Assessing Cumulative Organophosphate Pesticide Exposure and Risk among Pregnant Women Living in an Agricultural Community

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Abbreviations:

BMD_{10}: Benchmark $Dose_{10}$

CDC: Centers for Disease Control and Prevention

CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas

DEP: diethylphosphate; DETP: diethylthiophosphate; DEDTP: diethyldithiophosphate

DMP: dimethylphosphate; DMTP: dimethylthiophosphate; DMDTP: dimethyldithiophosphate

DPR: California Department of Pesticide Regulation, California Environmental Protection Agency

EPA: Environmental Protection Agency

FQPA: Food Quality Protection Act of 1996

LOD: Limits of detection

MOE: Margin of exposure

NIEHS: National Institute of Environmental Health Sciences

NOAEL: No-observable-adverse-effect level

OP: Organophosphate

POD: Point of Departure

PUR: California Department of Pesticide Regulation's pesticide use reporting system

QC: Quality Control

RPF: Relative Potency Factor

ABSTRACT

Approximately 225,000 kilograms of organophosphate (OP) pesticides are used annually in California's Salinas Valley, which is intensively farmed for vegetables and fruit. These activities have raised concerns about pesticide exposures to area residents. As part of a prospective cohort study, we collected three spot urine samples from 462 pregnant women and analyzed them for six dialkyl phosphate metabolites. Based on these urinary metabolite concentrations, we estimated OP pesticide doses with deterministic steady-state models using two methods: the first method assumed the pesticide metabolites were attributable entirely to a single diethyl or dimethyl OP pesticide; the second method adapted U.S. EPA draft guidelines for cumulative risk assessment to estimate dose from a mixture of OP pesticides that share a common mechanism of toxicity. We used pesticide use reporting data for the Salinas Valley to quantify the likely mixture to which the women were exposed.

Based on average OP pesticide dose estimates that assumed exposure to a single OP pesticide (Method 1), between 0% and 36.0% of study participants' exposures exceeded the U.S. EPA oral benchmark dose$_{10}$ (BMD_{10}) divided by a 100-fold uncertainty factor, depending on the assumption made about the parent compound. These BMD_{10} values were derived from studies of brain cholinesterase inhibition in rats. 14.7% of the participants' average cumulative OP pesticide dose estimates (Method 2) exceeded the BMD_{10} of the selected index chemical divided by a 100-fold uncertainty factor, regardless of index chemical chosen. An uncertainty analysis of the pesticide mixture parameter suggests that this point estimate could range from 1%-38%. Because our reference value (BMD_{10}/100) may not account for the special sensitivity of the developing fetus, this research points to the need for modeling approaches to estimate fetal exposures and assess risk from prenatal OP pesticide exposure.

INTRODUCTION

The Food Quality Protection Act (FQPA) of 1996 requires the U.S. Environmental Protection Agency (EPA) to consider the cumulative effects on human health that can result from exposure to mixtures of pesticides. In response, the U.S. EPA Office of Pesticide Programs, in consultation with the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel, has developed guidelines for the cumulative risk assessment of pesticides that share a common mechanism of toxicity (U.S. EPA 2002a, 2002d). The approach is conceptually similar to methods developed by the U.S. EPA for estimating exposures to mixtures of dioxins and dibenzofurans using toxicity equivalence factors to normalize the toxicity of each member of the group with respect to that of a single chemical (U.S. EPA 1989).

We have used these new cumulative risk assessment guidelines to estimate pregnant women's cumulative exposure in an agricultural region based on urinary OP metabolite levels (U.S. EPA 2002a, 2002b). This method makes use of the finding that OP pesticides share a common mechanism of toxicity (i.e., the same toxic effect occurs in or at the same organ or tissue by essentially the same sequence of major biochemical events), the inhibition of cholinesterase activity (Mileson et al. 1998; U.S. EPA 1999, 2001).

Recent biological monitoring data suggest that resident farm and farmworker families have higher exposures than reference families, and in particular children from families of pesticide applicators and those living less than 200 feet from a treated orchard have higher exposures compared to children living farther away (Fenske et al. 2000; Loewenherz et al. 1997; Lu et al. 2000; O'Rourke et al. 2000). Fenske et al. (2000) proposed that the measurement of dialkyl phosphate metabolites in children's urine has utility for estimating dose ranges for the OP

pesticides and thus can provide useful information to discussions of pesticide health risks. They reported that farmworker children were more likely than nonfarmworker children to be exposed to the OP pesticides azinphos-methyl and phosmet at levels exceeding U.S. EPA chronic dietary reference doses.

Recent federal and state initiatives have fostered a number of research projects focusing on pesticide exposures to children, however, little research on pesticide exposure to pregnant women has been performed. To date, no studies have evaluated OP pesticide exposure in pregnant women living in rural communities, although substantial toxicological evidence suggests that repeated low-level exposure to OP pesticides affects neurodevelopment and growth in developing animals, particularly when exposure occurs prenatally (Chanda and Pope 1996; Dam et al. 1998; Eskenazi et al. 1999; Gupta et al. 1985; Muto et al. 1992; Schulz et al. 1995; Whitney et al. 1995).

This study uses the proposed U.S. EPA Cumulative Risk Assessment Guidelines (US EPA 2002a, 2002d) to examine potential health risks to pregnant women participating in the CHAMACOS study (Center for the Health Assessment of Mother's and Children of Salinas), one of the eight Centers for Children's Environmental Health Research funded by the U.S. EPA and NIEHS in 1998. All women assessed as part of the CHAMACOS study had some exposure to OP pesticides, which is likely to be from a mixture of compounds with varying toxicities and usage patterns (Figure 1). Recognizing that people living in rural communities are potentially exposed to mixtures of chemicals with varying toxicities, the present study seeks to expand the work of Fenske et al. (2000) on children by evaluating pregnant women's exposure to multiple OP pesticides and assessing the cumulative risk of these exposures. Our aim is to determine whether pregnant women living in this region are potentially exposed to OP pesticides in excess

of health-based reference values, and thus whether their fetuses may be at an increased risk of adverse health outcomes.

MATERIALS AND METHODS

Study Area and Population Recruitment

The Salinas Valley of Monterey County is an agricultural area located in northern California, a few kilometers from the Pacific Ocean. Organophosphate (OP) insecticides are used on a variety of crops including lettuce, broccoli, cauliflower and strawberries. This region is approximately 25 kilometers wide and 110 kilometers long--extending from Castroville in the north to King City in the south. The temperate climate makes agricultural production possible almost year-round.

This study is based on serial cross-sectional data collected from pregnant women participating in the CHAMACOS study, a prospective cohort study of children's environmental health. Pregnant women were eligible for enrollment in the CHAMACOS study if they entered prenatal care between September 1999 and November 2000 at either of two community clinics in the area (Clinica de Salud del Valle de Salinas and Natividad Medical Center). At enrollment, all participants were at least 18 years of age, eligible for Medi-Cal health insurance, less than 20 weeks gestation, fluent in English or Spanish and planning to deliver their child at Natividad Medical Center. The CHAMACOS study population is 94% Mexican or Mexican-American, with 96% of participants living within 200% of the poverty line. 37% of study participants performed farm field or other agricultural work after becoming pregnant, and 75% lived in households where at least one adult member worked in agriculture.

Informed consent was obtained from all study participants following procedures established by the University of California Berkeley Human Subjects Review Board and the

Natividad Medical Center. Maternal body weight and fetal gestational age data were abstracted from study participants' medical records.

Urine Collection, Storage and Analysis

We collected urine samples from women at two times during pregnancy (~14 weeks gestation (n=593), ~26 weeks gestation (n=503)) and shortly after delivery (n=494). Specimens were stored at –80º C until shipment to the Centers for Disease Control (CDC), where six non-specific urinary OP metabolites were measured: dimethylphosphate (DMP); dimethyldithiophosphate (DMDTP); dimethylthiophosphate (DMTP); diethylphosphate (DEP); diethyldithiophosphate (DEDTP); and diethylthiophosphate (DETP). These metabolites derive from approximately 40 OP compounds, 28 of which are U.S. EPA-registered for use in the U.S., falling into the general categories of dimethyl and diethyl OP pesticides (Table 1).

The laboratory methods for dialkyl phosphate quantification employed the isotope dilution technique combined with gas chromatography and mass spectrometry (GC-MS/MS) (Bravo et al. 2002). Isotope dilution is widely regarded as the definitive technique for trace analysis with dialkyl phosphate metabolite detection limits of 1 ppb or less (Barr et al. 1999). Creatinine concentrations in urine were determined using a commercially available diagnostic enzyme method (Vitros CREA slides, Ortho Clinical Diagnostics, Raritan, NJ).

Laboratory quality control (QC) was established by the repeat analysis of two in-house urine pools enriched with known amounts of pesticide residues whose target values and confidence limits were previously determined. An analytical run was considered "out-of control" if the QC value failed to meet the requirements of the Westgard QC multi-rules (www.westgard.com). Data were not reported from runs considered "out-of-control."

Data Analysis

We used the following reporting convention for the six dialkyl phosphate metabolites: samples with no analytical response were considered nondetectable and were assigned a value of zero; samples with a detectable peak were reported as numerical values in micrograms per liter urine (μg/L). The creatinine concentration in each urine sample was reported in units of milligram creatinine per deciliter urine (mg/dL). One participant with missing creatinine concentration data, and four participants with urinary creatinine levels that implied unreasonably high fluid consumption rates (<10 mg/dL) were excluded from our final analyses. Two participants with missing body weight data were also excluded.

Pesticide Use

In California, growers and applicators are required to report all pesticide use to the County Agricultural Commissioner who then reports it to the California Department of Pesticide Regulation (DPR). We obtained the pesticide use report (PUR) datasets for 1999 and 2000 from the DPR. For agricultural use, crop, active ingredient, date, pounds applied, and the location of use, identified to a one-mile (1.6 kilometer) square section, are reported. To define agricultural OP pesticide use in the Salinas Valley, we selected all one-mile (1.6 kilometer) sections within a 200-foot (61-meter) elevation contour from the Salinas River with reported pesticide use. 99% of CHAMACOS participants lived within this area. Agricultural pesticide applications identified by the DPR as likely errors (e.g., due to key entry mistakes, etc.) in Monterey County were 3% of total OP pesticide use and were replaced by median use per application for the crop and pesticide product for the county. Other pesticide uses, including landscape (i.e., golf courses, parks, cemeteries), structural and roadside use, are reported by month and are not geographically identified. These other uses accounted for 0.8% and 1.0% of Monterey County OP pesticide use

for 1999 and 2000 respectively. We accounted for these other uses in our weekly and annual summaries of all 1999-2000 OP pesticide applications in the region as follows: to describe annual usage, they were simply added to the total; for weekly usage, monthly usage of these non-agricultural uses were divided by four and added to the agriculture use information.

Dose Calculations

OP pesticide doses were estimated based on urinary metabolite concentration data using two methods: the first method assumed metabolite levels were the result of exposure to a single pesticide (Fenske et al. 2000); the second method assumed exposure to a mixture of OP pesticides that share a common mechanism of toxicity and similar dose-response curves (U.S. EPA 2001, 2002b, 2002d).

Underlying our dose estimation models are the following assumptions:

(1) Urinary concentrations are representative of steady-state conditions over a 24-hour period. Under this steady-state assumption, we estimated a full day's urinary excretion of metabolites based on a spot urine sample using creatinine as an index of total daily urinary output volume. The relationship between 24-hour urine output volume and urinary creatinine is given by the following formula:

$$V_i = Ccr_i / Cc_i \qquad \text{(Eq 1)}$$

where, V_i = expected 24-hour urine output volume for i^{th} pregnant women (L/day); Ccr_i = reference value for pregnant women's daily creatinine excretion (mg/day) (Knuppel et al 1979; Davison et al. 1980; Davison and Noble 1981); Cc_i = creatinine concentration in i^{th} urine sample (mg/L).

(2) 100% of absorbed maternal OP pesticide dose is expressed in urine as diethyl and dimethyl phosphate metabolites.

(3) The six urinary OP metabolites were the result of exposure to OP pesticides used for agriculture, structural pest control or landscape purposes in Monterey County.

(4) OP metabolite concentrations are equivalent to internal doses on a molar basis. Because each OP pesticide molecule devolves into exactly one of its possible dialkyl phosphate metabolites, the molar sum of metabolite equals the molar concentration of OP pesticide.

Method 1. Single Chemical Approach

Single dose estimates were calculated assuming 100% of the exposure was from a single diethyl or dimethyl OP pesticide. We assumed that urinary diethyl phosphate metabolites were attributable to chlorpyrifos, diazinon or disulfoton, and that dimethyl phosphate metabolites were attributable to dimethoate, malathion, methidathion, naled or oxydemeton-methyl. These OP compounds are consistently among the most heavily used pesticides in Monterey County (i.e., they account for 100% and 99% of total diethyl and dimethyl OP pesticide use, respectively), according to the California DPR's pesticide use reporting system (DPR 1999, 2000). Dose estimates were not aggregated across diethyl and dimethyl OP pesticide classes. Only the relevant metabolites for each compound were considered in the dose calculations, e.g., chlorpyrifos dose calculations were based upon DEP and DETP concentrations only (Table 1). This method provides a reasonable upper bound estimate of dose from exposure to specific chemicals, and it is consistent with current regulatory methods.

Dose estimates from single diethyl and dimethyl OP pesticides were calculated with the following equations:

Diethyls

$$D_{DiEthyl} = \left(\left(\frac{C_{DEP}}{MW_{DEP}} + \frac{C_{DETP}}{MW_{DETP}} + \frac{C_{DEDTP}}{MW_{DEDTP}} \right) * MW_{DiEt\_OP} * \left(\frac{Cr_{Ex}}{Cr_{Conc}} \right) \right) \Big/ BW \quad \text{(Eq 2)}$$

Dimethyls

$$D_{DiMethyl} = \left(\left(\frac{C_{DMP}}{MW_{DMP}} + \frac{C_{DMTP}}{MW_{DMTP}} + \frac{C_{DMDTP}}{MW_{DMDTP}} \right) * MW_{DiMet\_OP} * \left(\frac{Cr_{Ex}}{Cr_{Conc}} \right) \right) \Big/ BW \quad \text{(Eq 3)}$$

where $D_{DiEthyl}$ = dose from diethyl OP pesticide (μg/kg/day); $D_{DiMethyl}$ = dose from dimethyl OP pesticide (μg/kg/day); C = urinary dialkyl phosphate metabolite concentration (μg/L); MW_{DiMet_OP} = molecular weight of dimethyl OP pesticide (g/mol); MW_{DiEt_OP} = molecular weight of diethyl OP pesticide (g/mol); Cr_{Ex} = expected daily urinary creatinine excretion (mg/day) from reference values for pregnant women (Knuppel et al 1979; Davison et al. 1980; Davison and Noble 1981); Cr_{Conc} = creatinine concentration (mg/L) derived from urine samples; BW = pregnant women's body weight (kg) around the time of urine sample collection.

Reported average doses are the arithmetic means of three single-day dose estimates (creatinine-adjusted) based on urine samples collected from participants at two times during pregnancy and at delivery.

Method 1: Risk Estimation

The toxicological reference value selected for comparison with dose estimates was the U.S. EPA oral BMD_{10} divided by a 100-fold uncertainty factor. This BMD_{10} has been developed for ingestion of OP pesticides, and is based on dose-response data for brain cholinesterase inhibition in female rats representing a 10% change in enzyme levels compared to controls,

derived from laboratory studies that lasted 21 days or longer (U.S. EPA 2002a, 2002b, 2002d). Current U.S. EPA oral BMD_{10} values for the OP pesticides used in Monterey County range from 0.07 to 313.9 mg/kg/day (Table 1) (U.S. EPA 2002d). The U.S. EPA is developing BMDs as an alternative to the no-observable-adverse-effect level (NOAEL) in dose-response assessments because they: (1) utilize all points on the dose-response curve; (2) are less sensitive to the number of animals used in a study; and (3) are not dependent on dose spacing. We selected the BMD_{10} as the point of departure for this analysis, and we applied a 100-fold uncertainty factor to the BMD_{10} to account for animal to human extrapolation and intrahuman variability. Using the BMD_{10}/100 as a reference value permits direct comparison of our Method 1 and Method 2 risk estimate results.

Method 2. Chemical Mixture Approach

An obvious limitation to the Method 1 approach is that women are probably exposed to the mixture of pesticides in their environment, rather than to a single compound. To estimate cumulative dose from exposure to mixtures of OP pesticides we converted each relevant pesticide into its index chemical toxicity equivalent using relative potency factors (RPFs). RPFs are the ratio of the toxic potency of a given chemical to that of an index chemical in the cumulative assessment group. We used U.S. EPA oral BMD_{10} values for brain cholinesterase inhibition as the measure of potency in our RPF calculations, and we selected chlorpyrifos as the index compound (Table 1) (U.S. EPA 2002d). A complete description of the methods and rational for this process can be found in the U.S. EPA cumulative risk assessment reports (U.S. EPA 2001, 2002a, 2002b, 2002d). As defined by the U.S. EPA,

$$RPF_n = \text{Measure of Potency}_{\text{index chemical}} / \text{Measure of Potency}_{\text{chemical n}} \quad \text{(Eq 4)}$$

where chemical n = a member of the cumulative assessment group; index chemical = the

chemical selected as the basis for standardization of toxicity of components in a mixture; Measure of Potency = BMD_{10}.

The pesticides in our cumulative assessment group are the 11 (3 diethyl and 8 dimethyl) OP pesticides commonly applied in the Salinas Valley that metabolize to dialkyl phosphate compounds (Table 1). PUR data for this region was used to describe the likely mixture to which the women were exposed. We selected chlorpyrifos as the index chemical because: (1) it is a compound in our cumulative assessment group for which complete hazard assessment and dose-response information is available; (2) it metabolizes into urinary dialkyl phosphate compounds; (3) it is commonly used in the Salinas Valley (DPR 1999, 2000); (4) its measure of potency, BMD_{10}, falls in the mid-range of BMD_{10} values for the OP pesticides in our cumulative assessment group. Method 2 risk estimates based on cumulative dose equivalents are insensitive to the choice of index chemical. Using RPFs, we calculated pregnant women's cumulative OP pesticide dose equivalents with the following equation:

$$D_{Cum} = \left(\mu Mol_{DiEthyl} \sum_{i=1}^{3} P_i MW_i RPF_i + \mu Mol_{DiMethyl} \sum_{i=1}^{8} P_i MW_i RPF_i \right) \Big/ BW \qquad \text{(Eq 5)}$$

where D_{Cum} = cumulative dose equivalents (μg/kg/day); $\mu Mol_{DiEthyl}$ = total micromoles of diethyl phosphate metabolites (DEP, DETP, DEDTP) excreted over a 24-hour period (see equation 1); $\mu Mol_{DiMethyl}$ = total micromoles of dimethyl phosphate metabolites (DMP, DMTP, DMDTP) excreted over a 24-hour period (see equation 1); P_i = proportion of pesticide i in mixture calculated from annual PUR data for the Salinas Valley; MW_i = molecular weight of i^{th} pesticide

(μg/μmol); RPF_i = relative potency factor of the i^{th} pesticide in the cumulative assessment group; BW = pregnant women's body weight (kg) around the time of urine sample collection.

For study participants that provided three urine samples, we estimated single-day cumulative dose equivalents (creatinine-adjusted) based on each sample. We then calculated the arithmetic mean, and selected the maximum dose equivalent from these estimates. For comparison with creatinine-adjusted estimates, we calculated volume-adjusted cumulative dose equivalents using reference values for pregnant women's total daily urine output volume (Cohen ed. 2000; Davison and Noble 1981).

Method 2: Uncertainty analysis

The parameter defining the mixture of OP pesticides to which study participants were potentially exposed is a source of uncertainty in our assessment. To evaluate the sensitivity of the model to this parameter, we used Monte Carlo simulation software (Crystal Ball, Decisioneering 2000 with Microsoft Excel 7.0) to vary the quantity of pesticides used to describe the assumed exposure mixture. We ran 5,000 simulations based on uniform sampling distributions of the weekly kilograms of pesticides applied in the CHAMACOS study area between 1999-2000 (DPR 1999, 2000). The uniform distributions were bounded by zero and by 110% of each pesticide's maximum reported weekly quantity applied.

Method 2: Cumulative Risk Estimation

To assess risk we compared estimated average and maximum cumulative dose equivalents to the index chemical's BMD_{10} divided by a 100-fold uncertainty factor (chlorpyrifos's $BMD_{10}/100$=14.8 μg/kg/day). Margins of exposure were calculated by taking the

ratio of the point of departure (POD), chlorpyrifos's BMD_{10}, to the estimated average and maximum single-day cumulative dose equivalents.

RESULTS

Urine samples were collected from pregnant women at two prenatal study visits (around 14 and 26 weeks gestation) and shortly after delivery; all three samples were obtained from 462 women. Summary statistics of the urinary OP metabolites for our final sample of 455 women are presented in Table 2. 43 of 1365 samples (3%) had no measurable metabolites, and we set concentrations for the six metabolites equal to zero. Among the dimethyl phosphate metabolites, the median concentrations were: DMP, 1.7 μg/L; DMTP, 6.2 μg/L; and DMDTP, 0.5 μg/L. Among the diethyl phosphate metabolites, the median concentrations were: DEP, 1.0 μg/L; DETP, 0.9 μg/L; and DEDTP, 0 μg/L. Overall, the dimethyl phosphate levels were higher than diethyl phosphate levels (Table 2). This is consistent with OP pesticide use patterns in Monterey County (DPR 1999, 2000).

Dose Calculations

Method 1: Single chemical approach

Table 3 summarizes the single OP pesticide dose calculation results (n=455) including geometric mean, median and range for this study population. Depending on the assumption made about the parent compound, creatinine-adjusted average dose estimates ranged from 0 to 45.7 μg/kg/day. Median dimethyl OP pesticide dose estimates ranged from 0.18-0.91 μg/kg/day, and median diethyl OP pesticide dose estimates ranged from 0.12-0.14 μg/kg/day.

Method 1: Risk Estimation

Depending on the choice of pesticide used in the dose calculation, we found that between 0% and 36.0% of participants had dose estimates that exceed 0.01 times the U.S. EPA BMD_{10} value, which corresponds to the BMD_{10} divided by a 100-fold uncertainty factor (Table 3). The large variability underscores the significance of the assumption made about which OP parent compound is responsible for the exposure. The results suggest, however, that a portion of pregnant women participating in the CHAMACOS study may have exposures exceeding an acceptable health-based margin of exposure for individual OP pesticides.

Method 2: Chemical mixture approach

Table 4 presents summary statistics for average cumulative OP pesticide dose equivalents derived from 3 spot urine samples. These dose estimates are log-normally distributed and range from 0.1-172.8 μg/kg/day (cumulative dose equivalents). Estimated dose equivalents from only the dimethyl OP pesticides ranged from 0.1-172.8 μg/kg/day, and estimated dose equivalents from only the diethyl OP pesticides ranged from 0.01-6.6 μg/kg/day. All study participants had average cumulative dose equivalent estimates greater than zero, and the geometric mean cumulative dose equivalent estimate for this population was 4.5 (95% CI: 4.1-5.0) μg/kg/day (Table 4). Maximum single-day cumulative dose equivalent estimates ranged from 0.3 to 511.5 μg/kg/day (geometric mean=9.0 (95% CI: 8.0-10.2) μg/kg/day).

We also generated cumulative dose equivalent estimates by adjusting for total daily urine volume based on reference values for pregnant women (Cohen ed. 2000; Davison and Noble 1981). Cumulative dose equivalents calculated from volume-adjusted data were highly correlated with creatinine-adjusted estimates ($R^2 = 0.8$), and results were very similar (not presented). The geometric mean and median volume-adjusted estimates were slightly higher

than creatinine-adjusted estimates, and the percentage of pregnant women with average cumulative doses that exceeded the BMD_{10}/100 were also higher (15.8% versus 14.7%).

Method 2: Cumulative Risk Estimation

Figure 2 presents the distribution of average cumulative dose for the entire population in index chemical (chlorpyrifos) toxicity equivalents (μg/kg/day). 14.7% of cumulative dose equivalent estimates exceeded the index chemical's BMD_{10} divided by a 100-fold safety factor (14.8 μg/kg/day). The percentage of pregnant women exceeding this reference value from exposure to only the dimethyl OP pesticides or only the diethyl OP pesticides were 14.1% and 0% respectively (Table 4). No participant had a cumulative dose equivalent estimate exceeding the chlorpyrifos POD (an oral BMD_{10} of 1480 μg/kg/day). Margins of exposure were calculated for the estimated average and maximum single-day cumulative dose equivalents. Figure 3 presents a range of margins of exposure at various percentiles on a logarithmic scale. 14.7% and 34.0% of calculated margins of exposure were less than 100 for women's average and maximum cumulative doses. A margin of exposure less than 100 implies that the estimated cumulative dose is greater than the POD divided by 100-fold uncertainty factor (e.g., index chemical's BMD_{10}/100).

The U.S. EPA is in the process of developing guidelines for formally incorporating standard uncertainty factors and FQPA safety factors into cumulative risk assessment for OP pesticides (U.S. EPA 2002c). Final consideration of the FQPA safety factor is pending (U.S. EPA 2002d).

Method 2: Uncertainty analysis

Based on 5000 simulations, the estimated average cumulative chlorpyrifos dose equivalents ranged from 0.03 to 320.2 μg/kg/day, and the population median ranged from 0.9-

10.2 (mean=4.4) μg/kg/day. The percent of participants with estimated average cumulative dose equivalents exceeding chlorpyrifos's BMD_{10}/100 ranged from 1% to 38% (mean=14%), and the 10^{th} and 90^{th} percentiles were 7% and 23%, respectively. While the simulations produced dose estimates that varied substantially, the simulation averages were consistent with the Method 2 point estimates presented in Table 4.

Method 1 and 2 comparisons

As expected, the Method 2 average risk estimate fell between the upper and lower risk estimates from Method 1. When assessing exposure to a single pesticide (Method 1), we attributed all diethyl or dimethyl OP pesticide dose to a particular compound. Because the chemicals in our cumulative assessment group have varying toxicities, when we estimate the number of people potentially exposed over the reference value using Method 1, we have estimates that range from the least to the greatest number possible, given our cumulative assessment group and the observed metabolite levels. Any mixture will have a cumulative toxicity, and consequently a risk level, somewhere between these two end points.

DISCUSSION

We estimated OP pesticide doses based on urinary OP metabolite levels using two methods. The first assumed exposure to a single pesticide; the second assumed exposure to a mixture of OP pesticides. We found that average cumulative dose estimates for 14.7% of CHAMACOS study participants exceed the index chemical's BMD_{10}/100-fold safety factor. Our uncertainty analysis of the pesticide mixture parameter suggests that this point estimate could range from 1%-38%.

This paper is one of the first case studies utilizing the U.S. EPA's new cumulative risk assessment framework for OP pesticides. Current U.S. EPA guidelines provide a methodology

for calculating cumulative dose using traditional exposure assessment methods that track exposure from source to dose (U.S. EPA 2002a, 2002b, 2002d). Such models rely on source-specific environmental concentration data, behavioral factors, and route-specific absorption factors. We have only utilized the portions of the guidelines dealing with dose aggregation because our dose estimations are based on biomonitoring data.

We have used U.S. EPA BMD_{10} values as the measure of toxic potency to calculate OP pesticide RPFs. These BMD_{10} values were derived from dose-response curves from studies of brain cholinesterase inhibition in female rats (U.S. EPA 2002b). Using these methods, cumulative dose estimates, which are toxicologically equivalent to a dose of the index chemical, will vary depending on the choice of the index chemical. The risk estimates based on these doses, however, will remain consistent regardless of the index chemical chosen from our cumulative assessment group.

Both methods of dose calculation presented here introduce uncertainty due to the assumption of parent compound(s), use of creatinine to estimate 24-hour urinary metabolite excretion, and intra-individual and temporal variability. Because our assessment was based on non-specific metabolite data, it was necessary to make assumptions about the mixture of pesticides to which each participant was exposed (i.e., the presence of urinary dialkyl phosphate metabolites indicates exposure to one or more OP pesticides, but does not indicate exposure to any particular pesticide). We incorporated pesticide use reporting data for the Salinas Valley into cumulative dose equivalent calculation models to describe the assumed mixture of parent compounds that resulted in an individual's urinary OP metabolites levels. Even for individuals living in rural areas, however, many potential sources of pesticide exposure exist in addition to agricultural pesticide use, including diet and home pesticide usage. In the CHAMACOS study

population, the percentage of women with OP pesticides in the home (7%) was small and not statistically correlated with urinary metabolite levels. Ideally, the assumed mixture in such analyses could be described by a complete multimedia assessment of all potential sources of pesticide exposure. In this analysis, however, we relied on pesticide use levels reported to the State of California to estimate the mixture of OP pesticides to which women were exposed. In future studies we plan to address this issue by basing cumulative OP pesticide dose equivalent estimates on chemical-specific biological monitoring data.

We recognize that the pesticide mixture is an uncertain parameter in our cumulative dose calculation model. Although the uncertainty analysis of this parameter produced cumulative dose estimates that varied substantially, simulation results were consistent with conclusions based on our point estimates.

In this assessment, we assumed that 100% of absorbed maternal OP pesticide dose is expressed in urine as diethyl and dimethyl phosphate metabolites. This assumption may underestimate dose because some metabolites will be excreted in other biological media besides urine (e.g., feces). Griffin et al. (1999) reported that on average 93% of administered chlorpyrifos was excreted in urine as dialkyl phosphate metabolites in 5 adult volunteers. While the kinetics of elimination vary among the dimethyl and diethyl phosphate metabolites, toxicological evidence suggests that the metabolites of many OP compounds are excreted primarily, but not exclusively, in the urine (Griffin et al. 2000; Krieger and Dinoff 2000). Further, total OP pesticide exposure may be underestimated because several OP pesticides, such as acephate, do not metabolize to any of the dialkyl phosphate metabolites and are therefore not included in our exposure-dose estimates. These compounds represent about 20% of total use in the CHAMACOS study area.

A potentially large source of variability in our models results from the urinary metabolite data itself. For these analyses we estimated dose based on three spot urine samples collected from each study participant over a six-month period. Thus, uncertainty due to intra-individual variability and temporal variation in dialkyl phosphate metabolite levels was introduced. To reduce this source of variability in future studies, multiple urine samples collected per day over several days are needed. Furthermore, urine samples were collected at various times throughout the day, at the convenience of the participants. The effect of this source of variability is unknown, but it is likely that both over- and under-estimates of actual daily doses were generated.

Adjusting metabolite concentration data for total daily urine volume was necessary because 24-hour urine samples are impractical in community-based studies and were not collected. Although creatinine adjustment is a common interpretive step in biological monitoring studies, its merits are debated in the scientific community (Boeniger et al. 1993). As a point of comparison, we also generated dose estimates by adjusting for total daily urine output volume based on reference values for pregnant women from the literature. Cumulative dose estimates calculated from volume-adjusted data were highly correlated with creatinine-adjusted estimates ($R^2 = 0.8$) and did not substantively change our findings. Since creatinine adjustments yielded reasonable estimates for total daily urine volumes, and they are expected to account for the relative concentrations/dilutions of the urine samples, we chose to report creatinine-adjusted dose estimates only.

It is also possible that these urinary metabolites represent exposure to the breakdown products of the parent compounds, rather than exposure to the OP pesticides themselves. If this were true, pesticide doses would tend to be overestimated (Fenske et al. 2000). To date, we have

found no data in the literature to indicate that this is the case for the dialkyl phosphate metabolites. In fact, the breakdown products are probably too polar to be effectively absorbed through the skin (Barr et al. 2002), potentially eliminating dermal absorption of the breakdown products as a contributor to the observed urinary metabolite concentrations. More research is needed on the kinetics of elimination of these compounds. Findings from one Japanese toxicological study suggest that exposure to diethyl phosphate metabolites would predominately result in the excretion of inorganic phosphate (Imaizumi et al. 1993). When rats were orally exposed to the diethyl phosphate metabolites DEP and DETP at a dose of 1 g/kg, DETP intensely inhibited cholinesterase in rat brain homogenate, and DEP weakly inhibited the cholinesterase activity. DEP-treated rats excreted inorganic phosphate and organic phosphate in the 24-hour urine at amounts of 53% and 13% of the dose respectively. DETP-treated animals excreted inorganic phosphate and organic phosphate in their urine at amounts of 18% and 10% of the dose respectively.

Risk evaluation of cumulative exposures would be improved by the development of suitable regulatory reference doses. Possible PODs for cumulative risk assessment of OP pesticides include the NOAEL and BMD_{10}. The U.S. EPA is in the process of refining guidelines for formally taking into account standard uncertainty factors and FQPA safety factors in cumulative risk assessment of chemicals sharing a common mechanism of toxicity (U.S. EPA 2002c). Following the general methodology used to derive RfDs from NOAELs, we have applied a 100-fold uncertainty factor to the index chemical's BMD_{10} to account for intra- and interspecies variability. This reference value may not be adequately protective for this population of pregnant women, and an additional FQPA safety factor may be necessary to account for the special sensitivity of the developing fetus to OP pesticide exposure. The U.S.

EPA has recently proposed applying a 3x FQPA safety factor to 6 of the 8 pesticides in our cumulative assessment group (U.S. EPA 2002d). When these 3x safety factors are applied at the RPF calculation stage (as proposed) the percent of CHAMACOS participants with cumulative exposures exceeding the $BMD_{10}/100$ increases from 14.7% to 42.6%.

We expect the human fetus to be particularly sensitive to OP pesticide exposure because during gestation the human brain is growing and developing very rapidly. Toxicological studies have shown that OP compounds can cross the placental and blood brain barriers (Chanda and Pope 1996; Gupta et al. 1985; Muto et al. 1992). As part of a prospective cohort study being conducted by the Columbia Center for Children's Environmental Health, Whyatt et al. (2002a, 2002b) analyzed 142 paired blood samples collected at birth from minority mothers and newborns for 29 pesticides. They reported that 8 of the 29 pesticides (including diazinon and chlorpyrifos) were detected in greater than 25% of the maternal and/or cord blood samples. In another study, Whyatt and Barr (2001) detected levels of dialkyl phosphate metabolites in meconium samples collected from 20 newborn infants. These findings suggest that OP pesticides are readily transferred from the mother to the developing human fetus (Perera et al. 2002).

For this assessment, we estimated absorbed OP pesticide dose to pregnant women. To calculate fetal dose, however, a better understanding of the physiological and pharmacokinetic processes that would determine transfer from the mother to fetus is required. Future research is needed to explore the feasibility of using physiologically-based pharmacokinetic modeling methods to reconstruct fetal dose from maternal biological monitoring data. Virtually no data are available regarding the absorption, metabolism, and excretion of OP pesticides in pregnant women.

In conclusion, our results suggest that a portion of pregnant women participating in the CHAMACOS study would have exposures exceeding a health-based reference value for aggregate exposure to a mixture of OP compounds, if one existed. The potential impact of these exposures to fetal health is unknown.

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Table 1. OP pesticide usage in the Salinas Valley[a] and associated urinary dialkyl phosphate metabolites, oral BMD_{10}s and relative potency factors (RPFs) for cumulative assessment group.

Pesticide[b]	Kilograms applied in 1999	% applied in 1999	Kilograms applied in 2000	% applied in 2000	BMD_{10}[c] (mg/kg/day)	RPF[d]	Metabolites
Chlorpyrifos	29,423	34.6	27,325	30.4	1.48	1.00	DEP, DETP
Diazinon	47,847	56.4	56,883	63.2	6.24	0.24	DEP, DETP
Disulfoton	7,613	9.0	5,763	6.4	0.07	21.14	DEP, DETP, DEDTP
Total diethyls	84,883	100.0	89,971	100.0			
Azinphos-methyl	626	0.6	101	0.1	0.86	1.72	DMP, DMTP, DMDTP
Dimethoate	19,232	18.4	16,115	15.1	0.25	5.92	DMP, DMTP, DMDTP
Malathion	35,188	33.6	45,727	42.8	313.91	0.005	DMP, DMTP, DMDTP
Methidathion	6,779	6.5	6,926	6.5	0.25	5.92	DMP, DMTP, DMDTP
Methyl parathion	66	0.1	0	0.0	0.67	2.21	DMP, DMTP
Naled	11,979	11.4	9,315	8.7	1.00	1.48	DMP
Oxydemeton-methyl	30,028	28.7	27,759	26.0	0.09	16.44	DMP, DMTP
Phosmet	743	0.7	909	0.9	3.56	0.42	DMP, DMTP, DMDTP
Total dimethyls	104,640	100.0	106,852	100.0			

[a]Includes agricultural, landscape maintenance, structural pest control and roadside pesticide usage (DPR 1999, 2000).
[b]OP pesticides that do not metabolize to dialkyl phosphate compounds (e.g., bensulide, acephate, etc.) are not listed.
[c]BMD_{10}: the benchmark dose with 10% change in brain cholinesterase inhibition compared to background response (U.S. EPA 2002a, 2002d).
[d]By definition, the RPF for the index chemical, chlorpyrifos, is 1.

Table 2. Urinary dialkyl phosphate metabolite levels, method limits of detection (μg/L), and percent samples assigned value of zero for 1365 urine samples collected from 455 women (3 samples each).

		Percentiles							
Analyte[a]	**Range**	**10th[b]**	**25th[b]**	**50th**	**75th**	**90th**	**Mean LOD[c]**	**% below LOD**	**% zero value**
DMP	0-2754	0	0	1.7	6.2	16.6	0.7	37.1	25.2
DMTP	0-2922	0	1.3	6.2	19.1	52.6	0.5	17.0	12.9
DMDTP	0-540	0	0	0.5	3.1	13.0	0.3	46.6	42.8
DEP	0-160	0	0	1.0	4.1	9.4	0.3	40.3	29.2
DETP	0-101	0	0	0.9	2.5	5.8	0.2	29.7	26.7
DEDTP	0-44	0	0	0	0.2	0.6	0.1	71.6	51.8

[a]DMDTP had 6 missing values; DMP and DEP had 2 missing values; DETP had 1 missing value (i.e., no result reported because of an unknown analytical interference in the urine sample).
[b]0 = No instrument response.
[c]Mean limits of detection derived from multiple batches of urine samples.

Table 3. Method 1 average OP pesticide dose estimates based on CHAMACOS data relative to U.S. EPA BMD_{10}s divided by 100-fold uncertainty factor (μg/kg/day)[a,b].

	Pregnant Women (n=455)							
	Dimethyl OP pesticides[c]					Diethyl OP pesticides		
	Dimethoate	**Malathion**	**Methidation**	**Naled**	**Oxydemeton-Methyl**	**Chlorpyrifos**	**Diazinon**	**Disulfoton**
10th percentile	0.14	0.20	0.18	0.02	0.12	0.04	0.04	0.03
25th percentile	0.30	0.44	0.40	0.07	0.27	0.08	0.07	0.06
50th percentile	0.63	0.91	0.84	0.18	0.57	0.14	0.13	0.12
75th percentile	1.49	2.15	1.96	0.48	1.33	0.27	0.24	0.23
90th percentile	3.09	4.45	4.07	1.07	2.92	0.48	0.42	0.40
Geometric Mean	0.65	0.94	0.86	0.18	0.58	0.14	0.12	0.12
(95% CI)	(0.58-0.73)	(0.84-1.05)	(0.77-0.96)	(0.16-0.21)	(0.52-0.65)	(0.13-0.16)	(0.11-0.14)	(0.11-0.13)
Range	0.02-27.81	0.02-40.07	0.02-36.67	0-45.67	0.01-29.86	0-4.36	0-3.78	0-3.45
Reference value (μg/kg/day)[d]	2.5	3139.1	2.5	10.0	0.9	14.8	62.4	0.7
Estimates exceeding reference value (%)	57 (12.5)	0 (0)	84 (18.5)	3 (0.7)	164 (36.0)	0 (0)	0 (0)	21 (4.6)

[a]Assumes 100% of OP pesticide dose is from single dimethyl or diethyl OP pesticide.
[b]Average dose estimates were derived from metabolite levels measured in 3 urine samples.
[c]Dose estimates for three dimethyl OP pesticides with usage less than 5,000 kilograms per year (i.e., azinphos-methyl, phosmet and methyl parathion) are not presented.
[d]Reference value: BMD_{10}/100.

Table 4. Method 2 estimated cumulative OP pesticide dose equivalents (μg/kg/day) for 455 pregnant women with chlorpyrifos as index chemical.

	Average cumulative doses[a]		
	Dimethyl	**Diethyl**	**OP Pesticides**
10th percentile	0.9	0.1	1.0
25th percentile	1.9	0.1	2.1
50th percentile	4.0	0.2	4.4
75th percentile	9.5	0.4	9.9
90th percentile	19.5	0.8	19.7
Geometric mean (95% CI)	4.1 (3.6-4.6)	0.2 (0.2-0.3)	4.5 (4.1-5.0)
Range	0.1-172.8	0.01-6.6	0.1-172.8
Estimates exceeding reference value (%)[b]	64 (14.1%)	0 (0%)	67 (14.7%)

[a]Average dose estimates were derived from metabolite levels measured in three urine samples.
[b]Reference value for index chemical, chlorpyrifos: 14.8 μg/kg/day (Benchmark $Dose_{10}$/100).

Figure 1. Relative hazard of agricultural OP pesticide use in the Salinas Valley[a].

Legend for Figure 1

Kilograms Applied (1999)

(BMD_{10}^{-1})*Kilograms OP Pesticide Applied

Notes for Figure 1

This chart provides a means to compare the relative hazard of OP pesticides used in the Salinas Valley, weighted by use and toxicity. For example, total use of malathion and oxydemeton-methyl were similar (DPR 1999), however, because oxydemeton-methyl is a much more potent inhibitor of cholinesterase (i.e., malathion oral BMD_{10}=313.9 mg/kg/day versus oxydemeton-methyl BMD_{10}=0.09 mg/kg/day), its potential hazard is much higher.

[a]Weighted by toxicity (U.S. EPA Benchmark $Dose_{10}$, BMD_{10}) and pesticide use (DPR. Pesticide Use Report, Annual 1999).

Figure 2. Distributions of average cumulative OP pesticide dose estimates for pregnant women in an agricultural community (n=455).

Legend for Figure 2

Frequency

Cumulative percentage

Figure 3. Margin of exposure (MOE) for estimated average and maximum cumulative dose equivalents for pregnant women (n=455).

Legend for Figure 3

MOEs based on average cumulative dose equivalents

MOEs based on maximum single-day cumulative dose equivalents

Figure 1.

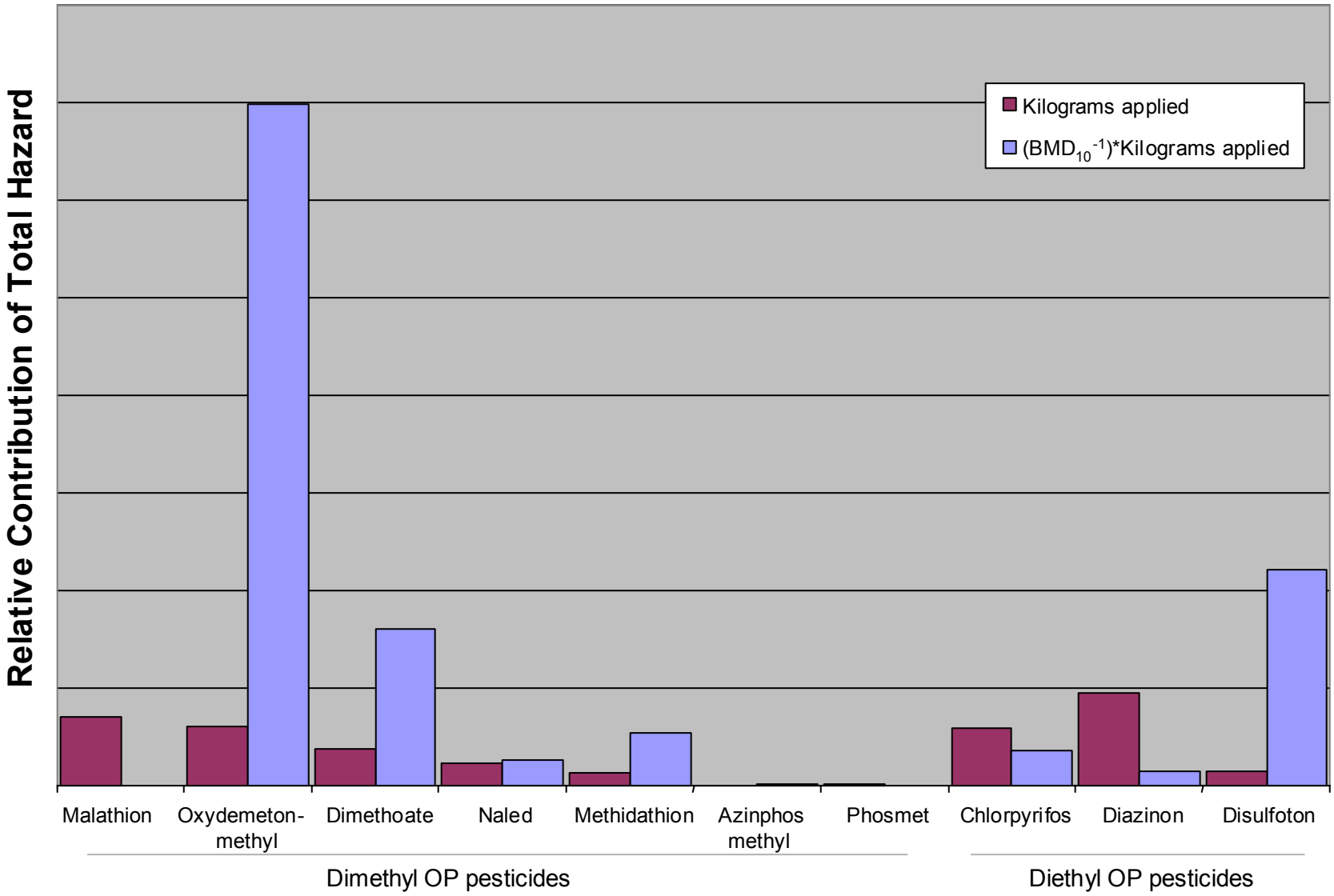
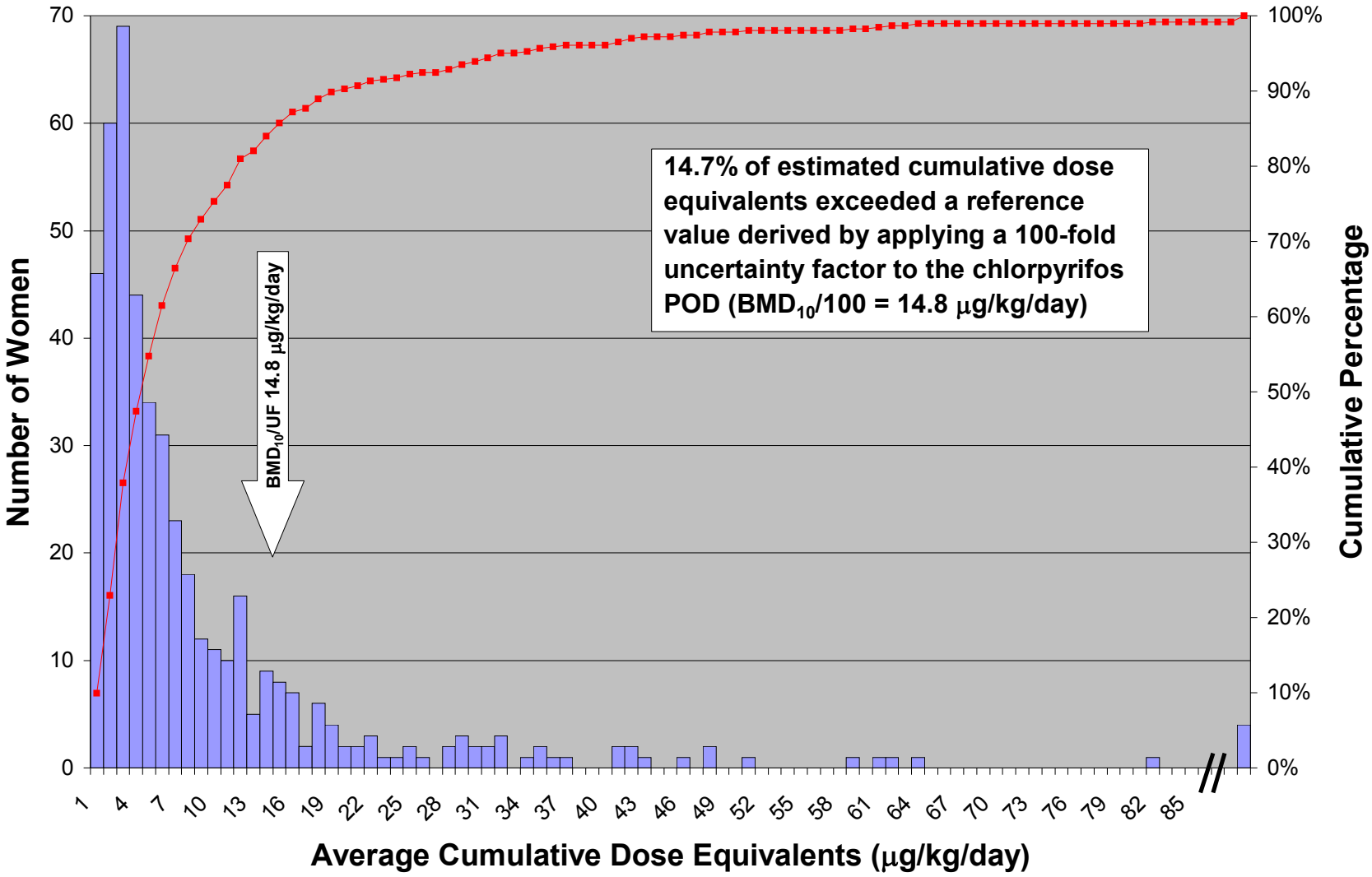

Relative Contribution of Total Hazard
Kilograms applied
(BMD10-1)*Kilograms applied
Malathion
Oxydemeton-methyl
Dimethoate
Naled
Methidathion
Azinphos methyl
Phosmet
Chlorpyrifos
Diazinon
Disulfoton
Dimethyl OP pesticides
Diethyl OP pesticides

Figure 2.


Number of Women
Cumulative Percentage
Average Cumulative Dose Equivalents (μg/kg/day)
BMD10/UF 14.8 μg/kg/day
14.7% of estimated cumulative dose equivalents exceeded a reference value derived by applying a 100-fold uncertainty factor to the chlorpyrifos POD (BMD10/100 = 14.8 μg/kg/day)
0
10
20
30
40
50
60
70
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
1
4
7
10
13
16
19
22
25
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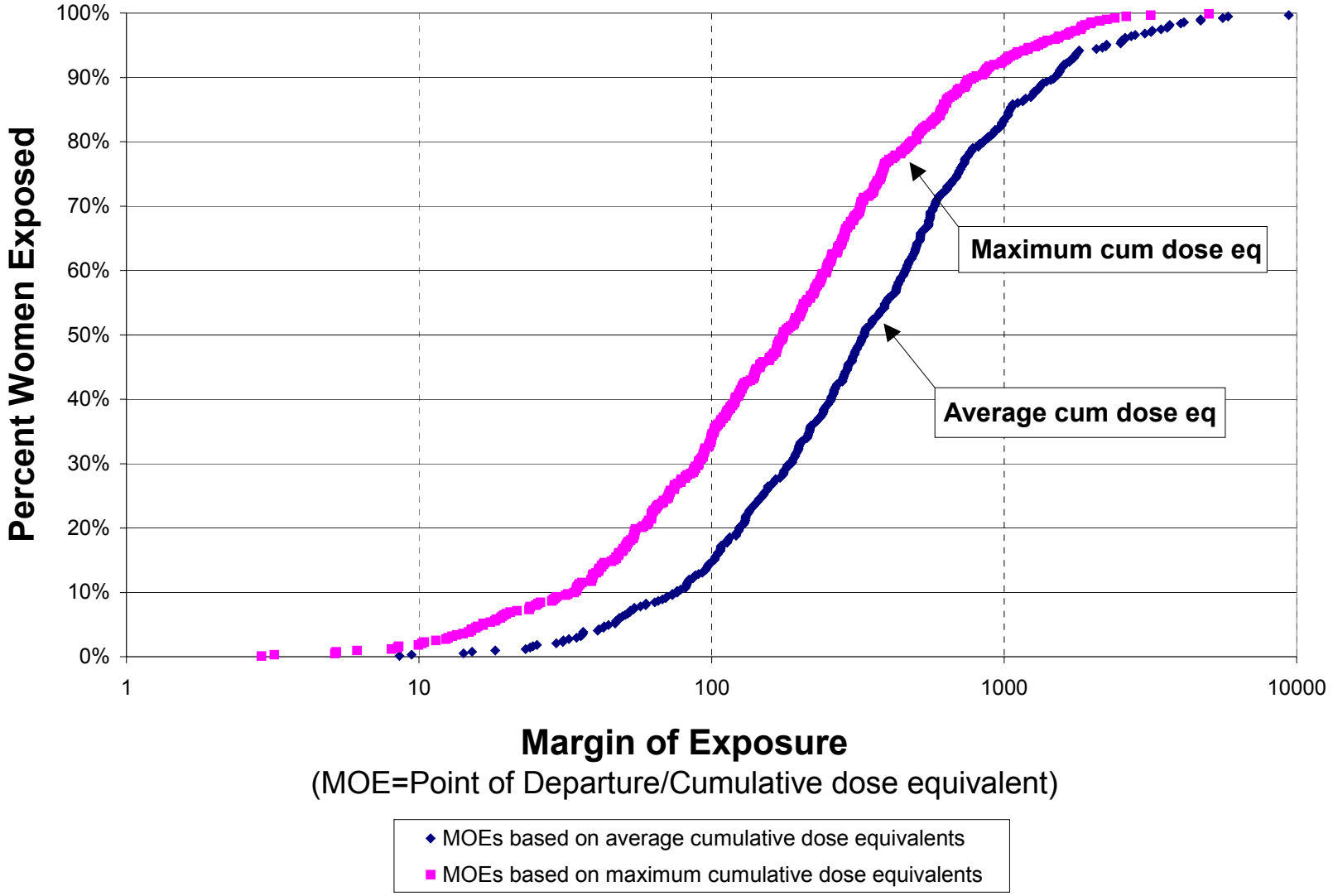
82
85

Figure 3.


Percent Women Exposed
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
Maximum cum dose eq
Average cum dose eq
Margin of Exposure
(MOE=Point of Departure/Cumulative dose equivalent)
MOEs based on average cumulative dose equivalents
MOEs based on maximum cumulative dose equivalents