Biologic Monitoring of Exposure to Organophosphorus Pesticides in 195 Italian Children

Cristina Aprea,[1] Mirella Strambi,[2] Maria Teresa Novelli,[3] Liana Lunghini,[1] and Nanda Bozzi[1]

[1]Department of Occupational Toxicology and Industrial Hygiene, Siena, Italy; [2]Istituto di Pediatria Preventiva e Neonatologia, Università degli Studi di Siena, Siena, Italy; [3]Agenzia Regionale per la Protezione Ambientale, Siena, Italy

One hundred ninety-five 6- to 7-year-old children who lived in the municipality of Siena (Tuscany, Italy) underwent biologic monitoring to evaluate urinary excretion of several alkylphosphates that are metabolites of organophosphorus pesticides. We evaluated dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). We obtained urine samples taken in the children's schools, and each sample was accompanied by a questionnaire about lifestyle and dietary habits. We found DMP and DMTP in detectable concentrations in the greatest number of samples (96 and 94%, respectively). The DMP values were geometric mean (GM) 116.7, [geometric standard deviation (GSD) 2.5], and a range of 7.4–1,471.5 nmol/g creatinine. The corresponding DMTP values were GM 104.3 (GSD 2.8) and a range of 4.0–1,526.0 nmol/g creatinine. DMDTP, DEP, DETP, and DEDTP concentrations were GM 14.1, (GSD 3.0), and a range of 3.3–754.6 nmol/g creatinine in 34% of the children; GM 33.2, (GSD 2.4), and a range of 5.1–360.1 nmol/g creatinine in 75% of the children; GM 16.0, (GSD 2.9), and a range of 3.1–284.7 in 48% of the children; and GM 7.7, (GSD 2.1), and a range of 2.3–140.1 in 12% of the children, respectively. The significant variable for urinary excretion of these metabolites in children was pest control operations performed inside or outside the house in the preceding month; however, the presence of a vegetable garden near the house rarely emerged. The urinary excretion of alkylphosphates in children was significantly higher than in a group of the adult population resident in the same province. *Key words*: biologic monitoring, children, general population, organophosphorus insecticides, urinary alkylphosphates. *Environ Health Perspect* 108:521–525 (2000). [Online 18 April 2000]
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The determination of pesticide residues or metabolites in biologic fluids of the general population has recently been the subject of many articles (*1–10*). Exposure of the general population to pesticides is due to residues in food and drink (dietary exposure), atmospheric dispersal of aerosols and vapors (respiratory exposure), and skin contact with contaminated articles (cutaneous exposure). Skin contamination may sometimes lead to oral nondietary exposure. Pesticide residues in indoor environments are not subject to degradation by sun, rain, and soil microbes and are therefore more persistent than in the environment at large.

Children's exposure to pesticides is potentially greater than that of adults for two reasons. First, depending on their age, children may spend much of their time on the floor, where they may come into contact with dust and soil. A substantial quantity of contaminated matter may be ingested through fingers and other objects placed in the mouth. Studies reported by U.S. Environmental Protection Agency investigators (*11*) estimate that children have a 12-times greater health risk than adults associated with the ingestion of dust and soil.

Dietary exposure to pesticide residues is also potentially higher for children. In relation to body weight, children drink more water, milk, and fruit juice than adults, and consume a large quantity of fresh foods.

Organochlorine compounds were the first to be studied in the general population because of their widespread use, persistence, and effects on health. However, in the last 20 years there has been a considerable increase in the use of less persistent compounds, such as organophosphorus insecticides, which have greater acute toxicity. The acute effects of the organophosphorous insecticides are well known, but the chronic effects are not well characterized and the available data are mainly for adults. Little is known about chronic toxicity in children (*12*) and no studies have been published on the neurotoxic effects of low levels of children's exposure.

Once organophosphorus pesticides (OPs) have entered the body, they are rapidly metab-olized and almost entirely excreted in the urine within 24 hr of absorption (*13*). Alkylphosphates [dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP)] are urinary metabolites of many phosphoric esters, and are derived by A-esterase-catalyzed hydrolysis (*14*).

Few studies regarding biologic monitoring of exposure of the general population to OPs by urinary alkylphosphate assay (*2,4,6*) have been published, and only one examines children (*6*). In this study, urinary excretion of DMTP in children living in families in which at least one member performed pest control operations with OPs was compared with that of a reference group consisting of children who lived far from agricultural environments and who had no member of the family working in agriculture.

The present study evaluated urinary excretion of six alkylphosphates in 195 children 6–7 years of age, who lived in Siena, a hill town in Tuscany (Italy). The collection of urine samples was accompanied by a questionnaire on lifestyle and dietary habits. We had three specific aims for the study. The first aim was to compare urinary excretion of these metabolites in the general infantile and adult populations [the adult population consisted of 124 subjects who lived in the same province and who were the subject of a previous study (*4*)]. The second aim was to determine whether dietary habits or lifestyle influence the urinary excretion of alkylphosphates by children in a statistically significant manner. The third aim was to determine whether children who ate one meal/day (lunch) at the school mensa, where all plant products (vegetables, fruit, cereals, legumes, vegetable oil, etc.) served were organic, had lower urinary excretion of alkylphosphates than those who ate lunch at home. "Organic" is defined as not treated with pesticides except copper sulfate and sulfur.

Methods

Study design and population recruitment. In May 1995, we obtained 195 spot urine samples from 195 children 6–7 years of age, who lived in Siena, a hill town in southwest Tuscany (central Italy). Siena has practically no major industries and the population consists mainly of bank, hospital, and university employees; shopkeepers; and professionals.

To obtain the population sample, we held preliminary meetings with the parents

of children enrolled in the first and second classes of all Siena elementary schools. At these meetings, we explained the study and the parents were given a questionnaire. The meeting was mainly spent explaining how to fill in the form and answering questions. The parents were then given the date that urine samples would be taken at the schools. Parents who agreed to participate had to return the completed questionnaire on the day of sampling. Urine samples were only obtained from children who returned the form.

Some of the children ate one meal/day (lunch) at the school mensa; others, who did not have school in the afternoon, ate all meals at home.

Urine sampling. On the day of sampling, health personnel went to the schools, collected the questionnaires, and gave each of the children a polyethylene container for the urine sample. Urine samples were produced between 0900 and 1200 hr. The urine was immediately refrigerated and was frozen as soon as it reached the laboratory.

Compilation of questionnaire. The questionnaire provided the parents' informed consent to their child's enrollment in the study. The details asked by the questionnaire concerned lifestyle and dietary habits, as follows:
• Surname
• Name
• Sex
• Date of birth
• Weight
• Height
• Address
• Telephone number
• School
• Class
• Father's occupation
• Mother's occupation
• Illnesses and hospitalization of child
• Do you have a garden or vegetable garden?
• Do you keep ornamental plants in the house?
• Do you buy cut flowers for the house?
• Do you keep domestic animals in the house?
• Do you use pesticides inside or outside the house?
• Food and drink ingested the day before urine collection.
If the questionnaire was incomplete, the parents were contacted by telephone to obtain the missing information.

Analysis of alkylphosphate metabolites. We analyzed alkylphosphates in the urine samples by gas chromatography with flame photometric detection after derivatization with pentafluorobenzylbromide and purification on SPE columns with CN-bound phase (*15*). Table 1 shows the recovery, reproducibility, and detection limits of the six compounds. The calibration curves, obtained by adding the six alkylphosphates to

urine, were linear ($r > 0.990$) for all compounds in the concentration interval between the detection limit and 1,500 µg/L.

The analytical results are expressed in nanomoles per gram creatinine. The creatinine assay was performed by the Larsen procedure (*16*) with a precision [coefficient of variation (CV%)] of 3.1%. Urinary creatinine concentrations formed a normal distribution in the range of 0.17–1.93 g/L.

Statistical analysis. Many urine samples had concentrations below detection limits for some metabolites. Our preliminary analysis therefore consisted of calculating the positivity percentages (% pos), i.e., the percentage of samples above detection limit for each analyte. Statistical analysis of the samples was then carried out, including a value half the detection limit for nondetectable analytes.

We used the Kolmogorov-Smirnov test to check the distribution of samples for the six alkylphosphates; we found a positive asymmetric distribution, which became normal after log transformation. Parametric analysis (multiple regression) was therefore used for subsequent comparisons. We used the Bonferroni/Dunn post hoc test (multiple comparisons) to examine whether the mean values of the dependent variables were different from each other for each level of the factors. Statistical significance was set at $\alpha = 0.05$.

We used some of the information obtained with the questionnaire (diet, occupation of parents, height, weight, and height/weight ratio) for qualitative classification of the population. Age was not considered because the children differed in age by no more than 1 year. The variables considered for subsequent statistical analysis were sex, the presence of a vegetable garden or garden near the house, ornamental plants or cut flowers (taken together) or pets in the house, pest control operations performed in the preceding month, and whether the child ate lunch at school on the day before sampling.

The influence of these variables was evaluated for single alkylphosphates and for the sum of dimethyl (DMP + DMTP + DMDTP), diethyl (DEP + DETP + DEDTP), and all metabolites expressed in nanomole per gram creatinine. We named these sums methyl, ethyl, and sum, respectively. To calculate these sums, analytes below the detection limit were counted as a value half the detection limit. None of the subjects had all of the metabolites below detection limit.

Results

Study participation was approximately 67%: of the 291 questionnaires distributed to parents, only 195 were completed and returned. The main reasons for low participation were forgetting to compile the form or return it,

absence from school, and lack of interest. Of the 195 children, 103 were girls (53%). None of the children had any particular diseases: there were two cases of allergy (one asthma and the other glomerulonephritis). The children were between 6 and 7 years of age; 119 children (61%) were 6 years of age.

Height, weight, and height/weight ratio [mean ± SD, (range)] were 125.7 ± 7.4, (100–143) cm; 26.7 ± 5.6, (16–47) kg, and 4.9 ± 0.8, (3.0–6.9), respectively. These parameters had a normal distribution.

The most common parental occupation was public servant (31 and 45% for mother and father, respectively), although a small percentage of parents were farmers (1 and 2% for mother and father, respectively). The latter occupation could result in paraoccupational (take-home) exposures of OP pesticides to children. In 115 cases (59.0%), the family had a vegetable garden or garden. In 170 cases the houses contained ornamental plants and/or cut flowers and in 46 cases (23.6%) the houses contained pets. In 29 cases (14.9%), pest-control operations had been performed in the previous month. The use of OPs was not declared in any questionnaire. Seventy-two children (36.9%) ate lunch at the mensa.

Urinary excretion of alkylphosphates (in nanomole per gram creatinine) is shown in Table 2. The geometric mean (GM) of DMP and DMTP was 3.5–15.2 times higher than that of the other metabolites. The % pos was approximately 95% for these two metabolites, 75% for DEP, and 48 and 12% for DETP and DEDTP, respectively. The highest values of DMTP and DMDTP in the ranges of concentration belonged to the same boy. The boy's father was a cook and the mother a white-collar worker. Their house had a vegetable garden and they kept ornamental plants and cut flowers in the house, as well as pets. They did not state that they used pesticides. The boy did not eat at the mensa and the day before sampling he consumed meat, fish, bread, pasta, smallgoods, cakes,

Table 1. Recovery, detection limits, and precision of the analytical method.

Alkyl-phosphate	Mean % recovery[a]	CV1%[b]	Detection limits[c] (µg/L)
DMTP Na	85.8	9.1	2
DMDTP K	90.0	11.9	2
DETP K	99.2	8.8	2
DEDTP K	101.0	9.8	2
DMP K	100.0	11.4	3
DEP K	87.9	7.9	3

Data from Aprea et al. (*15*).
[a]Recovery was evaluated at the added concentration of 62.5 µg/L. [b]Coefficient of variation of the whole analysis (the six alkylphosphates added to 10 aliquots of the same urine sample) including purification. [c]The detection limit was calculated on the basis of a signal 3 times the background noise. The data are for sodium and potassium salts of the six alkylphosphates.

fruit juice, tea, and bottled mineral water. The highest values of DMP, DEP, DETP, and DEDTP in the concentration ranges were in four boys and in one girl, whose parents were mostly white-collar workers. In all of the cases with the highest values, the house had a vegetable garden or contained ornamental plants/cut flowers or pets. In one case the occasional flea treatment of pets was stated; however, the product did not contain OPs. Two boys ate at the mensa and the other two went home for lunch. All of these children drank bottled mineral water and ate meat, pasta, bread, cheese, vegetables, fresh fruit, and fruit juice.

Comparison (Student's t-test) of the data in Table 2 with that of a group of 124 adults in the general population of southwest Tuscany (4) showed significantly higher values of all metabolites in the children. The values in adults were GM 62.8 (GSD 2.5), and a range of 5.5–720.7 (DMP); GM 70.7 (GSD 2.7), and a range of 5.5–643.9 (DMTP); GM 21.1 (GSD 2.3), and a range of 3.6–133.2 (DMDTP); GM 27.4 (GSD 2.5), and a range of 2.5–530.2 (DEP); GM 22.8 (GSD 2.2), and a range of 3.4–97.6 (DETP); and GM 13.7 (GSD 1.9), and a range of 6.3–54.9 (DEDTP) nmol/g creatinine. The % pos observed in the adults were 87, 99, 48, 82, 73, and 7, respectively, which were well correlated with those of the children.

The results of multiple regression, based on a model that used sex, mensa, vegetable garden, plants and flowers, pest control, and pets as independent variables, are shown in Table 3. The model was significant for DMTP, DMDTP, methyl, and sum, indicating that together, these variables influenced urinary excretion of the metabolites considered. The R^2 values were in the range of 0.044–0.080 and the variance explained by the models was in the range of 4.4–8.0%.

GM values and % pos of single analytes and the sums, divided according to variable, are given in Tables 4 and 5. For DMP, DEP, DEDTP, and ethyl, none of the variables was significantly related to urinary concentration (Bonferroni/Dunn post hoc test). The pest-control variable, however, was significantly related to the urinary excretion of DMTP, DETP, methyl, and sum, and the variable garden was related to the urinary excretion of DMDTP (Bonferroni/Dunn post hoc test).

Discussion

Alkylphosphates can be detected in the urine of subjects occupationally exposed to OPs (14,17–22) and in the general population (2,4): dimethyl OPs produce dimethyl metabolites (DMP, DMTP, and DMDTP) and diethyl OPs produce diethyl metabolites (DEP, DETP, and DEDTP). Depending on the chemical structure of the pesticide, various alkylphosphates can be detected (13,17): phosphorodithioates, such as azinphos-methyl, azinphos-ethyl, and dimethoate, give rise to -phosphates (DMP or DEP), -thiophosphates (DMTP or DETP), and -dithiophosphates (DEDTP or DEDTP); phosphorothioates such as chlorpyrifos, chlorpyrifos-methyl, fenitrothion, and omethoate give rise to -phosphates (DMP or DEP) and -thiophosphates (DMTP or DETP); and phosphates such as chlorfenvinphos, dichlorvos, and monocrotophos give rise to -phosphates (DMP or DEP). Alkylphosphates are therefore specific metabolites of OP pesticides in general, but their presence in urine may indicate exposure to different compounds of the group.

Coye et al. (17) reported that residues of DMP and DEP are directly associated with exposure to OPs, whereas DMDTP and DEDTP are less directly associated with exposure because they break down rapidly to the corresponding monosulfates (DMTP and DETP) and phosphates (DMP and DEP). This may explain the low % pos of the two disulfate metabolites (DMDTP and DEDTP) in the urine of the 195 children.

Urinary alkylphosphates can be detected in urine at exposure levels much less than those affecting cholinesterase activity (17). These metabolites are quickly eliminated and maximum excretion usually occurs within 24 hr of exposure (13). Because of this rapid excretion, the data on food and drink consumption obtained with the questionnaire regard the day before sampling.

There have been few studies on biologic monitoring of exposure of the general population to OPs based on assays of urinary alkylphosphates (2,4,6) and only one of the studies (6) considered children. The Loewenherz et al. (6) study was conducted in the state of Washington, and used biologic monitoring to determine the exposure of children of farm workers to OPs. DMTP emerged as the biologic indicator of exposure. Urinary

levels of this metabolite were significantly greater in the children of pest-control operators than in reference children (from families in which no one worked in agriculture and the house was at considerable distance from cultivated fields). Median concentrations of DMTP in the children of pest-control operators and in reference children were 0.021 and 0.005 µg/mL, respectively; maximum concentrations were 0.44 and 0.10 µg/mL, respectively. The percentage of detectable samples was 47 and 27%, respectively, for a detection limit of 15 µg/L. Younger children had higher urinary excretion than older ones, indicating that children's activities are a major factor for exposure to these substances. The other metabolite analyzed, DMDTP, was not detectable in 80.3% of samples, for a detection limit of 13 µg/L. Results from our study show a median value of detectable samples of 0.01 µg/mL and a maximum of 0.16 µg/mL. These values are lower than the pest-control operators' children, but slightly above the reference child population.

The high percentage of positive samples in the present study is due to the detection limit of the analytical method used. A previous study based on analysis of 5,976 samples obtained in the period 1976–1980 from adults and children living in 64 areas of the United States (including the second National Health and Nutrition Examina-tion Survey sampling areas) found lower alkylphosphate positivity percentages; however, the detection limits were 20 µg/L (2). If we exclude concentrations < 20 µg/L from the results of the present study, the % pos become 26.0% for DMP, 28.0% for DMTP, 2.0% for DMDTP, 0.5% for DEP, 2.0% for DETP, and 0.5% for DEDTP. These percentages are higher than those published for dimethyl metabolites (12% for DMP, 6% for DMTP, and < 1% for DMDTP) and lower than those for diethyl metabolites (7% for DEP, 6% for DETP, and < 1% for DEDTP) (2). The difference is presumably due to the greater use of dimethyl OPs than diethyl OPs in Italy.

Table 3. Results of multiple regression performed using sex, garden, plants and flowers, animals, and pest control as independent variables.

Dependent variable	Significance of regression	R^2
DMP	Not significant	0.044
DMTP	Significant ($p < 0.05$)	0.079
DMDTP	Significant ($p < 0.05$)	0.080
DEP	Not significant	0.049
DETP	Not significant	0.055
DEDTP	Not significant	0.062
Methyl	Significant ($p < 0.05$)	0.067
Ethyl	Not significant	0.056
Sum	Significant ($p < 0.05$)	0.072

The dependent variables are expressed as nmol/g creatinine.

Table 2. Concentrations of alkylphosphates (nmol/g creatinine) in the urine of 195 children living in Siena (Tuscany, Italy).

Metabolite	% pos	Mean ± SD	GM (GSD)	25th percentile	Median	75th percentile	Range
DMP	96	178.6 ± 196.6	116.7 (2.5)	65.0	109.8	222.1	7.4–1,471.5
DMTP	94	170.2 ± 194.0	104.3 (2.8)	57.9	99.3	189.8	4.0–1,526.0
DMDTP	34	30.5 ± 69.3	14.1 (3.0)	6.0	9.6	29.9	3.3–754.6
DEP	75	48.0 ± 46.3	33.2 (2.4)	17.4	36.0	63.4	5.1–360.1
DETP	48	28.9 ± 37.8	16.0 (2.9)	6.3	14.8	33.8	3.1–284.7
DEDTP	12	11.6 ± 16.8	7.7 (2.1)	4.6	6.1	11.1	2.3–140.1

Table 4. Mean values and % pos of variables for the six analytes (nmol/g creatinine).

Variable (n)	DMP		DMTP		DMDTP		DEP		DETP		DEDTP	
	GM (GSD)	% pos	GM (GSD)	% pos	GM (GSD)	% pos	GM (GSD)	% pos	GM (GSD)	% pos	GM (GSD)	% pos
Sex												
Female (103)	122.0 (2.4)	94	98.1 (2.5)	94	12.3 (2.5)	30	33.8 (2.3)	76	15.0 (2.8)	42	7.7 (2.1)	10
Male (92)	111.1 (2.7)	98	111.7 (3.0)	95	16.4 (3.4)	39	32.6 (2.6)	75	17.3 (2.9)	55	7.8 (2.3)	14
Garden												
No (80)	118.5 (2.3)	96	91.5 (2.8)	93	11.6 (2.6)*	28	33.0 (2.3)	79	15.7 (2.7)	54	7.3 (2.0)	11
Yes (115)	115.5 (2.7)	96	114.2 (2.8)	96	16.1 (3.2)*	39	33.3 (2.5)	73	16.3 (3.0)	44	8.1 (2.2)	12
Plants and flowers												
No (25)	90.6 (2.4)	92	109.7 (2.7)	96	14.9 (2.9)	40	35.7 (2.3)	80	14.5 (2.7)	52	6.8 (1.8)	12
Yes (170)	121.1 (2.6)	96	103.5 (2.8)	94	14.0 (3.0)	34	32.8 (2.4)	75	16.3 (2.9)	48	7.9 (2.2)	12
Animals												
No (149)	122.6 (2.6)	95	106.0 (2.8)	94	14.9 (2.6)	35	36.2 (2.4)	78	16.1 (2.9)	47	8.1 (2.2)	11
Yes (46)	99.7 (2.4)	98	98.9 (2.7)	96	11.9 (3.1)	33	25.1 (2.4)	67	15.8 (2.8)	52	6.6 (1.9)	13
Pest control												
No (166)	113.5 (2.5)	96	96.9 (2.8)*	93	13.3 (2.8)	33	32.7 (2.4)	75	14.9 (2.9)*	46	7.4 (2.1)	10
Yes (29)	136.9 (3.0)	96	159.0 (2.6)*	100	19.7 (3.8)	41	36.4 (2.4)	79	24.3 (2.7)*	62	9.9 (2.4)	21
School mensa												
No (123)	111.9 (2.5)	97	95.0 (2.9)	93	15.1 (3.2)	36	33.4 (2.4)	74	14.7 (2.6)	46	8.2 (2.2)	13
Yes (72)	125.4 (2.7)	94	122.4 (2.5)	96	12.5 (2.6)	32	32.9 (2.4)	78	18.7 (3.3)	53	7.0 (2.1)	10

*The variable was significant (Bonferroni/Dunn post hoc test).

Table 5. Mean values and % pos of variables for the methylated, ethylated, and sum of all metabolites (nmol/g creatinine).

Variable (n)	Methyl		Ethyl		Sum	
	GM (GSD)	% pos	GM (GSD)	% pos	GM (GSD)	% pos
Sex						
Female (103)	258.6 (2.1)	98	63.5 (2.1)	80	334.8 (2.0)	98
Male (92)	277.0 (2.5)	100	66.3 (2.3)	87	356.4 (2.3)	100
Garden						
No (80)	247.8 (2.1)	98	62.2 (2.1)	84	321.4 (2.0)	98
Yes (115)	281.5 (2.4)	99	66.6 (2.3)	83	362.1 (2.2)	100
Plants and flowers						
No (25)	248.7 (2.1)	100	66.1 (1.9)	88	327.9 (1.9)	100
Yes (170)	270.0 (2.3)	98	64.6 (2.3)	82	347.4 (2.2)	99
Animals						
No (149)	276.6 (2.3)	99	68.4 (2.2)	84	359.3 (2.2)	99
Yes (46)	238.8 (2.1)	98	54.4 (2.1)	80	301.9 (2.0)	100
Pest control						
No (166)	254.0 (2.2)*	99	62.2 (2.2)	82	329.0 (2.1)*	99
Yes (29)	356.7 (2.6)*	96	81.5 (2.2)	90	451.0 (2.4)*	100
School mensa						
No (123)	251.4 (2.3)	100	63.3 (2.2)	81	327.3 (2.2)	100
Yes (72)	296.5 (2.2)	96	67.4 (2.3)	86	376.9 (2.1)	97

*The variable was significant (Bonferroni/Dunn post hoc test).

The results of the present study are significantly higher than those obtained with a population of 124 adults who lived in southwest Tuscany and were sampled in the same period (4). There may be a number of reasons for this difference. Exposure to pesticide residues in food may be greater for children than for adults; for example, children tend to eat more fresh products, and they drink more water, milk, and fruit juice than adults in proportion to body weight. The food eaten by the children the day before sampling confirms this observation: approximately 85, 43, 66, 51, 41, and 36%, respectively, had eaten fresh fruit, milk, cooked vegetables, fruit juice, and infusions such as tea. The observation is nevertheless exclusively qualitative, as these foods may or may not be contaminated by different types of pesticides.

Consumption of food containing OPs is nevertheless a potential source of human exposure. In a recently published study, daily dietary exposure to chlorpyrifos, diazinon, and malathion in 1990 was evaluated in approximately 120,000 adults in the United States (23). Women's exposure to chlorpyrifos, diazinon, and malathion was GM 0.8 (GSD 1.47), and a range of 0.12–5.6 µg/day; GM 0.5 (GSD 1.36), and a range of 0.1–2.0 µg/day; and GM 4.7 (GSD 1.77), and a range of 0.15–50.8 µg/day, respectively. The same values for men were GM 0.8 (GSD 1.51), and a range of 0.03–6.0 µg/day; GM 0.5 (GSD 1.39), and a range of 0.02–2.7 µg/day; and GM 5.2 (GSD 1.80), and a range of 0.03–56.9 µg/day, respectively. Although exposure was similar in males and females, it varied substantially from person to person. This underlines the importance of

examining range of exposure when considering risk to public health due to contaminants in food. The uncertainty of the values was only calculated for malathion (CV% = 49%); it could not be evaluated for the other two pesticides because many samples were below detection limits. There do not seem to be any similar studies in children. The results of the present study showed that when one meal a day was eaten at the school mensa, where organically grown plant products were served, urinary excretion of the six alkylphosphates was not affected (Tables 4 and 5).

Other types of exposure may be greater for children than for adults. For example, children may be more exposed than adults to pesticide residues in the house because they play on the floor and put things in their mouths (oral nondietary and cutaneous exposure). Pesticides may be present in house dust (e.g., due to the use of pesticides in the house or in the garden), on dirt brought into the house on shoes or by pets, or on cut flowers and ornamental plants. The results of the present study confirm that pest control operations performed inside or outside the house in the preceding month and the presence of a vegetable garden near the house affect urinary excretion of methyl alkylphosphates in a significant manner. Information from the questionnaire does not indicate the use of OP insecticides at houses with gardens. However, houses with gardens are usually associated with other houses with gardens where OP pesticides could be used. These compounds are often used in gardens with flowers such as roses.

Pesticides are used for domestic purposes in the United States in approximately 90% of homes (24). One of the most widely used products is chlorpyrifos, which has replaced compounds such as aldrin, dieldrin, and chlordane. The main use is against termites,

and in many cases, spraying is carried out by residents of the homes. Two apartments were evaluated for the accumulation of chlorpyrifos on toys after the safety period. The compound distributes in two phases and may accumulate on toys and other surfaces such as pillows, and may be a considerable source of exposure, for 2 weeks after application. The total nondietary dose of chlorpyrifos may reach 208 µg/kg/day in children 3–6 years of age. Potential respiratory exposure was negligible, whereas dermal and oral nondietary doses were 39 and 61% of the total dose. For children who often put their fingers in their mouths, the daily nondietary dose may reach 356 µg/kg/day.

A study carried out in the state of Washington (25) studied whether children between 1 and 6 years of age who belonged to farming families and who lived in farming areas were more exposed to pesticides than children whose parents did not work in agriculture and who did not live in farming areas (reference families). House dust and soil samples were obtained where the children played. The samples were analyzed for four OPs commonly used on fruit trees (azinphos-methyl, chlorpyrifos, parathion, and phosmet). Pesticide concentrations were greater in house dust than in soil samples in all cases. Levels ranged from nondetectable to 930 ng/g in soil and from nondetectable to 17,000 ng/g in dust of houses near orchards or in which the parents worked in agriculture. All four compounds were detectable in 62% of dust samples and two-thirds of the houses near orchards contained at least one insecticide at concentrations > 1,000 ng/g. Residues were detected less often in reference houses, and concentrations were always < 1,000 ng/g. These results showed that children from farming families have a higher potential exposure than children from nonfarming families. Azinphos-methyl, which is only registered for use in agriculture, was found regularly in the samples, suggesting widespread exposure (25).

Based on urinary concentrations of alkylphosphates, it is difficult to estimate the daily dose of OPs to which the children were exposed because the same metabolites may be derived from hydrolytic cleavage of various compounds, which may have very different physicochemical, toxicologic, and metabolic characteristics, although they are all phosphoric esters. The problem is further complicated by the fact that absorption may also be due to cutaneous, oral, and respi-ratory exposure. Measures of urinary alkyl-phosphates can therefore only be used as a qualitative indication of exposure to OPs (26).

The present results seem quite significant. Our statistical sample was probably rather small to evaluate all of the variables considered. Moreover, the classes considered for each variable consisted of a different number of samples, which may also partially reduce the validity of the significance levels. It seems worthwhile to extend the study to the whole Italian population for confirmation of our findings and to detect differences between different areas.

In conclusion, the presence of these metabolites in the biologic fluids of adults and children is an excellent indicator of widespread environmental contamination and is more sensitive than evaluations of contamination of environmental matrices (air, water, food, drinks, etc.). In fact, analysis of environmental matrices and food sometimes provides results below detection limits, leading to the erroneous conclusion that these substances are not present in the environment and are therefore not dangerous for humans. The fact that detection limits are not reached for single matrices does not mean that OPs are absent or that they may not occur in increasing concentrations in living organisms at progressively higher positions in the food chain. Because humans do not have a direct relation with a single matrix, rather with all environmental compartments, they may act as concentrator-accumulators. Humans can therefore be regarded as one of the best indicators of diffuse contamination.

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