, 2001 [113]	19, 21–24	Serum/plasma	Mixed-phase SPE	GC–HRMS	Stable isotope analogues	30–80	0.001–0.030	8–30
Koch, 2001 [95]	7	Urine	Acid hydrolysis; steam distillation; silylation	GC–MS	Surrogate	104	0.05	4
Hunter, 1982 [217]	25	Serum	Dilution	Competitive inhibition enzyme immunoassay	None	n/a	0.3	[c]
Shackelford, 1999 [218]	7	Urine	Acid hydrolysis, C_{18} SPE; dilution	Enzyme immunoassay	None	[c]	3	[c]
MacKenzie, 2000 [219]	7	Urine	Dilution	Enzyme immunoassay	None	[c]	[c]	[c]
Olsson, 2001 [99]	7–9, 11–17	Urine	Enzyme hydrolysis, mixed bed SPE	HPLC–ESI-MS–MS	Stable isotope analogues	30–95	0.2–20	<15

LOD=Limit of detection; RSD=relative standard deviation; GC–FPD=gas chromatography–flame photometric detection; GC–FID=gas chromatography–flame ionization detection; GC–AFID=gas chromatography–alkali flame ionization detection; GC–ECD=gas chromatography–electron-capture detection; GC–MS=gas chromatography–mass spectrometry; GC–MS–MS=gas chromatography–tandem mass spectrometry; GC–HRMS=gas chromatography–high-resolution mass spectrometry; HPLC–ESI-MS–MS=high-performance liquid chromatography–electrospray ionization tandem mass spectrometry; HPLC–APCI-MS–MS=high-performance liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry; SPE=solid-phase extraction; HPLC=high-performance liquid chromatography with UV detection; CH-SPE=cyclohexyl phase solid-phase extraction; SAX-SPE=strong anion-exchange solid-phase extraction; CN-SPE=cyanopropyl phase solid-phase extraction; PFB=pentafluorobenzylation; I.S.=internal standard; n/a=not applicable

[a] 1=Dimethylphosphate; 2=dimethylthiophosphate; 3=dimethyldithiophosphate; 4=diethylphosphate; 5=diethylthiophosphate; 6=diethydithiophosphate; 7=3,5,6-trichloro-2-pyridinol (methyl/ethyl chlorpyrifos metabolite); 8=4-nitrophenol (methyl/ethyl parathion, EPN metabolites); 9=malathion dicarboxylic acid; 10=malathion monocarboxylic acid isomers; 11=acephate; 12=methamidaphos (methamidaphos, acephate metabolite); 13=2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY; diazinon metabolite); 14=3-hydroxymethyl-1,2,3-benzotriazin-4-(3H)-one (HMBT; azinphos methyl metabolite); 15=3-chloro-4-methyl-7-hydroxycoumarin (coumaphos metabolite); 16=2-diethylamino-6-methyl-4-pyrimidinol (pirimiphos methyl metabolite); 17=5-chloro-1-isopropyl-1H-1,2,4-triazol-3-ol (isazaphos methyl metabolite); 18=fenthion; 19=diazinon; 20=azinphos methyl; 21=parathion; 22=methylparathion; 23=dichlorvos; 24=chlorpyrifos; 25=paraoxon.

[b] Pseudo-solvent extraction involves adsorption of urine matrix and contents in diatomaceous earth and washing with solvent. This is the extraction for ChemElut, IsoElut, and Kieselguhr columns.

[c] Not given.

[d] Recovery refers only to the absolute recoveries from extraction or isolation of the analytes.

[e] Unable to obtain full article. Details taken from abstract. Missing details may be available in full article.

Organophosphates, carbamates, synthetic pyrethroids, phenoxyacid herbicides, triazine herbicides, chloroacetanilide herbicides are among the classes included in this pesticide grouping.

3.1. Insecticides

3.1.1. Organophosphates

Organophosphate (OP) pesticides are comprised of a phosphate (or thio- or dithiophosphate) moiety and an organic moiety. In most cases, the phosphate moiety is *O*,*O*-dialkyl substituted. These pesticides are potent cholinesterase inhibitors. They can reversibly or irreversibly bind covalently with the serine residue in the active site of acetyl cholinesterase and prevent its natural function in catabolism of neurotransmitters. This action is not unique to insects, but can produce the same effects in wildlife and humans.

Once human exposure occurs, OP insecticides are usually metabolized to the more reactive oxon form which may bind to cholinesterase or be hydrolyzed to a dialkyl phosphate and a hydroxylated organic moiety specific to the pesticide. As a result of binding to cholinesterase, the organic portion of the molecule is released. The cholinesterase-bound phosphate group may be ''aged'' by the loss of the *O*,*O*-dialkyl groups, or may be hydrolyzed to regenerate the active enzyme. These metabolites and hydrolysis products are then excreted in the urine, either in free form or bound to sugars or sulfates. Alternatively, the intact pesticide may undergo hydrolysis prior to any conversion to the oxon form and the polar metabolites are excreted. This metabolic pathway is shown schematically in Fig. 2, using methyl parathion as a representative OP. In any instance, a series of polar metabolites are excreted in the urine.

Six dialkyl phosphate (DAP) metabolites of OP pesticides are the most commonly measured [67–83]. These methods, which are outlined in Table 2, use liquid–liquid extraction with polar solvents such as ethyl acetate or diethyl ether, cyclohexyl solid-phase extraction, azeotropic distillation, or lyophilization to isolate the DAPs from the urine matrix. The DAPs are derivatized using a variety of reagents but most often pentafluorobenzyl bromide (PFB). Those methods that derivatize using methylating agents such as diazomethane cannot obtain an accurate analysis of dimethyl phosphate, since endogenous inorganic phosphate produces the same trimethyl derivative [71,74,75]. The derivatized extracts are analyzed using GC coupled with flame photometric detection (FPD) [67–73,76,77,79,80], flame ionization detection (FID) [74], mass spectrometry [75,78] or tandem mass spectrometry [81–83]. Many of these methods have LODs in the mid-μg/l (parts per billion, ppb) range [67–69,75], but several can detect levels in the low- [70,71,73,76,77,80] or sub-μg/l range [81–83]. The data generated from these analyses do not provide unequivocal identification of a single pesticide, but rather a cumulative index of exposure to most of the members of the class of OPs. It is important to note that DAPs may be possible metabolites of some industrial chemicals [84] and pharmaceuticals [85–88], but it is generally believed that most urinary DAP results from OP exposure or exposure to OP hydrolysis products.

In addition, DAPs have been measured in postpartum meconium using a simple methanol extraction, derivatization and analysis by GC–MS–MS [81]. The limits of detection are in the mid-ng/l range. Intact OP pesticides have also been measured in this unique matrix by Ostrea Jr. [89] and Ramirez [90]. The use of meconium as a matrix for biological monitoring is discussed in more detail in the Discussion section.

Pesticide-specific metabolites of OPs are also frequently measured (Table 2). The most common metabolite measured is 3,5,6-trichloro-2-pyridinol (3,5,6-TCPy), a metabolite of chlorpyrifos. Methods that measure 3,5,6-TCPy usually include an acid or enzyme hydrolysis followed by SPE or a liquid–liquid extraction. The extracted analytes are then derivatized, with the most popular derivatizing agents being PFB and diazomethane. The derivatized analytes are analyzed using GC–ECD [91–94], GC–MS [93,95], and GC–MS–MS [62,96,97]. Alternatively, the underivatized 3,5,6-TCP is analyzed using HPLC [98] or HPLC–electrospray ionization (ESI)-MS–MS [99].

Specific malathion metabolites, malathion dicarboxylic acid and α and β isomers of malathion monocarboxylic acid, have also been measured [70,100–103]. The metabolites are either measured as the intact metabolite [100–103] or are subjected to a base hydrolysis to form dimethylphosphate and

Table 3
Analytical methods for measuring various pesticides and/or metabolites in biological matrices

Method	Analytes	Matrix	Analysis method	LOD (μg/l)
Smith, 2001 [115]	Carbaryl/naphthalene metabolite	Urine	GC–HRMS	0.05
Duck, 1985 [117]	Carbaryl	Postmortem samples	HPLC	1000
Ward, 1987 [116]	Carbaryl and metabolites	Plasma, urine	HPLC	5–10
Hussain, 1990 [220]	Carbofuran	Urine	HPLC	[b]
Driskell, 1991 [124]	Methomyl	Blood	HPLC–TSP-MS–MS	[b]
Leenheers, 1992 [120]	Propoxur metabolite	Urine	GC–MS	6
Hardt, 1999 [121]	Propoxur metabolite (2-isopropoxyphenol)	Urine	GC–MS	0.5
Hardt, 1999 [123]	Pirimicarb metabolites (hydroxypyrimidines)	Urine	GC–MS	0.5–4
Harper, 1998 [122]	Aldicarb and metabolites	Urine	HPLC	[b]
Lavy, 1993 [119]	Glyphosate, metabolites of captan, carbaryl, and benomyl	Urine	GC–NPD, HPLC	2–100
Leng, 1999 [126]	Bioallethrin metabolites	Urine	GC–MS	10–20
Angerer, 1997 [127]	Pyrethroid metabolites	Urine	GC–MS	0.3–0.5
Aprea, 1997 [125]	Pyrethroid metabolites	Urine	GC–ECD	0.5
Leng, 1996 [128]	Pyrethroid metabolites	Urine	GC–MS	0.5
Baker, 2001 [131]	Pyrethroid metabolites	Urine	HPLC–APCI-MS–MS	0.3–0.7
Tuomainen, 1996 [129]	Deltamethrin metabolite	Urine	GC–ECD	2
Yao, 1992 [221]	Deltamethrin metabolites	Urine	HPLC	[b]
Junting, 1991 [222]	Pyrethroids	Urine, blood	GC–FID	[b]
DFG, 2001 [66]	Pyrethroid metabolites	Urine	GC–MS	0.1–0.5
Cowell, 1987 [159]	Alachlor metabolites (diethylaniline, hydroxydiethylaniline)	Urine	HPLC	5
Sanderson, 1995 [160]	Alachlor metabolites (diethylaniline, hydroxydiethylaniline)	Urine	HPLC	51
Biagini, 1995 [223]	Alachlor	Urine	ELISA immunoassay	1.1
Catenacci, 1990 [138]	Atrazine	Urine	GC–MS	0.5
Catenacci, 1993 [137]	Atrazine dealkylated metabolites	Urine	GC–NPD	0.5
Erickson, 1979 [139]	Atrazine	Urine	GC–HECD	0.06
Onnerfjord, 1998 [224,225]	Atrazine	Urine	Competitive flow immunoassay	0.3
Barr, 2001 [113]	Triazines, acetanilides, organophosphates, carbamates, pyrethroids, fungicides, DEET, others	Serum, plasma	GC–HRMS	0.001–0.030
Barr 1998 [114]	Carbaryl, triazines, acetanilides	Serum	GC–HRMS	0.03–0.1
Bees,on 1999 [103]	Atrazine mercapturate, 2,4-D, malathion metabolites	Urine	HPLC–APCI-MS–MS	0.3–0.5
Baker, 2000 [102]	Atrazine mercapturate, 2,4-D, malathion, diazinon, pyrethroid metabolites	Urine	HPLC–APCI-MS–MS	0.02–0.5
Gilman, 1998 [136]	Atrazine metabolites	Urine	HPLC–AMS	0.0005
Aprea, 1997 [226]	2,4-D, MCPA	Urine	HPLC–DAD	15
Libich, 1984 [140]	2,4-D	Urine	GC–HECD	[b]
Vural, 1984 [141]	2,4-D, 2,4,5-T	Urine	GC–ECD	1
Grover, 1985 [142]	2,4-D, dicamba	Urine	GC–ECD	17–50
Sell, 1983 [143]	2,4D	Urine	GC–ECD	70
Lyubimov, 2000 [227]	2,4-D	Urine	Enzyme immunoassay	12
De Beer, 1979 [228]	Mecoprop	Urine, plasma, tissue	GC–ECD	100
Draper, 1982 [147]	2,4-D, 2,4,5-T, dicamba, picloram, pronamide, pentachlorophenol	Urine	GC–ECD	50–100
Smith, 1979 [144]	2,4-D	Urine	GC–ECD	100
Thompson, 1996 [145]	2,4-D	Urine	GC–MS	0.75
Kohli, 1974 [148]	2,4-D	Urine, plasma	GC–FID	[b]
DFG, 2001 [66]	2,4-D	Urine		
Kolmodin-Hedman, 1980 [149]	2,4-D, 2,4,5-T	Urine, plasma	GC–ECD	50
Rivers, 1970 [150]	2,4-D, dicamba	Urine, plasma	GC–ECD	10–20

Table 3. Continued

Method	Analytes	Matrix	Analysis method	LOD (μg/l)
Nigg, 1983 [151]	2,4-D	Urine	GC–ECD	[b]
Frank, 1985 [152]	2,4-D	Urine	GC–ECD	0.1
De Felip, 1989 [154]	MCPA	Urine	GC–MS	10–25
Van Peteghem, 1976 [146]	2,4-D	Urine	GC–MS	10
Kawase, 1984 [162]	Paraquat, diquat	Blood, urine	GC–FID	[b]
Nakagiri, 1989 [163]	Paraquat, diquat	Serum, urine	HPLC	100
Krieger, 2000 [177]	Captan metabolite	Urine	GC–CECD	5
van Welie, 1991 [178]	Captan metabolites	Urine	GC–HRMS	3–110
Geyer, 1987 [180]	Chlordimeform metabolite (4-chloro-*o*-toluidine)	Urine	HPLC	20
Levy, 1981 [181]	Chlorobenzylate	Urine	GC–ECD	2
Weiss, 1999 [176]	Dithiocarbamate metabolite (2-thiazolidinethione-4-carboxylic acid)	Urine	GC–MS	0.5
Kurttio, 1998 [175]	Dithiocarbamate metabolite (ETU)	Urine	HPLC	0.2
Prince, 1985 [174]	Dithiocarbamate metabolite (ETU)	Urine	HPLC–EC	25
Kurttio, 1992 [173]	Dithiocarbamate metabolite (ETU)	Urine	HPLC–TSP-MS	0.2
Gomez-Catalan, 1987 [45]	Pentachlorophenol	Urine, serum	GC–ECD	0.1
Holler, 1989 [61]	Chlorinated phenols[a] phenoxy herbicides	Urine	GC–MS–MS	1
Hill, 1995 [62]	Chlorinated phenols[a] phenoxy herbicides carbamate/OP metabolites	Urine	GC–MS–MS	1–2
Shealy, 1996 [96]	Chlorinated phenols[a] Herbicides carbamate/OP metabolites	Urine	GC–MS–MS	0.5–1.2
DFG, 2001 [66]	Chlorinated phenols	Urine	GC–MSD	0.1–0.5
Rick, 1982 [164]	Pentachlorophenol	Urine, plasma	GC–ECD	1
Cline 1989 [165]	Pentachlorophenol	Urine, serum	GC–ECD	[b]
Uhl, 1986 [24]	Pentachlorophenol	Urine, plasma	GC–ECD	[b]
Butte, 1987 [229]	Pentachlorophenol	Urine, serum	GC–ECD	2–3
Needham, 1981 [167]	Pentachlorophenol	Urine, whole blood, serum	GC–ECD	1–2
Thompson, 1994 [170]	Pentachlorophenol	Urine	GC–MS	0.2
Colosio, 1993 [168]	Pentachlorophenol	Urine, plasma	GC–ECD	1
Noren, 1987 [169]	Pentachlorophenol	Urine	GC–ECD	0.2
DFG, 2001 [66]	Pentachlorophenol	Urine, serum/plasma	GC–ECD	2
Fraser, 1995 [183]	DEET	Urine, serum	GC–MS	200
Smallwood, 1992 [184]	DEET	Urine, serum	HPLC	90
Will, 1995 [179]	Vinclozolin metabolites	Urine	HPLC–EC	5
DFG, 2001 [66]	Vinclozolin (as 3,5-dichloroaniline)	Urine	HPLC–EC	5
Schettgen, 2001 [161]	Phenmedipham metabolite (*m*-toluidine)	Urine	GC–MS	0.1

MCPA=2-Methyl-4-chlorophenoxyacetic acid; 2,4-D=2,4-dichloroacetic acid; 2,4,5-T=2,4,5-trichloroacetic acid; DNBP=2-*sec*.-butyl-4,6-dinitrophenol; LOD=limit of detection; GC–FPD=gas chromatography–flame photometric detection; GC–FID=gas chromatography–flame ionization detection; GC–AFID=gas chromatography–alkali flame ionization detection; GC–ECD=gas chromatography–electron-capture detection; GC–HECD=gas chromatography–Hall electroconductivity detection; GC–CECD=gas chromatography–Coulson electroconductivity detection; HPLC=high-performance liquid chromatography with UV detection; HPLC–EC=high-performance liquid chromatography–electrochemical detection; GC–MS=gas chromatography–mass spectrometry; GC–HRMS=gas chromatography–high-resolution mass spectrometry; GC–MS–MS=gas chromatography–tandem mass spectrometry; HPLC–APCI-MS–MS=high-performance liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry; HPLC–TSP-MS=high-performance liquid chromatography–thermospray ionization mass spectrometry; ELISA=enzyme linked immunosorbent assay; AMS=accelerator mass spectrometry.

[a] Metabolites of chlorinated pesticides including organochlorines and organophosphates.

[b] Not specified.

dimethylthiophosphate which can then be analyzed using the DAP methodology [70].

Other less frequently measured specific OP metabolites include 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), a metabolite of diazinon, and 4-nitrophenol, which is a metabolite of methyl and ethyl parathion, EPN, and other non OP chemicals such as 4-aminophenol. These metabolites are quan-

tified using a solvent extraction or solid-phase extraction and analysis using high-performance liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry (HPLC–APCI-MS–MS) [102,104]. Alternatively, 4-nitrophenol can be derivatized and quantified using GC–MS–MS [62,96].

Several methods have been reported that measure the intact OP pesticides in blood, serum, or plasma (Table 2) [51,105–113]. The vast majority of these methods were developed for forensic applications or for diagnosis of acute pesticide intoxication and have limits of detection in the μg/l to the mg/l range. Most of these methods lack the sensitivity and/or the selectivity to measure pesticides in blood or blood products resulting from incidental exposures. One method can measure these intact pesticides in the general population [113]; however, it requires the use of GC–high-resolution mass spectrometry (HRMS) which is often too expensive for most laboratories to utilize.

3.1.2. Carbamates

Carbamate insecticides have the same mechanism of toxicity action as the OP insecticides, except their effects are more reversible and less severe. The most popular of these pesticides for residential uses are carbaryl (Sevin) and propoxur (Baygon). Many carbamates such as aldicarb and methomyl are also used in agricultural applications.

Carbaryl exposure has been estimated based upon urinary measurements of 1-naphthol, its most abundant metabolite. 1-Naphthol has been measured using several different methods which are shown in Table 3. These methods employ a solvent extraction or solid-phase microextraction (SPME) with analysis using GC–MS–MS [62,96], GC–HRMS [114,115] or HPLC [116,117]. However, 1-naphthol, as well as 2-naphthol, is a metabolite also of naphthalene, a ubiquitous polyaromatic hydrocarbon. Thus, the measurement of 1-naphthol does not distinguish these two sources. Measurement of other less abundant metabolites of carbaryl, such as 4-hydroxy-carbarylglucuronide [28] may help to circumvent this problem. Indirectly, researchers have examined the correlate of the prevalence of 2-naphthol and 1-naphthol in order to discern the contributions of carbaryl and naphthalene exposure [118].

Other carbamate metabolites that have been measured in urine include benomyl [119], carbofuran [62,96,119], carbosulfan [62,96], propoxur [62,96, 120,121], aldicarb [122], and pirimicarb [123] (Table 3).

In addition, several carbamates have been measured in serum and plasma [113–115]. The carbamates, in general, are particularly unstable in blood so sometimes their metabolites must be measured as well. For instance, carbaryl is hydrolyzed rapidly in blood to its major metabolite, 1-naphthol; therefore, 1-naphthol is usually measured in serum or plasma [113,114]. In addition, a propoxur metabolite, 2-isopropoxyphenol, can be successful quantified in serum or plasma [113]. Methomyl was measured in the whole blood of a pilot who died during aerial application the pesticide [124].

3.1.3. Pyrethroids

Pyrethrins are naturally-occurring chemicals that are produced by chrysanthemums which exhibit a pesticidal effect on insects. Natural pyrethrins are comprised of many isomeric forms and are usually classified as the pyrethrin I and II isomer sets. Synthetic pyrethroids are man-made chemicals that are produced to mimic the effective action of natural pyrethrins. Their chemical structures are typically comprised of a chrysanthemic acid analogue that is esterified most often with a ringed structure. Pyrethroids are non-systemic pesticides that have contact and stomach action. Some pyrethroids also have a slight repellent effect. In most formulations, piperonyl butoxide is added as a synergist. In the past several years, the use of synthetic pyrethroids has escalated as the use of the more toxic OP and carbamate insecticides has been curtailed. Many products such as Raid brand pesticides that are commonly found in retail stores for home use contain pyrethroids such as permethrin and deltamethrin for eliminating household pests such as ants and spiders.

During metabolism of the pyrethroids, the chrysanthemic acid ester is usually cleaved via esterase or mixed function oxidase activity and any resulting alcohol moieties are converted to their corresponding acids. These metabolites are partly conjugated to glucuronide and both the conjugates and free acids are excreted in the urine.

To our knowledge, no methods exist to measure natural pyrethrins or piperonyl butoxide in human

matrices. This may be due to the logistically difficult task of measuring multiple isomers for exposure assessment of one pesticide product or it may be the lack of priority due to its inherently low human toxicity or the relative security individuals feel when using a ''natural'' product.

Several methods exist for the measurement of synthetic pyrethroid metabolites in human urine (Table 3). The metabolites of permethrin, cypermethrin, deltamethrin, and cyfluthrin are most commonly measured. 3-Phenoxybenzoic acid (3PBA) is a metabolite that is common to as many as 20 synthetic pyrethroids. It has been measured alone [125], with other non pyrethroid pesticides [102], or as a part of a suite of pyrethroid metabolites [126–128]. Most of these methods employ an acid hydrolysis, solvent or SPE extraction, and derivatization to the pentafluorobenzyl or methyl ester followed by analysis using GC–ECD [125,129] or GC–MS [126–128]. The LODs are in the low-μg/l range. Another method, with similar LODs, uses an enzyme hydrolysis and a solvent extraction followed by analysis using HPLC–APCI-MS–MS [130].

Other more specific metabolites of synthetic pyrethroids have also been measured in urine. *cis*- and *trans*-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*- and *trans*-DCCA) are metabolites of permethrin, cypermethrin, and cyfluthrin; *cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) is a metabolites of deltamethrin; and 4-fluoro-3-phenoxybenzoic acid (4F3PBA) is a metabolite of cyfluthrin. These metabolites were measured along with 3PBA in several of the methods mentioned above [127,128]. The German Research Foundation (Deutsche Forschungsgemeinschaft; DFG) has published a compendium of analytical methods for measuring a variety of hazardous substances in biological materials including these pyrethroid metabolites [66]. More recently, a method has been developed which measures these same five metabolites using an enzyme hydrolysis, a simple solid-phase extraction with a mixed bed polymer and analysis using HPLC–electrospray ionization MS–MS analysis [131]. The LODs of this method are in the mid-ng/l range with relative standard deviations (RSDs) around 10% or less.

Synthetic pyrethroids have also been measured in serum and plasma [113,132]. Leng et al. observed a dramatic decrease in the concentrations of permethrin and several other pyrethroids in spiked serum (60 μg/l) when stored at 4 °C over 8 days [132]. By adding 1% formic acid before storing the spiked serum, the deterioration, presumably due to esterase activity, was diminished for permethrin and markedly reduced for the other pyrethroids. Barr et al. did not observe this decrease in permethrin concentrations in spiked serum (50 and 15 pg/g) stored at −70 °C over 4 months [113]. However, more variability was observed in the analysis of permethrin isomers in these stored serum samples after about 1 month and other pesticides that are metabolized via esterase activity (i.e., carbamates and some reactive OPs) did show marked decreases. This area warrants further investigations if serum measurements will continue to be made.

3.2. Herbicides

3.2.1. Triazine herbicides

Triazines are pre- and post-emergence herbicides used to control broad-leafed weeds and some annual grasses. These herbicides inhibit the photosynthetic electron transport in certain plants. Human exposure to triazines has been linked with the development of ovarian cancer [133]. The chemical structures of triazine herbicides are permutations of alkyl substituted 2,4-diamines of chlorotriazine. Upon entering the body, they are metabolized via the glutathione detoxification pathway or by simple dealkylation. For glutathione detoxification, the chlorine atom on the triazine herbicide is subject to an enzymatic-catalyzed substitution by the free –SH on the internal cysteine residue of the glutathione tripeptide. The terminal peptides are enzymatically cleaved and the cysteine is *N*-acetylated. The mercapturate and dealkylation metabolites are then excreted into the urine.

Atrazine is the most studied triazine herbicide. It was also the single most heavily applied pesticide in the US in 1997 [134]. Although dealkylated metabolites can also be formed, atrazine mercapturate was identified as the major human metabolite of atrazine [135]. Three methods have been published for the measurement of atrazine mercapturate in humans [102,103,136]. Two methods use a solvent extraction followed by analysis using HPLC–APCI-MS–MS with LODs in the mid-ng/l range and RSDs typically

less than 10% while the third method uses accelerator mass spectrometry.

Dealkylated metabolites of triazine herbicides can be formed and excreted in the urine. These metabolites are not specific for a single triazine, but provide class exposure information. These metabolites can be measured using GC–NPD [137]. Free atrazine has also been measured in urine using GC–MS [138] and GC–Hall electroconductivity detection (HECD) [139].

Triazines can also be measured as the intact pesticide in blood products. Atrazine has been measured at the low-ng/l level using SPE and GC–HRMS [113,114].

3.2.2. Phenoxyacid herbicides

Phenoxyacid herbicides are post-emergence growth inhibitors used to eliminate unwanted foliage or weeds. The most common phenoxyacid herbicides are 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). These two herbicides were combined in equal proportions to make Agent Orange, the herbicide applied in the jungles of Vietnam, Laos, and Cambodia along with agricultural regions of Vietnam in the late 1960s and early 1970s during the Vietnam War. Because it is contaminated with the highly toxic and persistent 2,3,7,8-tetrachlorodibenzo-*p*-dioxin along with other chlorinated dioxins and furans, 2,4,5-T has been banned for most applications. Although 2,4-D also contains small amounts of persistent chlorinated dioxins and furans, it is still the most abundantly applied residential pesticide [2]. In its ester or salt forms, it is commonly found in home and garden stores in combination with other herbicides such as dicamba or mecoprop for application on lawns.

2,4-D is excreted in the urine as the unmetabolized intact pesticide and its esters are hydrolyzed to 2,4-D prior to excretion. It has been measured routinely in urine by many researchers using several techniques [66,139–152]. The most common techniques involve conversion of the free acid to its protected ester form (usually methyl or pentafluorobenzyl esters) then analysis using GC–HECD [140], GC–ECD [141–144,147,149–152], GC–FID [148], GC–MS [145,146], or GC–MS–MS [62,96]. It has also been measured using HPLC–diode array detection (DAD) [139]. In addition to 2,4-D, mecoprop, dichlorprop, MCPA (2-methyl-4-chloro-phenoxyacetic acid), and 2,4,5-T have been measured using some of these methods [139,141,147,153,154].

3.2.3. Chloroacetanilides

Chloroacetanilides are pre-emergence systemic herbicides that work by preventing protein synthesis and root elongation in plants [155]. The herbicides are *N*,*N*-disubstituted anilines. The individual chloroacetanilides usually differ by their alkyl substituents on the aniline ring. Metolachlor and alachlor are two of the most abundantly applied herbicides in the US [134]. Although detailed metabolism has not been studied on many of herbicides in this class, Coleman et al. [156] observed than many of them, with the unusual exception of metolachlor, form diethylaniline or methylethylaniline intermediates human liver microsomes that are capable of reacting with biomolecules. The author suggests two possible mechanisms for the formation of these reactive intermediates: (1) cytochrome P450-mediated formation of the *N*-monosubstituted acetamide followed by arylamidase reaction; or (2) glutathione conjugation and subsequent amide hydrolysis. In humans, the major urinary metabolite of alachlor has been identified as its mercapturate [157] and preliminary studies suggest that the same is true for metolachlor [29,158]. These metabolites are not inconsistent with the suggested metabolic pathways.

These mercapturate metabolites have been measured in urine using HPLC–MS–MS [29,157]. The substituted aniline metabolites have also been measured in urine using HPLC [159,160]. In addition, three intact chloroacetanilide pesticides have been measured in serum and plasma using GC–HRMS [113] with limits of detection in the low-ng/l range.

3.2.4. Other herbicides

Other herbicides that do not conveniently fit into any other category have also been measured in humans. Dicamba, which is often used in conjunction with 2,4-D in garden applications, has been measured in urine using GC–ECD [142,147,150] and GC–MS–MS [96] at the low- to mid-μg/l range. Urinary *m*-toluidine has been measured as a biomarker of phenmedipham exposure using GC–MS with a limit of detection of 0.1 μg/l [161]. Additionally, paraquat and diquat have been measured in urine, blood, and serum using GC–FID [162] and HPLC [163]. Chlorthal-dimethyl and trifluralin have

been measured in plasma and serum down to 1 ng/l using GC–HRMS [113].

3.3. Fungicides

Fungicides, although widely used, are not the most common class of pesticides typically measured in humans. Hexachlorobenzene is an industrial chemical but also a fungicide; it was discussed with the organochlorine pesticides. Pentachlorophenol (PCP) has also been widely used as a preventive fungicide, insecticide, and herbicide. Previously, it was commonly applied on wood products to prevent termite infestation and mildew development.

Many methods have been reported that measure PCP in serum, plasma, and urine. These methods typically employ some deconjugation step, a solvent or solid-phase extraction, and analysis using GC–ECD [24,164–170], GC–MS [171,172] or GC–MS–MS [61,62,96]. Many of these methods measure PCP in conjunction with other chlorinated phenols. The LODs of these methods are in the low- or sub-μg/l range.

Metabolites of alkylene bisdithiocarbamates have also been measured in humans. Ethylene bisdithiocarbamates, such as maneb, mancozeb, and ziram, are metabolized to ethylenethiourea (ETU). ETU itself has been shown to be carcinogenic in animals [172]. It is also used as an accelerator in rubber production [173].

ETU has been measured in human urine using resin chromatography to isolate the analyte and analysis by HPLC–electrochemical detection (EC) [174], HPLC–DAD [175] and HPLC–thermospray (TSP)-MS [173]. The limits of detection of these methods are 25, 0.2 and 200 μg/l, respectively. The HPLC–TSP-MS method had a much higher LOD but did offer increased selectivity. Because TSP in inherently inefficient at transferring analytes from the liquid phase in HPLC into the gas phase in MS and, as such, has been replaced with more efficient HPLC–MS interfaces, such as electrospray and atmospheric pressure chemical ionization, it is likely the LOD could be dramatically improved with the more efficient interfaces.

Another metabolite of alkylene bisdithiocarbamates is 2-thiazolidinethione-4-carboxylic acid (TTCA). TTCA has been measured in urine using a liquid extraction followed by ethyl esterification and analysis by GC–MS [176].

Other fungicides that have been measured in biological matrices include captan, folpet, dichloran, chlorothalonil, metalaxyl and vinclozolin. Captan and folpet have been measured as their major metabolites, tetrahydrophthalimide (THPI) and phthalimide (PI), respectively, in urine and serum samples. THPI has been measured in urine using GC–HRMS [113,177] and GC–Coulson electroconductivity detection (CECD) [178] with LODs in the low-μg/l range. In addition, THPI, PI, dichloran, chlorothalonil, and metalaxyl have been measured in serum and plasma using GC–HRMS with LODs in the low-ng/μl range [113]. Vinclozolin metabolites have been measured by base hydrolysis to 3,5-dichloroaniline and the measurement of 3,5-dichloroaniline by HPLC–EC with detection near 900 μg/l in urine [179] and 5 μg/l in urine [66].

3.4. Other pesticides

Chlordimeform and chlorobenzilate are acaricides. The major urinary metabolite of chlordimeform, 4-chloro-*o*-toluidine, has been quantified to levels near 20 μg/l after a base hydrolysis using HPLC [180]. The major metabolite of chlorobenzilate, *p,p′*-dichlorobenzophenone, has been quantified in urine to 2 μg/l using GC–ECD [181].

p-Dichlorobenzene and naphthalene are fumigants that are used as insecticides or disinfectants. The major urinary metabolites of both pesticides, 2,5-dichlorophenol and 1- and 2-naphthol, respectively, have been measured in urine by GC–MS–MS following a solvent extraction and derivatization [62,96] and/or by GC–HRMS following SPME and gas–solid-phase derivatization [115]. In addition, *p*-dichlorobenzene has been measured in whole blood using purge-and-trap with GC–HRMS [182].

DEET (diethyl-*m*-toluamide) is commonly used as a mosquito repellent in commercially available formulations such as OFF. Although it is used extensively, it has rarely been measured in humans. Fraser et al. [183] report a method for measuring DEET in the serum and urine of a non-fatal poisoning case using GC–MS. Smallwood et al. measured DEET in human serum and urine as low as 90 μg/l using HPLC [184]. Additionally, DEET has been measured in serum down to 10 ng/l using GC–

HRMS [113] and in urine at the mid- to low-ng/l level using HPLC–APCI-MS–MS [131].

4. Discussion

Although much research has provided methods for measuring a variety of pesticides in biological matrices, there is still much work left to be done. As pesticides are banned or their use is limited, manufacturers are compelled to create and mass produce effective yet less toxic pesticides. As this occurs, there will be a gradual yet steady shift in the pesticides used worldwide, most assuredly accompanied by a transitional lag in developing countries. In essence, this leaves researchers performing biological monitoring of exposure in a perpetual state of method development in an effort to keep up with the growing and changing face of pesticides. With this in mind, we would like to point out several considerations and/or complications when developing methods and several areas that warrant exploration.

4.1. Matrix considerations

The choice of matrix for biomonitoring of the persistent pesticides is usually fairly straightforward. Most of the persistent pesticides are best measured in serum, plasma or other lipophilic matrices such as breast milk as their biological half lives are quite long (Fig. 3). Since these pesticides are inherently lipophilic, they tend to sequester into the fat stores of the body; therefore, their concentration in serum, plasma, or breast milk is dependent upon the lipid content of the matrix. For this reason, persistent pesticide levels, like other persistent organic pollutants, are often normalized on the lipid content of the individual sample [185–187]. This is especially useful for intersample comparisons.

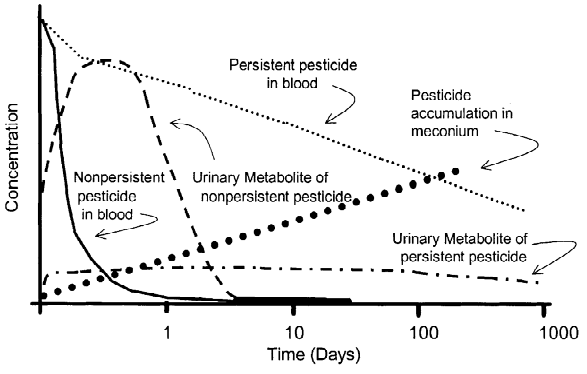

Fig. 3. Schematic representation of the typical fate and lifetimes of persistent and nonpersistent pesticides in human blood, urine, and fetal meconium.

As shown in Fig. 3, a small portion of persistent pesticides may be metabolized and excreted, at a fairly steady state, in urine over a long time span, depending largely on the pesticide half life. These metabolites can be measured in urine; however, the data must generally be corrected for urine dilution if a 24-h sample is not obtained (see the discussion below for more details).

Unfortunately, because of the long half lives of persistent pesticides, it is usually difficult or impossible to distinguish recent exposures from exposures that occurred decades ago. One possible indicator of a recent exposure may be a level elevated above the range that is normally seen. In addition, serial measurements over several days may show a spike in concentrations that may be due to more recent exposures.

The choice of matrix for biomonitoring for the contemporary or nonpersistent pesticides is dependent upon a number of variables including the toxicokinetics of the toxicant (Fig. 3), the availability of the matrix, the ease of matrix manipulation, and the LOD of the analytical method. Contemporary pesticides are usually monitored in urine and less frequently in blood.

Measuring the internal dose of toxicants in blood has several advantages over measuring it in urine. Generally, the parent compound, instead of a metabolite, can be directly monitored in blood products such as whole blood, plasma, or serum; therefore, the development of a blood measurement technique may not require detailed information on the metabolism.

Because blood is a regulated fluid (i.e., the volume does not vary with water intake or other factors), no corrections for dilution are necessary. As with the persistent pesticides, dependent upon the lipophilicity of the pesticide, lipid corrections may be necessary for intersample comparisons; however, this is usually not necessary with contemporary pesticides. Blood concentrations of the toxicant are often at a maximum directly following exposure, so the preferred time range for sampling may be clearer than

with urine. However, blood concentrations of toxicants may vary with the exposure route; ingested toxicants usually require more time to reach the blood stream than inhaled or dermally absorbed doses. Furthermore, blood measurements are more likely than urine measurements to reflect the dose available for the target site [188] since the measured dose has not yet been eliminated from the body.

The major disadvantages of blood measurements are the venipuncture required to obtain the sample and the low toxicant concentrations. Unfortunately, the invasive nature of venipuncture sampling limits researchers' ability to obtain samples from children or, in some instances, to get high participation rates in large-scale studies. In addition, when samples can be obtained, the amount of blood available to perform the analysis is often limited; therefore, ultrasensitive analytical techniques may be required. For nonpersistent pesticides, analysis of blood is further complicated by the inherently low toxicant concentrations that are generally present in blood (ng/l or parts per trillion) when compared with urinary metabolite concentrations (μg/l or parts per billion).

An obvious advantage of biological monitoring in urine is its ease of availability. This is especially advantageous when multiple samples are required or when biological monitoring of children is necessary. Generally, the participation rate in large-scale studies is higher when urine samples are requested instead of blood.

Another advantage of urine is the amount of sample available for analysis. The analysis is not usually limited by the volume of sample available, except perhaps with very small children; therefore, less sensitive instruments could be used by compensating for the decreased instrument sensitivity with increased sample. The analysis of urine is further enhanced because the concentrations of toxicants or metabolites are higher in urine than in blood due to their relatively rapid metabolism and excretion. However, an increase in the sample size is generally accompanied by an increase in background noise of the sample.

Because urine analyses usually require the measurement of a metabolite instead of the parent pesticide, detailed information regarding the toxicant's metabolism is necessary to determine the appropriate biomarker of exposure. Unfortunately, detailed metabolic information is sometimes not available for pesticides, and in many cases where it is available, the reported metabolism applies only to a particular species of animals. In these cases, studies to determine the major human metabolites of the pesticides must be conducted or the best available information on animal metabolism must be used. Unfortunately, when animal metabolic information is used in developing an analytical method, the metabolite may not be detected in human samples. In these cases, these data do not necessarily indicate low or no internal dose of the toxicant; they may also indicate the wrong metabolite was monitored.

Because urine is a nonregulated body fluid, the concentration of toxicants or metabolites may vary, even if the internal dose remains constant. For this reason, either 24-h urine samples must be obtained for analysis or ''spot'' or ''grab'' samples must be corrected for dilution. Because 24-h urine samples are usually not practical, ''spot'' or ''grab'' samples or, for more concentrated samples, first morning voids are generally obtained, and their concentrations are normalized on the creatinine concentration, specific gravity or osmolality of the urine [189–191]. However, these correction methods do not necessarily correct for urine dilution because the metabolites may not be treated similar to creatinine in the body and because creatinine excretion can vary based upon several factors including seasonal and diurnal variations [192] and those related to muscle mass such as age, weight, sex, and pregnancy [192,193]. This problem with creatinine correction is highlighted when comparing adult metabolite levels to children metabolite levels. The inherently lower creatinine concentrations in children may cause the dilution to be ''over corrected'' which, in turn, may give the false appearance of elevated levels (when compared to adults) in children. However, to date, creatinine correction is the most widely accepted method for normalizing urine metabolite concentrations.

In some cases, particular metabolites may originate from more than one pesticide, which inhibits specific identification of the source of the original exposure. One example of a nonspecific metabolite is a dialkylphosphate, which may be derived from a variety of organophosphate pesticides. Dialkylphosphate concentrations provide nonspecific information about exposure to a class of pesticides instead of to a

single compound. Such information is certainly useful when determining exposure prevalence to most members of a class of compounds; however, it may not accurately reflect the toxicity associated with the exposure. An exception to this may be when the nonspecific metabolite is the toxic compound, such as with ETU metabolites of dithiocarbamates.

Although saliva has been used as a matrix for biomonitoring other xenobiotics, very little work has focused on saliva as a matrix for pesticide measurements. Although no chromatographic techniques were employed and is outside the scope of this review, measurements of pesticides in saliva or oral fluids have been performed using immunoassay. Lu et al. explored the feasibility of detection of pesticides in rats [194] and, recently, Denovan et al. reported saliva measurements of atrazine in herbicide applicators. Where saliva measurements are shown to correlate with plasma or serum measurements, saliva may be a good matrix for biomonitoring of pesticides [195].

Saliva measurements offer several distinct advantages. Saliva is likely to be a much cleaner matrix than urine or serum, since those compounds that cannot easily diffuse across cell membranes will be excluded from this matrix [196]. In addition, saliva is plentiful with the average adult secreting from 500 to 1500 ml/day [196] and collection is easy, noninvasive, and does not require privacy. To avoid the unpleasantness of spitting, some commercially available collection tubes include a cotton or polyfiber plug which may be chewed for several minutes to collect saliva. Using these special saliva tubes, collection may be done independently and shipped to the researcher. Biomonitoring of pesticides in saliva is an area worth more development.

A limitation in measuring nonpersistent chemicals as a whole is the transient nature of the pesticides in the body. In most instances, measurements in urine, blood, or saliva will only be indicative of recent exposure (Fig. 3). If sampling is not timed correctly, an exposure event might even be totally missed. As more interest has been directed toward children, both pre- and post-natal, and the potential relationship between pesticide exposures and developmental effects (e.g., decreased physiological and psychological development, congenital defects, etc.), the transient exposure information severely limits these studies.

Measurements in meconium offer a potential solution to this problem in studies observing in utero exposure effects. Meconium is a greenish–black tar-like substance that begins to accumulate in the intestines of a fetus during the second trimester of pregnancy and is expelled shortly after birth as the newborn's first few bowel movements. Theoretically for xenobiotics that cross the placental barrier and enter the fetus, a portion may be partitioned either as the parent compound or a metabolite into the meconium while the remainder is mostly metabolized and excreted into the amniotic fluid. Those metabolites that end up in the amniotic fluid can be swallowed or inhaled by the fetus and again a portion partitioned into the meconium. This cycle may continue until birth; thereby allowing a cumulative dosimeter of in utero exposure. Meconium has primarily been used to measure fetal exposure to illicit drugs, nicotine, and alcohol [197–199]. However, more recently, it has been explored as a potential matrix for biomonitoring fetal pesticide exposure [81,89]. These initial studies show promising potential for using meconium measurements in epidemiologic studies. However, more work needs to be done in calibrating levels in meconium with known levels of exposure.

4.2. Quality assurance/quality control

A vital component of biological monitoring is a sound quality assurance/control program (QA/QC). QA/QC programs are typically comprised of multiple testing procedures that easily allow the detection of systematic failures in the methodology. These testing procedures can include proficiency testing to ensure accuracy as measured against a known reference material, repeat measurements of known biological materials to confirm the validity of an analytical run and to measure analytical precision, ''round robin'' studies to confirm reproducible measurement values among laboratories analyzing for pesticides and/or metabolites, regular verification of instrument calibration, daily assurance of minimal laboratory contamination by analyzing ''blank'' samples, and cross validations to ensure that multiple analysts and instruments obtain similar analytical values. Many laboratories have adopted comprehensive QA/QC programs to ensure valid measurement results [200,201]. For instance, some public health laboratories in the US have been certified by the

Health Care Finance Administration (HFCA) to comply with all QA/QC parameters outlined in the Clinical Laboratories Improvement Amendment of 1988 (CLIA '88) [202]. The Federal Republic of Germany has chosen to implement a rigorous internal and external quality assurance program for environmental and toxicological analyses [201,203, 204]. Many parameters for implementing or improving a quality assurance program have been published [204,205].

4.3. Reference values

As the LODs of biological monitoring methods continue to decrease, a phenomenon often called the ''vanishing zero'', the frequency of detection of monitored pesticides increases accordingly. Consequently, the interpretation of internal dose information becomes more difficult. Although all detectable internal dose measurements represent exposure to the pesticides monitored, it is difficult to distinguish between overt exposures and background exposures. To aid in the differentiation of these exposures, reference values can be employed. Reference range values can be considered analogous to normal clinical values. They are determined by measuring the pesticide of concern or its metabolite in a large number of individuals with no known exposure to the compound. The distribution of the data from these measurements determines the reference range, usually defined as the 5th to the 95th percentile of the reference population. Increasingly, reference data are being reported on many pesticides that are currently measured in humans [93,95,206–209]; however, much reference data is still missing.

Although reference range values are extremely useful in evaluating environmental exposures to contemporary-use pesticides, there are some restrictions on their utility. Occupational exposures above ''background'' levels are generally expected in industrial settings; therefore, reference ranges are not necessarily useful in regulating occupational exposures. In addition, the age (and other demographic variables, if available) of the reference population must be taken into consideration. Extrapolation of adult data to children and vice versa, should be done with caution. Reference ranges are not foolproof exposure indices, but they are valuable tools for evaluating the extent of an individual's exposure as compared with that of individuals with no attributable exposure.

4.4. Biological exposure indices/tolerance values

Because reference values established for incidental exposures have limited utility when evaluating occupational exposures, the American Conference of Governmental Industrial Hygienists (ACGIH) and the DFG have established reference values for occupational exposures to toxic chemicals known as biological exposure indices (BEI) and biological tolerance values (BAT), respectively [210,211]. BEI and BAT publications provide extensive lists of chemicals and the acceptable levels of the chemical and/or its metabolite in urine [212,213]. These values can be used as reference values to which urinary concentrations in occupationally exposed individuals can be compared. For the most part, the BEI and BAT are in agreement with minor differences in the approaches taken to determine the values and to interpret them [214]. Scientists are actively working to resolve these minor differences so there can be international agreement on these occupational exposure guidelines [214].

5. Conclusions

Biological monitoring is a useful tool for assessing exposure to pesticides. The data gleaned from biological monitoring studies can be used successfully, in conjunction with other data, in risk assessment and risk management. Although many methods have been published for measuring pesticides in human matrices, there will be a continual need for methods that assess exposure to emerging pesticides. With this in mind, we would like to recommend standard criteria for reporting these methods. During validation, we recommend that researchers evaluate and report the following method specifications, including information on how each was determined: (1) limit of detection; (2) limit of quantification; (3) precision (specify concentration); (4) accuracy of the measurements; (5) extraction recoveries; (6) efficiency of derivatization reaction, if applicable; (7) linearity (including error about calibration slope and correlation coefficient of regression lines); (8) stability of analyte in the matrix tested; and (9) applicability to

human samples. With these standard data included in each report, we think that researchers will be better able to evaluate the methods for use in their particular applications.

6. Note

The use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service, the Department of Health and Human Services, or the Centers for Disease Control and Prevention.

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