Cumulative Organophosphate Pesticide Exposure and Risk Assessment among Pregnant Women Living in an Agricultural Community: A Case Study from the CHAMACOS Cohort

Rosemary Castorina,1 Asa Bradman,1 Thomas E. McKone,1,2 Dana B. Barr,3 Martha E. Harnly,4 and Brenda Eskenazi1

1Center for Children’s Environmental Health Research, School of Public Health, University of California, and 2Lawrence Berkeley National Laboratory and University of California, Berkeley, California, USA; 3National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 4Environmental Health Investigations Branch, California Department of Health Services, Oakland, California, USA

Approximately 230,000 kg of organophosphate (OP) pesticides are applied annually in California’s Salinas Valley. These activities have raised concerns about exposures to area residents. We collected three spot urine samples from pregnant women (between 1999 and 2001) enrolled in CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas), a longitudinal birth cohort study, and analyzed them for six dialkyl phosphate metabolites. We used urine from 446 pregnant women to estimate OP pesticide doses with two deterministic steady-state modeling methods: method 1, which assumed the metabolites were attributable entirely to a single diethyl or dimethyl OP pesticide; and method 2, which adapted U.S. Environmental Protection Agency (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 approximate the 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 36.1% of study participants’ doses failed to attain a margin of exposure (MOE) of 100 relative to the U.S. EPA oral benchmark dose (BMD)10, depending on the assumption made about the parent compound. These BMD10 values are doses expected to produce a 10% reduction in brain cholinesterase activity compared with background response in rats. Given the participants’ average cumulative OP pesticide dose estimates (method 2) and regardless of the index chemical selected, we found that 14.8% of the doses failed to attain an MOE of 100 relative to the BMD10 of the selected index. An uncertainty analysis of the pesticide mixture parameter, which is extrapolated from pesticide application data for the study area and not directly quantified for each individual, suggests that this point estimate could range from 1 to 34%. In future analyses, we will use pesticide-specific urinary metabolites, when available, to evaluate cumulative OP pesticide exposures. Key words: cumulative dose, exposure, mixtures, organophosphate pesticides, pregnancy, prenatal, risk, urinary metabolites, women. Environ Health Perspect 111:1640–1648 (2003). doi:10.1289/ehp.5887 available via http://dx.doi.org/ [Online 16 June 2003]

Substantial toxicologic evidence suggests that low-level exposure to organophosphate (OP) pesticides affects neurodevelopment and growth in developing animals (Chanda and Pope 1996; Dam et al. 1998; Eskenazi et al. 1999; Gupta et al. 1985; Muto et al. 1992; Schulz et al. 1995; Song et al. 1997; Whitney et al. 1995). Recent biologic monitoring studies, which include pregnant women and children, show that there is widespread OP pesticide exposure in the U.S. population (Berkowitz et al. 2003; CDC 2003; Fenske et al. 2000; O’Rourke et al. 2000). To address concerns about the potential health effects of pre- and postnatal exposure of children to pesticides, the Food Quality Protection Act (FQPA) was passed in 1996, significantly amending the U.S. laws that regulate pesticides: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act. The FQPA established a stringent health-based standard (“a reasonable certainty of no harm”) for pesticide residues in foods to assure protection from unacceptable pesticide exposure and to strengthen health protections from pesticide risks for sensitive populations. In addition, the FQPA required the U.S. Environmental Protection Agency (U.S. EPA) to consider the cumulative effects on human health that may result from exposure to mixtures of pesticides (FQPA 1996). In response, the U.S. EPA Office of Pesticide Programs, in consultation with the FIFRA 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 (i.e., binding to the aryl hydrocarbon receptor) of each member of the group with respect to that of a single chemical (U.S. EPA 1989).

These new cumulative risk assessment guidelines use the finding that OP pesticides share a common mechanism of toxicity, the inhibition of cholinesterase activity (Miles et al. 1998; U.S. EPA 1999a, 2001). The U.S. EPA has determined oral benchmark doses (BMD10) values based on a 10% reduction in brain cholinesterase activity compared to controls for 33 OP pesticides (U.S. EPA 2002d). These BMD10 values can be used to calculate relative potency factors (RPFs) for cumulative risk assessments of OP pesticide exposure. RPFs are the ratio of the toxic potency of a given chemical to that of an index chemical in the cumulative assessment group. Thus, when biologic measures of internal OP pesticide dose are available, cumulative OP pesticide dose equivalents can be calculated for a population by converting individual OP pesticide doses to index chemical toxicity equivalent doses, using RPFs, and then summing them.

Received 16 July 2002; accepted 16 June 2003.

Address correspondence to B. Eskenazi, Center for Children’s Environmental Health, Research/CHAMACOS, School of Public Health, University of California, Berkeley, 2150 Shattuck Ave, Ste 600, Berkeley, CA 94720-7380 USA. Telephone: (510) 642-3496. Fax: (510) 642-9083. E-mail: eskenazi@uclink4.berkeley.edu

We thank E. Weltzien, C. Salemme, and R. Bravo for their technical assistance; R. McLaughlin and R. Scafl of the California Department of Health Services Environmental Health Investigations Branch for summarizing pesticide use information; and the CHAMACOS field and laboratory staff and all the women who participated in this study for their valuable time and commitment. This research was jointly funded by the U.S. Environmental Protection Agency and the National Institute of Environmental Health Sciences (awards R82679 and PO1ES09605). This work was also supported in part by the University of California (UC) Toxic Substances Research and Training Program, UC Berkeley Superfund Training Core, and UC Agricultural Health and Safety Center at Davis. T.E.M. was supported in part by the U.S. EPA National Exposure Research Laboratory through Interagency Agreement DW-988-38190-01-0 with Lawrence Berkeley National Laboratory through the U.S. Department of Energy under contract grant DE-AC03-76SF00098.
Children’s Health | Cumulative pesticide dose estimates for pregnant women

Fenske et al. (2000) proposed that the measurement of dialkyl phosphate metabolites in children’s urine has utility for estimating single OP pesticide dose ranges and thus can provide information useful to discussions of pesticide health risks. They reported that children of farmworkers were more likely than non-farmworker children to be exposed to the OP pesticides azinphos-methyl and phosmet at levels exceeding U.S. EPA chronic dietary reference doses (RfDs).

Another vulnerable population is pregnant women and their fetuses. The present study uses the proposed U.S. EPA cumulative risk assessment guidelines (U.S. EPA 2002a, 2002d) to examine potential health risks to pregnant women participating in the CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) study, a birth cohort study in an agricultural area. All women assessed as part of the CHAMACOS study had detectable levels of urinary OP metabolites at some point in their pregnancy, which are likely to be from a mixture of compounds with varying toxicities and use patterns (Figure 1). Recognizing that people living in rural communities are potentially exposed to mixtures of chemicals with varying toxicities, this 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 a health-protective reference value.

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. OP insecticides are used on a variety of crops, including lettuce, broccoli, cauliflower, and strawberries. This region is approximately 25 km wide and 110 km 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 spot urine samples collected over 6 months from pregnant women participating in the CHAMACOS study, a prospective cohort study of children’s environmental health. The CHAMACOS study methods have been described previously (Eskanazi et al. 2003). Pregnant women were eligible for enrollment if they entered prenatal care between September 1999 and November 2000 at either of two community clinics in the area (Clínica 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, at least 20 weeks gestation, fluent in English or Spanish, and planning to deliver their child at Natividad Medical Center. Medi-Cal is California’s Medicaid health care program, and eligibility is based on economic need. The CHAMACOS study population is 94% Mexican or Mexican American, with 96% of participants living within 200% of the federal poverty line (U.S. Bureau of the Census 2000). Forty-four percent of study participants performed field or other agricultural work after becoming pregnant, and 87% 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 institutional review boards at the University of California, Berkeley, 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 twice during pregnancy, at approximately 14 weeks gestation (n = 593) and approximately 26 weeks gestation (n = 500), and after delivery (n = 493). All three urine samples were collected from 460 women. Specimens were stored at −80°C until shipment to the Centers for Disease Control and Prevention, where six non-specific urinary OP metabolites were measured: dimethyl phosphate (DMP), dimethylphosphonate (DMDP), dimethylphosphite (DMT), diethyl phosphate (DEP), diethylphosphite (DEDP), and diethylphosphite (DETP). These metabolites derive from approximately 40 OP compounds, 28 of which are registered with the U.S. EPA for use in the United States, 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 (Bravo et al. 2002). Isotope dilution is widely regarded as the state-of-the-art 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 multirules (Westgard 2002). Data were not reported from runs considered “out of control.”

Data analysis. We statistically analyzed urinary metabolite data from only the women who provided three urine samples. 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 of urine.

![Figure 1. Relative hazard of agricultural OP pesticide use in the Salinas Valley. This chart allows comparison of the relative hazard of OP pesticides used in the Salinas Valley, weighted by pesticide use (DPR 2000) and toxicity (BMD₉₀). For example, total use of malathion is greater than that of oxydemeton-methyl (DPR 2000); however, because oxydemeton-methyl is a much more potent inhibitor of cholinesterase (i.e., malathion oral BMD₉₀ = 313.9 mg/kg/day vs. oxydemeton-methyl BMD₉₀ = 0.09 mg/kg/day), its potential hazard is much higher.](image-url)
urine. We calculated Spearman R values to assess the correlation of the urinary diethyl and dimethyl phosphate molar concentrations between the different sampling points. The creatinine concentration in each urine sample was reported as milligrams of creatinine per deciliter of urine. 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 and seven participants who provided urine samples more than 60 days after delivery were also excluded, leaving a final sample size of 446 women.

**Pesticide use data.** In California, all agricultural pesticide use is reported to the county agricultural commissioner, who then reports it to the California Department of Pesticide Regulation (DPR; Sacramento, CA). For agricultural use, crop, active ingredient, date, pounds applied, and the location of use, identified to a one-mile (1.6 km) square section, are reported. We obtained the pesticide use report (PUR) data sets for 1999, 2000, and 2001 from the DPR. To define agricultural OP pesticide use in the Salinas Valley, we selected all 1-mile (1.6 km) sections within a 200-foot (61-m) elevation contour from the Salinas River with reported pesticide use. Ninety-nine percent of CHAMACOS participants lived within this area. Other pesticide uses, including for landscape (i.e., golf courses, parks, cemeteries), structural, and right-of-way purposes, are reported by month and are not geographically identified (reported at county level only). These nonagricultural uses accounted for 0.8%, 1.0%, and 1.1% of Monterey County OP pesticide use for 1999, 2000, and 2001, respectively. We accounted for these other uses in our weekly and annual summaries of all 1999, 2000, and 2001 OP pesticide applications in the region as follows: To describe annual use, they were simply added to the total; for weekly use, monthly instances of these nonagricultural uses were divided by four and added to the agriculture use information.

The PUR data we used for dose estimation were concurrent with the year in which urine samples were collected. Two factors support our choice to use annual data to represent the mixtures, rather than daily, weekly, monthly, or other time periods more proximate to the urine collection. First, the annual PUR data are more likely to approximate mixtures across all sources and times. Second, mapping of PUR data in the Salinas Valley reveals that pesticide use is relatively uniform and that there are no apparent "hot spots."

**Dose calculations.** We used two methods to estimate OP pesticide doses from the urinary metabolite concentration data. For method 1, we assumed that metabolite levels were the result of exposure to a single pesticide (Fenske et al. 2000). For method 2, we assumed that observed metabolites were attributable to 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: a) Urinary concentrations represent steady-state conditions over a 24-hr 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-hr urine output volume and urinary creatinine is given by the following formula:

$$V_t = \frac{C_{cr}}{C_{ce}}$$

where $V_t$ is the expected 24-hr urine output volume for the $i$th pregnant woman (liters per day), $C_{cr}$ is the reference value for the $i$th pregnant woman’s daily creatinine excretion (milligrams per day) (Davison et al. 1980; Davison and Noble 1981; Knuppel et al. 1979), and $C_{ce}$ is the creatinine concentration in the $i$th woman’s urine sample (milligrams per liter).

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

d) The parent compounds of the six urinary OP metabolites depend strongly on the mixture of OP pesticides used for agriculture, structural pest control, right-of-way, or landscape purposes in the Salinas Valley. That is to say, the proportion of chemicals applied is a reasonable surrogate for the mixture of OP pesticides to which the women were exposed from all sources.

d) OP metabolite concentrations are equivalent to internal doses on a molar basis. Because each OP pesticide molecule devolutes into exactly one of its possible dialkyl phosphates, the molar sum of metabolite equals the molar concentration of OP pesticide.

**Method 1: single chemical approach.** We calculated single dose estimates 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 the Salinas Valley (i.e., they account for 100% and 99% of total diethyl and dimethyl OP pesticide use, respectively), according to the California DPR’s PUR (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; for example, chlorpyrifos dose calculations were based

---

**Table 1.** OP pesticide usage in the Salinas Valley and associated urinary dialkyl phosphate metabolites, oral BMD$_{10}$ values, and RPFs for cumulative assessment group.

| Pesticide | 1999 | 2000 | 2001 |
|-----------|------|------|------|
| Kilograms applied | Percent | Kilograms applied | Percent | Kilograms applied | Percent |
| Azinphos-methyl | 626 | 0.6 | 101 | 0.1 | 56 | 0.1 |
| Dimethoate | 19,272 | 18.4 | 16,115 | 15.1 | 15,523 | 15.3 |
| Malathion | 36,188 | 33.6 | 45,727 | 42.8 | 44,181 | 43.5 |
| Methidathion | 6,779 | 6.5 | 6,926 | 6.5 | 6,449 | 6.3 |
| Methyl parathion | 66 | 0.1 | 0 | 0.0 | 0 | 0.0 |
| Naled | 11,979 | 11.4 | 9,315 | 8.7 | 7,749 | 7.6 |
| Oxydemeton-methyl | 30,028 | 28.7 | 27,759 | 26.0 | 26,244 | 25.8 |
| Phosmet | 745 | 0.7 | 999 | 0.9 | 1,436 | 1.4 |
| Total dimethylestes | 104,640 | 100.0 | 106,852 | 100.0 | 101,638 | 100.0 |
| Chlorpyrifos | 29,423 | 34.6 | 27,325 | 30.4 | 25,283 | 27.5 |
| Diazinon | 47,847 | 56.4 | 56,883 | 63.2 | 61,944 | 67.4 |
| Disulfoton | 7,613 | 9.0 | 5,763 | 6.4 | 4,634 | 5.1 |
| Total diethyl | 84,883 | 100.0 | 89,571 | 100.0 | 91,861 | 100.0 |

*Includes agricultural, landscape maintenance, structural pest control, and right-of-way pesticide use (DPR 1999, 2000, 2001). Pesticide use is reported in kilograms of active ingredient. OP pesticides that do not metabolize to dialkyl phosphate compounds (e.g., bensoide, acephate) are not listed. By definition, the RPF for the index chemical, chlorpyrifos, is 1.
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:

For diethy1s,

$$D_{\text{Diethyl}} = \left( \frac{C_{\text{DEP}}}{MW_{\text{DEP}}} + \frac{C_{\text{DETP}}}{MW_{\text{DETP}}} \right) \times \frac{C_{\text{DiEt OP Conc}}}{BW}$$

where $D_{\text{Diethyl}}$ is the dose from diethyl OP pesticide (micrograms per kilogram per day), $C_{\text{DiEt OP Conc}}$ is the concentration of the parent pesticide in the saliva sample (micrograms per liter), $MW$ is the molecular weight of an OP pesticide (grams per mole). $BW$ is the woman’s body weight (kilograms) 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 twice during pregnancy and once postpartum.

Method 1: risk estimation. We used the U.S. EPA BMD10 as the basis for our risk estimates. This BMD10 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 with controls, derived from laboratory studies that lasted 21 days or longer (U.S. EPA 2002a, 2002b, 2002d). Current U.S. EPA oral BMD10 values for the OP pesticides used in the Salinas Valley range from 0.07 to 313.9 mg/kg/day (Table 1) (U.S. EPA 2002d). The BMD is the dose that corresponds to a specified level of increased response (the benchmark response) compared with background. This dose is calculated by fitting a mathematical model to the dose–response data.

The U.S. EPA is developing BMDs as an alternative to the no-observable-adverse-effect level (NOAEL) and lowest-observable-adverse-effect level as points of departure for deriving RfDs for noncancer risk assessments because they use all points on the dose–response curve, are less sensitive to the number of animals used in a study, and do not depend on dose spacing. The hazard quotient, defined as the ratio of an observed dose to an RfD, is used to express risk relative to an RfD. In contrast to the hazard quotient, the U.S. EPA uses a margin of exposure (MOE), the ratio of a point of departure (i.e., BMD) to an observed dose, to express risk using the BMD. Although an acceptable hazard quotient should be less than 1, an acceptable MOE should be significantly greater than 1, typically 100 or more, depending on the health end point and the quality and source of data used to obtain the BMD.

We selected a health-protective dose for our population based on the U.S. EPA oral BMD10 and an MOE of 100. Thus, the dose exceeding the BMD10 divided by 100 is a dose of concern. We selected the BMD10 as the point of departure for this analysis, and used an MOE of 100 to account for animal to human extrapolation and intrahuman variability. The point of departure is meant to represent the lowest dose with a specified response within the range of experimental data. Risk associated with lower, environmentally relevant human exposures can be extrapolated from the point of departure. Using doses exceeding the BMD10/100 as doses of concern permits direct comparison of our method 1 and method 2 risk estimate results.

The BMD10 is an appropriate measure of toxicity for cumulative risk assessments of OP pesticides because it represents the common end point and mechanism of action for the cumulative assessment group. The acute and chronic U.S. EPA RfDs for individual OP pesticides, however, are based on NOAELs found for the most sensitive end point in the most sensitive species. These RfDs and NOAELs were derived for use in single chemical assessments, and thus are not recommended for use in cumulative assessments because they may not toxicologically represent the common mechanism of action (U.S. EPA 2002a).

Method 2: chemical mixture approach. An obvious limitation to method 1 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 RPFs, which 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 BMD10 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 rationale 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,

$$RPF = \frac{\text{Measure of potency}_{\text{index chemical}}}{\text{Measure of potency}_{\text{chemical}}}$$

where chemical $n$ is a member of the cumulative assessment group, index chemical is the chemical selected as the basis for standardization of toxicity of components in a mixture, and measure of potency is the BMD10.

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 were used to describe the likely mixture to which the women were exposed. We selected chlorpyrifos as the index chemical because it is a compound in our cumulative assessment group for which complete hazard assessment and dose–response information is available, it metabolizes into urinary dialkyl phosphate compounds, it is commonly used in the Salinas Valley (DPR 1999, 2000), and its measure of relative toxicity, as expressed by the BMD10, falls in the mid-range of BMD10 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 a pregnant woman’s cumulative OP pesticide dose equivalent with the following equation:

$$D_{\text{Cum}} = \frac{\sum_{i=1}^{n} \frac{\mu \text{mol}_{\text{Diethyl}} \text{DiEt OP Conc}_{i}}{BW} \times \text{MW}_{\text{DiEt OP}} \times RPF_{i}}{BW} + \frac{\sum_{i=1}^{n} \frac{\mu \text{mol}_{\text{Dimethyl}} \text{DiM OP Conc}_{i}}{BW} \times \text{MW}_{\text{DiM OP}} \times RPF_{i}}{BW}$$

where $D_{\text{Cum}}$ is the cumulative dose equivalent (micrograms per kilogram per day), $\mu \text{mol}_{\text{Diethyl}}$ is total micromoles of diethyl phosphate metabolites (DEP, DETP, DEDTP) excreted over a
24-hr period (see Equation 1), \( \mu \text{Mol}_{\text{Dimethyl}} \) is total micromoles of dimethyl phosphate metabolites (DMP, DMTP, DMDTP) excreted over a 24-hr period (see Equation 1), \( P_i \) is the proportion of pesticide \( i \) in the mixture calculated from annual PUR data for the Salinas Valley, \( MW \) is the molecular weight of the \( i^{th} \) pesticide (micrograms per micromole), \( RPF \) is the relative potency factor of the \( i^{th} \) pesticide in the cumulative assessment group, and \( BW \) is the pregnant woman’s body weight (kilograms) around the time of urine sample collection.

To estimate three single-day cumulative dose equivalents (creatinine-adjusted), we used measured metabolite levels in the three urine samples obtained from all study participants. We then calculated the arithmetic mean and summarized the minimum, median, and 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 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 key source of uncertainty in our assessment. To evaluate the sensitivity of the model to this parameter, we used Monte Carlo simulation software (Crystal Ball Standard Edition 2000; Decisioneering, Inc., Denver, CO) 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 from 1999 through 2001 (DPR 1999, 2000, 2001). 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 minimum, average, and maximum cumulative dose equivalents with an MOE of 100 relative to the index chemical’s BMD_{10} (for chlorpyrifos, this value is BMD_{10} = 14.8 \mu g/kg/day). Our intent is to imply that women with cumulative dose equivalents that exceed the index chemical’s BMD_{10}/100 are potentially affected. Thus, our concept of risk is the fraction of the study population with cumulative OP pesticide dose estimates above what we consider a health-protective dose (the index chemical’s BMD_{10}/100).

We calculated MOEs by taking the ratio of the point of departure, chlorpyrifos’s BMD_{10}, to the estimated minimum, average, and maximum single-day cumulative dose equivalents (Figure 2). The MOE is specifically identified by the U.S. EPA Office of Pesticide Programs as an appropriate metric for characterizing risk from exposure to mixtures of OP pesticides (U.S. EPA 2002b).

Results
Urine samples were collected from pregnant women at two prenatal study visits (around 14 and 26 weeks’ gestation) and after delivery; all three samples were obtained from 460 women. Summary statistics of the urinary OP metabolites for our final sample of 446 women are presented in Table 2. Forty-two of 1,338 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 \mu g/L; DMTP, 6.3 \mu g/L; and DMDTP, 0.5 \mu g/L. Among the diethyl phosphate metabolites, the median concentrations were DEP, 1.1 \mu g/L; DETP, 0.9 \mu g/L; and DEDTP, 0 \mu g/L. Overall, the dimethyl phosphate levels were higher than diethyl phosphate levels (Table 2).

Dose calculations. Method 1: single chemical approach. Table 3 summarizes the results for the single OP pesticide dose calculations (\( n = 446 \)), including geometric mean, median, and range, for this study population. We present average dose estimates calculated by taking the arithmetic mean of three single-day doses. Depending on the assumption made about the parent compound, creatinine-adjusted average dose estimates ranged from 0 to 45.7 \mu g/kg/day. Minimum and median single-day cumulative dose equivalent estimates ranged from 0.1 to 171.4 \mu g/kg/day. Minimum and median daily cumulative dose equivalent estimates ranged from 0 to 20.6 \mu g/kg/day [geometric mean = 0.7 (95% CI, 0.6–0.8) \mu g/kg/day] and from 0 to 32.3 [geometric mean = 2.5 (95% CI, 2.2–2.7) \mu g/kg/day], respectively.

Spearman rank correlation analyses indicate little or no correlation between the urinary metabolite levels at the three different sampling times. Spearman \( R \) values ranged from 0.04 to 0.11. The highest correlation coefficient (\( R = 0.11 \)), found between the dimethyl phosphate metabolite concentrations in samples collected at ~14 weeks and ~26 weeks gestation, was statistically significant because of the large sample size (\( p = 0.02 \)) but was low in magnitude. The average of the ranges in the women’s urinary dialkyl

Figure 2. MOEs for estimated maximum, average, and minimum cumulative dose equivalents for pregnant women (\( n = 446 \)).
found that 14.8% of estimated cumulative releases (micrograms per kilogram per day). We index chemical (chlorpyrifos) toxicity equivalent cumulative dose for the entire population in Figure 3 presents the distribution of average risk estimates.

Method 2: cumulative risk estimation. Figure 3 presents the distribution of average risk estimates for the entire population in index chemical (chlorpyrifos) toxicity equivalents (micrograms per kilogram per day). We found that 14.8% of estimated cumulative dose equivalents exceeded the index chemical’s BMD10/100 was also higher (15.9 vs. 14.8%).

We performed additional analyses to investigate the impact of the maximum single-day cumulative dose estimate on our average cumulative dose estimates. We found that 30% of the 66 women with average cumulative dose estimates exceeding the index chemical’s BMD10/100 had two or more single-day estimates that were greater than the BMD10/100. Therefore, the average cumulative dose estimates could have been shifted upward by the maximum single-day high values, if they were rare events. Extending this analysis to the entire study population, we found that 26 of 446 (5.8%) women had two or more single-day estimates greater than the index chemical’s BMD10/100. The geometric mean of the estimated cumulative dose equivalents for this group (22.6 µg/kg/day; 95% CI, 18.8–27.1) exceeded the index chemical’s BMD10/100 (14.8 µg/kg/day). Further, we found that 12 of 446 (28.3%) women had one single-day estimate and 294 of 446 (65.9%) women had no single-day estimate greater than the index chemical’s BMD10/100.

Table 2. Urinary dialkyl phosphate metabolite and creatinine levels, method limits of detection (µg/L), and percentage of samples assigned a value of zero for 1,338 urine samples collected from 446 women.

| Analyte | LOD | 10th | 25th | 50th | 75th | 90th | Mean | < LOD | < LOD | < LOD |
|---------|-----|------|------|------|------|------|------|-------|-------|-------|
| DMP     | 0.25 | 0    | 0.17 | 0.63 | 1.67 | 16.7 | 0.73 | 37.2  | 25.5  |
| DMTP    | 0.25 | 0    | 1.3  | 10.6 | 26.8 | 52.8 | 0.5  | 17.0  | 12.8  |
| DMDTP   | 0.25 | 0    | 0.35 | 3.1  | 13.3 | 13.3 | 0.3  | 46.8  | 43.0  |
| DEP     | 0.25 | 0    | 1.4  | 1.4  | 9.5  | 9.5  | 0.3  | 40.1  | 29.1  |
| DETP    | 0.25 | 0    | 0.9  | 2.5  | 5.8  | 5.8  | 0.2  | 29.7  | 26.6  |
| DEDTP   | 0.25 | 0    | 0    | 0.2  | 0.5  | 0.5  | 0.1  | 71.4  | 51.5  |
| Creatinine | 11.2-46.1 | 34.8 | 53.8 | 88.2 | 129.5 | 176.1 | 5.0 | 0 | 0 |

*Three samples each. **DMTP had six missing values; DMP and DEP had two missing values; DETP had one missing value (i.e., no result reported because of an unknown analytical interference in the urine sample). *No instrument response. 

Table 3. Method 1 average OP pesticide dose estimates for pregnant women (n = 446) based on CHAMACOS data relative to U.S. EPA BMD10 values divided by a 100-fold uncertainty factor (µg/kg/day).

| Dimethyl OP pesticides | Diethyl OP pesticides |
|------------------------|------------------------|
| Dimethoate | Malathion | Methidathion | Naled | Oxydemeton-methyl | Chlorpyrifos | Diazinon | Disulfoton |
| 10th percentile | 0.14 | 0.20 | 0.18 | 0.02 | 0.12 | 0.04 | 0.04 | 0.04 |
| 25th percentile | 0.31 | 0.44 | 0.41 | 0.07 | 0.27 | 0.08 | 0.07 | 0.06 |
| 50th percentile | 0.64 | 0.92 | 0.84 | 0.18 | 0.57 | 0.15 | 0.13 | 0.12 |
| 75th percentile | 1.50 | 2.16 | 1.97 | 0.48 | 1.34 | 0.28 | 0.24 | 0.23 |
| 90th percentile | 3.14 | 4.52 | 4.14 | 1.08 | 3.00 | 0.48 | 0.42 | 0.40 |
| Geometric mean | 0.66 | 0.92 | 0.86 | 0.18 | 0.58 | 0.15 | 0.13 | 0.12 |
| (95% CI) | (0.58–0.74) | (0.84–1.06) | (0.77–0.97) | (0.16–0.21) | (0.50–0.66) | (0.13–0.16) | (0.12–0.14) | (0.11–0.13) |
| Range | 0.02 to 27.81 | 0.02 to 40.07 | 0.02 to 36.97 | 0.01 to 45.67 | 0.01 to 29.96 | 0.01 to 29.96 | 0.01 to 29.96 | 0.01 to 29.96 |
| BMD10/100 (µg/kg/day) | 2.5 | 313.1 | 2.5 | 10.0 | 0.9 | 14.8 | 62.4 | 0.7 |
| Estimates exceeding BMD10/100 (%) | 57 (12.8) | 0 (0) | 83 (18.6) | 3 (0.7) | 161 (36.1) | 0 (0) | 0 (0) | 21 (4.7) |
| U.S. EPA chronic RfD (µg/kg/day) | 0.5 | 24 | 1.5 | 2 | 0.125 | 0.3 | 0.2 | 0.13 |

*Method 1 assumes 100% of OP pesticide dose is from single dimethyl or diethyl OP pesticide. Average dose estimates were derived from metabolite levels measured in three urine samples. *Dose estimates for three dimethyl OP pesticides with use < 2,000 kg/yr (i.e., azinphos-methyl, phosmet, and methyl parathion) are not presented. 

Environmental Health Perspectives • VOLUME 111 • NUMBER 13 • OCTOBER 2003
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 equivalents for 14.8% of CHAMACOS study participants were below the MOE of 100, which was used to define a dose of concern. Our uncertainty analysis of the pesticide mixture parameter suggests that this point estimate could range from 1 to 34%.

This report presents one of the first case studies using 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 used only the portions of the guidelines dealing with dose aggregation because our dose estimations are based on biomonitoring data.

We 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). With the use of 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-hr urinary metabolite excretion, and intraindividual and temporal variability. Because our assessment was based on nonspecific metabolite data, it was necessary to make assumptions about the mixture of pesticides to which each participant was exposed. Different doses of the same compound may have different toxic potencies.

**Table 4.** Method 2 estimated average cumulative OP pesticide dose equivalents (µg/kg/day) for 446 pregnant women with chlorpyrifos as index chemical.

| Percentile  | Dimethyls only | Diethyls only | Total$^a$ |
|------------|----------------|---------------|-----------|
| 10th       | 0.9            | 0.1           | 1.0       |
| 25th       | 1.9            | 0.1           | 2.1       |
| 50th       | 4.0            | 0.2           | 4.4       |
| 75th       | 9.5            | 0.4           | 10.0      |
| 90th       | 19.8           | 0.8           | 20.1      |
| Geometric mean (95% CI) | 4.1 (3.6–4.6) | 0.2 (0.2–0.3) | 4.5 (4.1–5.1) |
| Range      | 0.1–171.3      | 0.01–6.6      | 0.1–171.4 |

*Average cumulative dose equivalents were derived from metabolite levels measured in three urine samples. Total dimethyl and diethyl OP pesticides. BMD$_{10}$/100 of index chemical (chlorpyrifos) = 14.8 µg/kg/day.

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 biologic 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 five adult volunteers. Although the kinetics of elimination vary among the dimethyl and diethyl phosphate metabolites, toxicologic evidence suggests that the metabolites of many OP compounds are excreted primarily, but not
solely, in the urine (Griffin et al. 1999; 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 urinary dialkyl phosphate metabolites and are therefore not included in our exposure–dose estimates. These OP pesticides, which do not devote into dialkyl phosphate metabolites, represent approximately 20% of total OP pesticide use in the CHAMACOS study area.

A potentially large source of variability in our models results from the urinary metabolite data themselves. For these analyses we estimated dose based on three spot urine samples collected from each study participant over a 6-month period. Thus, uncertainty due to intrindividual 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 underestimates of actual daily doses were generated.

Adjusting metabolite concentration data for total daily urine volume was necessary because 24-hr urine samples are impractical in community-based studies and were not collected. Although creatinine adjustment is a common interpretive step in biologic 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. Because 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 toxicologic 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. DETP-treated rats excreted inorganic phosphate and organic phosphate in the 24-hr 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 (Imaizumi et al. 1993).

Risk evaluation of cumulative exposures would be improved by the development of suitable regulatory reference doses. Possible points of departure for cumulative risk assessment of OP pesticides include the NOAEL and BMD$_{10}$. The U.S. EPA is refining guidelines for formally considering standard uncertainty factors and the FQPA safety factor in cumulative risk assessments 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 health-protective dose level 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 the RfPs for six of the eight pesticides in our cumulative assessment group (U.S. EPA 2002d). When these 3x safety factors are applied at the RfP calculation stage (as proposed), the percentage of CHAMACOS participants with cumulative exposures exceeding the BMD$_{10}$/100 increases from 14.8 to 41.9%.

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. Toxicologic 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. (2003) 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 > 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 for pregnant women. To calculate fetal dose, however, a better understanding of the physiologic 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 to reconstruct fetal dose from maternal biologic 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 small portion of pregnant women participating in the CHAMACOS study may have cumulative OP pesticide exposures exceeding a health-protective reference value. The potential impact of these exposures to fetal health is unknown.

**References**

Barr DB, Barr JR, Driskell WJ, Hill RH, Ashley DL, Needham LL, et al. 1999. Strategies for biological monitoring of exposure for contemporary-use pesticides. Toxicol Ind Health 15(1):161–179.

Barr DB, Turner WE, DiPietro E, McClure PC, Baker SE, Barr JR, et al. 2002. Measurement of p-nitrophenol in the urine of residents whose homes were contaminated with methyl parathion. Environ Health Perspect 110(suppl 6):1085–1091.

Berkowitz GS, Obel J, Deych E, Lapiniski R, Godbold J, Liu Z, et al. 2003. Exposure to indoor pesticides during pregnancy in a multietnic urban cohort. Environ Health Perspect 111:79–84.

Boeniger MF, Lowry LR, Rosenberg J. 1993. Interpretation of urinary results used to assess chemical exposure with emphasis on cholinesterase adjustments: a review. Am Ind Hyg Assoc J 54(10):427.

Brau R, Driskell WJ, Whitehead RD Jr, Needham LL, Barr DB. 2002. Quantification of dialkyl phosphate metabolites of organophosphate pesticides in human urine using GC-MS/MS with isotopic internal standards. J Anal Toxicol 26(5):245–252.

CDC. 2003. Second National Report on Human Exposure to Environmental Chemicals. NCEH Pub No. 03-0022. Atlanta, GA:Centers for Disease Control and Prevention. Available: http://www.cdc.gov/exposurerreport/ [accessed 11 March 2003].

Chanda SM, Pope CN. 1996. Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. Pharmacol Biochem Behav 54(4):771–776.

Cohen WR, ed. 2000. Cherry and Merkatz’s Complications of Pregnancy, 5th ed. Philadelphia: Lippincott Williams & Wilkins.

Dam K, Seidler FJ, Slotkin TA. 1998. Developmental neurotoxicity of chlorpyrifos: delayed targeting of DNA synthesis after repeated administration. Brain Res Dev Brain Res 108(1–2):29–45.

Davison JM, Dunlop W, Ennolkka M. 1980. 24-Hour creatinine clearance during the third trimester of normal pregnancy. Br J Obstet Gynaecol 87:106–109.

Davison JM, Noble, MC. 1981. Serial changes in 24-hour creatinine clearance during normal menstrual cycles and the first trimester of pregnancy. Br J Obstet Gynaecol 88(1):10–17.

DPR. 1999. Pesticide Use Report, Annual 1999, Indexed by Chemical and by Crop. Sacramento, CA:Department of Pesticide Regulation, California Environmental Protection Agency.
Perera FP, Illman SM, Kinney PL, Whyatt RM, Kelvin EA, Shepard Muto MA, Lobelle F Jr, Bidanset JH, Wurpel JN. 1992. Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure and creatinine variation among children in an agricultural community: evidence of prenatal exposure to Dursban. Vet Hum Toxicol 34(6):498–501.

Knuppel RA, Sbarra AJ, Cetrulo CL, Kappy KA, Ingardia CJ, Imaizumi H, Nagamatsu K, Hasegawa A, Ohno Y, Takanaka A. 1985. Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Eskenazi B, Bradman A, Gladstone EA, Jaramillo S, Birch K, Castorina R. 1999. Exposure of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect 107(suppl 3):406–419.

Eskenazi B, Bradman A, Gladstone EA, Jaramillo S, Birch K, Holland N. 2003. CHAMACOS, a longitudinal birth cohort study: lessons from the fields. J Child Health 11(1):3–27.

Fenske RA, Kassel JC, Lu C, Kalmian D, Simcox NJ, Allen E et al. 2000. Biologically based pesticide dose estimates for children in an agricultural community. Environ Health Perspect 108:519–520.

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

Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985.

Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.

Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. 1985. Griffin P, Mason H, Heywood K, Cocker J. 1999. Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56(1):10–13.