Use of Biomarkers to Indicate Exposure of Children to Organophosphate Pesticides: Implications for a Longitudinal Study of Children's Environmental Health

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Because of their history of widespread use in the United States and unknown long-term health effects, organophosphate pesticides (OPs) are being considered as a chemical class of interest in planning for the National Children's Study, a longitudinal study of children's environmental health. The availability and appropriate use of biomarkers to determine absorbed doses of environmental chemicals such as OPs are critical issues. Biomarkers of OP exposure are typically measured in blood and urine; however, postpartum meconium has been shown to be a promising matrix for assessing cumulative *in utero* exposure to the fetus, and studies are currently in progress to determine the utility of using saliva and amniotic fluid as matrices. In this article, we discuss the advantages and disadvantages of the currently available OP exposure monitoring methods (cholinesterase inhibition in blood, pesticides in blood, metabolites in urine and alternative matrices); study design issues for a large, long-term study of children's environmental health; and current research and future research needs. Because OPs are rapidly metabolized and excreted, the utility of one-time spot measurements of OP biomarkers is questionable unless background exposure levels are relatively stable over time or a specific time frame of interest for the study is identified and samples are collected accordingly. Biomarkers of OP exposure can be a valuable tool in epidemiology of children's environmental health, as long as they are applied and interpreted appropriately. *Key words:* biomarkers, blood, children, exposure, meconium, organophosphate, pesticides, study design, urine. *Environ Health Perspect* 111:1939–1946 (2003). doi:10.1289/ehp.6179 available via *http://dx.doi.org/* [Online 10 September 2003]

The Children's Health Act of 2000 authorized the National Children's Study (NCS), a large, multiagency, long-term study of environmental influences on children's health and development (Children's Health Act 2000). The NCS will examine about 100,000 children across the United States and follow them during prenatal development, through birth and childhood, and into adulthood (Branum et al. 2003). The NCS Exposure to Chemical Agents Working Group has identified nonpersistent pesticides, including synthetic pyrethroid and organophosphate pesticides (OPs), as chemical classes of study for potential adverse neurodevelopmental outcomes (National Children's Study 2001). The use of biomarkers to determine absorbed doses of environmental chemicals such as OPs is a critical issue for implementing the NCS.

OPs became widely used as the environmentally persistent organochlorine pesticides were banned in the 1970s. OPs gained popularity in the early 1980s because they were relatively inexpensive, readily available, less persistent in the environment, and less susceptible to pest resistance. OPs are used primarily in agriculture on crops, but are also used in residential settings for pest control and for public health protection against vector-borne diseases (Table 1). Approximately 60 million pounds of OPs are applied to U.S. crops annually; nonagricultural uses account for an additional 17 million pounds [U.S.

Environmental Protection Agency (U.S. EPA) 1999]. A survey conducted for the U.S. EPA found that nearly half of U.S. households with a child younger than 5 years had a pesticide stored within reach of children (Whitmore et al. 1992). OPs account for about half of all insecticides used in the United States by amount sold. In outdoor settings, OPs are relatively nonpersistent because they are degraded by photochemical and microbiologic actions. However, when used indoors or as a part of structural treatments, these compounds can remain stable for extended periods of time (i.e., months to years; Fenske et al. 2000) and can remain potentially available for repeated exposure to both adults and children.

The safety of OPs has come under increasing scrutiny after the release of the National Research Council's (1993) report focusing on dietary pesticide exposure among infants and children. Consequently, passage of the Food Quality Protection Act of 1996 led the U.S. EPA to consider childhood pesticide exposure in aggregate and reassess all pesticide residue tolerances (Food Quality Protection Act 1996). OPs were the first class of pesticides whose tolerances were reassessed because of their common mode of toxicity, widespread use, and unknown long-term health effects. Because of increasing concern regarding the safety of these pesticides to children, many OP uses are being phased out (Table 1).

Acute effects of OP exposures are well documented and well understood (Kwong 2002). Because they are powerful inhibitors of carboxylic ester hydrolases, including acetylcholinesterase (AChE; found in nerve tissues and erythrocytes) and butyrylcholinesterase (plasma or pseudocholinesterase), individuals exposed to high levels of OPs can develop acute cholinergic syndrome, which is characterized by a variety of symptoms including rhinorrhea, salivation, lachrymation, tachycardia, headache, convulsions, and death (Karalliedde et al. 2001). In addition, these individuals can also develop a proximal and reversible paralysis called intermediate syndrome, organophosphate-induced delayed polyneuropathy, or long-term neurologic sequelae. Although adverse effects of chronic low-level OP exposure are suspected, they have not been conclusively determined (Eskenazi et al. 1999; Ray and Richards 2001).

Because exposure to OPs is multiroute and the dominant route of childhood exposure depends on a variety of dietary and behavioral factors, quantification of exposure is not a trivial process. Therefore, in many epidemiologic studies, markers of exposure in biologic samples have been measured to provide an estimation of absorbed dose (Aprea et al. 2000; Heudorf and Angerer 2001; Loewenherz et al. 1997; Lu et al. 2001; Mills and Zahm 2001; Moate et al. 1999; O'Rourke et al. 2000; Whyatt and Barr 2001). Although blood and urine are the primary human specimens that have been used for biologic monitoring of OP

exposure, postpartum meconium has been shown to be a promising matrix for assessing cumulative *in utero* exposures to the fetus, and studies are in progress to determine the utility of using saliva and amniotic fluid as matrices (Bradman et al. 2003; Whyatt and Barr 2001).

OP Metabolism

Our ability to incorporate biomarkers of OPs successfully into epidemiologic studies depends on our knowledge of OP metabolism. OPs all have the same general structure and mode of toxicity (Mileson et al. 1998). They are composed of a phosphate (or phosphorothioate or phosphorodithioate) moiety, which in most cases is *O,O*-dialkyl substituted, where the alkyl groups are either dimethyl or diethyl; and an organic group that is specific to each pesticide (Figure 1). For instance, chlorpyrifos is composed of an *O,O*-diethyl phosphorothioate to which a 3,5,6-trichloropyridinyl group is attached. Once entering the body, OPs can be enzymatically converted to their oxon form and then react with available cholinesterase. The oxon can also be enzymatically or spontaneously hydrolyzed to form a dialkyl phosphate (DAP) metabolite and a specific metabolite moiety. In the case of chlorpyrifos, diethylphosphate and 3,5,6-trichloro-2-pyridinol (TCPY) are formed. If the pesticide is

Table 1. Common OPs, their metabolites, and uses, with implications for biomarker assessment in children.

Pesticides	DMP	DMTP	DMDTP	DEP	DETP	DEDTP	Specific OP metabolites	Analysis of OP in blood	Insecticidal uses[a]
Acephate	—	—	—	—	—	—	Acephate, methamidophos	—	Crops, food handling, methamidophos ornamentals, residential
Azinphos-methyl	X	X	X	—	—	—	BTA, MSMB	—	Crops, trees, ornamentals.
Bensulide	—	—	—	—	—	—	—	—	Crops, lawn/turf, ornamental
Cadusafos	—	—	—	—	—	—	—	—	Import tolerances only (bananas)
Chlorethoxyphos	—	—	—	X	X	—	—	—	Crops (corn)
Chlorpyrifos	—	—	—	X	X	—	3,5,6-TCPY	X	Crop, lawn/turf, termiticide, ornamentals, pasture, livestock
Chlorpyrifos-methyl	X	X	—	—	—	—	3,5,6-TCPY	—	Stored grain; undergoing voluntary cancellation (2004 for use, 2008 for tolerances)
Coumaphos	—	—	—	X	X	—	CMHC	—	Livestock
Diazinon	—	—	—	X	X	—	IMPY	X	Crop, lawn/turf, residential/commercial; residential uses being phased out
Dichlorvos (DDVP)	X	—	—	—	—	—	—	X	Pest strips, residential, food, storage/processing, livestock
Dicrotophos	X	—	—	—	—	—	—	—	Crops (cotton), trees
Dimethoate	X	X	X	—	—	—	—	—	Crops, ornamentals
Disulfoton	—	—	—	X	X	X	—	—	Crops, ornamentals
Ethion	—	—	—	X	X	X	—	—	Crops (citrus), livestock; undergoing voluntary cancellation
Ethoprop	—	—	—	—	—	—	—	—	Crops, ornamentals, trees
Ethyl parathion	—	—	—	X	X	—	*p*-Nitrophenol	X	Crops; undergoing voluntary cancellation (by 2003)
Fenamiphos	—	—	—	—	—	—	—	—	Crops, ornamental, turf; undergoing voluntary cancellation (by 2007)
Fenitrothion	X	X	—	—	—	—	—	—	Residential/commercial ant/roach bait; imported wheat (Australia)
Fenthion	X	X	—	—	—	—	—	—	Livestock, mosquito control (Florida)
Malathion	X	X	X	—	—	—	Malathion dicarboxylic acid; Malathion mono-carboxylic acid	X	Crops, livestock, lawn/turf, mosquito
Methamidophos	—	—	—	—	—	—	Methamidophos	—	Crops
Methidathion	X	X	X	—	—	—	—	—	Crops, ornamentals
Methyl parathion	X	X	—	—	—	—	*p*-Nitrophenol	X	Crops
Mevinphos	X	—	—	—	—	—	—	—	Import tolerances only
Naled	X	—	—	—	—	—	—	—	Crops, greenhouse
Oxydemeton-methyl	X	X	—	—	—	—	—	—	Crops
Phorate	—	—	—	X	X	X	—	X	Crops
Phosalone	—	—	—	X	X	X	—	—	Import tolerances only
Phosmet	X	X	X	—	—	—	—	—	Crops, ornamental, forestry, livestock
Phostebupirim (tebupirimphos)	—	—	—	—	—	—	—	—	Crops (corn)
Pirimiphos-methyl	X	X	—	—	—	—	DEAMPY	—	Stored corn, seed, grain, livestock, bulbs
Profenofos	—	—	—	—	—	—	—	—	Crops (cotton)
Propetamphos	—	—	—	—	—	—	—	—	Indoor ant control
Sulfotepp	—	—	—	X	X	—	—	—	Greenhouses, ornamental; undergoing voluntary cancellation (by ~2004)
Temephos	X	X	—	—	—	—	—	—	Mosquito larva
Terbufos	—	—	—	X	X	X	—	X	Crops
Tetrachlorvinphos	X	—	—	—	—	—	—	—	Livestock, domestic animals (dogs/cats)
Tribufos	—	—	—	X	X	—	—	—	Crops (cotton)
Trichlorfon	X	—	—	—	—	—	—	—	Ornamentals, turf, agricultural premises, nurseries, ants

Abbreviations: —, not applicable; BTA, 1,2,3-benzotriazin-4(3*H*)-one/ol; CIT, 5-chloro-1-isopropyl-(1*H*)-1,2,4-triazol-3-ol/one; CMHC, 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one/ol; DEAMPY, 2-(diethylamino)-6-methylpyrimidin-4-ol/one; DEDTP, diethyldithiophosphate; DMDTP, dimethyldithiophosphate; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine; MSMB, methylsulfonylmethylbenzazimide; X, exposure to the pesticide listed.
[a]Sources on insecticidal uses from U.S. EPA. (2003)

not converted to its oxon form, it can undergo hydrolysis to its specific metabolite and dialkylthionate metabolites (i.e., dialkylthiophosphate and/or dialkyldithiophosphate). For chlorpyrifos, these metabolites are diethylthiophosphate and TCPY. These metabolites and/or their glucuronide or sulfate conjugates are excreted in urine.

Biomarkers of Effect: Monitoring Cholinesterase Inhibition in Blood

Examining cholinesterase inhibition as a biomarker of effect is one potential strategy. Acetylcholine (ACh) transmits electrochemical signals across neuronal synapses and neuromuscular junctions and is hydrolyzed by the action of the enzyme AChE. A serine residue with a free hydroxyl group in the active site of AChE covalently reacts with ACh, acetylating the serine while releasing the choline group. Within microseconds, the serine residue is deacetylated by hydrolysis and is free to degrade another ACh molecule. OPs in the oxon form (i.e., phosphate form) can react similarly with the serine residue; however, the dephosphorylation process is much slower, along the order of hours to days, than that for deacetylation (Karalliedde et al. 2001). Therefore, the serine residue on the phosphorylated AChE is not available to break down ACh. The toxic effects of OPs result from their ability to inhibit the action of AChE in the nervous system, causing a buildup of ACh,

overstimulating the nervous system (Karalliedde et al. 2001). OP poisoning is diagnosed by measuring reduced cholinesterase activity in red blood cells (RBCs).

Although cholinesterase monitoring has the advantage of providing a measure of physiologic response, it has disadvantages as well. Interpretation of AChE monitoring results is complicated by inter- and intraindividual variation in enzymatic activity and confounding factors such as cholinesterase suppression resulting from health conditions, and/or exposure to other cholinesterase-inhibiting pesticides (e.g., carbamate pesticides; Bissbort et al. 2001; Lessenger and Reese 1999). Because of fluctuations in AChE levels, a baseline AChE level is needed to determine if suppression has occurred. Genetic influences not related to sex, race, or age account for 23% of variation in AChE activity levels among humans (Lessenger and Reese 1999). AChE levels in children younger than 4 months have been shown to be lower than for adults, whereas levels in children older than 4 months were comparable with those of adults (Karlsen et al. 1981). Pregnancy, diseases, medications, and illegal drugs affect AChE levels in adults (De Peyster et al. 1993; Lessenger and Reese 1999). Because of the tremendous amount of variation in AChE due to endogenous and exogenous variables, a monitoring program that does not account for variation due to these factors will produce data that will be difficult, if not impossible, to interpret. Another drawback of AChE monitoring is that

large doses are required for significant AChE inhibition to occur. Therefore, AChE monitoring is used more appropriately as an indicator of toxicity at high exposure levels and is rather insensitive at low exposure levels (He 1999).

The inhibition of RBC AChE and plasma cholinesterase (PChE) is highly correlated with intensity and duration of exposure to OPs. RBC AChE, the same molecular target as that responsible for acute OP toxicity in the nervous system, is a more specific indicator than is PChE. However, a few of the OP compounds (e.g., malathion, diazinon, and dichlorvos) are earlier inhibitors of PChE than of AChE. In this case, PChE might be a more sensitive indicator of exposure than is AChE but may not be associated with symptoms and signs of toxicity (Jeyaratnam and Maroni 1994).

AChE measurements have been used extensively in occupational monitoring of pesticide applicators (Magnotti et al. 1988) but have not been used widely in general population exposure studies. The U.S. EPA considers blood AChE inhibition data to be appropriate for deriving reference doses or concentrations as part of a weight of evidence analysis for pesticide toxicity but notes that reliability of these measures depends on the quality of the available data (U.S. EPA 2000).

The electrometric and colorimetric methods, which measure change in pH and light absorbance, respectively, are used most often to measure AChE suppression. Both methods can be used to measure both serum and erythrocyte cholinesterases and are relatively simple, inexpensive, and reproducible (Vandekar 1980). Even with modern testing kits and methods, the determination of serum and erythrocyte AChE activity levels depends greatly on technician experience and skill. Aware of such sources of error, California—the only U.S. state requiring AChE testing for pesticide applicators—requires laboratory certification of its AChE analysts (CEPA 2002).

Because OPs are metabolized rapidly, there is some potential benefit of a marker of biologic effect (e.g., AChE suppression) that may integrate exposure over a longer time frame. However, children are generally believed to be exposed to OPs at levels much lower than encountered occupationally. Their exposure is more likely to be intermittent or variable in intensity, and they are not likely to have baseline AChE levels available for comparison. All of these factors make blood measurements of AChE less viable as a biomarker for children's OP exposure.

A potential alternative measurement is to measure the bound cholinesterase itself. Researchers in the Netherlands have developed a sensitive analytic technique to accurately measure butyrylcholinesterase bound to organophosphate nerve agents (Fidder et al. 2002). Their technique involves the enzymatic
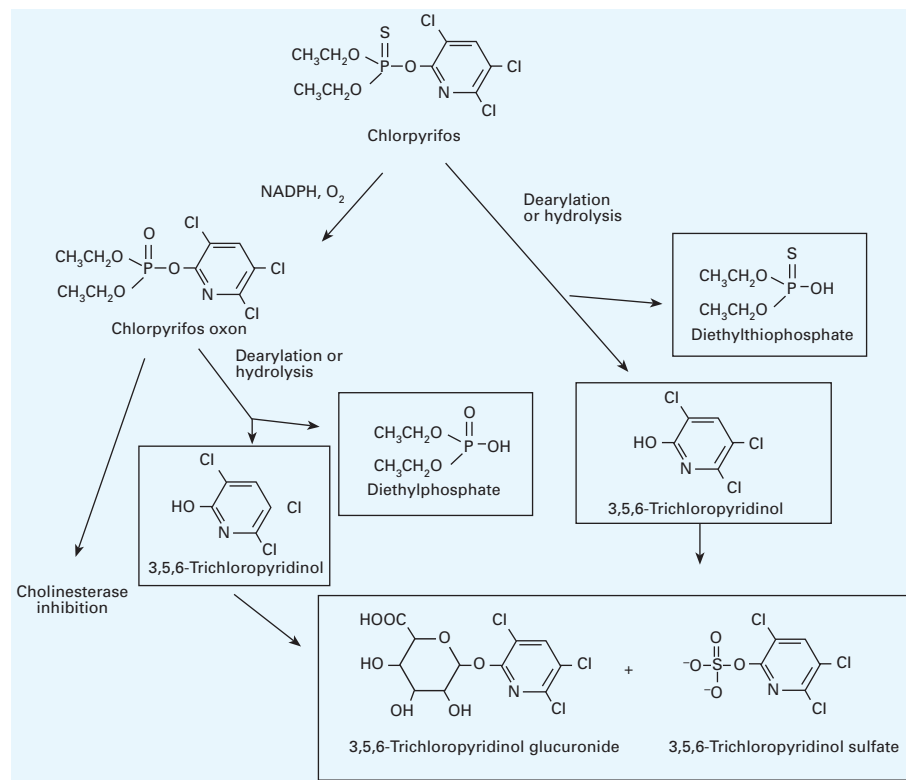

Figure 1. Metabolism of chlorpyrifos. Chemicals enclosed in boxes are excreted in the urine.

digestion of butyrylcholinesterase to form a nonapeptide. The amount of the phosphorylated peptide is quantified relative to the nonphosphorylated peptide. Using this method, exposure could be detected at levels that caused only about 1% suppression of cholinesterase activity—a level much too low to detect using standard cholinesterase assays. This measurement would likely reflect the clinically relevant dose for acute toxicity, but it is less clear whether this method would provide a useful biomarker for potential OP-related health effects that may not rely on this mechanism of action.

Biomarkers of Exposure: Monitoring Pesticides and/or Metabolites in Biologic Samples

Blood. Monitoring OP concentrations in blood or blood products (e.g., serum, plasma) offers several advantages. The parent compounds can be monitored directly in blood products instead of their metabolites, which are usually measured in urine. Therefore, detailed information regarding the metabolism of the pesticide is less critical, and toxicant concentrations for specific OPs are known rather than inferred from metabolites. This information is especially beneficial because not all OPs are equally toxic. Blood measurements provide an estimation of the dose available for the target site, allowing for prediction of dose–response relationships. Furthermore, because blood is a regulated fluid (i.e., the volume does not vary substantially with water intake or other factors), the blood concentrations of toxicants measured at a specified time interval after exposure will remain the same as long as the absorbed amounts are constant; therefore, no corrections for dilution are necessary. Blood concentrations of the toxicant are often at a maximum directly after exposure, so if exposure events are known, the preferred time range for sampling may be clearer than with urine. However, in a large study such as the NCS, biologic sample collection will likely be based on other considerations (e.g., clinic visits) rather than occurrence of an exposure event.

The major disadvantages related to blood measurements are the venipuncture and associated risks (e.g., bruising, discomfort) required to obtain the sample, and the analytic challenge of measuring low toxicant concentrations. If available, umbilical cord blood can overcome some of these concerns for measuring recent *in utero* exposures, because venipuncture is not needed and relatively large quantities of blood (> 30 mL) can be collected. The invasive nature of venipuncture puts some limits on researchers' ability to obtain samples from children and pregnant women or to get high participation rates in large-scale studies. In addition, the amount of blood available to perform the analysis is often limited; therefore, ultrasensitive analytic techniques may be required. Analysis is further complicated by the inherently low concentrations of OPs present in the blood (typically seen in the nanogram per liter or parts per trillion range) when compared with urinary metabolite concentrations (typically seen in the microgram per liter or parts per billion range).

Several laboratory methods have been reported that measure intact OPs in blood (Fournier et al. 1978; Frenzel et al. 2000; Kawasaki et al. 1992; Liu et al. 1989; Maroni et al. 1990; Marques 1990; Meyer et al. 1998; Sharma et al. 1990). Unfortunately, most of these methods have limits of detection (LODs) that would prevent their use in measuring incidental exposures. For example, Frenzel et al. (2000) reported a method to measure methamidophos and methyl parathion in blood with LODs of about 25 µg/L. However, data reported by Whyatt et al. (2003) indicate that levels in pregnant women and cord blood were about 3 orders of magnitude lower. Recent advances in analytic instrumentation have facilitated the development of highly sensitive methods (Barr et al. 2002; Liu and Pleil 2002); however, these methods are often complicated and costly, precluding their use for routine analysis.

Urine. An obvious advantage of biologic monitoring in urine is the ease of sample collection, the high concentration of analytes, and the greater amount of sample available for analysis compared with blood. However, these advantages may be diminished somewhat for infants and very small children for whom urine collection procedures require special consideration. Appropriate sample collection apparatus, such as urine collection bags or toilet inserts, must be provided to collect urine samples from children who are not toilet trained.

One of the disadvantages of urinary analysis is that urine output varies. Many factors influence daily urinary output, such as water, urea, salt, specific gravity, and osmolality. Consequently, the concentration of toxicants or metabolites may vary, even if the internal dose remains constant. For this reason, either 24-hr urine samples must be obtained for analysis, or "spot" or "grab" samples must be corrected for dilution. Because 24-hr urine samples are not practical in large-scale population-based studies of children, spot samples or first morning voids (for more concentrated samples) can be obtained, and their concentration normalized using creatinine (CRE) concentration or specific gravity. However, CRE yield has been shown to be variable among children (Freeman et al. 1995; O'Rourke et al. 2000). Metabolite results are considered questionable for samples with CRE < 0.3 or > 3.0 g/L (Lauwerys and Hoet 1993; O'Rourke et al. 2000; WHO 1996); however, no data are reported on which these limits are based. Furthermore, because CRE excretion depends on muscle mass, children inherently excrete less CRE. In fact, about 35% of the children's samples analyzed at the Centers for Disease Control and Prevention (CDC) had CRE concentrations less than 0.3 g/L (CDC. Unpublished data). This makes comparisons between CRE-adjusted adults' and children's urinary toxicant concentrations subject to great error because of "overcorrection" of children's samples.

An alternative though less widely used method for adjusting urine dilution is based on urinary specific gravity. Specific gravity measurements take into account all solids dissolved in a urine sample, not just CRE. Typically, urine samples are adjusted to a specific gravity of 1.024. Additional research is required to determine whether this is a viable alternative for routine urinary adjustments.

Variations in metabolite concentrations due to changing water content in urine can also be eliminated using urinary excretion rate calculations (Rigas et al. 2001). However, because the void volume and times of previous and current void are required, this approach may not be practical for young children.

Of the OPs registered with the U.S. EPA for use in the United States, about 75% metabolize to form from one to three of the six DAP metabolites. These six metabolites are dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate, diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate. The OP pesticides registered with the U.S. EPA and their potential common metabolites are listed in Table 1. Pesticide-specific information cannot be derived from the quantitative measurement of these metabolites; however, a cumulative dose measure of OPs as a class of pesticides can be obtained.

The pesticide-specific metabolites can also be measured. The quantitative measurement of these metabolites provides a measure of dose for a specific pesticide. For instance, the measurement of TCPY provides dose information specific to chlorpyrifos or chlorpyrifos-methyl. It is important to note that not all pesticide-specific metabolites are derived solely from OPs. For example, 4-nitrophenol, a specific metabolite of parathion or methyl parathion, is also a widely used chemical and can enter the body from other exposure sources.

Measurements of either the common OP metabolites or the specific metabolites have advantages and disadvantages. Both the common and specific metabolites are also the hydrolysis or breakdown products of OPs in environmental media (Heudorf and Angerer 2001). Therefore, the measured concentrations of these metabolites can result from

exposure to only the pesticide breakdown products in environmental media. The common metabolite measurements provide class-specific data that take into account not only aggregate exposures (i.e., exposure to a single pesticide from multiple sources) but also cumulative exposures (i.e., exposure to two or more pesticides with the same mechanism of toxicity). However, because individual pesticides differ in acute toxicities, these data are not good indicators of the toxicity of the cumulative dose. DAPs may be metabolites of some industrial chemicals and rare pharmaceuticals, but it is generally believed that most DAPs result from OP exposure or exposure to OP hydrolysis products (Barr et al. 2002). The DAP data can be used to "screen" for total OP exposure, and follow-up analyses of the specific metabolites can be performed if the common metabolites levels appear elevated. In addition, these data can help distinguish between the individual pesticides in OP pairs (e.g., methyl parathion from parathion, chlorpyrifos from chlorpyrifos-methyl).

The specific metabolite measurements provide dose data on individual pesticides. However, because of the large number of OPs, the chemical nature of some of the OP metabolites, and the lack of availability of analytic standards, it is not feasible to measure all of the specific metabolites at this time. Therefore, cumulative data cannot be obtained from specific metabolite measurements because so many metabolites cannot be measured. We believe that the data obtained from measuring both the common and specific metabolites, where available, complement each other. Together, these data can provide the most complete OP internal dose information.

Urine metabolite levels are generally considered a much more sensitive and specific indicator of OP exposure than is AChE monitoring, and have been reported in several studies of children (Adgate et al. 2001; Aprea et al. 2000; Fenske et al. 2002; Loewenherz et al. 1997; Lu et al. 2001; Mills and Zahm 2001; Moate et al. 1999; O'Rourke et al. 2000). DAPs can be detected in urine at exposure levels below those affecting cholinesterase activity (Coye et al. 1986). However, because all OPs differ in toxicity, the measured DAP levels are not direct measures of toxicologic potential.

It is necessary to understand the kinetics of metabolite excretion to know the optimum time for urine collection (Griffin et al. 1999). Also, not all OPs are metabolized to a measurable level of DAPs; therefore, exposure may occur but not be detected by current laboratory methods. Because of variation in metabolic rates from individual to individual, optimal sampling times will vary. OPs are metabolized and preferentially excreted in the urine usually within 24–48 hr of exposure (WHO 1996).

At least seven laboratories in North America and Europe are routinely analyzing DAPs in epidemiologic studies. These laboratories use methodologies usually developed in-house and employ gas chromatography with some sort of selective detection technique such as nitrogen-phosphorus detection or mass spectrometric detection. The LODs of these methods are in the low picogram to the low nanogram per millimeter range. Surprisingly, even though so many laboratories are measuring these DAP concentrations in a variety of studies and are reporting the values in peer-reviewed literature, the first interlaboratory comparison of analyses was performed just recently. To confirm that data originating from the various laboratories are directly comparable, the U.S. EPA initiated an interlaboratory comparison study among the North American laboratories performing DAP analyses. The results of this study will allow us to determine whether results across laboratories are comparable; and if they are not, it will allow us to standardize methodologies to promote harmonization among laboratory data.

Several studies have shown higher DAP levels in children than adults. In a study of 1,194 subjects in Germany, Heudorf and Angerer (2001) found significantly higher metabolite concentrations per gram of CRE in children younger than 6 years than in any other age group (6 to < 14 years, 14 to < 20 years, ≥ 20 years). The researchers concluded that this was a result of the children's lower urinary CRE content, but allowed that children's exposure may still be higher than that of adults. In a study of farmworkers and their children in California, Mills and Zahm (2001) found DMP, DMTP, and DETP more often in the urine of children than in that of their farmworker parents. However, DEP was detected more frequently among adults than among children. A study of 195 Italian children found significantly higher values of all OP metabolites in children than in 124 adults sampled in a previous study in a nearby region (Aprea et al. 2000).

Specific OP metabolites have also been measured in several studies involving children. In the Minnesota Children's Pesticide Exposure Study (MNCPES), higher levels of TCPY were found among urban children than among those living in nonurban areas (Adgate et al. 2001). Malathion dicarboxylic acid showed a similar, although only marginally significant, trend among this study population. Fenske et al. (2002) measured both TCPY and *para*-nitrophenol, a metabolite of parathion, methyl parathion, and other chemicals, in the urine of children of farmworkers and pesticide applicators. Although the mean urinary concentrations of both metabolites were greater in children living within 200 feet of pesticide-treated farmland, these differences

were not statistically significant. MacIntosh et al. (2001) found that dietary chlorpyrifos levels were significantly correlated with mean urinary TCPY excretion in the National Human Exposure Assessment Survey in Maryland and that the dietary chlorpyrifos accounted for approximately 7% of the urinary TCPY.

Meconium. Only recently has the use of meconium been investigated as a potential matrix for testing of exposure to OPs. Meconium has previously been used to identify fetal exposure to illicit drugs, alcohol, and tobacco (Bearer et al. 1999; Browne et al. 1994; Callahan et al. 1992; Clark et al. 1992; Dempsey et al. 1999; Maynard et al. 1991; Moore et al. 1998; Ostrea 1999; Ryan et al. 1994) as well as the more persistent environmental toxicants, such as organochlorines and heavy metals (Hong et al. 2002; Ramirez et al. 2000). Meconium begins to accumulate in the bowels of human fetuses at approximately 16 weeks gestation and is generally not excreted until after delivery (Moriya et al. 1994). Meconium is a complex matrix, consisting mainly of water but also containing mucopolysaccharides, lipids, proteins, bile acids and salts, epithelial cells, cholesterol and sterol precursors, blood-group substances, squamous cells, residual amniotic fluid, and enzymes (Moore et al. 1998). Xenobiotics appear to enter the meconium as a consequence of bile excretion into the intestines and/or of swallowing of amniotic fluid by the fetus (Ostrea et al. 1993). Evidence suggests that the half-life of xenobiotics in meconium can be protracted and that measured levels may reflect exposures from the second trimester of pregnancy through delivery (Bearer et al. 1999; Ostrea 1999).

Whyatt and Barr (2001) analyzed 20 meconium samples from newborns and found some level of OP metabolites in all 20 samples. The researchers concluded that measurement of OP metabolites in meconium showed promise as a biomarker of prenatal exposure. Detection limits for the OP metabolites were low and comparable with or better than those seen with adult urine. One of the biggest advantages of meconium analysis is the ease and noninvasive nature of meconium collection. Whyatt and Barr (2001) also found that pesticide metabolites were stable in meconium for more than 12 hr at room temperature. Given these initial promising findings, further research is needed to determine the time frame of exposure represented by pesticide levels in meconium and evaluate the dose–response relationship (Whyatt and Barr 2001). Additionally, the same issues as for urinary analysis must be addressed in meconium analysis, such as kinetics of metabolism, pass-through of metabolites, normalization of measured concentrations on water content, and establishment of reference concentrations.

Study Design Issues

Given the scope, complexity, and magnitude of the NCS, one cost-effective, time-saving strategy to assess potential health effects associated with OP exposure would be to devise a screening method to identify and oversample the most highly exposed children. Identification of more highly exposed children would limit the number of results reported below detection and/or quantification limits of analytic methods. Limited evidence exists to identify children at higher risk for OP exposure, and a recent report from the MNCPES highlights the challenges of screening for general pesticide use when specific target compounds are of interest (Sexton et al. 2003). The following groups have been identified as having higher urinary biomarker levels for OPs: children of pesticide applicators (Loewenherz et al. 1997), younger children within the 0–6 year age range (Loewenherz et al. 1997), children living closer to pesticide-treated orchards (Loewenherz et al. 1997), children living in urban areas (Adgate et al. 2001), and those living where pesticides are used inside or outside the home (Aprea et al. 2000; Lu et al. 2001). In a study such as the NCS, a first step might include questionnaire data to indicate the likelihood that the child is exposed and a qualitative ranking of the exposure (e.g., low, moderate, high). Biologic sampling could then be targeted at specifically ranked groups rather than applied to everyone with the same intensity. Where chemical analyses are resource intensive, other strategies can be used. If compounds are stable after long-term storage, then specimens can be analyzed case by case for children in special studies, such as nested case–control studies of childhood diseases. Callahan et al. (1995) discuss statistical methods and issues regarding stratification and oversampling for target populations that could be used to evaluate health risks associated with low-level or infrequent exposures.

The MNCPES is an example of a study conducted in this manner. The MNCPES used a probability-based sampling strategy to identify and oversample children who were potentially exposed to targeted pesticides (Quackenboss et al. 2000). Using probability-based sampling reduced the potential for a selection bias, allowed the calculation of weighted population statistics, and allowed the results to be generalized to the population. The MNCPES was conducted in three phases: *a*) identification of households with age-eligible children (3–12 years) and more frequent pesticide use, *b*) screening of households selected in phase 1 using a questionnaire and an inventory of pesticide product storage and use, and *c*) intensive monitoring of the children with the highest potential for exposure as identified in phase 2. Whether or not the NCS is a probability sample, sampling units can still be derived to enhance the internal validity of OP exposure measurements based on identifying highly exposed children and subsets of children with lower levels of exposure for comparison purposes.

Current Research and Future Research Needs

Much research is currently in progress to evaluate children's exposure to OPs, health effects resulting from OP exposure, and other biologic monitoring methods. For example, many of the jointly funded National Institute of Environmental Health Sciences/U.S. EPA Centers for Children's Environmental Health and Disease Prevention Research are conducting studies in which women are enrolled during early pregnancy and their children are followed for the first several years of life (Castorina et al. 2003). These studies will further define current OP exposure levels in children, help determine the most appropriate matrix or matrices for exposure measurements, facilitate the development of additional biomonitoring methods, provide information about the frequency of collections needed to estimate chronic exposures and to detect acute or peak exposures for individuals, evaluate and/or validate questionnaires, and elucidate health end points (e.g., neurodevelopment, growth, and respiratory health). A great deal of research related to OP biomarkers in blood, urine, and other matrices remains to be completed before the NCS begins. Research needs for each of the currently available biomonitoring methods are discussed below.

To identify specific OP concentrations in blood, laboratory analysis methods must be developed for all OPs currently registered for use in the United States or having allowable tolerances on imported food products (Table 1). This may not be a feasible expectation, given the chemical properties of the particular pesticides; however, it is still a critical need for a comprehensive OP exposure assessment. A long-term study such as the NCS will then have the information needed to develop dose–response curves, correlating health end points with measured toxicant concentrations.

Research is needed to identify physical and biologic mechanisms of degradation of OPs and fate of metabolites in the environment and in the human body. OPs degrade in foodstuff before ingestion, but the amount of these metabolites and any biologic effect they might have as they pass through the human body are unknown. Currently, the U.S. EPA and the CDC are jointly engaged in research to identify the relative contributions of exposure to environmental degradation products to urinary metabolite levels. Multiroute (e.g., duplicate diet, dust, and air) environmental exposures are being measured for both intact pesticides and their degradation products as well as monitoring blood, urine, and saliva. These data should help us understand better the potential confounding of biomarker data from exposure to pesticides with exposure to the environmental degradates.

Easier, less costly, but still high-quality laboratory methods for DAP measurements would allow more samples to be analyzed in less time. Currently, the specific metabolites for only 10 OPs have been measured in urine (Table 1). Identification of specific metabolic products for each OP, if possible, would allow the use of urine, an easier matrix to obtain than blood, in prediction of dose–response curves. Research to establish the half-life for each OP would allow better estimation of the appropriate sampling time frame during human studies and allow for dose estimations.

Further research is needed to establish reference-range CRE concentrations based on age, race, sex, and seasonal variation. Research of specific gravity (and/or osmolality) would indicate whether it is a viable alternative to adjust for urine dilution.

Use of postpartum meconium shows promise, and further research could firmly establish it as a biomarker for OP exposure. Research is needed to determine the time frame of exposure represented by metabolite levels, kinetics of metabolism, fate of metabolites, best methods to normalize measured concentrations on water content, and reference concentrations.

It would be valuable to know if there is significant degradation of the parent compounds and/or metabolites in biologic samples over time and how storage conditions are related to potential degradation. Many important exposure–health outcome studies may be done after years of specimen storage—for example, nested case–control studies to evaluate the potential risk of early life pesticide exposures associated with an uncommon child health outcome.

Conclusions

Biomarkers are an effective way to determine exposure to OPs, particularly for known exposure events. A challenge of all monitoring methods discussed here is that OPs are so rapidly metabolized and excreted. It is vital for investigators to consider the relationship between the exposure time frame reflected in an OP biomarker and the research questions. It would not be advisable to assume that biologic measures of OP exposure levels are relatively constant over time.

In a longitudinal study, to characterize intermittent exposures as well as the chronic background OP level, repeated biologic samples may be needed. Specimens could be collected at a number of potential contact points where OP exposure could be assessed, such as clinical exams to assess child development and routine prenatal visits. Those analyses should

be conducted if the investigators believe the time frame is important or representative of a vulnerable period in child development, recognizing that the spot sample cannot provide an integrated measure of exposure over time. Critical windows of vulnerability in the developing child have been identified for many health outcomes (Selevan et al. 2000). When the relevant exposure time frame is known, researchers can try to obtain biologic specimens during those time windows. Alternatively, research questions about exposure events, such as accidental poisoning or nearby agricultural spraying, may be used to develop protocols that bring children in after an acute event and follow them to observe potential effects.

For hypotheses that relate *in utero* exposure to child health outcomes, meconium may give an integrated measure that probably reflects aggregate OP exposure over the second half of pregnancy. This time frame is important for many child health end points but would not directly cover birth defects or other outcomes with first-trimester origins. Maternal samples collected prenatally might also provide an indication of short-term acute fetal exposure. Maternal blood samples collected at delivery have OP levels that correlate well with those measured in their umbilical cord blood (Whyatt et al. 2003), suggesting that maternal pesticide levels may accurately reflect the dose being transported to the fetus through the placenta.

Measurement of AChE in blood is not currently a viable method for monitoring long-term, low-level exposure in children. However, monitoring specific OPs in blood offers useful information because health end points can be correlated to specific toxicant concentrations. A powerful tool will be available once laboratory methods have been developed to measure all OPs used in the United States. In the meantime, measurement of specific metabolites in blood used in concert with urine metabolite measurements (general and specific) will allow for prediction of dose–response relationships for many of the OPs. Some research questions may be more confidently answered with other measures of usual or typical exposure, such as residential pesticide use or time–activity questionnaires.

If the relevant time frame for exposure is past or uncertain, the utility of a one-time measurement for OP biomarkers is questionable. Most OP biomarkers reflect recent exposure via all pathways over a very short time frame, from several hours to days. One-time biomarker measures could be useful in populations of children with relatively stable background levels of OP exposure. Children who are subject to exposure variation associated with intermittent uses, such as in agricultural areas or periodic residential applications, are likely to be poorly classified on the basis of one spot measurement. As we learn more about the contribution of various sources of exposure for children, we may be better able to identify groups of children that can be classified accurately using OP biomarkers. Unless the researchers have some confidence about the relation between the historical and current levels of exposure, a biomarker of current exposure might prove misleading.

Lack of precision and potential misclassification of exposure are common shortcomings in environmental epidemiology. Although this limitation can be easily overcome when the risk relationships under study are robust or the exposures are relatively uncommon, it is a greater challenge for exploratory investigations of health effects associated with ubiquitous, low-level exposures such as a typical child's OP exposure scenario. Answering current research questions on the stability of stored samples over time, interlaboratory analytic comparisons, and further descriptions of the characteristics of children and families where exposures are more common and more stable over time will help us determine the appropriate strategies for incorporating OP biomarkers into the NCS.

As in any observational study where the participants receive little direct benefit, biologic specimens represent a significant contribution of the participants toward the advancement of science and the public good. These contributions should be used to enhance hazard identification and elucidate risk relationships where the underlying scientific rationale suggests they will be of greatest public health benefit. Biomarkers of OP exposure can be a valuable tool in epidemiology of children's environmental health. They can improve exposure assessment and reduce misclassification, thereby strengthening our confidence in any exposure–outcome relationships observed. The challenge is to apply and interpret those biomarkers appropriately.

REFERENCES

Adgate JL, Barr DB, Clayton CA, Eberly LE, Freeman NC, Lioy PJ, et al. 2001. Measurement of children's exposure to pesticides: analysis of urinary metabolite levels in a probability-based sample. Environ Health Perspect 109:583–590.

Aprea C, Strabi M, Novelli MT, Lunghini L, Bozzi N. 2000. Biologic monitoring of exposure to organophosphorus pesticides in 195 Italian children. Environ Health Perspect 108:521–525.

Barr DB, Barr JR, Maggio VL, Whitehead RD Jr, Sadowski MA, Whatt RM, et al. 2002. A multi-analyte method for quantification of contemporary pesticides in human serum and plasma using high resolution mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 778:99–111.

Bearer CF, Lee S, Salvator AE, Minnes S, Swick A, Yamashita T, et al. 1999. Ethyl linoleate in meconium: a biomarker for prenatal ethanol exposure. Alcohol Clin Exp Res 23:487–493.

Bissbort SH, Vermaak WJ, Elias J, Bester MJ, Dhatt GS, Pum JK. 2001. Novel test and its automation for the determination of erythrocyte acetylcholinesterase and its application to organophosphate exposure. Clin Chim Acta 303:139–145.

Bradman A, Barr DB, Claus Henn BG, Brumheller T, Curry C, Eskenazi B. 2003. Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study. Environ Health Perspect 111:1779–1782.

Branum AM, Collman GW, Correa A, Keim SA, Kessel W, Kimmel CA, et al. 2003. The National Children's Study of environmental effects on child health and development. Environ Health Perspect 111:642–646.

Browne S, Moore C, Negrusz A, Tebbett I, Covert R, Dusick A. 1994. Detection of cocaine, norcocaine, and cocaethylene in the meconium of premature neonates. J Forensic Sci 39:1515–1519.

Callahan CM, Grant TM, Phipps P, Clark G, Novack AH, Streissguth AP, et al. 1992. Measurement of gestational cocaine exposure: sensitivity of infants' hair, meconium, and urine. J Pediatr 120:763–768.

Callahan MA, Clickner RP, Whitmore RW, Kalton G, Sexton K. 1995. Overview of important design issues for a National Human Exposure Assessment Survey. J Expo Anal Environ Epidemiol 5:257–282.

Castorina R, Bradman A, McKone TE, Barr DB, Harnly ME, Eskenazi B. 2003. Cumulative organophosphate pesticide exposure and risk assessment among pregnant women living in an agricultural community: a case study from the CHAMACOS cohort. Environ Health Perspect 111:1640–1648.

CEPA. 2002. Guidelines for Physicians Who Supervise Workers Exposed to Cholinesterase-Inhibiting Pesticides. 4th ed. Sacramento, CA:California Environmental Protection Agency.

Children's Health Act of 2000. 2000. Public Law 106-310.

Clark GD, Rosenzweig IB, Raisys VA, Callahan CM, Grant TM, Streissguth AP. 1992. The analysis of cocaine and benzoyl-ecgonine in meconium. J Anal Toxicol 16:261–263.

Coye MJ, Lowe JA, Maddy KJ. 1986. Biological monitoring of agricultural workers exposed to pesticides. II. Monitoring of intact pesticides and their metabolites. J Occup Med 28:628–636.

Dempsey D, Moore C, Deitermann D, Lewis D, Feeley B, Niedbala RS. 1999. The detection of cotinine in hydrolyzed meconium samples. Forensic Sci Int 102:167–171.

De Peyster A, Willis WO, Molgaard CA, MacKendrick TM, Walker C. 1993. Cholinesterase and self-reported pesticide exposure among pregnant women. Arch Environ Health 48:348–352.

Eskenazi B, Bradman A, Castorina R. 1999. Exposure of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect 107(suppl 3):409–419.

Fenske RA, Lu C, Barr D, Needham L. 2002. Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. Environ Health Perspect 110:549–553.

Fenske RA, Lu C, Simcox NJ, Loewenherz C, Touchstone J, Moate TF, et al. 2000. Strategies for assessing children's organophosphorus pesticide exposures in agricultural communities. J Expo Anal Environ Epidemiol 10:662–671.

Fidder A, Hulst AG, Noort D, de Ruiter R, van der Schans MJ, Benschop HP, et al. 2002. Restrospective detection of exposure to organophosphorus anti-cholinesterases: mass spectrometric analysis of phosphylated human butyryl-cholinesterase. Chem Res Toxicol 15:582–590.

Food Quality Protection Act of 1996. 1996. Public Law 104-170.

Fournier E, Sonnier M, Dally S. 1978. Detection and assay of organophosphate pesticides in human blood by gas chromatography. Clin Toxicol 12:457–462.

Freeman NC, Wainman T, Lioy PJ, Stern AH, Shupack SI. 1995. The effect of remediation of chromium waste sites on chromium levels in urine of children living in the surrounding neighborhood. J Air Waste Manag Assoc 45:604–614.

Frenzel T, Sochor H, Speer K, Uihlein M. 2000. Rapid multimethod for verification and determination of toxic pesticides in whole blood by means of capillary GC-MS. J Anal Toxicol 24:365–371.

Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56:10–13.

He F. 1999. Biological monitoring of exposures to pesticides: current issues. Toxicol Lett 108:277–283.

Heudorf U, Angerer J. 2001. Metabolites of organophosphorous insecticides in urine specimens from inhabitants of a residential area. Environ Res 86:80–87.

Hong Z, Gunter M, Randow FF. 2002. Meconium: a matrix reflecting potential fetal exposure to organochlorine pesticides and its metabolites. Ecotoxicol Environ Saf 51:60–64.

Jeyaratnam J, Maroni M. 1994. Organophosphorous compounds. Toxicology 91:15–27.

Karalliedde L, Feldman S, Henry J, Marrs T, eds. 2001. Organophosphates and Health. River Edge, NJ:World Scientific Publishing.

Karlsen RL, Sterri S, Lyngaas S, Fonnum F. 1981. Reference values for erythrocyte acetylcholinesterase and plasma cholinesterase activities in children, implications for organophosphate intoxication. Scand J Clin Lab Invest 41:301–302.

Kawasaki S, Ueda H, Itoh H, Tadano J. 1992. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Chromatogr 595:193–202.

Kwong TC. 2002. Organophosphate pesticides: biochemistry and clinical toxicology. Ther Drug Monit 24:144–149.

Lauwerys RR, Hoet P. 1993. Industrial Chemical Exposure: Guidelines for Biological Monitoring. Boca Raton, FL:Lewis Publishers.

Lessenger JE, Reese BE. 1999. Rational use of cholinesterase activity testing in pesticide poisoning. J Am Board Fam Pract 12:307–314.

Liu J, Suzuki O, Kumazawa T, Seno H. 1989. Rapid isolation with Sep-Pak C18 cartridges and wide-bore capillary gas chromatography of organophosphate pesticides. Forensic Sci Int 41:67–72.

Liu S, Pleil JD. 2002. Human blood and environmental media screening method for pesticides and polychlorinated biphenyl compounds using liquid extraction and gas chromatography-mass spectrometry analysis. J Chromatogr B Analyt Technol Biomed Life Sci 769:155–167.

Loewenherz C, Fenske RA, Simcox NJ, Bellamy G, Kalman D. 1997. Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington State. Environ Health Perspect 105:1344–1353.

Lu C, Knutson DE, Fisker-Andersen J, Fenske RA. 2001. Biological monitoring survey of organophosphorus pesticide exposure among pre-school children in the Seattle metropolitan area. Environ Health Perspect 109:299–303.

MacIntosh DL, Kabiru C, Echols SL, Ryan PB. 2001. Dietary exposure to chlorpyrifos and levels of 3,5,6-trichloro-2-pyridinol in urine. J Expo Anal Environ Epidemiol 11:279–285.

Magnotti RA Jr, Dowling K, Eberly JP, McConnell RS. 1988. Field measurement of plasma and erythrocyte cholinesterases. Clin Chim Acta 176:315–332.

Maroni M, Catenacci G, Galli D, Cavallo D, Ravazzani G. 1990. Biological monitoring of human exposure to acephate. Arch Environ Contam Toxicol 19:782–788.

Marques EG. 1990. Acute intoxication by azinphos-ethyl. J Anal Toxicol 14:243–246.

Maynard EC, Amoruso LP, Oh W. 1991. Meconium for drug testing. Am J Dis Child 145:650–652.

Meyer E, Borrey D, Lambert W, Van Peteghem C, Piette M, De Leenheer A. 1998. Analysis of fenthion in postmortem samples by HPLC with diode-array detection and GC-MS using solid-phase extraction. J Anal Toxicol 22:248–252.

Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT, et al. 1998. Common mechanism of toxicity: a case study of organophosphorus pesticides. Toxicol Sci 41:8–20.

Mills PK, Zahm SH. 2001. Organophosphate pesticide residues in urine of farmworkers and their children in Fresno County, California. Am J Ind Med 40:571–577.

Moate TF, Lu C, Fenske RA, Hahne RM, Kalman DA. 1999. Improved cleanup and determination of dialkyl phosphates in urine of children exposed to organophosphorus insecticides. J Anal Toxicol 23:230–236.

Moore C, Negrusz A, Lews D. 1998. Determination of drugs of abuse in meconium. J Chromatogr B Biomed Sci Appl 713:137–146.

Moriya F, Chan KM, Noguchi TT, Wu PY. 1994. Testing for drugs of abuse in meconium of newborn infants. J Anal Toxicol 18:41–45.

National Children's Study October Assembly Meeting. 2001. Exposure to Chemical Agents Working Group Report. Washington, DC:National Institute of Child Health and Development. Available: http://nationalchildrensstudy.gov/committees/chemical_agents/report.cfm [accessed 14 August 2002].

National Research Council. 1993. Pesticides in the Diets of Infants and Children. Washington, DC:National Academy Press.

O'Rourke MK, Lizardi PS, Rogan SP, Freeman NC, Aguirre A, Saint CG. 2000. Pesticide exposure and creatinine variation among young children. J Expo Anal Environ Epidemiol 10:672–681.

Ostrea EM Jr. 1999. Testing for exposure to illicit drugs and other agents in the neonate: a review of laboratory methods and the role of meconium analysis. Curr Probl Pediatr 29:37–56.

Ostrea EM Jr, Romero A, Yee H. 1993. Adaptation of the meconium drug test for mass screening. J Pediatr 122:152–154.

Quackenboss JJ, Pellizzari ED, Shubat P, Whitmore RW, Adgate JL, Thomas KW, et al. 2000. Design strategy for assessing multi-pathway exposure for children: the Minnesota Children's Pesticide Exposure Study (MNCPES). J Expo Anal Environ Epidemiol 10:145–158.

Ramirez GB, Cruz MC, Pagulayan O, Ostrea E, Dalisay C. 2000. The Tagum study I: analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium, and infants' hair. Pediatrics 106:774–781.

Ray DE, Richards PG. 2001. The potential for toxic effects of chronic, low-dose exposure to organophosphates. Toxicol Lett 120:343–351.

Rigas ML, Okino MS, Quakenboss JJ. 2001. Use of a pharmacokinetic model to assess chlorpyrifos exposure and dose in children, based on urinary biomarker measurements. Toxicol Sci 61:374–381.

Ryan RM, Wagner CL, Schultz JM, Varley J, DiPreta J, Sherer DM, et al. 1994. Meconium analysis for improved identification of infants exposed to cocaine in utero. J Pediatr 125:435–440.

Selevan SG, Kimmel CA, Mendola P. 2000. Identifying critical windows of exposure for children's health. Environ Health Perspect 108(suppl 3):451–455.

Sexton K, Adgate JL, Eberly LE, Clayton CA, Whitmore RW, Pellizzari ED, et al. 2003. Predicting children's short-term exposure to pesticides: results of a questionnaire screening approach. Environ Health Perspect 110:123–128.

Sharma VK, Jadhav RK, Rao GJ, Saraf AK, Chandra H. 1990. High performance liquid chromatographic method for the analysis of organophosphorus and carbamate pesticides. Forensic Sci Int 48:21–25.

U.S. EPA. 1999. Organophosphorous Pesticides in Food—A Primer on Reassessment of Residue Limits. 735-F-99-014. Washington, DC:U.S. Environmental Protection Agency.

———. 2000. Science Policy on the Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides. Washington, DC:U.S. Environmental Protection Agency, Office of Pesticide Programs. Available: http://www.epa.gov/pesticides/trac/science/cholin.pdf [accessed 12 November 2002].

U.S. EPA. 2003. Organophosphate Review: Chemical Status. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.epa.gov/oppsrrd1/cumulativenew/status.htm [accessed 24 October 2003].

Vandekar M. 1980. Minimizing occupational exposure to pesticides: cholinesterase determination and organophosphorus poisoning. Residue Rev 75:67–80.

Whitmore RW, Kelly JE, Reading PL. 1992. National Home and Garden Pesticide Survey: Final Report. Vol 1. Research Triangle Park, NC:Research Triangle Institute.

Whyatt RM, Barr DB. 2001. Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: a validation study. Environ Health Perspect 109:417–420.

Whyatt RM, Barr DB, Camann DE, Kinney PL, Barr JR, Andrews HF, et al. 2003. Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect 111:749–756.

WHO. 1996. Biological Monitoring of Chemical Exposure in the Workplace. Vol 1. Geneva:World Health Organization.