Organophosphate Pesticide Residues in Urine of Farmworkers and Their Children in Fresno County, California

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Background *Childhood cancer, notably leukemia, brain cancer, non-Hodgkin's lymphoma, soft tissue sarcoma, and Hodgkin's disease, has been associated with pesticide exposure, often with greater relative risks than among exposed adults, suggesting greater susceptibility in children. These differences in risk may be due to developmental factors or differences in pesticide exposure.*

Methods *A feasibility study was conducted to determine levels of pesticide metabolites in urine of adults (n = 18) and children (n = 9) in Fresno County, California, an intensely agricultural county in the Central San Joaquin Valley. Spot urine samples were obtained and analyzed for six metabolites of organophosphate (OP) pesticides using gas chromatography with flame photometric detection methods. The metabolites of OP pesticides included DMP, DEP, DMTP, DMDTP, DETP, and DEDTP.*

Results *Levels were generally low for both adults and children for most metabolites tested. Frequencies of detection ranged from 0 to 37%, with mean levels ranging from non-detectable to 13.22 ppb. However, levels of several metabolites were higher in children than in adults. The most frequently detected metabolite, DMP, was found among 44% of the children and 33% of the adults. DMTP was detected among 33% of the children and 28% of the adults.*

Conclusions *These results are difficult to interpret given the sampling variation associated with the small sample size. Nevertheless, because OP pesticides have been associated with increased cancer risk in animal and human studies, these results indicate a need to closely monitor children's exposure to environmental chemicals.*
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INTRODUCTION

Although farmers experience lower overall mortality and lower total cancer incidence than the general population, they experience elevated risks of non-Hodgkin's lymphoma, leukemia, multiple myeloma, and cancers of the prostate, brain, stomach, lip, and skin [Zahm et al., 1997]. Some of these excesses may be due to exposure to agricultural chemicals.

Children of farmers and farmworkers have not been studied extensively even though they also live or work on farms with their parents and come into contact with agricultural chemicals through direct contact with plants, soil, carpeting, and other fomites, as well as through physical contact with their parents. By virtue of their age, children are exposed while they are rapidly developing and may be more susceptible to the effects of toxins such as pesticides. In addition, physiological differences between adults and children affect the amount of chemicals absorbed in the body. Compared to adults, children have a greater surface area to body weight ratio, which may lead to greater relative amounts of absorption of chemicals through the skin. Children also have higher circulatory flow rates that

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can impact the distribution of toxic chemicals in the body. During the developmental years, changes occur in liver enzyme systems that can increase or decrease the toxicity of environmental chemicals in children, causing the same amount of chemical exposure in a child to have a greater proportional impact than in an adult. Children's immature immune systems may also be less effective than adults' for detoxifying and eliminating hazardous agents [Guzelian, 1992; Bearer, 1995].

In the central valley of California, an excess of childhood cancers has been observed in the farming town of McFarland [Moses, 1989]. The incidence rate of childhood cancer in this town is about twice as high as expected. Although, the United States Environmental Protection Agency is continuing an investigation into the quality of the air, soil, and water in the area, no explanation for the elevated childhood cancer has been identified conclusively as yet.

Although little research on farmworker children has been conducted to date, there have been many studies which have examined pesticides and cancer among children in the general population [Zahm and Ward, 1998]. Cancers associated with pesticides among these children include leukemia, brain cancer, non-Hodgkin's lymphoma, soft tissue sarcoma, and Hodgkin's disease. These same cancers are repeatedly associated with pesticide exposure among adults [Zahm et al., 1997], only the magnitude of the risks associated with pesticides is often greater in children than in adults, suggesting greater susceptibility in children [Zahm and Ward, 1998]. These differences in risk prompted us to examine levels of pesticide metabolites in urine of children and adults in a farming area of California.

We chose to study organophosphate (OP) insecticides because they are important agricultural chemicals and are associated with cancer in animals and in epidemiologic studies, and their metabolites are well characterized and measurable non-invasively in urine.

In recent years, while organochlorine use fell from 70% of synthetic organic pesticide use (by pounds active ingredient) in 1966 to only 6% in 1982, OP use rose from about 20% of the total in 1966 to almost 70% in 1982 [Osteen and Szmedra, 1989]. The switch over from organochlorines to OP is partly due to OPs' lack of environmental persistence, a major problem with organochlorine pesticides. Also, OPs are relatively inexpensive, are broad spectrum pesticides, and, so far, have not led to many OP-resistant insects. However, OPs have serious acute toxic health effects [Davies and Peterson, 1997]. Chronic health effects, including cancer, have been less intensively studied.

The results of animal bioassay testing of OP pesticides have been reviewed and evaluated by the National Toxicology Program and the International Agency for Research on Cancer. These reviews cite evidence for carcinogenicity of several OP pesticides. For example, dichlorvos, piperonyl sulfoxide, and tetrachlorvinphos have demonstrated evidence of carcinogenicity in rats and mice in the NTP bioassay program [Ashby and Tennant, 1988].

There is also some epidemiological evidence for the carcinogenicity of OP pesticides in humans. Studies conducted by the National Cancer Institute among farmers in Minnesota and Iowa, e.g., found elevated risks for non-Hodgkin's lymphoma associated with use of diazinon, dichorovos, and malathion [Cantor et al., 1992] and increased leukemia risk associated with exposure to crotoxyphos, dichlorvos, and famphur [Brown et al., 1990]. A study completed in eastern Nebraska found significant positive associations between diazinon and malathion use and risk of non-Hodgkin's lymphoma [Zahm et al., 1990].

Exposure to OPs can be measured by determination of urinary alkylphosphates. OPs are hydrolyzed rapidly with six alkylphosphate metabolites detectable in the urine for several days after exposure. These metabolites can be found in association with lesser levels of exposure than that necessary to produce cholinesterase inhibition [Coye et al., 1986].

Therefore, a feasibility study was undertaken to obtain urine samples from farmworkers and their children in an area of extensive OP use to test for urinary metabolites of OP, and to compare urinary OP metabolite levels in adult farmworkers to the metabolite levels in their children.

METHODS

Fresno County, California is the leading agricultural county in the United States with more than three billion dollars in crops and commodities produced annually. Approximately 40 million pounds of pesticides are applied annually in Fresno County [California Department of Pesticide Regulation, 1997]. In 1995, more than 750,000 pounds of OP were used in production agriculture, including 575,000 pounds of chlorpyrifos, 165,000 pounds of diazinon, and 7,600 pounds of malathion.

Farmworker families were recruited in October, 1997, from western Fresno County, California, a rural area characterized by intensive grape and cotton farming with heavy OP pesticide use. A convenience sample of nine farmworker families was selected for study in October of 1997. These families were known to be farmworkers and had participated in previous health surveys conducted by the Cancer Registry. Thirty-five dollars compensation was offered to the families for participation in the study. From each family, adult male, an adult female, and one of their minor children were included in the study. (The total sample, therefore, comprised of 27 subjects.) The study was approved by the institutional review boards of the Public Health Institute of Berkeley and the National Cancer Institute.

A comprehensive lifetime occupational questionnaire was administered in Spanish to the farmworker parents in a domestic setting by trained, bilingual interviewers. The questionnaire used an icon/calendar method [Engel et al., 2001, this issue; Zahm et al., 2001, this issue]. Spot urine samples were collected from all adults ($n = 18$) and children ($n = 9$). The entire process took approximately 2 h to complete per family.

The collected urines were immediately placed on ice, and were brought to the Veterans Administration Medical Center in Fresno for freezing at $-16°C$. Samples were collected over a 4-week period during October and November, 1997. After collection was completed, all samples were forwarded to the California Department of Food and Agriculture Center for Analytical Chemistry in Sacramento.

The urine samples were tested for six metabolites of OP pesticides using a HP gas chromatograph (GC) with flame photometric detection [Weisskopf and Seiber, 1987]. The determination of urinary alkylphophates is used to monitor occupational exposure to OP pesticides [Aprea et al., 1996]. These metabolites included dimethylphosphate (DMP), diethylphosphate (DEP), dimethylphosphorthionate (DMDP), diethylphosphorothionate (DETP), dimethylphosphorodithionate (DMDTP), and diethylphosphorodithionate (DEDTP).

The Limit of Quantification (LOQ) of the GC method was 25 parts per billion (ppb) or about three times the limit of detection (LOD) of the individual metabolite. Trace levels were those for which the peak height was greater than zero and at least 75% of LOD. Peak heights less than 75% of the LOD are considered ''none detected'' because of the extreme unreliability of peak identification at such a low integration level.

Analysis of the data was based on comparing frequencies of detection between metabolites for adults and children and comparing mean levels of metabolites in adults and children where data were available.

For comparison purposes, data on urinary OP metabolite levels were obtained from the Second National Health and Nutrition Examination Survey (NHANES II) conducted between 1976 and 1980 [Murphy et al., 1983]. These general population data included urinary metabolites from a national probability sample of persons aged 12–74 from 64 locations throughout the United States. Comparison data were also obtained from a recent survey of farmworker children in central Washington State [Loewenhertz et al., 1997].

RESULTS

Levels of OP metabolites were generally very low in both adults and children in this survey. The LOD for the six metabolites ranged from 4.8 to 7.5 ppb with no metabolite exceeding the LOQ (25 ppb) in value (Table I). DMP and DMTP were the most frequently detected metabolites, although frequencies of non-detectable ranged from 63% for DMP to 100% for DMDTP and DEDTP. The highest frequencies of detection were found for DMP (37%), DMTP (30%), and DETP (7%) with mean levels of 8.24 ppb for DMP, 13.22 ppb for DMTP, and 6.85 ppb for DETP, after excluding the trace and non-detectable subjects. Frequencies of detection were higher among children than among adults for these three metabolites (Fig. 1).

Mean levels of metabolites for DMP and DMTP were slightly higher among children than adults. DMP levels were 8.36 ppb among children and 8.11 ppb among adults. DMTP levels were 13.63 and 12.92 ppb, respectively. DETP levels were higher among adults (7.98 ppb) than children (5.73 ppb). No formal statistical tests of these differences were conducted due to the extremely small size of these samples.

The frequencies of detection of DMP, DMTP, and DETP were higher among the Fresno-area farmworkers and their children than among the general population sampled in from the second National Health and Nutrition Examination Survey (NHANES II) conducted between 1976 and 1980 [Murphy et al., 1983] (Fig. 2). Trace levels of DEP and no

TABLE I. Limits of Detection (LOD), Frequencies of Detection, and Mean Levels of Six Alkylphosphate Metabolites in Urine of Farmworker Adults ($n = 18$) and Their Children ($n = 9$) in Rural Fresno County, California, 1997

Metric	DMP	DEP	DMTP	DMDTP	DETP	DEDTP
LOD (ppb)	5.9	6.3	7.5	7.5	4.8	6
Frequency of detection (% overall)	37	7	30	0	7	0
Adults	33	11	28	0	6	0
Children	44	0	33	0	11	0
Total mean level (ppb)	8.24	ND	13.22	ND	6.85	ND
Adults	8.11	ND	12.92	ND	7.98	ND
Children	8.36	ND	13.63	ND	5.73	ND

DMP, Dimethylphosphate; DEP, Diethylphosphate; DMTP, Dimethylphosphorthionate; DMDTP, Dimethylphosphorodithionate; DETP, Diethylphosphorothionate; DEDTP, Diethylphosphorodithionate; ND, Non-detectable.
All subjects with DEP had trace levels only.

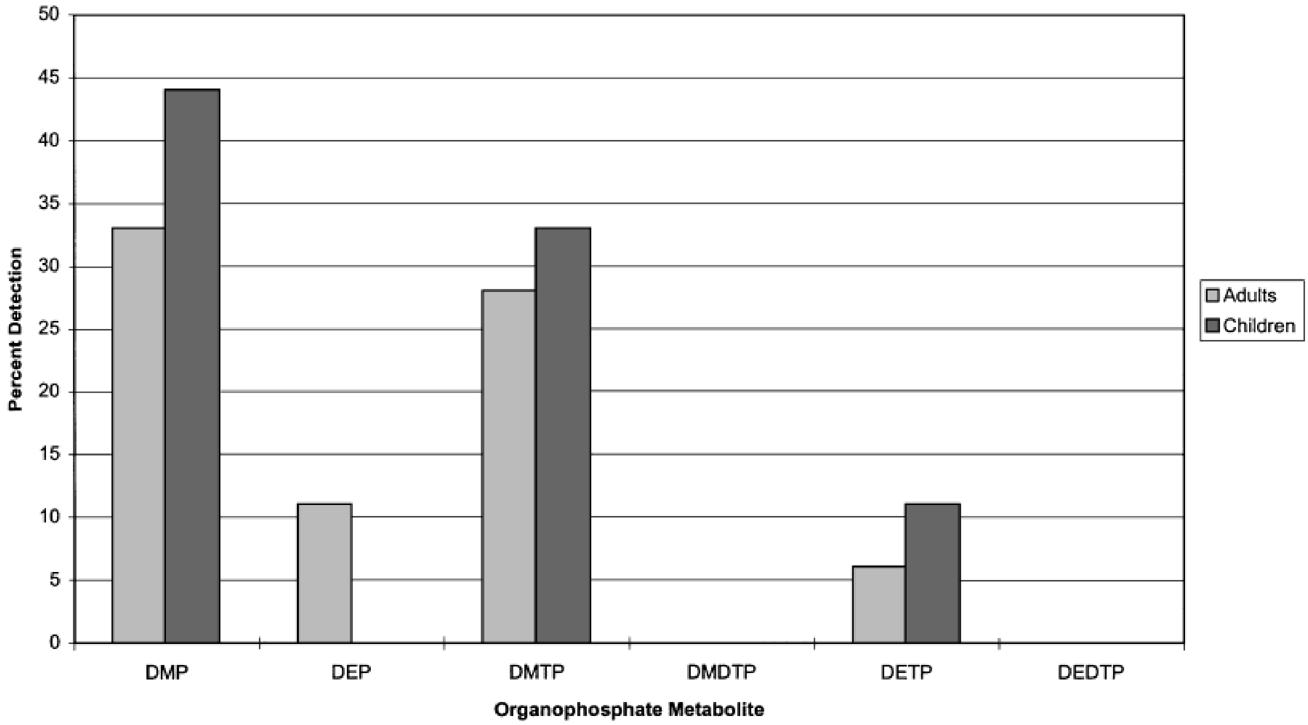


FIGURE 1. Frequency of detection of organophosphate metabolites in urine of farmworker adults ($n = 18$) and children ($n = 9$) in Fresno California, 1997.

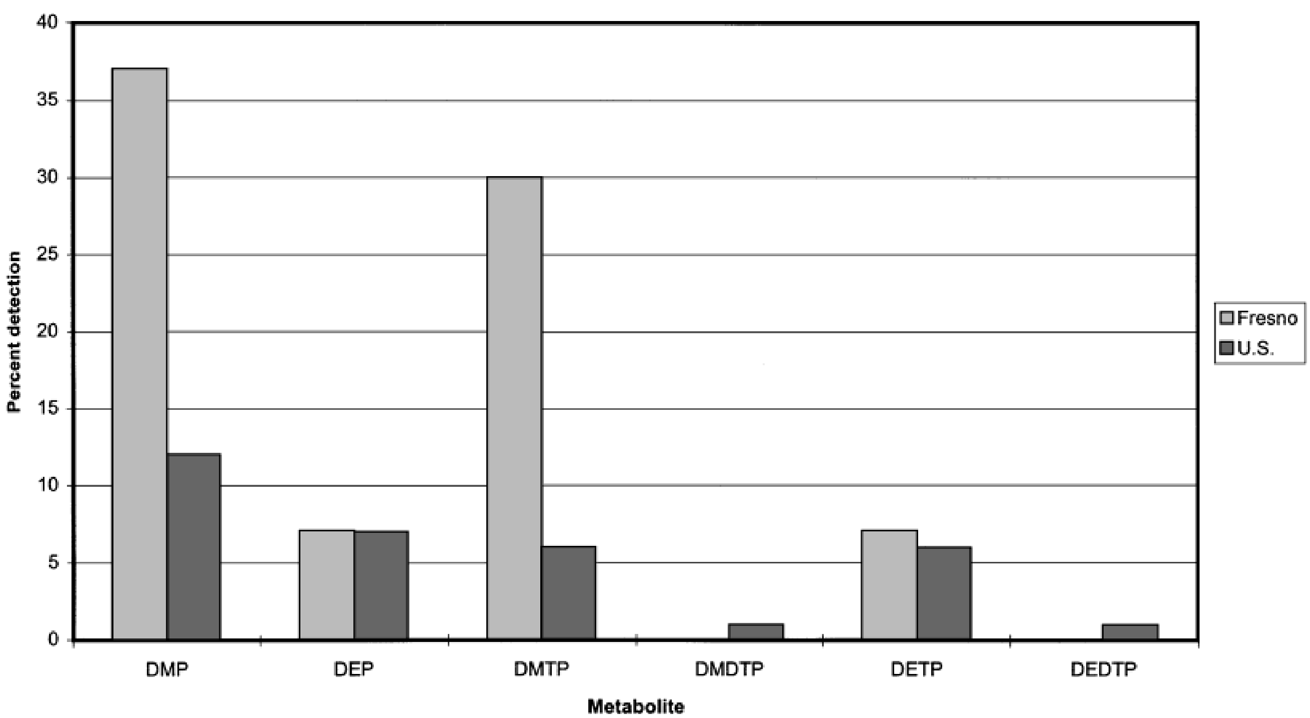


FIGURE 2. Frequency of detection of dialkylphosphate residues in urine of farmworkers and children in Fresno, California (1997) vs. the general US population (1976–1980).

levels DMDTP and DEDTP were detected in members of the Fresno area sample, whereas about 1–7% of the general U.S. population sample had detectable levels of these metabolites.

The frequencies of detection and mean levels (ppb) of DMTP among Fresno children were intermediate between Washington State children of pesticide applicators and Washington State reference (i.e., non-farmworker) children (Fig. 3).

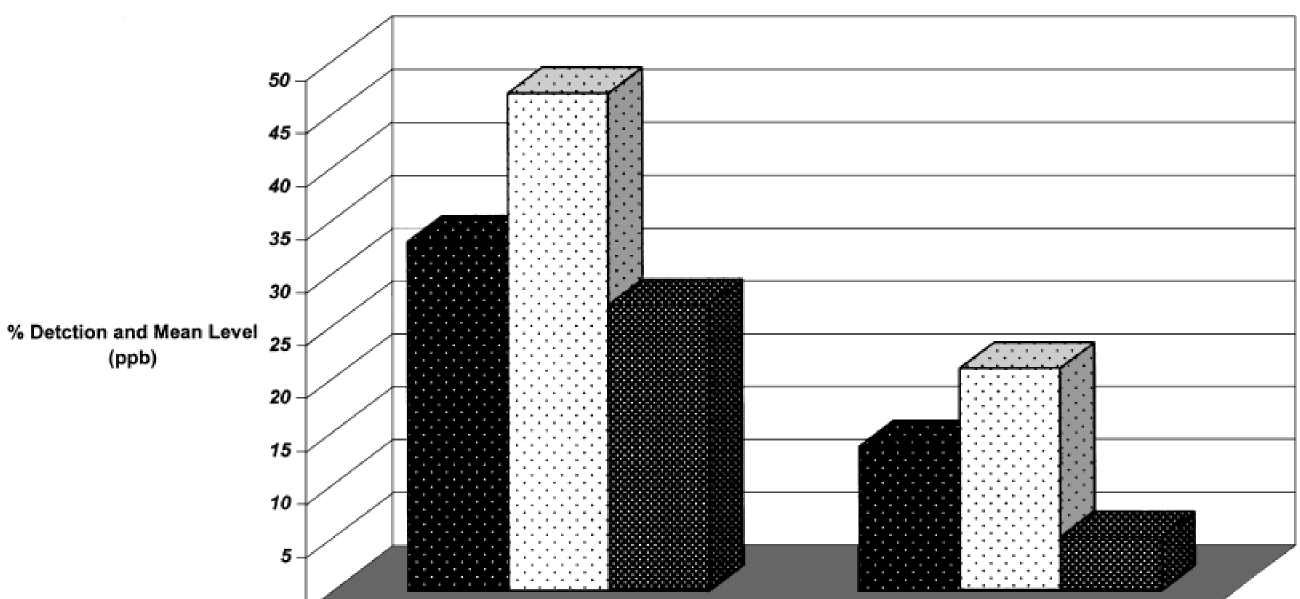
DISCUSSION

In this small survey, we found some OP metabolites more often in urine of farmworker children than of their farmworker parents. This was true for three of the metabolites for which we tested (DMP, DMTP, and DETP). DEP was detected more frequently among adults than children. DMDTP and DEDTP were not detected among any subjects. The pesticide metabolite found at highest concentration in this study was DMP, which, along with DEP, is thought to be directly attributable to OP pesticide exposure. DMDTP and DEDTP metabolites are less directly associated with pesticide exposure and were not found in the present study. Dietary and other sources may contribute to levels of DMTP and DETP metabolites [Coye et al., 1986].

The pesticide metabolite levels detected in this Fresno study were quite low, which may reflect low exposure or may have been due to timing of sample collection (in the fall when agricultural pesticide use is limited) and the relatively rapid elimination of OP metabolites from the body. Alternatively, heavy exposure may have occurred but was missed by chance due to our small sample and random variation. The low levels detected are unlikely to be due to an unusually low use of OPs in the area of study. Agricultural use of OP pesticides has been extensive in this area of California for decades, and use during 1997 was typical of most years. Despite the large proportion of non-detectable levels, the frequency of detection for three of the six OPs measured in 1997 greatly exceeded the NHANES levels measured in 1976–1980.

The small sample size limits conclusions concerning exposure differences by age, however, the greater levels detected among children than adults for some chemicals were consistent with a study of residential exposure that reported that children absorbed 2 to 10-times more insecticide than the parents as a result of contact with treated surfaces [Krieger et al., 1999]. Another study of urinary alkyl phosphate metabolites among farmers' families in El Salvador showed high frequency of exposure among children of farmers and field workers [Azaroff, 1999]. In that study, detection of metabolites was greater if the head of the household applied pesticides within 2 years or if the mother reported OP use in the home or yard.

The greater frequencies of detection of DMP and DMTP among farmworkers' children than among their parents merit comment in light of a recent Italian study in



	Frequency of Detection (%)	Mean Level (ppb)
Fresno Children	33	13.6
WA -Appl.Children	47	21
WA-Ref.Children	27	5

FIGURE 3. Frequency of detection and mean levels of dimethylthiophosphate in urine of children in Fresno and Washington State, 1997.

which DMP and DMTP were also commonly found in urine samples of children in a farming region of Tuscany [Aprea et al., 2000]. In that study the excretion of OP metabolites in children was significantly higher than in adults residing in the same provinces, results which are concordant with those reported in this study in California. These findings raise concerns about health effects of environmental chemicals in children, concerns which have been reviewed previously [Schaffer, 1994]. Children may have greater potential for exposure to pesticides than adults through certain routes of exposure. Children's normal activities may expose them to higher levels of pesticides applied in and around the home [Fenske, 1990]. House dust in agricultural areas can have high levels of pesticides that are not readily degraded indoors by sun, rain, and microbes [Simcox et al., 1995]. A report concerning exposure routes among children to the pesticide chlorpyrifos noted that the sorbent surfaces of such objects as toys and pillows can become contaminated with household pesticides and that dermal and non-dietary oral doses from playing with toys can be substantial [Gurunathan et al., 1998]. In addition, the National Research Council noted that dietary intake of pesticides per unit body weight may be greater among children than among adults [National Research Council, 1993]. Children between the age group of 1 and 5 years eat three to five times more food per pound than the average American adult, particularly fruits and vegetables, which generally have greater levels of pesticides than other foods. Young children (of less than 1 year) consume 21 times as much apple juice and eat 2–7 times more grapes, bananas, pears, carrots, and broccoli than the average adult.

A recent study completed by the Environmental Health Investigations Branch of the Department of Health Services of California found household dust in farmworker family homes in the Central Valley of California to have higher levels of OP pesticides (including diazinon and chlorpyrifos) than non-farmworker family homes [Bradman et al., 1997]. The health implications of these findings are uncertain although dust ingestion by children in that study may have exceeded the United States Environmental Protection Agency Office of Pesticide Program's chronic reference dose for diazinon.

This study has demonstrated the feasibility of collecting urine samples from farmworkers and their children in an intensively agricultural area. It has also demonstrated that children have biologic measures of exposure to agricultural chemicals that are as high or higher than their farmworker parents do. These findings support the need for large-scale epidemiologic studies of cancer and other health outcomes among farmworkers' children.

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REFERENCES

Aprea C, Sciarra G, Orsi D, Boccalon P, Sartorelli P, Sartorelli E. 1996. Urinary excretion of alkylphosphates in the general population (Italy). Sci Total Environ 177:37–41.

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

Ashby J, Tennant RW. 1988. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. Mutat Res 204:17–115.

Azaroff LS. 1999. Biomarkers of exposure to organophosphorous insecticides among farmers' families in rural El Salvador: factors associated with exposure. Environ Res A80:138–147.

Bearer CF. 1995. How are children different from adults? Env Health Perspect 103:(Suppl. 6)7–12.

Bradman MA, Harnley ME, Draper W, Siedel S, Teran S, Wakeman D, Neutra R. 1997. Pesticide exposures to children from California's Central Valley; results of a pilot study. J Exp Anal Environ Epidemiol 7:217–234.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50:6585–6591.

California Department of Pesticide Regulation. 1997. Pesticide use reports, 1995. Sacramento: California Department of Pesticide Regulation.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, Schoman LM, Dick FR. 1992. Pesticides and other risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 52:2447–2455.

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

Davies JE, Peterson JC. 1997. Surveillance of occupational, accidental, and incidental exposure to organophosphate pesticides using urine alkyl phospahate and phenolic metabolite measurements. Ann NY Acad Sciences 837:257–268.

Engel LS, Keifer MC, Zahm SH. 2001. Comparison of a traditional questionnaire with an icon/calendar-based questionnaire to assess occupational history. Am J Ind Med 40:502–511 (this issue).

Fenske R, Black KG, Elkner KP, Lee C, Methner M, Soto R. 1990. Potential exposure and health effects of infants following residential pesticide applications. Am J Public Health 80:689–693.

Gurunathan S, Robson M, Freeman N, Buckley B, Roy A, Meyer R, Bukowski J, Lioy PJ. 1998. Accumulation of chlorpyrifos on residential surfaces and toys accessible to children. Environ Health Perspect 106:109:9–16.

Guzelian PS, Henry CJ, Olin SS, editors. 1992. Similarities and differences between children and adults: implications for risk assessment. Washington, D.C: ILSI Press.

Krieger RI, Dinoff TM, Bernard CE, Williams RL. 1999. Clarification of human pesticide exposures in risk assessment and risk management.

Presented at “the role of human exposure assessment in the prevention of environmental disease.” National Institute of Environmental Health Sciences, September 22–24, 1999, Rockville, MD.

Loewenhertz C, Fenske R, Simcox N, Bellamy G, Kalman D. 1997. Biological monitoring of organophosphate pesticide exposure among children of agricultural workers in central Washington state. Environ Health Perspect 105:1344–1353.

Moses M. 1989. Pesticide-related health problems and farmworkers. Am Assoc Occup Health Nurses J 37:115–130.

Murphy RS, Kutz FW, Strassman S. 1983. Selected pesticide residues or metabolites in blood and urine specimens from a general population survey. Environ Health Perspect 48:81–86.

National Research Council. 1993. Pesticides in the diets of infants and children. Washington, D.C: National Academy Press.

Osteen C, Szmedra P. 1989. Agricultural pesticide use trends and policy issues. Washington, D.C: US Department of Agriculture Economic Research Service.

Schaffer M. 1994. Children and toxic substances: confronting a major public health challenge. Environ Health Perspect 102(Suppl. 2):155–156.

Simcox NJ, Fenske RA, Wolz SA, Lee IG, Kalman ND. 1995. Pesticides in household dust and soil: exposure pathway for children of agricultural families. Environ Health Perspect 103:1126–1134.

Weisskopf CP, Seiber JN. 1987. In: Wang RGM, Franklin CA, Honycutt RC, Reinert JC, Editors. ACS symposium series No. 382. Washington, D.C: American Chemical Society. p 206.

Zahm SH, Weisenburger DD, Babbit PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiol 1:349–356.

Zahm SH, Ward MH, Blair A. 1997. Pesticides and cancer. In: Keifer M, editor. Pesticides. Philadelphia: Hanley and Belfus, Inc. Occupational medicine: state of the art reviews 12:269–289.

Zahm SH, Ward MH. 1998. Pesticides and childhood cancer. Environ Health Perspect 106(Suppl. 3):893–908.

Zahm SH, Colt J, Engel LS, Keifer MC, Alvarado AJ, Burau K, Butterfield P, Caldera S, Cooper S, Garcia D, Hanis C, Hendrikson E, Heyer N, Hunt L, Krauska M, MacNaughton N, McDonnell CJ, Mills PK, Mull D, Nordstrom D, Outterson B, Slesinger DP, Smith MA, Stallones L, Stephens C, Sweeney A, Sweitzer K, Vernon S, Blair A. 2001. Development of a life events/icon calendar questionnaire to ascertain occupational histories and other characteristics of migrant farmworkers. Am J Ind Med 40:490–501(this issue).