8.0 URINARY BIOMARKER MEASUREMENTS

Biological markers are indicators of the actual body burden of a chemical. As such, they reflect all routes of exposure, as well as inter-individual differences in absorption and metabolism. Moreover, they are often more directly related to potential adverse health effects than the external concentrations (Lowry, 1986: Hulka and Margolin, 1992). In human observational measurement studies involving young children, urine is the primary vehicle for biomonitoring. Urine is advantageous over blood because of its noninvasiveness, ease of collection, and large available quantity. Urinary biomarkers, however, also have disadvantages related to uncertainties in the fraction of the absorbed compound that is eliminated and in the precision of the measurements.

The relationship between a biological marker and external exposure is influenced by factors related both to the environment and to human physiology. Factors related to the environment include spatial and temporal variability in exposure concentrations (as discussed in earlier chapters of this report) and effects of the presence of other chemicals (Coble et al, 2005). Factors related to human physiology include differences, both over time and across individuals, in the rates of absorption, distribution, metabolism, and excretion (Droz, 1989). When biological monitoring and exposure monitoring are used together, the relationship between the two may be evaluated to investigate the relative contribution of the various exposure routes.

Evaluating the relative contribution of exposure routes to aggregate intake is subject to error related to estimates of exposure and of aggregate intake. Issues related to route-specific exposure estimates have been discussed earlier. Dependable information on the toxicokinetics (absorption, distribution, metabolism, and excretion) of a compound are necessary for reliable estimates of aggregate intake, whether those estimates are derived from the sum of route-specific absorption estimates or from excreted biomarker levels. To accurately estimate aggregate intake from excreted biomarker levels, urinary biomarker output rates must be calculated from the biomarker levels. Such calculations require information on the entire urine volume and elapsed time since previous void - information that has rarely been collected in field studies.

8.1 Toxicokinetics of Organophosphate and Pyrethroid Pesticides

Some understanding of organophosphate and pyrethroid pesticide toxicokinetics is necessary to meaningfully compare the environmental and dietary concentrations presented in the previous chapters with the urinary biomarker concentrations presented in this chapter. Despite extensive usage, remarkably little is available from the scientific literature on kinetic parameters in humans. Parameters reported for absorption of parent compounds and elimination of urinary metabolites following pesticide exposure are summarized in Table 8.1.

Absorption

Inhalation studies with a variety of gases have shown that even the most efficiently absorbed low molecular weight, highly water soluble compounds rarely exceed 70% uptake. No studies reporting the fraction of organophosphate pesticides absorbed through inhalation were found, but

Leng *et al.* (1997) reported that only about 16% of the pyrethroid cyfluthrin was absorbed through inhalation.

The importance of the dietary contribution to aggregate exposure among infants and young children is well known (NRC, 1993), but few studies have investigated what fraction of ingested pesticide residue is absorbed. For organophosphates, Nolan *et al.* (1984) estimated 70% absorption of chlorpyrifos based on urinary 3,5,6-trichloro-2-pyridinol (TCPy), whereas others estimated 60% to 93% absorption based on dialkylphosphate (DAP) metabolites (Garfitt *et al.*, 2002; Griffin *et al.*, 1999). Diet reportedly affects absorption (Timchalk *et al.*, 2002). As for pyrethroids, Woollen *et al.* (1992) estimated that 27-57% of cypermethrin was absorbed, while Eadsforth and colleagues (1983; 1988) estimated 45-49% and 72-78% for the *cis* and *trans* isomers, respectively.

Dermal absorption is typically low due to loss by washing, evaporation, or exfoliation (Feldmann and Maibach, 1974). For organophosphate pesticides, absorption of chlorpyrifos was estimated, based on its primary metabolite TCPy, to be 1.28% of an applied dose of 4 mg/cm² (over 12-20 hr) (Nolan *et al.,* 1984), and 1.2% and 4.3% of applied doses of 0.15 and 0.05 mg/cm² (over 4 hr), respectively (Meuling *et al.*, 2005). Absorption of both chlorpyrifos and diazinon was estimated to be about 1% of applied doses of about 0.4 and 1.3 mg/cm² (over 8 hr), respectively, based on DAP metabolites (Griffin *et al.,* 1999; Garfitt *et al*., 2002). The percent that is absorbed increases as the applied dose (per cm²) decreases. Large differences have been reported by anatomical area (Maibach *et al.*, 1971) and among individuals (Feldmann and Maibach, 1974). For pyrethroids, Bartelt and Hubbell (1987) found only about 2% of applied permethrin to be absorbed within 24 h. Wester *et al.* (1994) observed that approximately 2% (forearm) and 7.5% (scalp) of radiolabeled pyrethrin was absorbed. The ATSDR (2001) has concluded that for pyrethroids in general, < 2% of the applied dermal dose is absorbed, at a rate much slower than that by the oral or inhaled routes.

Due to the paucity of available information on absorption from human studies, simple default values based on human studies, animal studies, and conservative assumptions are often required. For small children (ages 1-6) the following route-specific absorption is often assumed: 50-100% for inhalation, 50% for ingestion, and 1-3% for dermal. In addition, a daily intake of 100 mg of house dust is assumed for indirect ingestion. These absorption assumptions are a source of substantial uncertainty in route-specific intake estimates. In fact, since dermal absorption increases with decreasing dermal loadings (as demonstrated above with organophosphates), default assumptions of less than 3% for dermal absorption may underestimate absorption at the very low levels measured in field studies

Distribution and Metabolism

Once in the bloodstream, organophosphate or pyrethroid pesticides are rapidly distributed and metabolized. A typical organophosphate (OP) pesticide is composed of a dialkyl (either dimethyl or diethyl) phosphate moiety and an organic group. Hydrolytic cleavage of the ester bond yields one dialkylphosphate (DAP) metabolite and one organic group moiety (Barr *et al.,* 2004). Dimethyl OPs (including malathion, phosmet, and azinphos-methyl) produce dimethyl metabolites and diethyl OPs (including chlorpyrifos and diazinon) produce diethyl metabolites

(Aprea *et al.,* 2002). The organic group metabolites, including 2-isopropyl-6-methyl-4-pyrimidinol (IMPy) for diazinon and 3,5,6-trichloro-2-pyridinol (TCPy) for chlorpyrifos, are considered to be semi-specific.

Pyrethroids are esters of chrysanthemic acid and benzyl alcohols. Hydrolytic cleavage of the ester bond yields a benzoic acid and a chrysanthemic acid derivative. The 3-phenoxybenzoic acid (3-PBA) metabolite is common to 10 of the 18 pyrethroids registered in the United States including permethrin, cypermethrin, deltamethrin, esfenvalerate (Baker *et al.,* 2004). Other benzoic acid metabolites analogous to 3-PBA are more specific and include 4-fluoro-3-phenoxybenzoic acid (4F3PBA) from cyfluthrin and 2-methyl-3-phenylbenzoic acid (MPA) from bifenthrin. These are not necessarily terminal metabolites; for example, as much as 38% of 3-PBA has been reported by Woollen *et al.* (1992) to undergo further oxidation to 3-(4'-hydroxyphenoxy) benzoic acid (4OH3PBA). The chrysanthemic acid derivative *cis*-2,2-dibromovinyl-2,2-dimethyl-cyclopropane-1-carboxylic acid (DBCA) is specific to deltamethrin while the *cis*- and *trans*- isomers of 2,2-dichlorovinyl-2,2-dimethyl- cyclopropane-1-carboxylic acid (DCCA) are common to permethrin, cypermethrin, and cyfluthrin.

Excretion

Both the OPs and the pyrethroids are rapidly eliminated in urine. Elimination appears to follow first-order kinetics, with elimination half-times in humans ranging from 2 to 41 hours for OPs and from 6.4 to 16.5 hours for pyrethroids, depending on both the compound and the route of exposure (ATSDR, 2001; Garfitt *et al.*, 2002; Meuling *et al.*, 2005). The elimination half-life of about 8 hours reported for 3-PBA among workers exposed to cypermethrin (Kuhn *et al.*, 1999) suggests that 88% of the metabolite is excreted within the first 24 hours following exposure.

Route-specific differences in the peak excretion of urinary OP pesticide metabolites have been reported (Griffin *et al.*, 1999; Garfitt *et al.*, 2002; Meuling *et al.*, 2005). Peak excretion is observed to occur 6 to 24 hours later when absorption is by the dermal route compared to when absorption is by the oral route, largely because of route-specific differences in absorption. Peak excretion may occur as late as 48 hours following dermal exposure, as observed among volunteers performing scripted "Jazzercise" activities (Krieger *et al.*, 2000). Extended peak excretion times suggest that chlorpyrifos may be retained by the skin and may remain systemically available for prolonged periods (Meuling *et al.*, 2005)

While the above toxicokinetic studies evaluate excreted mass or mass rates, our past field studies have largely evaluated only biomarker concentrations. In the future, all studies should include information on void volumes and times to allow excreted mass to be calculated. Relevant transformations can be found in Rigas *et al.* (2001) and are currently incorporated in the SHEDS model.

Table 8.1 Absorption and elimination characteristics for pesticides and urinary biomarkers of pesticide exposure.

COMPOUND	ABSORPTION OF PARENT COMPOUND			ELIMINATION OF METABOLITES		
	ORAL	DERMAL	INHALATION	ORAL	DERMAL	INHALATION
Chlorpyrifos	Volunteer studies: 70% of oral dose excreted in urine as TCPy (Nolan *et al.,* 1984), 93% of oral dose excreted in urine as dialkyl-phosphates (Griffin *et al.,* 1999). Absorption factor estimated at 0.90 (ATSDR).	Volunteer studies: 1.3% of dermal dose excreted in urine as TCPy (Nolan *et al.,* 1984). 1% of dermal dose excreted as dialkyl-phosphates (Griffin *et al.,* 1999), 1.2 – 4.3% of dermal dose excreted as TCPy (Meuling *et al.*, 2005).	No Information.	Volunteer study, 27 h oral (Nolan *et al.,* 1984). Volunteer study, approx 15.5 h oral (Griffin *et al.,* 1999).	Volunteer study, 27 h dermal (Nolan *et al.,* 1984). Volunteer study, approx 30 h dermal (Griffin *et al.,* 1999). Volunteer study, approx 41 h dermal (Meuling *et al.,* 2005).	No Information.
Diazinon	Human oral absorption approx. 60% (Garfitt *et al.,* 2002). Default oral absorption factor of 0.85 (ATSDR).	Human dermal absorption rate: 456 ng/cm²/h (Garfitt *et al.,* 2002).	No Information.	Human study, 2 h oral (Garfitt *et al.,* 2002).	Human study, 9 h dermal (Garfitt *et al.,* 2002).	No Information.
Pyrethroids (as a group)	Absorption is incomplete, minimum estimate 40 - 60%, but first- pass metabolism may underestimate absorption (ATSDR, 2001).	<2% of the applied dermal dose is absorbed, rate of absorption much slower than by the oral or inhaled routes; may be stored in skin and then slowly released into the systemic circulation (ATSDR, 2001).	Rapidly absorbed in humans following inhalation, but no estimates of fraction absorbed are available (ATSDR, 2001).	Elimination appears to follow first-order kinetics, with elimination half-times in humans ranging from 6.4 to 16.5 hours, depending upon the specific pyrethroid and exposure route studied (ATSDR, 2001).		
Permethrin	Oral absorption factor of 0.70 suggested (NRC).	Poor dermal absorption: ~2% of applied dose absorbed/24 h (Bartelt and Hubbell, 1987); 7.5% (scalp) and 1.9% (forearm) of applied dose (Wester *et al*., 1994).	No Information.	No Information.	No Information.	No Information.
Cyfluthrin	No Information.	No Information.	Human data suggest ~15% absorption (Leng et al, 1997).	Human oral dosing produced t-½ of 6.4 h (Leng et al, 1997b).	No Information.	Human ½-lives of 6.9 h (c-DCCA), 6.2 h (t-DCCA), 5.3 h (FPBA) (Leng et al, 1997).
Cypermethrin	Human volunteer study 27-57% (mean 36%) cypermethrin absorbed (Woollen *et al.*, 1992).	No Information.	No Information.	Human oral dosing, urinary metabolites have mean ½-life of 16.5 h (Woollen *et al.,* 1992).	Human dermal dosing, excretion rates peaked at 12-36 h, mean ½-life was 13 h (Woollen *et al.,* 1992).	No Information.

8.2 Measurements of Pesticide Metabolites in Urine

Urinary biomarkers were measured in several large-scale and pilot-scale children's observational measurement studies described in Table 8.2. These include the MNCPES, CTEPP, NHEXAS-AZ, CPPAES, JAX, CHAMACOS, PET, and DIYC studies. All urine samples were collected exclusively at the children's homes except for the CTEPP study, in which urine samples were also collected at daycare centers. Urine collection followed outdoor turf applications in the PET study and routine professional indoor applications in the DIYC and CPPAES studies.

Spot urine samples, mainly first morning voids, were collected using age-appropriate methods including under-toilet seat bonnets (CTEPP, PET), collection cup (NHEXAS-AZ, MNCPES), diaper insert (DIYC), and "potty chair" (CPPAES). Table 8.3 presents selected organophosphate (OP) and pyrethroid metabolites that were measured in the children's urine samples in multiple studies. The pesticide metabolites are 3,5,6-trichloro-2-pyridinol (TCPy), 2-isopropyl-6-methyl-4-pyrimidinol (IMP), and 3-phenoxybenzoic acid (3-PBA).

Sample collection was performed by the children's caregivers following protocols provided by the investigators. Chemical analysis of urinary metabolites in nearly all included studies was performed by the National Center for Environmental Health of the Centers for Disease Control and Prevention in Atlanta, GA, using validated tandem mass spectroscopy techniques (Baker *et al.,* 2000; Baker *et al.,* 2004; Beeson *et al.,* 1999; Hill *et al.,* 1995). Chemical analysis for the CTEPP study was performed by Battelle Institute using validated gas chromatography/mass spectroscopy techniques.

Limits of detection for each pesticide metabolite are given by study in Table 8.4. Detection frequencies are provided in Figure 8.1. Concentrations for the median and 95th percentiles for each urinary metabolite are presented by study in Table 8.5. Figure 8.2 shows the log probability plots of urinary TCPy and 3-PBA concentrations for children across large observational field studies. Figure 8.3 presents the box-and-whisker plots that graphically depict the urinary TCPy and 3-PBA concentrations for both the large-scale and pilot-scale children's observational measurement studies.

The National Health and Nutrition Examination Survey (NHANES) includes an ongoing assessment of the exposure of the U.S. population to environmental chemicals through the measurement of biomarkers. Spot measurements of urinary pesticide biomarkers among children 6 to 12 years old from both the 1999-2000 and the 2001-2002 cycles are included for comparison with results from our studies. Please note that NHANES does not report results by region or by season.

- The chlorpyrifos metabolite TCPy was detected in over 90% of the children's urine samples in all listed studies. The pyrethroid metabolite 3-PBA was detected in over 60% of the CTEPP-OH samples and over 90% of the JAX samples (Figure 8.1).
- The urinary TCPy concentrations were at least an order of magnitude higher than the urinary 3-PBA concentrations across studies (Figure 8.2).

- There is virtually no difference in urinary TCPy concentrations measured in CTEPP NC and OH, but the concentrations from Minnesota and Arizona are substantially higher (Figure 8.2, all unweighted). Higher levels in MNCPES and NHEXAS-AZ may reflect intentional oversampling of pesticide-using households in MNCPES, and greater use of chlorpyrifos at the time that MNCPES and NHEXAS-AZ were conducted.
- Compared to values for children under 12 years old collected in the 1999-2002 NHANES (Figure 8.2), the median TCPy values were higher in all of our studies, but the 95th percentile values were only higher for MNCPES.
- The children in JAX had levels of 3-PBA that were at least seven times higher than those of children in CTEPP-OH (Figure 8.3). All urine data from JAX participants suggest that JAX is a high pesticide usage area.
- The median 3-PBA value in CTEPP (0.3 ng/mL) was similar to NHANES (0.3 ng/mL), but the median JAX value (2.2 ng/mL) was much higher (Figure 8.3).
- Levels of IMP were about an order of magnitude higher in DIYC compared to PET or NHANES (Figure 8.3).
- The median urinary TCPy concentration was the highest for the NHEXAS-AZ and JAX studies and the lowest for the CTEPP-NC and CTEPP-OH studies (Table 8.5).
- In the CPPAES study, the intensity of the crack and crevice applications of chlorpyrifos was described as either high (n = 7) or low (n = 3), with mean air concentrations resulting from "high" applications five orders of magnitude higher than those from "low" applications. Figure 8.4 shows that the urinary TCPy concentrations over time were not much different for the children in the high versus low application groups.
- For children in the "high" application group in CPPAES, the median urinary TCPy concentration one day before application of chlorpyrifos was higher than on the first two days following application (Figure 8.4). Crack and crevice applications of chlorpyrifos at these homes did not substantially increase the children's urinary TCPy concentrations.
- The concentration-time profiles for urinary TCPy levels in CPPAES did not mirror the environmental concentration time profiles (Figure 8.5).

Table 8.2 Summary of the children's urinary biomarker collection methods.

Study	N	Age Range	Sampling Device	Collection Strategy	Collection After Pesticide Use	Collection Frequency	Analytes [a]	Urinary Output Correction Factors
NHEXAS-AZ (subset)	21	5 to12 yr	Urine collection cup	Morning void	No	Once (in 3-day monitoring period)	TCPy	Creatinine
MNCPES	102	3 to 13 yr	Urine collection cup	Morning void	No	Days 3, 5, and 7 of sampling period	TCPy	Creatinine
CTEPP	257	2 to 5 yr	Bonnet for children, urine collection cup for adults	Morning void, after lunch, after dinner/ before bedtime	Only for some homes (~15%)	Over a 48-hr period	TCPy, 3-PBA (Ohio, only)	Creatinine, Specific gravity
JAX	9	4 to 6 yr	Plastic cup	Morning void	Yes, indoor	1 day	TCPy, IMP, 3-PBA	Creatinine
CHAMACOS	20	6 to 24 mo	Cotton diaper and Infant urine collection bag or commode container	One overnight and one spot sample	No	Once	Dialkyl Phosphate metabolites	Creatinine
CPPAES	10	2 to 4 yr	Toys R' Us child's potty, plastic cup	Morning void	Yes, indoor	Pre- and days 1, 2, 3, 5, 7, 9, and 11 post-application	TCPy	Creatinine
PET Pilot	6	5 to 12 yr	Urine collection bottle or urine bonnet	Morning void	Yes, outdoor	Pre- and days 1, 2, 4, and 8 post-application	IMP	Creatinine
DIYC	3	1 to 3 yr	Diaper insert or collection cups	Morning void and other spot samples	Yes, indoor	Days 3, 5, and 7 post-application	IMP	Creatinine

[a] Analytes relevant to interstudy comparison. Most studies included additional metabolites.

Table 8.3 Urinary metabolites of organophosphate and pyrethroid pesticides measured in the children's observational measurement studies.

Metabolite	Parent Compound
3,5,6-Trichloro-2-pyridinol (TCPy)	Chlorpyrifos [a]
2-Isopropyl-6-methyl-4-pyrimidinol (IMP)	Diazinon
3-Phenoxybenzoic acid (3-PBA)	Permethrin [b]

[a] TCPy is also a metabolite of chlorpyrifos-methyl, which may occur in children's diet.
[b] Several other pyrethroids are metabolized into 3-PBA including cypermethrin, deltamethrin, fenvalerate, fluvalinate, permethrin, sumithrin.

Table 8.4 Limits of detection (ng/mL) for each pesticide metabolite measured in the children's urine samples by study.

Study	TCPy	IMP	3-PBA
NHEXAS-AZ	1.0	NA	NA
MNCPES	1.4	NA	NA
CTEPP-NC	1.0	NA	NA
CTEPP-OH	1.0	NA	0.2
JAX	0.4	2.0	0.5
CPPAES	1.0	NA	NA
PET	NA	0.3	NA
DIYC	NA	1.0	NA

NA, Not Applicable.

Table 8.5 Median and 95th percentile values (ng/mL) for the pesticide metabolites TCPy, IMP, and 3-PBA measured in the children's urine samples by study.

Study	TCPy		IMP		3-PBA	
	P50	P95	P50	P95	P50	P95
NHEXAS-AZ	12.0	26.0	NA	NA	NA	NA
MNCPES	7.2	23.0	NA	NA	NA	NA
CTEPP-NC	5.3	15.5	NA	NA	NA	NA
CTEPP-OH	5.1	12.3	NA	NA	0.3	1.8
JAX	9.8	21.2	<MDL	<MDL	2.2	98.7
CPPAES	7.7	18.0	NA	NA	NA	NA
PET	NA	NA	0.71	6.58	NA	NA
DIYC	NA	NA	7.1	27.0	NA	NA

NA, Not Applicable.

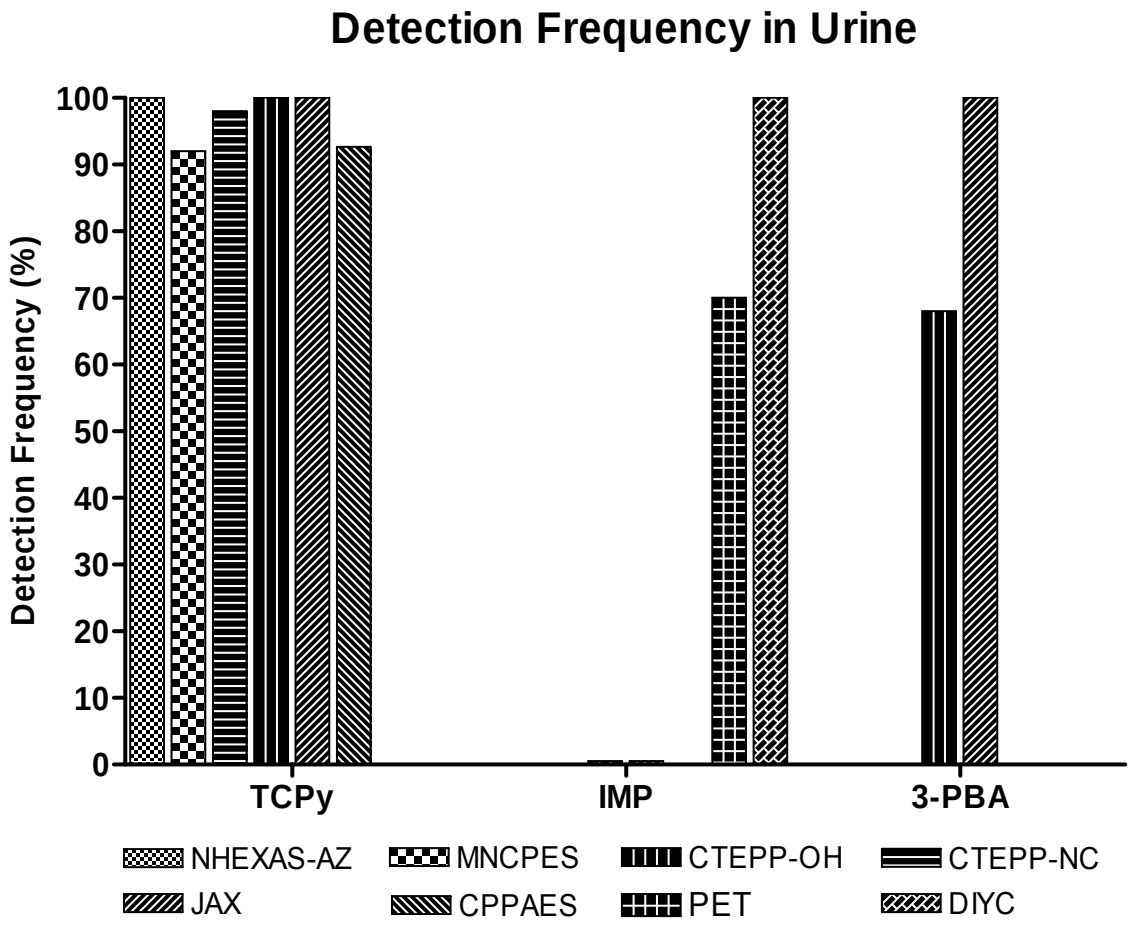

Figure 8.1 Detection frequencies of pesticide metabolites in the children's urines samples by study.

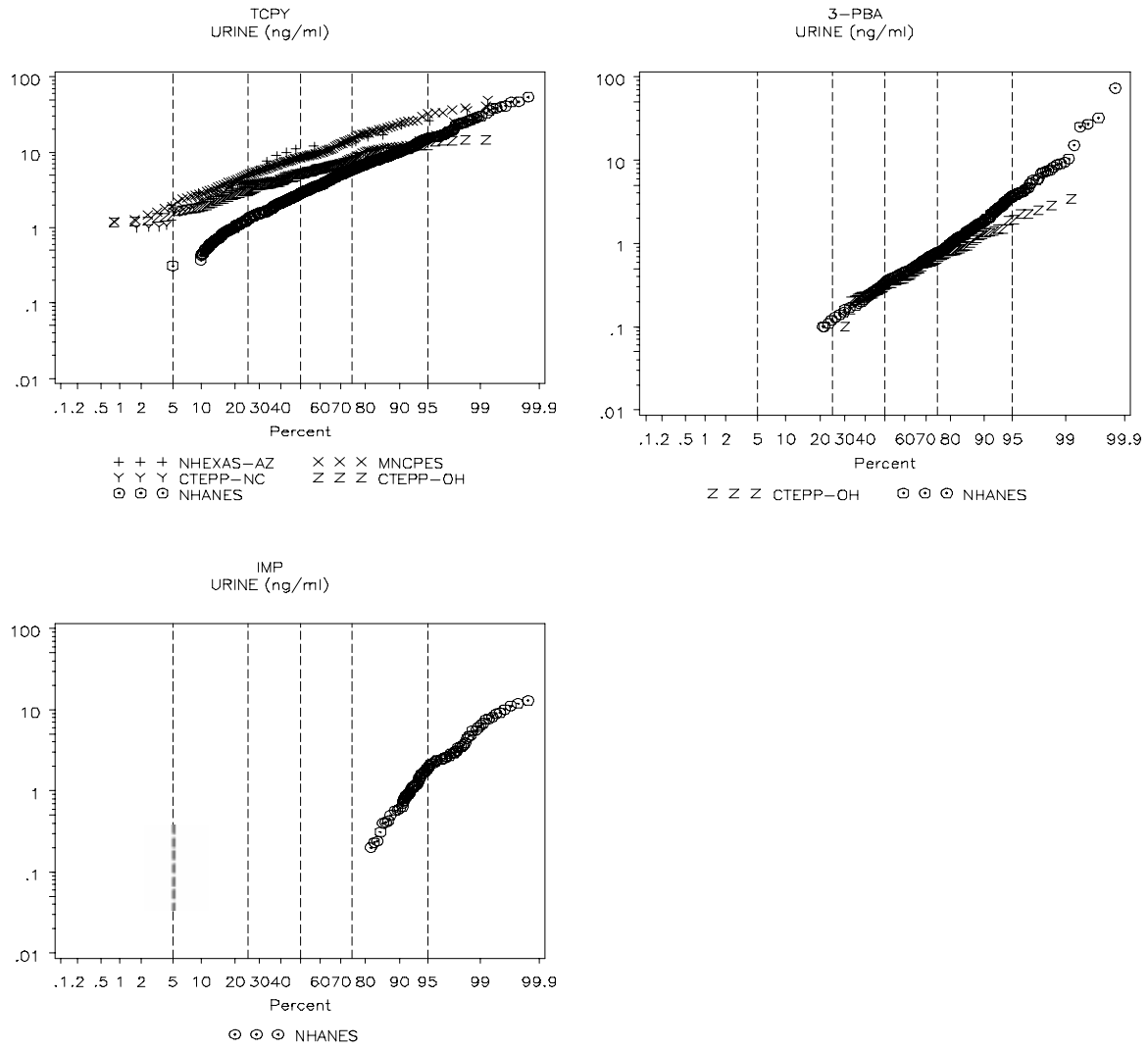

Figure 8.2 Log probability plots of urinary TCPy, 3-PBA, and IMP concentrations across large observational field studies. NHANES results are included for comparison.

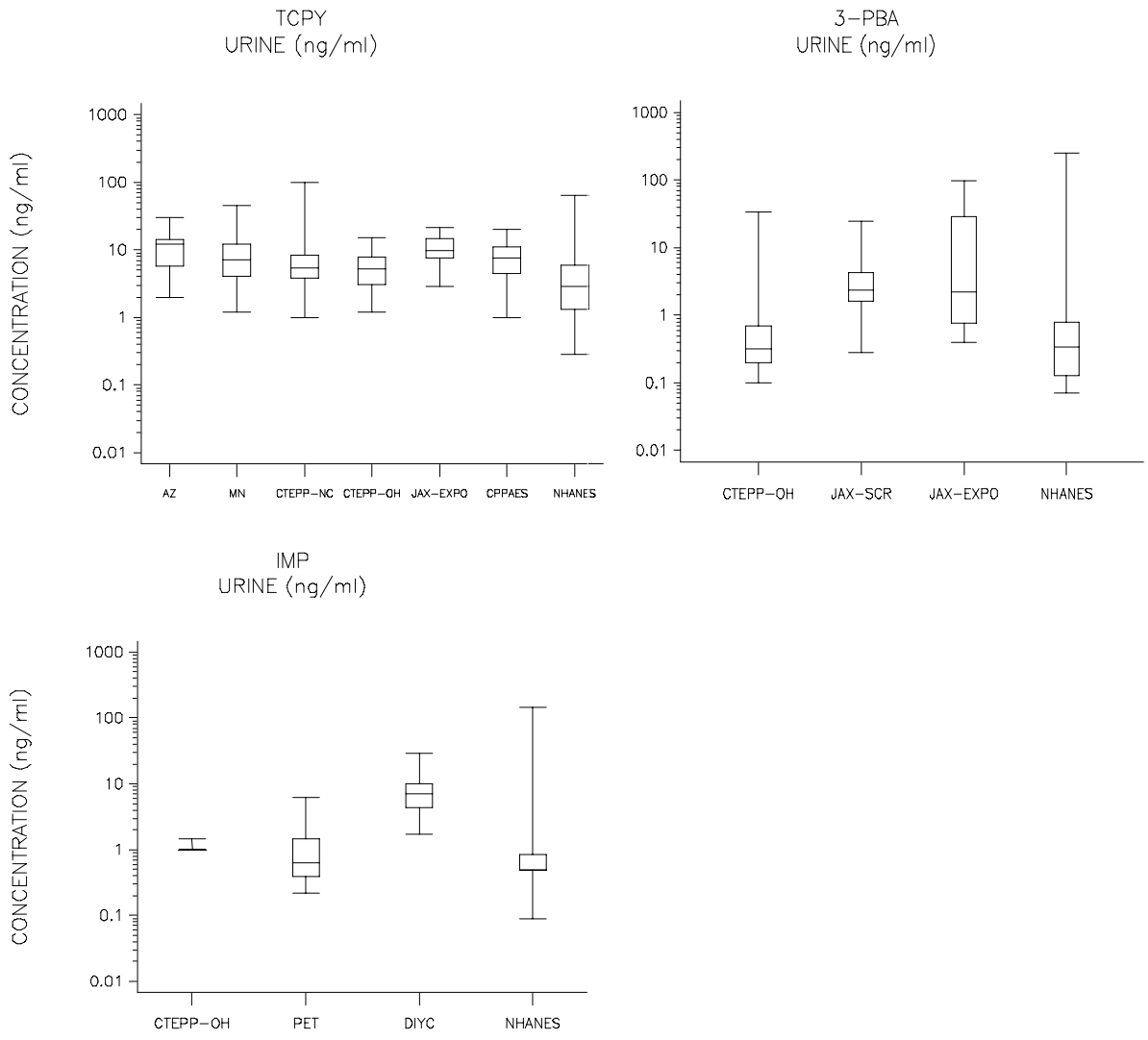

Figure 8.3 Box-and-whisker plots comparing the urinary TCPy and 3-PBA concentrations across studies.

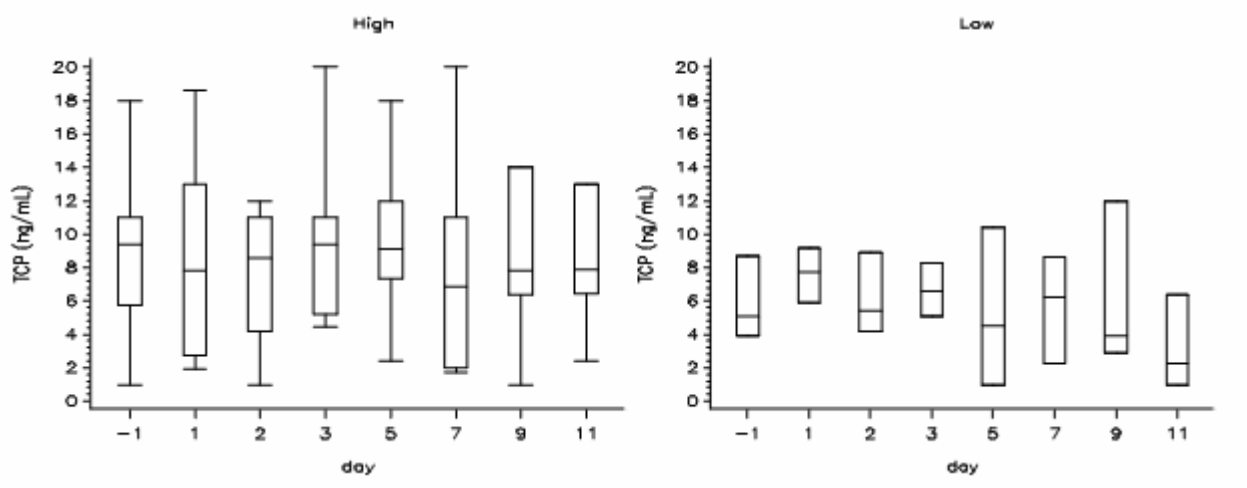

Figure 8.4 Urinary TCPy concentrations (ng/mL) over time for the children in the high and low application groups in CPPAES.

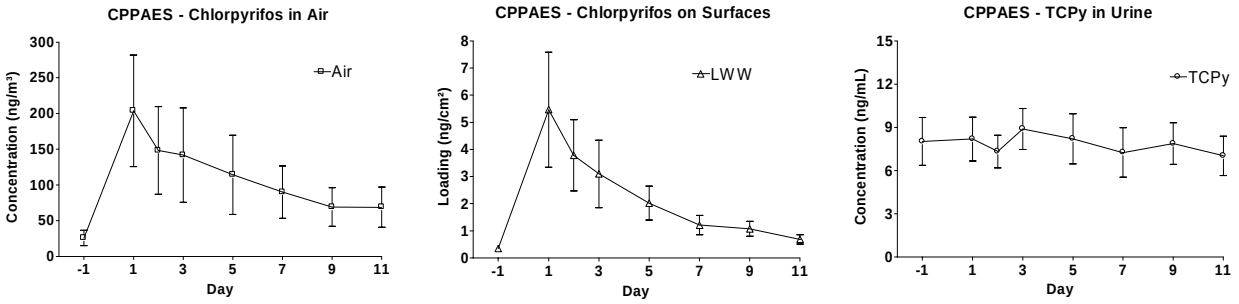

Figure 8.5 Time profiles for chlorpyrifos in environmental media and TCPy concentrations in urine for all children in the CPPAES.

8.3 Temporal Variability in Biomarker Measurements

In the CTEPP study, the children's spot urine samples (up to six per child) were analyzed separately for pesticide metabolites if the participants reported that a pesticide had been used in their homes within seven days of field monitoring. Figure 8.6 shows the variability of urinary TCPy concentrations in the children's urine samples over a 48-h period.

Intraclass correlation coefficients (ICCs) for urinary TCPy and 3-PBA concentrations in NC and OH children in the CTEPP study are provided in Table 8.6. The between and within-person geometric standard deviations (GSDs) for logged urinary concentrations of TCPy and 3-PBA for the NC and OH children in the CTEPP study are given in Table 8.7. Concentration-time profiles for TCPy and 3-PBA among CTEPP children are provided in Figure 8.6 and for IMP among PET study children in Figure 8.7.

- Relatively low ICCs (Table 8.6) indicate that a single measurement may not adequately represent the mean of the 48-hr sampling period for 3-PBA among adults and TCPy among children. Consistency of urinary metabolite concentrations over even short periods of time appears to be dependent on both the metabolite and the study population.
- Within-person GSDs are equal to or nearly equal to between-person GSDs for both TCPy and 3-PBA in urine measured in CTEPP (Table 8.7). This indicates that a single spot urine measurement is not sufficient to differentiate among children over a 48-hr time frame.
- Spot urine measurements over 48 hours among CTEPP participants reporting recent pesticide applications show large sample-to-sample variability and large differences among individuals (Figure 8.6).
- Adjustment of urinary metabolite values by specific gravity did not meaningfully reduce within-person variability of TCPy (Figure 8.6).
- While no statistically significant difference was observed between pre- and post-application urinary IMP concentrations in the PET study, the time-concentration profile clearly shows an observable decay in children's urinary biomarker concentrations in the eight days following the outdoor lawn application (Figure 8.7). The pattern among adults is not consistent with that among children.
- Comparing first morning voids (FMV) to other spot samples collected among a subsample of CTEPP children (data not presented), the median concentration in FMV is substantially (43%) higher than the median of the non-FMV samples for TCPy, and slightly (35%) higher for 3-PBA, due to longer urine accumulation time in the bladder.
- In CHAMACOS, concentrations in overnight diapers were compared to concentrations in spot samples (Bradman *et al.*, 2006; data not presented). In all cases, diethyl phosphates were lower in overnight diaper samples than in spot samples, while for toddlers dimethyl phosphates were higher in overnight diaper samples. Median total DAP concentrations for all children were higher in the overnight samples compared to the spot samples (140 *vs.* 100 nmol/l), but the differences were not statistically significant (Wilcoxon test).

- Spearman correlations were calculated for CHAMACOS spot and overnight samples by age (Bradman *et al.*, 2006). Spot and overnight urine concentrations were significantly correlated in CHAMACOS (Bradman *et al.*, 2006): dimethyl phosphate (Spearman rho=0.53; p=0.02), diethyl phosphate (Spearman rho=0.48; p=0.03), and total DAP metabolites (Spearman rho=0.57; p=0.009).

Table 8.6 Intraclass correlation coefficients (ICC) for logged CTEPP urinary metabolites. [a]

Metabolite	NC Children	OH Children
3-PBA	-- [b]	0.70
TCPy	0.65	0.48

[a] An ICC of 0.80 indicates that a single measurement reliably represents the average of a set of measurements.
[b] -- = no data.

Table 8.7 Between- and within-person geometric standard deviations (GSDs) for logged urinary concentrations from children in the CTEPP study.

Metabolite	Measure	NC Children	OH Children
3-PBA	Between-person GSD	-- [a]	1.5
	Within-person GSD	--	1.2
TCPy	Between-person GSD	1.5	1.5
	Within-person GSD	1.3	1.5

[a] -- = no data.

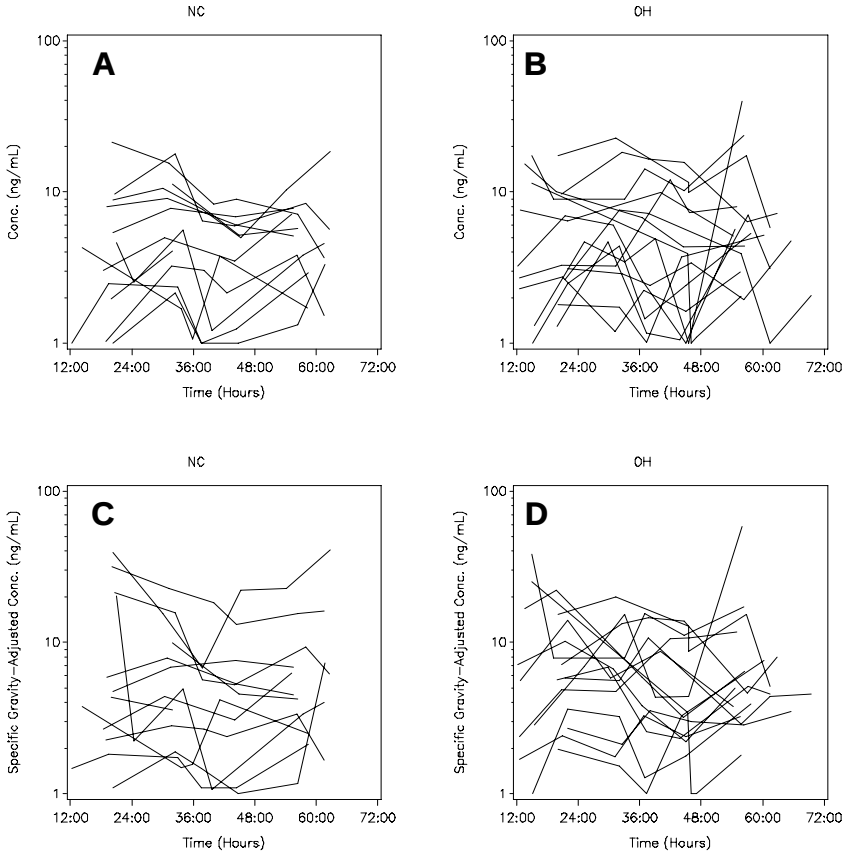

Figure 8.6 Concentration versus time plots for urinary TCPy measurements among CTEPP-NC and CTEPP-OH participants reporting a recent pesticide application. Urines in panels A and B are without adjustment. Urines in panels C and D are adjusted by specific gravity. Note that not all voids within the 48 hour period were collected.

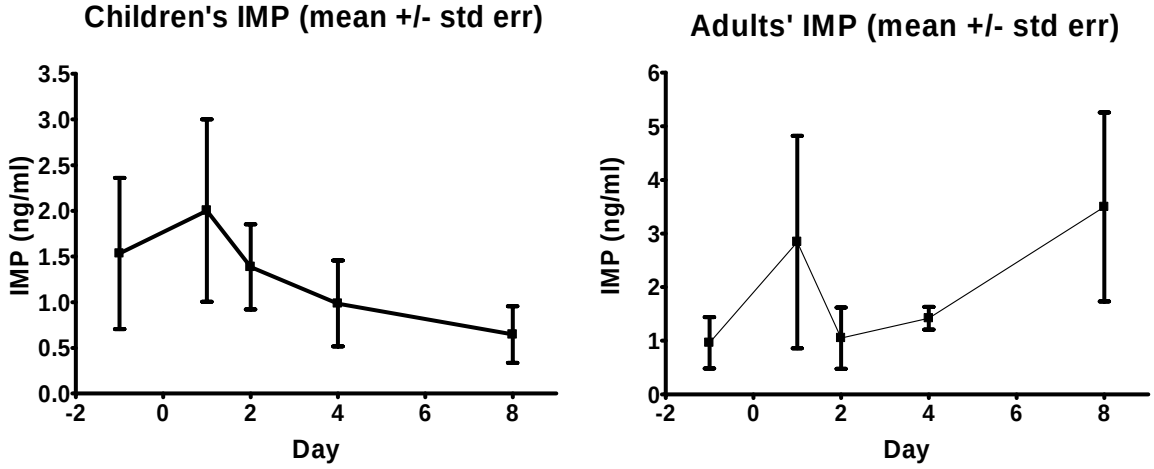

Figure 8.7 Time-concentration profile for urinary IMP measurements among child and adult PET study participants following an outdoor granular turf pesticide application.

8.4 Urine and Creatinine Excretion among Children

Urine output varies with water intake, urea, salt, specific gravity, and osmolality (Wessels *et al.,* 2003). Consequently, the concentration of metabolites in spot urine samples may vary, even if the internal dose remains constant. Since collecting 24-h urine samples from children is often impractical, spot urine samples are commonly collected and normalized using creatinine (CRE) concentration. However, CRE yield has been shown to be variable among children (Freeman *et al.*, 1995; O'Rourke *et al.*, 2000). Furthermore, because CRE excretion is dependent upon muscle mass, children inherently excrete less CRE than adults. This makes comparisons between CRE-adjusted adult and children urinary biomarker concentrations subject to error due to "over-correction" of children's samples. Age-dependent differences in daily creatinine clearance must also be considered when comparing young children and older ones (Krieger *et al.,* 2001; Wessels et al, 2003), as differences are great even for 1-year olds (0.08 g creatinine/day) relative to 5-year olds (0.4 g creatinine/day).

Alternative approaches for adjusting for urine dilution are based on urinary specific gravity and on urinary output. Specific gravity adjustment accounts for all dissolved solids, with a specific gravity of 1.024 considered normal for adults. Both specific gravity and creatinine were measured in CTEPP urine samples.

Urinary output among young children is often estimated with equations from the Exposure Factors Handbook. Zartarian et al (2000) estimated daily urinary output volumes of 500 and 800 mL for the children 0–4 and 5–9 years of age, respectively, based on Geigy Scientific Tables. Estimated daily urinary output and creatinine excretion for children 3-12 years of age based on first morning void measurements and recorded ancillary information from the MNCPES are presented in Figure 8.8.

- In unpooled samples from CTEPP, specific gravity of children's urine averaged 1.020, significantly different than the 1.024 of adult urine (t-test, $p < 0.001$).
- In the MNCPES study, the daily urine output rates (mean ± SD) increased from 13 ± 6 mL/hr for 3-4 year olds to 19 ± 7 mL/hr for 11-12 year olds (Figure 8.8) based on first morning void samples with known volumes and void times.
- In the MNCPES study, creatinine excretion rates (mean ± SD) increased from 10 ± 4 mg/hr for 3-4 year olds up to 24 ± 12 mg/hr for 11-12 year olds (Figure 8.8).
- There was neither a substantial nor consistent difference between sexes for either daily urine output or daily creatinine excretion rate, suggesting that sex is not an important predictor of creatinine excretion for pre-pubescent children (Figure 8.8).
- Failure to appropriately account for creatinine excretion results in "over-correction" of children's samples when making comparisons between CRE-adjusted adult and children urinary metabolite concentrations, making child levels appear higher by comparison.
- An alternate approach for avoiding issues with variable urine volumes is to calculate biomarker excretion rates. This requires collection of complete voids, void volume measurements, and recording previous and final void times.

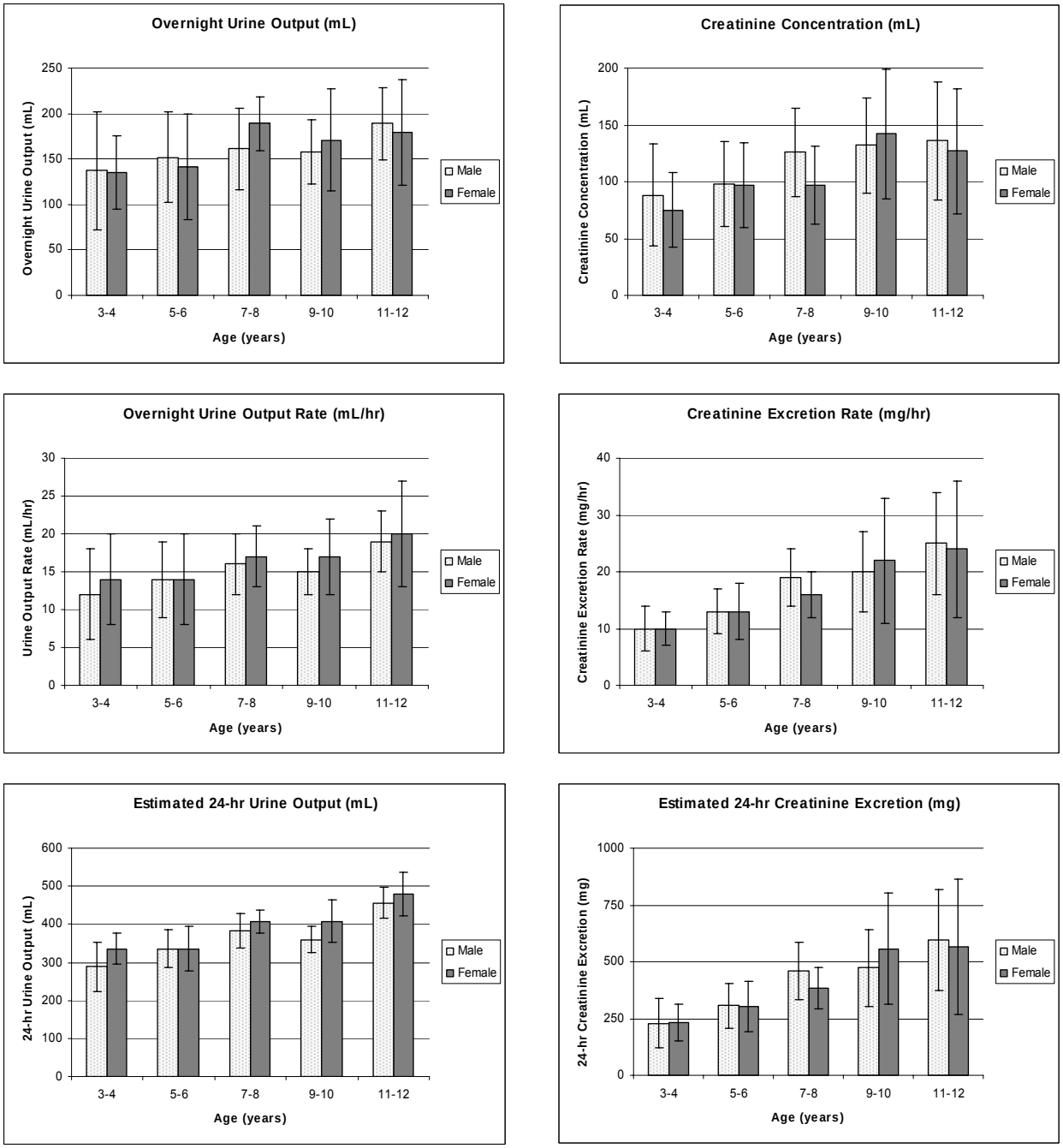

Figure 8.8 Estimates of age-specific urinary output and creatinine excretion, based on data from the MNCPES.

8.5 Relative Importance of Exposure Routes

The relative importance of the dietary ingestion, indirect ingestion, dermal, and inhalation routes of exposure with respect to aggregate intake has been investigated with data from both the MNCPES and CTEPP studies. Daily inhalation and dietary intake estimates (ng/kg/day) for chlorpyrifos among children in MNCPES are available in Clayton *et al.* (2003). Estimated relative importance of the inhalation, dietary ingestion, and indirect ingestion routes of exposure to OPs and pyrethroids among children in CTEPP are presented in Morgan *et al.* (2004).

- MNCPES chlorpyrifos data showed that ingestion was a more dominant route of intake than inhalation. Urinary metabolite levels, however, showed a stronger association with air ($r=0.42$, $p<0.01$) than with dietary ($r=0.22$, $p<0.05$) measurements.
- Using MNCPES data as an input, the SHEDS model suggested (data not presented) that the dominant pathway for highly exposed chlorpyrifos users was non-dietary ingestion, followed by dietary ingestion. The model also suggested that the relative contribution of exposure pathways may differ by pesticide.
- TCPy was found in several environmental media in CTEPP, particularly in solid food samples. Estimated intake of TCPy (Figure 8.9) was about 12 times higher than intake of chlorpyrifos for CTEPP children. Even when environmental TCPy is considered, nearly 60% of the TCPy excreted in urine remained unaccounted for. This suggests that either a major pathway of children's exposure to chlorpyrifos and TCPy remains unaccounted for in our algorithms or that some underlying assumptions are incorrect.
- Despite indications that intake of TCPy from solid food may be responsible for the bulk of TCPy intake, intake from solid food and excretion are poorly correlated ($r^2=0.01$, Figure 8.10). The absorption rate for TCPy remains unknown, as does whether or not it is metabolized to other products in the body.
- Based on exposure algorithms (with absorption assumed to be 50% by each route), the primary route of exposure and intake for chlorpyrifos and permethrin among CTEPP children was dietary ingestion (Table 8.8 and Figure 8.11). Inhalation was the secondary route for chlorpyrifos and diazinon (organophosphates); while indirect ingestion was the secondary route for permethrin (pyrethroid).
- Based on algorithms, the contribution of diet to aggregate intake generally decreases as intake increases (Figure 8.12). Conversely, nondietary ingestion becomes increasingly important with increasing aggregate intake.
- Unlike with TCPy, the estimated aggregate intake of *cis*- and *trans*-permethrin among CTEPP-OH children was close to the excreted amount of 3-PBA (Figure 8.12). However, children may have also been exposed to other pyrethroids that are metabolized into 3-PBA and could have contributed to the excreted amounts measured.
- Our studies consistently report a low correlation between concentrations of urinary biomarkers of pesticide exposure and environmental concentrations. Algorithm-based estimates of aggregate intake do little to improve the correlation. A better understanding of how differences in activities between children affects intake may be needed.
- Figures 8.14 and 8.15 present environmental and dietary levels of chlorpyrifos and

urinary concentrations of TCPy by study. There is little evidence that differences in environmental media concentrations translate into differences in urinary concentrations. The pattern is most similar between food and urine concentrations (Figure 8.15).

Table 8.8 Estimated relative importance of the inhalation, dietary ingestion, and indirect ingestion routes of exposure among children in CTEPP NC and OH.

Class	Pollutants	Apportionment of Aggregated Exposure/Dose
OP Insecticide	Chlorpyrifos and Diazinon	***NC:*** dietary ingestion ≈ inhalation > indirect ingestion ***OH:*** dietary ingestion > inhalation > indirect ingestion
Pyrethroid Insecticide	*cis*- and *trans*-Permethrin	***NC:*** dietary ingestion ≈ indirect ingestion > inhalation ***OH:*** dietary ingestion > indirect ingestion > inhalation

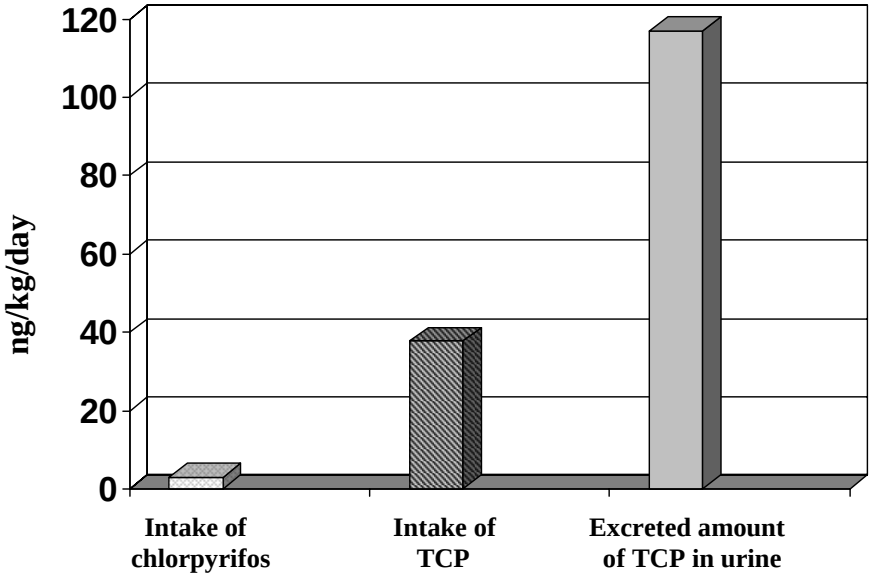

Figure 8.9 The median estimated intakes of chlorpyrifos and TCPy in CTEPP-NC compared with the excreted median amounts of TCPy in the preschool children's urine (Morgan *et al.*, 2005).

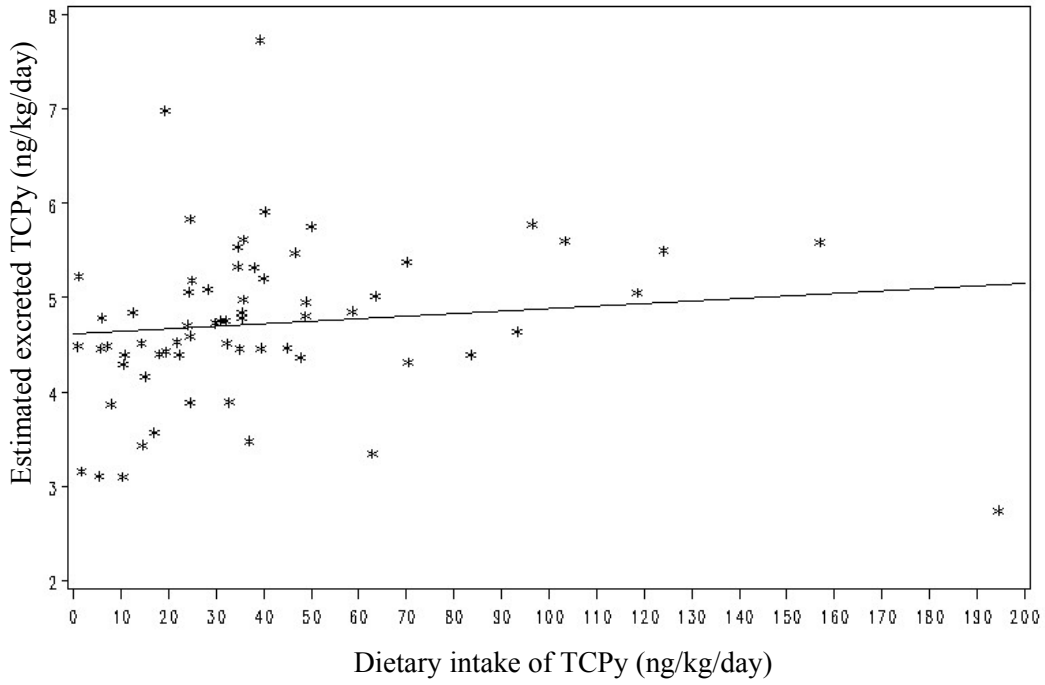

Figure 8.10 Intake of environmental TCPy through the dietary route correlated poorly (r^2=0.01) with the amount of TCPy excreted in the urine of CTEPP-NC preschool children.

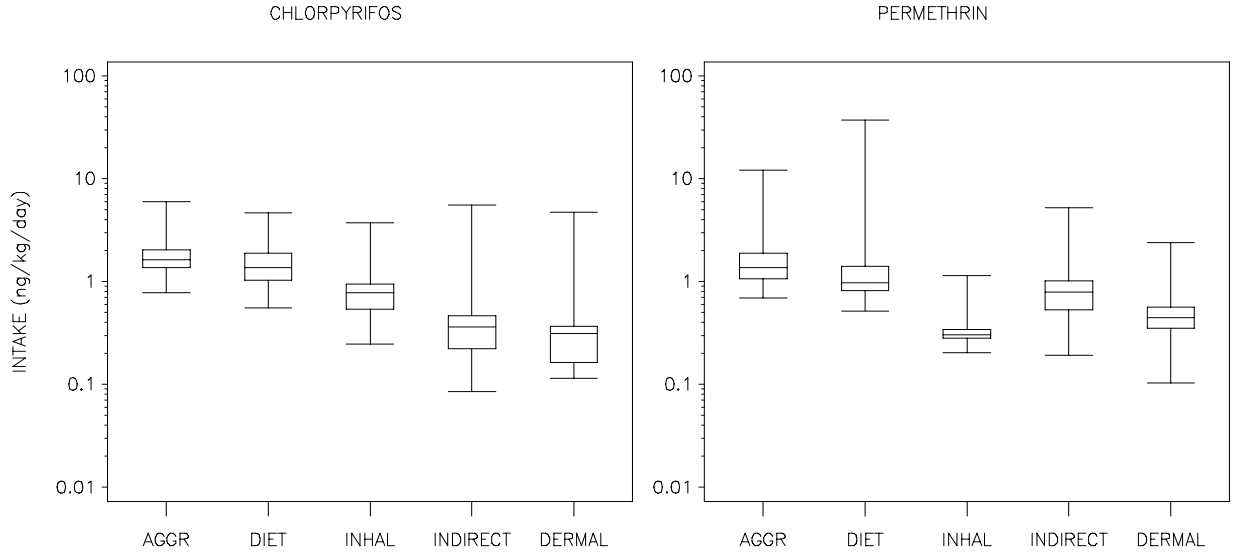

Figure 8.11 Estimated distributions of aggregate intake ("AGGR") of chlorpyrifos and permethrin (ng/kg/day) and estimated distributions of the four contributing routes (diet, inhalation, indirect ingestion, and dermal) among CTEPP-OH children.

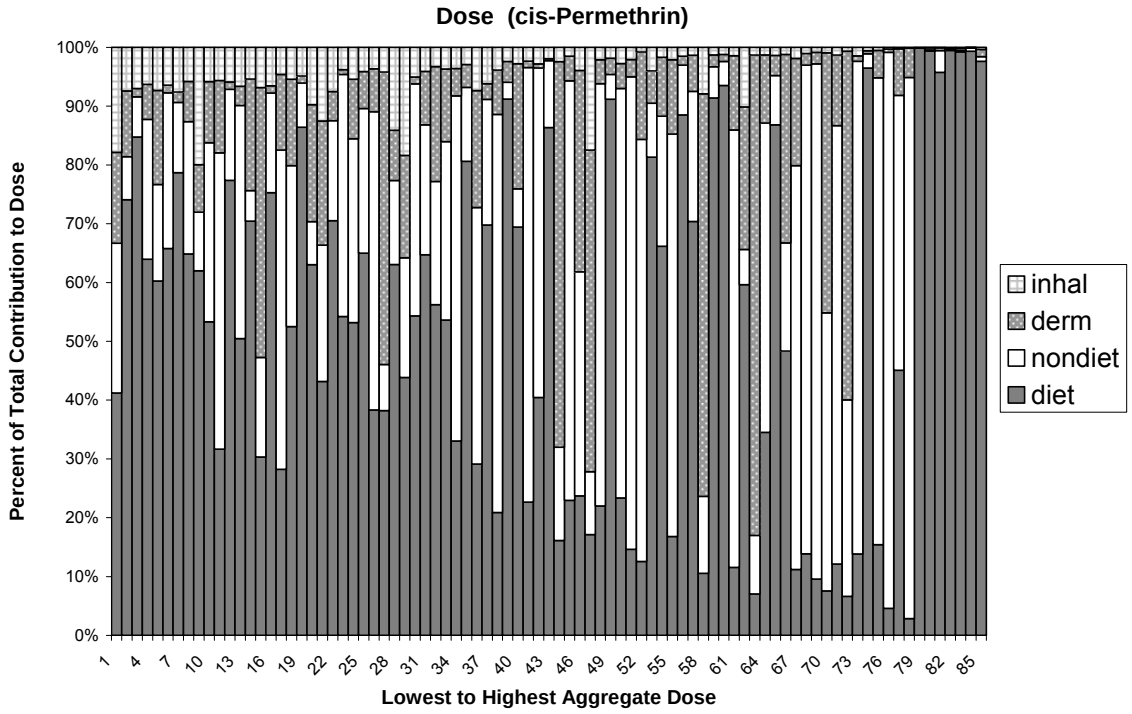

Figure 8.12 The contributions of inhalation, dermal absorption, diet, and nondietary ingestion to aggregate intake of *cis*-permethrin.

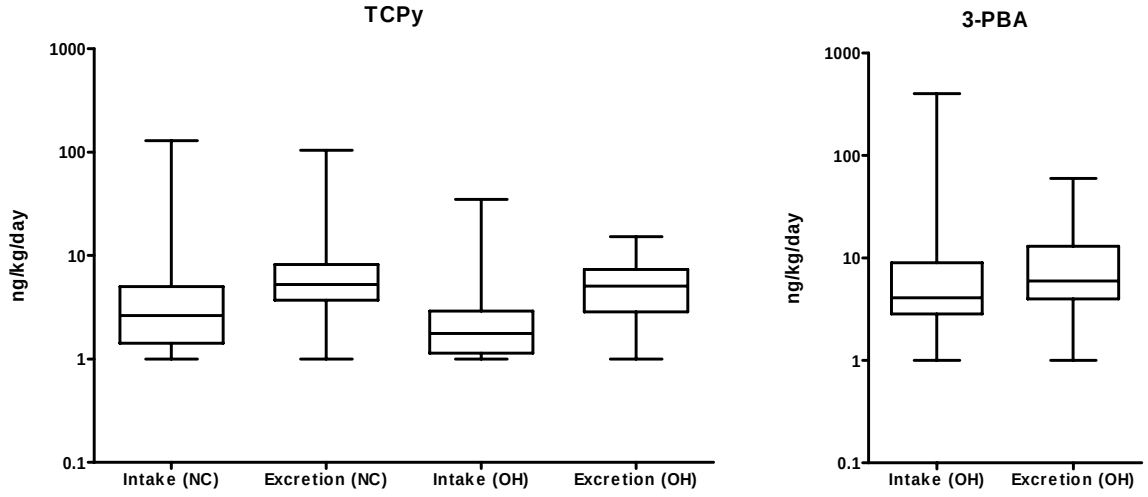

Figure 8.13 Children's estimated aggregate intake of chlorpyrifos and permethrin compared to their measured urinary metabolites (CTEPP).

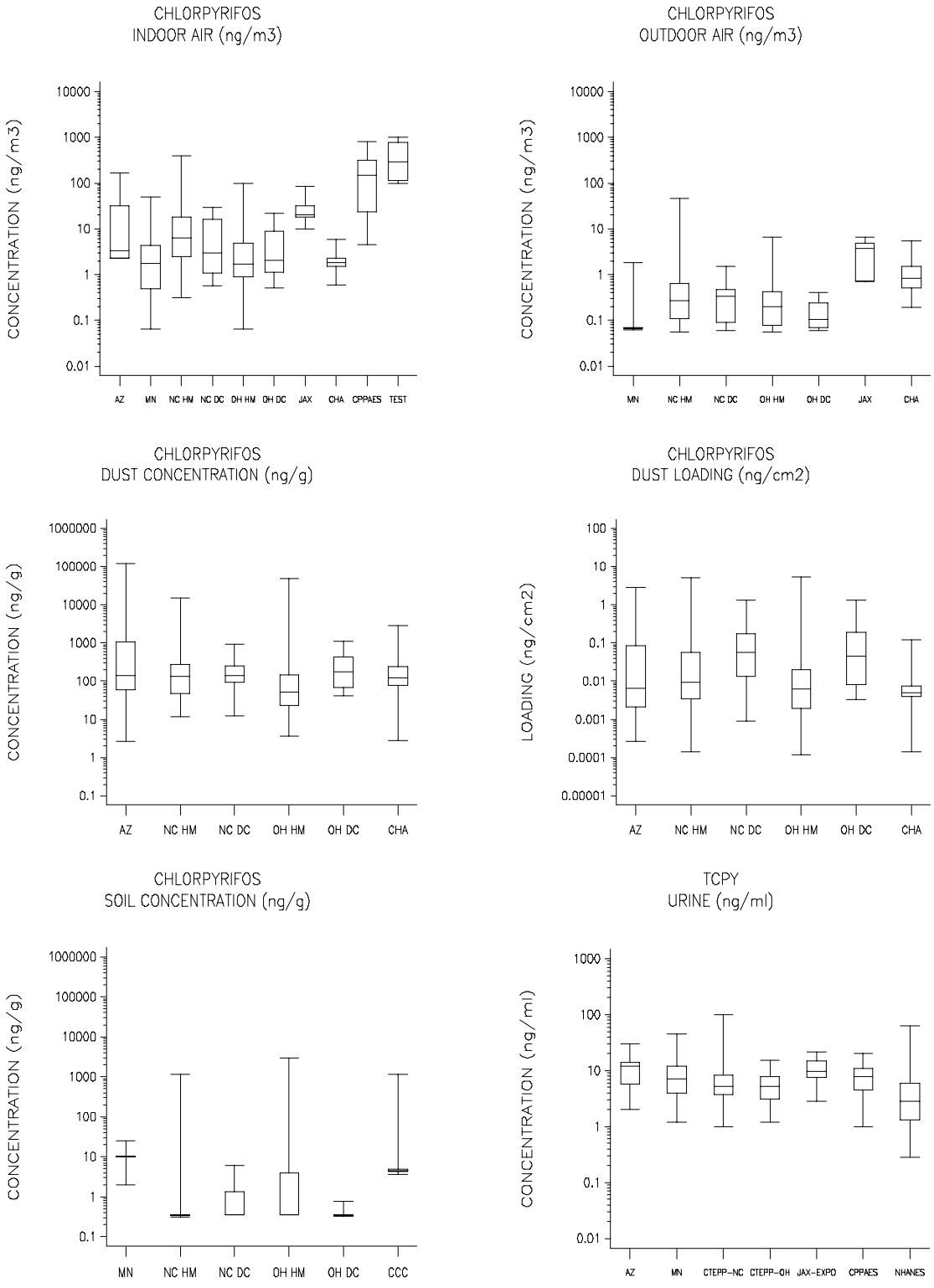

Figure 8.14 Distributions of TCPy in urine across studies (bottom right panel) in comparison to distributions of chlorpyrifos in indoor air, outdoor air, dust, and soil across studies.

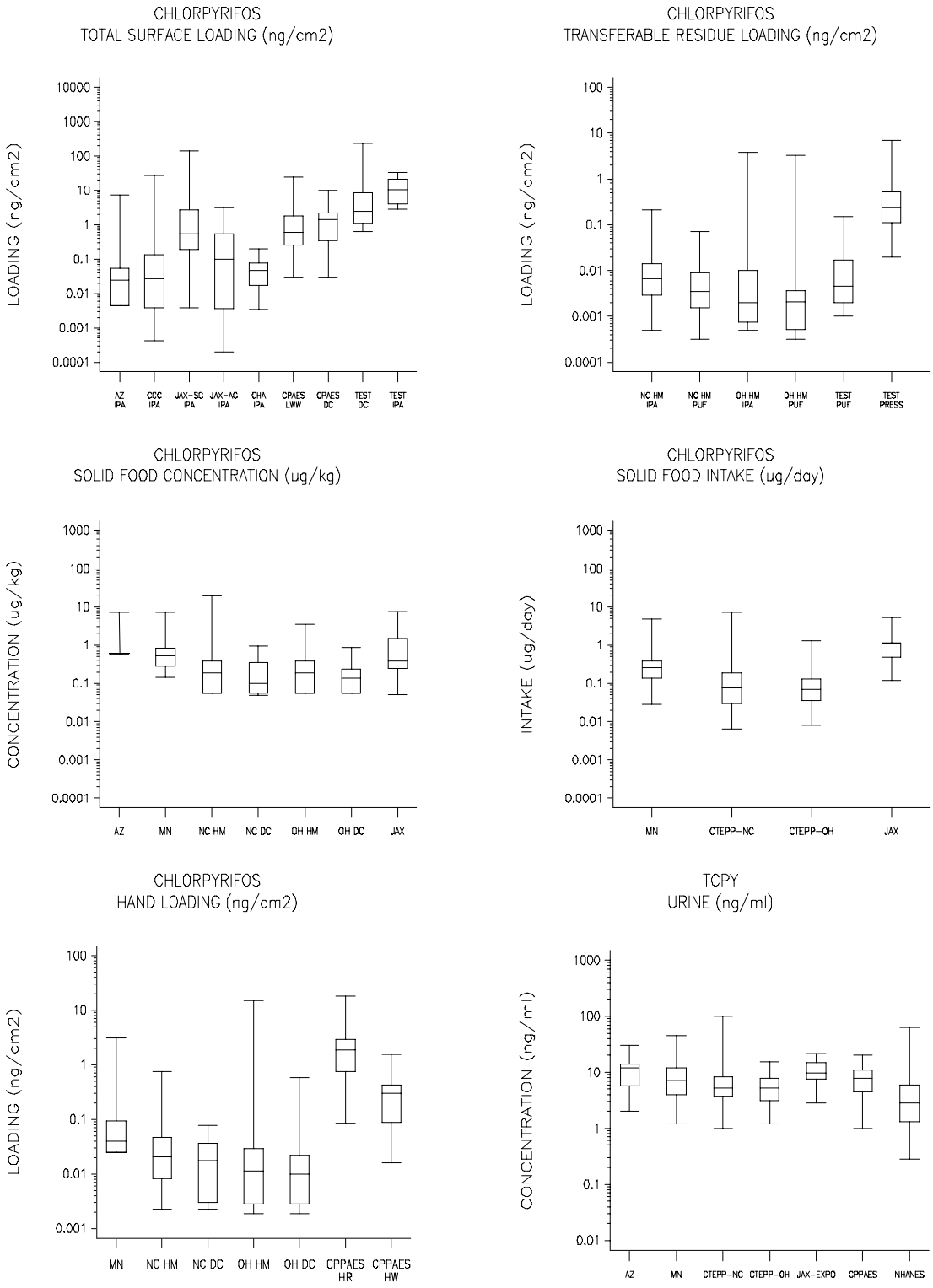

Figure 8.15 Distributions of TCPy in urine across studies (bottom right panel) in comparison to distributions of chlorpyrifos on surfaces, in solid food, and on hands across studies.

8.6 Model Predictions

The Stochastic Human Exposure and Dose Simulation (SHEDS) model (Zartarian *et al.*, 2000) provides route-specific estimates of aggregate exposures, relying on input data from assorted data sets, including those described in this report. The Safe Foods Project is currently developing an exposure-dose-response model to address cumulative risks associated with exposures to multiple pyrethroids. The Project intends to use the Exposure Related Dose Estimating Model (ERDEM) (Blancato *et al.*, 2004) to predict internal dose based on cumulative exposure estimates from SHEDS. The model will be used to identify critical pathways of human exposure and dose. A meaningful discussion of SHEDS and ERDEM is beyond the scope of this report, but an example of an important application of SHEDS is described below.

- Use of the SHEDS model with MNCPES data (Figure 8.16) helped reveal the importance of accounting for exposures to the metabolite/degradate TCPy in environmental media. Without such accounting, the model under-predicted urinary TCPy concentrations.
- SHEDS found that urinary biomarker concentrations depend mainly on dietary intake. An uncertainty analysis (independent of dietary) found other important factors to be: applied pesticide mass; surface area of treated rooms; time in treated rooms; air and residue decay rates; surface-to-skin transfer efficiency; dermal transfer coefficient; saliva removal efficiency; fraction hands mouthed; daily hand wash events; removal efficiency; maximum dermal loading; dermal absorption rate; and frequency of hand-mouth activity.
- By identifying critical pathways of human exposure and dose (and their associated uncertainties), models such as SHEDS and ERDEM guide the planning for future measurement studies so that newly identified data gaps may be filled with real-world measurement data.
- Applying SHEDS to different pesticide classes will provide information on degree to which factors that affect exposure differ across pesticide classes (*e.g.*, pyrethroids *vs.* organophosphates).

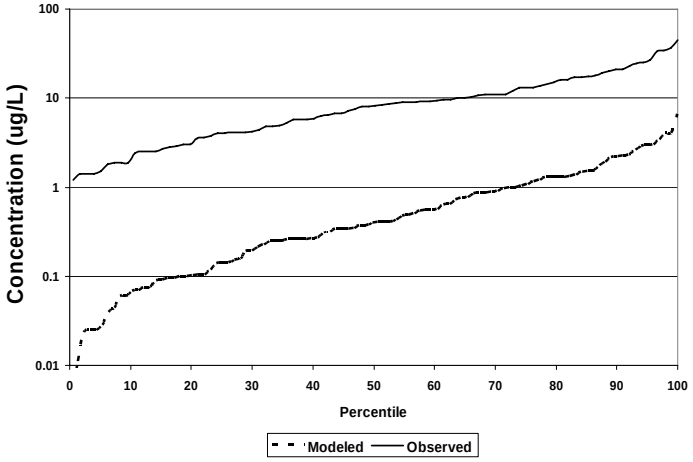

Figure 8.16 Comparison of TCPy in urine between SHEDS model and observed MNCPES data when TCPy in the environment is not considered (Source: Xue *et al.,* 2004).