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Pesticide dose estimates for children of Iowa farmers and non-farmers

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Abstract

Farm children have the potential to be exposed to pesticides. Biological monitoring is often employed to assess this exposure; however, the significance of the exposure is uncertain unless doses are estimated. In the spring and summer of 2001, 118 children (66 farm, 52 non-farm) of Iowa farm and non-farm households were recruited to participate in a study investigating potential take-home pesticide exposure. Each child provided an evening and morning urine sample at two visits spaced approximately 1 month apart, with the first sample collection taken within a few days after pesticide application. Estimated doses were calculated for atrazine, metolachlor, chlorpyrifos, and glyphosate from urinary metabolite concentrations derived from the spot urine samples and compared to EPA reference doses. For all pesticides except glyphosate, the doses from farm children were higher than doses from the non-farm children. The difference was statistically significant for atrazine ($p < 0.0001$) but only marginally significant for chlorpyrifos and metolachlor ($p = 0.07$ and 0.1, respectively). Among farm children, geometric mean doses were higher for children on farms where a particular pesticide was applied compared to farms where that pesticide was not applied for all pesticides except glyphosate; results were significant for atrazine ($p = 0.030$) and metolachlor ($p = 0.042$), and marginally significant for chlorpyrifos ($p = 0.057$). The highest estimated doses for atrazine, chlorpyrifos, metolachlor, and glyphosate were 0.085, 1.96, 3.16, and 0.34 μg/kg/day, respectively. None of the doses exceeded any of the EPA reference values for atrazine, metolachlor, and glyphosate; however, all of the doses for chlorpyrifos exceeded the EPA chronic population adjusted reference value. Doses were similar for male and female children. A trend of decreasing dose with increasing age was observed for chlorpyrifos.
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1. Introduction

Children of farmers have the potential to be exposed to agricultural pesticides via the take-home pathway. That is, farmers may inadvertently bring pesticides into the home on their clothing and shoes, which can be deposited into dust and onto surfaces or they may directly deposit pesticides on their children if they handle their children prior to washing. Children, especially children less than 6 years old, spend more time indoors and on the floors and may be exposed via hand and object to mouth contact. Black et al. (2005) observed an hourly median hand to mouth contact frequency of 10–19 and an hourly median object to mouth contact frequency of 6–18 for children aged 7–53 months. Furthermore, farm children may have the opportunity to be exposed to agricultural pesticides by playing or working in treated fields, contact with treated animals, contact with contaminated farm vehicles, equipment or storage areas, and even through direct handling of

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pesticides. Parental occupation involving pesticide application has been associated with childhood cancers (Daniels et al., 1997; Flower et al., 2004; Zahm and Ward, 1998) and household pesticide use has been associated with childhood leukemia (Ma et al., 2002).

Several papers have been published investigating farm children's exposure to pesticides using biological monitoring (Acquavella et al., 2004; Coronado et al., 2004; Curl et al., 2002; Curwin et al., 2007; Fenske et al., 2000, 2002; Koch et al., 2002; Loewenherz et al., 1997; Lu et al., 2000; Thompson et al., 2003). However, only a few have estimated pesticide dose to ascertain the potential health significance of these exposures (Acquavella et al., 2004; Fenske et al., 2000). Biological monitoring has the advantage of aggregating exposures from all sources and routes, a current requirement for pesticide health risk assessment in the United States as mandated by the Food Quality Protection Act of 1996 (FQPA, 1996). Biological monitoring data are often collected in the form of urinary metabolite concentrations. While this is useful, an estimate of dose would be helpful in ascertaining the risk associated with the urinary metabolite concentrations. Mage et al. (2004) described an approach to estimating pesticide dose from urinary metabolite concentration for adults and suggested that a similar approach could be used for children, provided that the appropriate equations are used for predicting a child's daily creatinine clearance rate.

The US Environmental Protection Agency (USEPA) sets reference doses (RfD), which reflect dietary risk, for pesticides during the registration process. The reference dose is derived from animal toxicity studies and is generally based on the most sensitive toxic endpoint (e.g. weight loss) in the most appropriate animal model. Different routes, such as oral and dermal, and duration of exposure are used in the toxicity studies and a weight of evidence approach may be taken when determining the RfD. The RfD incorporates an uncertainty factor of 100 to account for inter- and intra-species variability and, in response to the FQPA, recent re-registration eligibility decisions have incorporated an additional FQPA safety factor where warranted. The FQPA has mandated that the EPA take into consideration sensitive subpopulations when establishing reference doses. Where toxicity data indicate a subpopulation (e.g. children) may be more sensitive to a pesticide, an additional safety factor up to 10 may be incorporated into the reference dose. The additional safety factor will depend on the endpoint of concern, the route of exposure, and the degree of sensitivity. Such a reference dose is called a population adjusted dose (PAD).

In 2001, a study was initiated in Iowa to investigate take-home exposure among farm families. The results of biological monitoring among these families have been reported previously (Curwin et al., 2005, 2007). In this paper, dose estimates for the children in the study have

Table 1
EPA acute reference doses

Pesticide	Acute RfD[a] (µg/kg/day)	Study	Toxicity endpoint
Atrazine	10[b]	Developmental toxicity study in rat and rabbit	Delayed ossification in fetuses; decreased body weight gain in adults
Chlorpyrifos	0.5[b]	Acute blood time course study in male rats	Plasma cholinesterase inhibition
Metolachlor	n/a[c]	n/a	n/a
Glyphosate	n/a	n/a	n/a

[a]The USEPA defines an acute RfD as an "estimate of a daily oral exposure for an acute duration (24 h or less) to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime".
[b]Denotes a population adjusted reference dose (PAD) which incorporates an additional FQPA safety factor of 10.
[c]n/a, not available.

Table 2
EPA chronic reference doses

Pesticide	Chronic RfD[a] (µg/kg/day)	Study	Toxicity endpoint
Atrazine	1.8[b]	Six-month LH surge study in rat	Attenuation of preovulatory luteinizing hormone (LH) surge
Chlorpyrifos	0.03[b]	Weight of evidence from five studies: 2-year dog, 90-day dog, 2-year rat, 90-day rat, developmental neurotoxicity in rat	Plasma and red blood cell cholinesterase inhibition
Metolachlor	100	1 year toxicity study in dogs	Decreased body weight gain
Glyphosate	2000	Developmental toxicity study in rabbit	Maternal death

[a]The USEPA defines a chronic RfD as an "estimate of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime".
[b]Denotes a population adjusted reference dose (PAD) which incorporates an additional FQPA safety factor of 10.

been calculated from the urine concentrations in an effort to determine the significance of the exposure levels observed. The purpose of this paper was twofold: (1) to calculate the dose estimates in children of farmers and non-farmers in Iowa for four pesticides: atrazine, metolachlor, chlorpyrifos, and glyphosate; and (2) to compare the dose estimates to EPA reference values for each pesticide. The acute and chronic reference doses and the studies and endpoints used to derive them for the four pesticides studied here can be found in Tables 1 and 2 (USEPA, 1993, 1995, 2002, 2003).

2. Methods

In the spring and summer of 2001, the children less than 16 years of age of farmers and non-farmers residing in 10 counties in central, eastern Iowa were recruited to participate in the study. Sixty-six farm children (29 female and 37 male) and 52 non-farm children (20 female and 32 male) were enrolled in the study. Participant recruitment has been described previously (Curwin et al., 2002). In short, recruitment was conducted by convenience sampling. To be eligible for the study, the non-farm families had to live in a home on land that was not used for farming, and where nobody in the household worked in agriculture or commercial pesticide application. The non-farm families came from both rural and small town environments. In some cases, the non-farm families lived near farms, but as long as their land was not used for farming, they were eligible. The farm families had to be using at least one of seven target pesticides—atrazine, acetochlor, metolachlor, alachlor, chlorpyrifos, glyphosate, and 2,4-D. The target pesticides were selected because of their extensive use in Iowa agriculture and are among those most commonly used in Iowa. All of the pesticides are corn or soybean herbicides, with the exception of chlorpyrifos which is an insecticide used on corn. Only the results for atrazine, metolachlor, chlorpyrifos, and glyphosate are reported due to limitations of the analytical methods for the urine samples. The National Institute for Occupational Safety and Health (NIOSH) Human Subject Review Board approved this study.

Sample collection and analysis has been described previously (Curwin et al., 2007). Briefly, the children were visited on two occasions and two spot urine samples were collected by the participants: one in the evening and the first void the following morning. For young children, a parent collected the urine samples. The samples were stored in a cooler or refrigerator until collected by research staff. Twenty-five milliliter aliquots were stored on dry ice and shipped frozen to the NIOSH laboratory. The total volume of each urine void was recorded. The metabolites or parent compound of four pesticides—atrazine (atrazine mercapturate), chlorpyrifos (3,5,6-trichloro2-pyridinol), metolachlor (metolachlor mercapturate), and glyphosate (parent glyphosate)—were analyzed in the urine samples using immunoassay techniques and reported in micrograms of pesticide per liter of urine (μg/L). The limits of detection for atrazine mercapturate, trichloropyridinol (TCP), metolachlor mercapturate, and glyphosate were 1.16, 3.32, 0.3, 0.9 μg/L, respectively. Urinary creatinine was measured in the urine samples using a commercially available enzyme slide technology (Vitros 250 Chemistry System, Ortho-Clinical Diagnostics). Urine pesticide concentrations by volume were normalized by creatinine to give a concentration in micrograms of pesticide per gram of creatinine (μg/g).

2.1. Dose estimates

The absorbed daily dose (ADD) in micrograms of pesticide per kilogram body weight per day (μg/kg/day) was calculated using formula (1). The doses were calculated from spot urine samples collected on two occasions and are assumed to represent daily dose:

$$\mathrm{ADD(\mu g/kg/day)} = \frac{(C)(Cn)(CF)(R_{mw})}{BW}, \tag{1}$$

where C is the concentration of metabolite or pesticide in urine per gram creatinine (μg/g), Cn is the calculated mass of creatinine excreted per day (g/day), CF is a correction factor, R_{mw} is the ratio of parent pesticide and pesticide metabolite molecular weights, and BW is the body weight (kg).

Since spot urine samples were collected from each subject, total daily (24-h) excretion of creatinine (Cn) was calculated using the following equation (adapted from Cockcroft and Gault, 1976):

$$\mathrm{Cn(g/day)} = \left(\frac{\mathrm{CnER} \times 1440 \ \mathrm{min/day}}{1.73} \right) \times \mathrm{BSA} \times \frac{1\,\mathrm{g}}{1000\,\mathrm{mg}}, \tag{2}$$

where Cn ER is the creatinine urinary excretion rate in mg/min per $1.73\,\mathrm{m}^2$ body surface area and BSA is body surface area (m^2).

The creatinine urinary excretion rate was calculated as a function of age using the following equation from Shull et al. (1978)

$$\mathrm{CnER} = 0.035 \times \mathrm{age\,(years)} + 0.236. \tag{3}$$

The Shull et al. formula, which averages male and female creatinine excretion rates, was used for all children in the study, even those who had reached puberty. After puberty, however, females with the same weight and height as males of the same age would be expected to have diminished creatinine excretion due to diminished muscle mass. Thus, the use of the Shull relation implicitly makes a simplifying assumption by using an average value for boys and girls after puberty.

BSA was calculated as a function of height and weight using the following equation from Mosteller (1987)

$$\mathrm{BSA(m^2)} = \left(\frac{\mathrm{ht(cm)} \times \mathrm{wt(kg)}}{3600} \right)^{0.5}. \tag{4}$$

BSA was estimated using the EPA Exposure Factors Handbook (1997) for four farm children of unknown height.

Correction factors were used to account for incomplete excretion of the pesticides in urine. Approximately 67% of atrazine is excreted via urine (Timchalk et al., 1990) with atrazine mercapturate accounting for approximately 80% of the excreted metabolites (Buchholtz et al., 1999) resulting in a correction factor of $(1/0.67)/0.8 = 1.9$. Approximately 50% of metolachlor is excreted in urine (Davison et al., 1994); however, estimates of the percentage of metolachlor mercapturate in urine were not available. Metolachlor is structurally similar to alachlor and the percentage of alachlor mercapturate in human urine has been shown to range from 25% to 62% (Driskell et al., 1996). Using a conservative estimate of 60% for the percentage of metolachlor mercapturate in urine resulted in a correction factor of $(1/0.5)/0.6 = 3.3$. For chlorpyrifos, approximately 70% is excreted as TCP in urine (Nolan et al., 1984) resulting in a correction factor of $1/0.7 = 1.4$. Finally, approximately 30% of glyphosate is excreted via the urine with almost 100% excreted as unchanged parent compound (Kennepohl and Munro, 2001) resulting in a correction factor of $(1/0.3)/1 = 3.3$.

The ratio of molecular weights (R_{mw}), calculated by dividing the pesticide parent molecular weight by the pesticide metabolite molecular weight, was 0.63, 0.69, 1.77, and 1.00 for atrazine, metolachlor, chlorpyrifos, and glyphosate, respectively. This calculation assumes that one molecule of the parent compound produces one molecule of the metabolite. The ratio of molecular weights corrects for the differences in mass between one molecule of the parent pesticide and one molecule of the metabolite.

2.2. Data analysis

All statistical analyses were performed using SAS 9 Software® (SAS Institute Inc., Cary, NC). Data analysis methods needed to address two primary concerns. First, since children from each household provided evening and morning urine samples at two visits and multiple children were sampled from each household, urinary pesticide concentrations could not be treated as independent. A second concern was that urinary pesticide concentrations were frequently below the analytical limit of detection

(LOD), particularly for atrazine, metolachlor, and glyphosate (Curwin et al., 2007). Of the 417 urine samples obtained from children, only 20% detected atrazine above the LOD, 61% detected metolachlor above the LOD and 84% detected glyphosate above the LOD; however, nearly all ($n = 416$) samples detected chlorpyrifos above the LOD. Furthermore, the laboratory did not censor urine samples below the LOD, rather, values were reported as non-detect, a positive level below the LOD, or a level greater than or equal to the LOD. Values reported below the LOD may be within the error around a zero value and are not as reliable as values above the LOD. Furthermore, these values are typically not reported as such, rather they are usually reported as left-censored at the LOD. Methods are commonly available for dealing with correlated data (i.e., mixed-effects regression modeling) and highly censored data (i.e., maximum likelihood estimation); however, methods are not readily available for dealing with both of these problems at the same time.

Initially, maximum likelihood estimation via the LIFEREG procedure in SAS was used to estimate geometric mean (GM) doses, adjusted for age and sex, separately for farm and non-farm children. In the maximum likelihood analysis, urinary metabolite concentrations reported as either a non-detect or a positive level below the LOD were considered to be left-censored at the LOD. The concentration for each urine void (identified by child, visit, and time) was adjusted for creatinine and used to calculate an estimated dose. Doses based on urinary metabolite concentrations censored at the LOD were considered to be left-censored at the dose level calculated using the LOD. The lognormal distribution was specified as the underlying distribution. Since the LIFEREG procedure assumes independence among the observations, standard errors were known to be underestimated by the procedure; therefore, the LIFEREG procedure was not used for significance testing.

Mixed-effects modeling via the MIXED procedure in SAS was used to test whether the GM dose for farm children was significantly different than the GM dose for non-farm children. The dependent variable was the natural log transformed dose; fixed effects were household type, age, and sex; and random effects were household and child nested within household. To simplify the models, the average of the evening and morning dose estimates was used as an estimate of dose for the visit. In these models, urinary metabolite concentrations reported as a positive level below the LOD were used to estimate pesticide dose (i.e., they were not censored at the LOD). For urinary metabolite concentrations reported as a non-detect, the minimum positive concentration divided by two was used to estimate pesticide dose. The mixed-effects model was given by

$$\ln(y_{ijk}) = \beta_0 + \beta_1(\text{household type}) + \beta_2(\text{sex}) + \beta_3(\text{age}) + \gamma_i + \gamma_{j(i)} + \varepsilon_{ijk}, \quad (5)$$

where y_{ijk} is the kth dose estimate for child j within household i, $i = 1$–50, $j = 1$–n_i, $k = 1$–2, n_i is the number of children in household i, γ_i is the random effect for household, $\gamma_{j(i)}$ is the random effect for child nested within household, and ε_{ijk} is the random error term. The model specified separate covariance parameter estimates for farm and non-farm households. Results are presented as adjusted GMs by taking the antilog of the adjusted log-transformed means. The covariance parameter estimates from the mixed-effects models provided estimates of the between-household, between-child, and within-child variance components. For each child, a single dose estimate was obtained by averaging the individual dose estimates for comparison to EPA reference doses.

In order to evaluate the accuracy of the immunoassay techniques used, 178 duplicate urine samples from 50 fathers of the children were analyzed by enzyme-linked immunosorbent assay (ELISA) techniques (i.e., the same immunoassay techniques used to analyze the children's samples) and also by high-performance liquid chromatography (HPLC)–tandem mass spectrometry. The HPLC method has been described previously (Curwin et al., 2005). Mixed-effects modeling was used to relate the urinary TCP concentrations derived from HPLC to the concentrations derived from ELISA and the observed relationship was used to correct the dose estimates for potential bias due to ELISA method.

3. Results

Farm and non-farm households were similar with respect to the number of children residing in the household (median 2 children per household, range 1–4 children per household). Approximately 60% of the children were male. Children ranged from less than 1 year to 15 years of age (median 7 years). The distributions of age, height, and weight were similar for farm and non-farm children (Table 3).

The estimated GM doses obtained using maximum likelihood methods and mixed-effects modeling are presented in Table 4. The two methods produced similar estimates for chlorpyrifos, which was expected since there was little censoring for chlorpyrifos. Estimates for metolachlor and glyphosate, which saw considerable censoring, were fairly close for the two methods. Estimates for atrazine, which saw the greatest amount of censoring, were similar for farm children, but approximately eight times higher based on mixed-effects modeling compared to maximum likelihood for non-farm children. Regardless of the method used to estimate the GM, for all pesticides except glyphosate, the GM dose for farm children was higher than the GM dose for non-farm children. The difference was statistically significant for atrazine ($p < 0.0001$) but only marginally significant for chlorpyrifos and metolachlor ($p = 0.07$ and 0.10, respectively). Non-farm children had slightly higher glyphosate doses, but the difference was not statistically significant. When comparing the children on farms where a particular pesticide was applied versus farms where that pesticide was not applied, the GM doses were higher for children where that pesticide was applied for all pesticides except glyphosate. This result was significant for atrazine ($p = 0.030$) and metolachlor ($p = 0.042$), and marginally significant for chlorpyrifos ($p = 0.057$).

A trend of decreasing dose with increasing age for all children combined was observed for chlorpyrifos ($p < 0.0001$); increasing age by 1 year was associated with a -4.0% change (95% confidence interval -5.4% to -2.6%) in the estimated GM chlorpyrifos dose. In a categorical analysis, the estimated least-squares GM

Table 3
Characteristics of the farm and non-farm children in the study

Characteristic	Farm children ($n = 66$)	Non-farm children ($n = 52$)
Male (%)	56	62
Age (years) median (range)	7.0 ($<$1–15.6)	7.2 ($<$1–15.7)
Height (cm) median (range)[a]	124 (61–178)	122 (79–175)
Weight (kg) median (range)[b]	27.2 (7.3–63.5)	22.7 (12.7–68.9)

[a]Height information was missing for five farm children and one non-farm child.

[b]Weight information was missing for one non-farm child.

Table 4
Geometric mean pesticide doses (µg/kg/day) for farm and non-farm children

Pesticide household type	Number of			Absorbed daily dose (µg/kg/day)				
	Homes	Children[a]	Samples	% <LOD[b] (%)	Range[c]	ML GM[d]	Mixed GM[e]	*p*-Value
Atrazine								
Farm	25	65	235	74	0.002–0.085	0.013	0.011	<0.0001
Non-farm	25	51	180	88	0.000–0.040	0.008	0.001	
Farm								
Applied	20	53	127	74	0.000–0.11	0.014	0.015	0.030
Not applied	5	12	108	75	0.000–0.055	0.012	0.007	
Chlorpyrifos								
Farm	25	65	235	<1	0.27–1.96	0.67	0.68	0.071
Non-farm	25	51	180	0	0.24–1.36	0.58	0.57	
Farm								
Applied	2	6	12	0	0.59–1.40	0.93	0.89	0.057
Not applied	23	59	223	<1	0.22–1.96	0.66	0.67	
Metolachlor								
Farm	25	65	235	37	0.000–3.16	0.016	0.015	0.10
Non-farm	25	51	180	42	0.000–0.072	0.013	0.008	
Farm								
Applied	7	17	40	10	0.008–6.30	0.038	0.030	0.042
Not applied	18	48	195	43	0.000–3.09	0.014	0.013	
Glyphosate								
Farm	25	65	235	19	0.013–0.34	0.11	0.10	0.23
Non-farm	25	51	180	12	0.037–0.33	0.13	0.12	
Farm								
Applied	17	40	98	16	0.001–0.33	0.11	0.090	0.32
Not applied	8	25	137	20	0.003–0.64	0.11	0.11	

[a] Urine samples were not provided for one farm and one non-farm child.

[b] Percent of samples with pesticide dose estimate censored at the limit of detection (LOD).

[c] For farm and non-farm, range of overall dose (one per child averaged over both visits); for farm-applied and farm-not applied, range of dose for each visit (one per child-visit).

[d] Estimated geometric mean (GM) dose based on maximum likelihood (ML) methods was adjusted for age and sex.

[e] Estimated GM dose and *p*-value for farm GM versus non-farm GM based on mixed-effect models with fixed effects of household type, age and sex, and random effects of household and child within household.

chlorpyrifos dose was significantly higher for children less than 10 years of age (0.67 µg/kg/day) when compared to children 10 years or older (0.49 µg/kg/day, $p < 0.0001$). Trends were not observed with age for atrazine, metolachlor, and glyphosate. Pesticide doses were similar for male and female children.

The highest dose estimates for farm children were 0.085, 1.96, 3.16, and 0.34 µg/kg/day and the highest dose estimates for non-farm children were 0.040, 1.36, 0.072, and 0.33 µg/kg/day for atrazine, chlorpyrifos, metolachlor, and glyphosate, respectively (Table 4). No child had an overall dose estimate that exceeded the EPA chronic reference values for atrazine, metolachlor, and glyphosate; however, every child's overall dose estimate exceeded the EPA population adjusted chronic reference value for chlorpyrifos (Table 5). About 97% and 92% of the estimated chlorpyrifos doses for farm and non-farm children, respectively, exceeded the EPA general population reference value of 0.3 (the reference dose not incorporating the extra safety factor of 10 for sensitive

subpopulations) and 83% and 74% of farm and non-farm children, respectively, exceeded the EPA population adjusted acute reference value (Table 5).

Results of the analysis of the fathers' urine samples analyzed by HPLC and immunoassay were previously reported (Curwin et al., 2005, 2007). When compared, TCP concentrations based on the ELISA method were three–four times higher compared to the HPLC method. The observed relationship was given by

$$\hat{C}_{HPLC} = -1.05 + 0.411 \times C_{ELISA},$$

where C_{HPLC} represents the concentration of chlorpyrifos in the urine based on the HPLC method and C_{ELISA} represents the concentration of chlorpyrifos in the urine based on the ELISA method. This relationship was used to correct the children's chlorpyrifos doses based on ELISA. The percentage of the corrected doses exceeding the EPA reference values are provided in Table 5.

Estimates of the between-household, between-child, and within-child variance components for the children's

Table 5
Percent of children with estimated dose exceeding reference values[a]

Reference value[b]	Atrazine		Chlorpyrifos		Metolachlor		Glyphosate	
	Farm (%)	Non-farm (%)	Farm (%)	Non-farm (%)	Farm (%)	Non-farm (%)	Farm (%)	Non-farm (%)
NOAEL	0	0	0 (0)[c]	0 (0)	0	0	0	0
Acute RfD	0	0	0 (0)	0 (0)	n/a[d]	n/a	n/a	n/a
Acute PAD	0	0	83 (3)	74 (2)	n/a	n/a	n/a	n/a
Chronic RfD	0	0	97 (31)	92 (14)	0	0	0	0
Chronic PAD	0	0	100 (100)	100 (100)	n/a	n/a	n/a	n/a

[a]Overall estimated dose based on the average of the available dose estimates for each child.
[b]NOAEL, no observable adverse effect level; RfD, reference dose; PAD, population adjusted reference dose.
[c]Percents in parentheses used the corrected dose estimates.
[d]n/a, not available.

Table 6
Within- and between-household variance components for children's pesticide doses

Pesticide household type	Estimated variance components[a]					
	Between-household		Between-child		Within-child	
	$\hat{\sigma}^2_{bh}$	%[b]	$\hat{\sigma}^2_{bc}$	%	$\hat{\sigma}^2_{wc}$	%
Atrazine						
Farm	0.54	17	0	0	2.64	83
Non-farm	3.36	33	0	0	6.76	67
Chlorpyrifos						
Farm	0.091	52	0.0015	1	0.081	47
Non-farm	0.066	44	0.017	11	0.069	45
Metolachlor						
Farm	1.16	39	0.35	12	1.45	49
Non-farm	1.36	51	0	0	1.33	49
Glyphosate						
Farm	0.45	42	0	0	0.61	58
Non-farm	0.13	30	0	0	0.31	70

[a]Variance components ($\hat{\sigma}^2_{bh}$, $\hat{\sigma}^2_{bc}$, $\hat{\sigma}^2_{wc}$) were estimated by modeling the natural log transformed doses using a mixed-effects model with fixed effects of household type, age and sex, and random effects of household and child within household. The model specified separate covariance parameter estimates for farm and non-farm households.
[b]% denotes the proportion of the total variance.

pesticide doses are provided in Table 6. Variance components were computed for farm and non-farm children after adjusting for age and sex. The within-child variance components were generally higher than the other variance components for both farm and non-farm children. The between-child variance contributed relatively little to the overall variance.

4. Discussion

The results presented here provide an indication of the significance of pesticide exposure among farm children. The doses were higher for farm children than non-farm children and for children on farms where a particular pesticide was applied than children on farms where that pesticide was not applied for all the pesticides except glyphosate. Glyphosate is used both agriculturally and residentially which may explain why the doses were similar. However, all of the dose estimates for atrazine, metolachlor, and glyphosate were well below the EPA chronic reference value for these pesticides.

Of concern, however, were the dose estimates for chlorpyrifos. All of the dose estimates for chlorpyrifos were above the EPA population adjusted chronic reference dose for children. The lowest estimated dose for chlorpyrifos was 0.24 µg/kg/day compared to the reference dose of 0.03 µg/kg/day. The EPA reference dose for chlorpyrifos was based on a no-observable-adverse-effect-level (NOAEL) of 30 µg/kg/day and incorporates a safety factor of 100 for inter- and intra-species variation and an additional safety factor of 10 for all children and for females between the ages of 13 and 50 years, both of which are considered to be sensitive subpopulations. Additionally, most of the estimated doses for farm and non-farm children exceeded the general population chronic reference dose without the additional safety factor and the population adjusted acute reference value of 0.5 µg/kg/day. None of the dose estimates exceeded the general population acute reference dose or the NOAEL.

It is probable that the chlorpyrifos doses were overestimated as a result of direct exposure to TCP, a metabolite of chlorpyrifos; however, the calculated chlorpyrifos doses assume that the TCP excreted in urine came entirely from chlorpyrifos exposure. TCP was observed in 100% of dust and wipe samples and in greater than 95% of food and air samples in a study investigating chlorpyrifos exposure among pre-school children (Morgan et al., 2005). Chlorpyrifos was also found in these media, and generally in higher amounts, except in food where TCP was found to be 12 times higher than chlorpyrifos. Wilson et al. (2003) in another study of pre-school children, conclude that the estimated intake of TCP from food can account for all of the TCP found in the children's urine. However, the children were not from farm environments so chlorpyrifos

exposures were much lower than TCP exposures. Direct TCP exposure is probably accounting for some of the TCP in the children's urine in this study but to what extent is unclear.

The chlorpyrifos doses may also be overestimated due to the ELISA analytical method used to determine TCP concentration in urine. If a bias exists with the ELISA method, resulting in higher urinary TCP concentrations, than the calculated doses would also be higher. The observed relationship between HPLC and ELISA for the fathers' urine samples was used to "correct" the ELISA concentrations for the children's urine samples which resulted in estimated doses for chlorpyrifos that were much lower. Using the adjusted concentrations, the percentage of farm children with doses exceeding the acute PAD, chronic, and chronic PAD reference values was 3%, 31%, and 100%, respectively. Similarly, the percent of non-farm children with doses exceeding the acute PAD, chronic, and chronic PAD reference doses was 2%, 14%, and 100%, respectively. Thus, 100% of the children had doses exceeding the chronic PAD dose based on the corrected urinary metabolite concentrations, but the percent of children exceeding the acute PAD and chronic reference doses was much reduced. This potential upward bias of the ELISA method was not explored for the other pesticides in this paper. However, all of the estimated doses for the other pesticides were below all of the reference values; therefore, correcting the estimated doses downward would not change this result.

Fenske et al. (2000) found that 56% of estimated azinphos-methyl doses for children of agricultural workers exceeded the EPA reference dose, while 44% of the doses for non-agricultural children exceeded the reference dose. Values were much lower for phosmet where less than 10% exceeded the EPA reference dose. Additionally, single day dose estimates were calculated for azinphos-methyl and 26% exceeded the EPA acute reference dose. None of the estimates exceeded the empirically derived NOAEL for these compounds. The authors concluded that the results indicated that children in agricultural communities have pesticide exposures of regulatory concern.

Fenske's results appear to contrast with our results. Our study would indicate, with the exception of chlorpyrifos, that children in agricultural communities may not have exposures of regulatory concern at least for atrazine, metolachlor, and glyphosate. It should be noted that Fenske's doses were derived by summing two urinary metabolites that are common to organophosphate exposure in general and are not specific to azinphos-methyl and phosmet. As a result, it is possible that they overestimated the dose for these pesticides. Acquavella et al. (2004) observed results similar to ours in that none of their estimated doses for glyphosate exceeded the EPA chronic reference dose.

An interesting result was the trend of decreasing dose with increasing age for chlorpyrifos; in particular, doses were higher among children under the age of 10 years.

Creatinine excretion is known to be positively associated with age among children (Barr et al., 2005). In our study, the amount of creatinine excreted per day was estimated from body surface area, which in turn was estimated from height and weight, which are both positively associated with age among children. Since body weight increases substantially as a child gets older, the normalized pesticide dose per kilogram body weight for a given urinary metabolite concentration is reduced. Therefore, the trend observed here may be partly an artifact of how the doses were estimated. Urinary metabolite concentrations among the children were negatively associated with age, but the associations were not significant (Curwin et al., 2007). However, it seems probable that younger children would have a higher dose for a given exposure than older children. Black et al. (2005) observed that the time children spent playing on the floor decreased with increasing age and that infants had the highest frequency of mouthing behavior. To better determine the effect of age on dose, 24-h urine samples should be collected over several days.

There are several limitations in our pesticide dose estimates that are common to the estimation of doses from spot urinary metabolite concentrations. First, it was assumed that the spot urine samples were representative of average daily pesticide excretion and that the doses estimate average daily doses. Depending on the timing of the urine collection with respect to pesticide application in the farm homes, the doses may be overestimated. Urine samples were not collected in the fall and winter months. Presumably, the urine concentrations would be lower during these months and therefore the average daily dose over a year could be lower. Second, the merits of creatinine adjustment for spot urine samples are being debated, especially in children (Barr et al., 2005; Boeniger et al., 1993). Lastly, it was assumed that the amount of pesticide metabolite excreted in urine was equivalent to an absorbed pesticide dose. However, chlorpyrifos doses due to chlorpyrifos exposure are probably overestimated due to direct exposure to TCP. Further, all the doses may underestimate exposure if the percentage of metabolite excreted in urine used to derive the correction factors is lower and conversely the dose would be overestimated if the percents excreted where higher. The correction factors were derived from the best estimate of the amount of metabolite excreted in urine from the current literature.

5. Conclusion

Farm children generally had higher pesticide dose estimates than non-farm children. However, with the exception of chlorpyrifos, all the estimates were below EPA chronic and acute reference doses. All chlorpyrifos dose estimates for both farm and non-farm children were above the EPA population adjusted chronic reference dose and most were above the population adjusted acute reference dose and are of concern. Estimation of pesticide dose from farm children's urine samples allows comparison

to EPA reference doses and therefore provides an indication of the significance of pesticide exposure. Additional longitudinal studies which better estimate daily pesticide doses over the course of a year are needed to truly determine the health significance of pesticide exposures.

Disclaimer

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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