Longitudinal Investigation of Dietary Exposure to Selected Pesticides

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Between September 1995 and September 1996, 4-day composite duplicate plate samples (379 solid food samples and 303 beverage samples) were obtained from a stratified random sample of 75 individuals in Maryland and analyzed for the presence of 10 pesticides. Samples were collected in each of six approximately equally spaced cycles as part of a larger pilot investigation of longitudinal exposure to pesticides and other elements. Chlorpyrifos was detected in 38.3% of the solid food samples, malathion in 75.2%, and *p,p´*-DDE in 21.4%. Other pesticides were detected in less than 10% of the solid food samples. Pesticide residues were not detected in duplicate beverage samples. In solid food samples, the mean concentration of chlorpyrifos was 0.7 (SD 1.7) µg/kg, 1.8 (2.1) for malathion, and 0.2 (0.6) for *p,p´*-DDE. The detection rate and mean concentration of chlorpyrifos, malathion, and *p,p´*-DDE varied by a factor of 2–3 among sampling cycles and significantly according to results from several statistical analyses. Co-occurrence of chlorpyrifos and malathion in solid food samples was found relatively frequently and also varied with time. Pesticides were detected in food samples with greatest frequency in spring and summer months and with lowest frequency in winter months. These results support the hypothesis that 4-day average exposure to chlorpyrifos and malathion varies over time for this population mean and for individual members of the population and that correlation between exposures to these two organophosphate pesticides can occur. The measurements of pesticide levels in duplicate plate samples presented here can be used to evaluate and set parameters for dietary exposure models.
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Passed into law in 1996, the U.S. Food Quality Protection Act (FQPA) requires a more comprehensive assessment than ever before of pesticide exposure, dose, and effects (*1,2*). In particular, the FQPA requires pesticide risk assessments to consider exposure to potentially sensitive subgroups in the population, coincident dietary and nondietary (i.e., aggregate) exposure, and contemporaneous multichemical (i.e., cumulative) exposure. These issues also are important to epidemiological studies designed to evaluate the associations between selected human health outcomes and pesticide exposure (*3–5*).

Traditional dietary exposure assessments for pesticides are based on food consumption data from population-based surveys and pesticide levels observed in food samples collected for surveillance monitoring or in a market-basket design (*6–13*). The utility of this approach is limited by incomplete information on the accuracy of the market-basket methodology, interindividual variability of dietary pesticide exposure, temporal aspects of dietary pesticide exposure, and cumulative pesticide exposure through food. In this paper, we present the results of an investigation of these issues for seven organochlorine insecticides, two organophosphorous insecticides, and one triazine herbicide. The objectives of the study were to *a*) determine pesticide levels in short-term composite food samples; *b*) evaluate variability in pesticide occurrence and levels by time of year; *c*) evaluate variability in pesticide occurrence and levels among individuals; and *d*) describe co-occurrence of multiple pesticides in short-term food samples. The data presented here are the product of a pilot investigation of temporal variation in human exposure to selected contaminants in multiple media—the National Human Exposure Assessment Survey in Maryland (NHEXAS–Maryland).

Methodology

Study population. A stratified probability sample of 80 individuals older than 10 years of age was selected from four contiguous counties in Maryland that compose the Baltimore metropolitan statistical area. The sampling strategy was designed to ensure adequate representation of urban, suburban, and rural residences as well as the racial diversity of the metropolitan Baltimore area. An additional contiguous county, Talbot County, was included to ensure adequate representation of the rural stratum. Details of the sampling strategy are reported elsewhere (*14*).

All participants provided informed consent under protocols approved by an institutional review board. Demographic characteristics of the study population are summarized elsewhere (*15*). Each individual participated in as many as six 1-week monitoring periods or cycles approximately equally spaced over 12 months. Cycles 1–6 correspond to 21 September–23 December 1995; 15 January–23 February 1996; 27 February–20 April 1996; 22 April–15 June 1996; 18 June–27 July 1996; and 30 July–18 September 1996, respectively. Field staff collected samples of environmental and biological media, including solid food and beverages, during a consecutive 7-day period within a cycle. Participants completed exposure-related questionnaires during each cycle as well.

Duplicate plate collection and analysis. Participants were requested to prepare a duplicate portion of meals consumed on 4 consecutive days during each sampling cycle. Participants were compensated to offset food costs and to provide an incentive. Duplicate portions were placed in precleaned, leakproof, 1-gallon high-density polyethylene containers. Beverages were collected and stored separate from solid food samples. Commencing with cycle 2, the weight of each 4-day solid food and beverage sample was recorded by a field technician. The samples were placed in Polyfoam packers with blue-ice and shipped overnight to a U.S. Food and Drug Administration (FDA) laboratory in Kansas City, Missouri.

Samples were homogenized and analyzed for 10 pesticides following established methods (*16*). The target pesticides were selected to represent three classes of pesticides: triazine herbicides, organophosphorus insecticides, and organochlorine insecticides. Briefly, samples were organic solvent extracted, cleaned up with Florisil (U.S. Silica, Berkeley Springs, WV), and analyzed

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by gas–liquid chromatography with flame photometric, electron capture, or electrolytic conductivity detection. For all samples containing a detectable amount of target analyte, the presence of the pesticide was confirmed using an auxiliary analytical technique as specified in the analytical method.

Quality assurance. We performed numerous quality assurance steps for concentration data of the target pesticides to ensure traceability and accuracy of the data. A chain of custody (COC) form followed each sample and questionnaire from the field to the laboratory and finally to the database manager. A food or beverage sample data point not accompanied by a completed COC was omitted from subsequent analysis. In the laboratory, we analyzed reagent blanks for the presence of target pesticides, and we determined detection limits and recovery efficiencies over the course of the investigation. Detection limits (DL), as follows, were identical in food and beverage samples and did not vary over the course of the study: atrazine, 0.17 µg/kg; *cis*-chlordane, *trans*-chlordane, dieldrin, and heptachlor, 0.05 µg/kg; chlorpyrifos and malathion, 0.1 µg/kg; *p,p´*-DDD, *p,p´*-DDE, and *p,p´*-DDT, 0.07 µg/kg. Recovery efficiency as determined by fortified samples (previously analyzed samples spiked with a known amount of analyte to a concentration in the range of 4.4–35.2 µg/kg and reanalyzed) approximated 100% for each pesticide and did not vary substantially or significantly among sampling cycles according to an analysis of variance test. The exception to the lack of intercycle variation was dieldrin in solid food samples, for which between cycle variability was marginally significant (p = 0.0433). However, the range of recoveries for dieldrin in solid food was relatively small (79.3–97.2%) (Table 1). Field blanks and replicate samples were not obtained.

Data analysis. To evaluate temporal variation in the detection rate of the pesticides, we restricted data analysis to observations from those individuals who participated in more than one cycle. An observation in the data set contained the DL and concentration of each pesticide in a duplicate plate sample (micrograms of analyte per kilogram of sample) and average daily mass of the duplicate plate (kilograms of sample per day). We computed average daily exposure to a pesticide (micrograms of analyte per day) as the product of pesticide concentration and mass of the duplicate plate. Concentrations of pesticides not detected in samples were assumed to be zero.

We determined statistical weights through reflection of the sampling design with appropriate weights reflecting differential probability of selection from the initial population for each stratum. Specific weights for each participant and cycle combination can be obtained from the authors. We generated population-weighted descriptive statistics for the pesticide concentrations in diet samples and associated exposure for each pesticide overall and for each sampling cycle. Mean pesticide concentrations and exposures across sampling cycles were calculated for each participant to estimate prolonged average concentrations in food and dietary exposures. All means reported and analyzed here are population-weighted arithmetic means.

For analytes found in more than 60% of the duplicate plate samples, we used a mixed generalized linear model (GLM) to test for significant variability of population-weighted mean pesticide detection frequency (binary: 0 = not detected; 1 = detected), concentration (micrograms per kilogram), and average daily exposure (micrograms per day) among sampling cycles (*17*). Also for those analytes, we used a two-way GLM to test for significant interindividual variability for each exposure metric controlling for the effect of sampling cycle. For pesticides found in less than 60% but in more than 20% of the samples, we tested significant intercycle and interindividual variability using the nonparametric Kruskal-Wallis (K-W) procedure. For this group of pesticides, we also used logistic regression to evaluate temporal variability in the rate of pesticide detection. For this analysis the detection rate in each cycle was compared to that in cycle 1:

$$\text{logit } X = \beta_1 + \beta_2 \text{ cycle } 2 + \beta_3 \text{ cycle } 3 + \beta_4 \text{ cycle } 4 + \beta_5 \text{ cycle } 5 + \beta_6 \text{ cycle } 6, \quad [1]$$

where X stands for logit of pesticide detection and the variables cycle N are dummy variables that are equal to 1 if the observation is in that cycle and 0 otherwise. No statistical tests were performed on data for analytes detected in fewer than 20% of the duplicate plate samples.

Cumulative exposure is defined as joint exposure to more than one substance with the same toxicological mechanism of action, and has received particular attention with regard to organophosphorus insecticides (*18*). Cumulative exposure to pesticides in this set of data was assessed as the frequency of duplicate plate samples that contained more than one pesticide. We used Spearman correlation analysis to describe the relationship between pesticide concentrations measured in the samples.

Table 1. Recovery efficiency (%) for pesticides in fortified duplicate solid food and beverage samples.

Pesticide	Fortified concentration[a] (µg/kg)	Cycle 1	2	3	4	5	6	All	*p*-Value[b]
Solid food samples (*n*)		9	6	7	5	6	6	39	
Atrazine	15.9–35.2	87.9	96.5	102.0	101.8	114.3	109.8	101.0	0.1626
cis-Chlordane	4.7–8.2	83.9	91.3	88.7	94.0	89.7	93.7	89.6	0.3999
trans-Chlordane	5.2–8.7	86.9	92.5	88.9	96.8	93.2	92.5	91.2	0.6066
Chlorpyrifos	10.9–18.6	91.7	90.2	84.7	85.2	95.8	87.8	89.4	0.5744
Dieldrin	4.9–8.7	79.3	82.7	80.3	97.2	93.5	91.0	86.3	0.0433
Heptachlor	4.6–8.2	76.4	89.7	82.4	88.6	86.5	84.2	83.8	0.0938
Malathion	10.5–19.9	83.9	73.3	77.3	78.2	91.0	87.8	82.1	0.1252
p,p´-DDD	6.9–12.0	84.0	93.5	89.4	93.2	101.0	94.7	91.9	0.1128
p,p´-DDE	6.5–16.2	91.1	108.3	100.1	105.6	105.5	110.8	102.5	0.1283
p,p´-DDT	9.5–16.0	83.4	89.7	83.4	91.4	90.8	91.7	87.8	0.4371
Beverage samples (*n*)		7	5	5	1	3	4	25	
Atrazine	16.0–32.9	91.1	113.8	114.8	90	105.7	112.5	105.5	0.0614
cis-Chlordane	5.3–7.9	87.8	84.0	84.0	73	89.3	86.0	85.6	0.7252
trans-Chlordane	5.5–7.6	88.4	85.0	88.2	76	89.3	86.5	87.0	0.7976
Chlorpyrifos	10.3–15.8	88.1	80.8	80.8	79	89.0	84.8	84.4	0.6489
Dieldrin	4.4–14.9	79.5	80.8	90.2	73	90.7	94.5	85.4	0.0930
Heptachlor	4.5–7.2	76.1	84.0	83.8	68	80.7	78.3	79.8	0.4387
Malathion	9.0–16.2	86.5	76.4	71.2	80	83.3	81.3	80.0	0.2098
p,p´-DDD	7.2–11.7	89.8	97.8	85.8	77	87.3	95.0	90.6	0.4738
p,p´-DDE	7.5–12.7	90.0	109.4	92.8	75	99.0	98.8	96.3	0.0541
p,p´-DDT	10.5–15.8	89.1	85.8	81.6	77	85.7	87.0	85.7	0.7641

[a]Range of concentration resulting from addition of standard to duplicate plate samples. [b]*p*-Value for general linear model test of significant variabilty of recovery among cycles.

Table 2. Demographic characteristics of NHEXAS–Maryland study population from whom dietary pesticide data were obtained.

Factor/level	Frequency	Percent
Sex		
Female	48	64.0
Male	27	36.0
Age (years)		
< 25	6	8.0
25–44	32	42.7
45–64	30	40.0
> 64	7	9.3
Race		
African American	14	18.7
Asian/Pacific Islander	1	1.3
Caucasian	60	80.0

Results

The final data set comprised 379 duplicate solid food samples from 75 individuals (Table 2). The distribution of observations among sampling cycles was 75, 69, 68, 61, 47, and 59 samples for cycles 1–6, respectively. Thirty-five individuals provided a duplicate solid food sample in all 6 cycles, 18 in 5 cycles, 14 in 4, 7 in 3 cycles, and 1 in 2 cycles. As discussed later, secondary data analyses indicated that the dropout apparent from the cycle-specific participation rates did not influence our findings in a meaningful way.

We obtained 303 duplicate beverage samples from 75 individuals and analyzed them for the target pesticides. One sample obtained in the first cycle contained *p,p´*-DDE at an estimated level of 0.6 µg/kg. Pesticides were not detected in the remaining beverage samples. The low detection rate for pesticides in beverages is consistent with findings from other studies (*19*).

Temporal variation of pesticides in solid food samples. Distributions of pesticide concentration that were observed in duplicate solid food samples are summarized in Table 3. Chlorpyrifos was detected in 38.3% of the samples, malathion in 75.2%, and *p,p´*-DDE in 21.4%. Each of the seven other pesticides was found in less than 10% of the samples. Cycle-specific occurrence frequency ranged over a factor of approximately 2 for chlorpyrifos, 1.5 for malathion, and 3 for *p,p´*-DDE (Table 4). For each of these pesticides, cycle-specific detection frequency varied significantly according to the mixed GLM (Table 4) and the K-W and two-way GLM procedures (not shown in Table 4). By the mixed GLM analysis, detection of chlorpyrifos and malathion was significantly ($p < 0.04$) greater in cycles 3 and 4, corresponding to March through mid-June 1996, than in the other sampling cycles. In the logistic regression analysis, chlorpyrifos was detected more frequently in cycle 3 than in cycle 1 [odds ratio (OR) = 2.9, $p = 0.0032$]. The occurrence of malathion was significantly greater in cycles 3 (OR = 5.8, $p = 0.0002$) and 4 (OR = 13.9, $p < 0.0001$) than in cycle 1 in the logistic regression analysis. The frequency of *p,p´*-DDE detection was significantly greater in cycle 3 than in cycles 2, 4, and 5 according to the mixed GLM and greater than in cycle 1 (OR = 2.7, $p = 0.0086$) for the logistic regression.

Mean (SD) concentrations for chlorpyrifos, malathion, and *p,p´*-DDE computed from all 379 observations made over the entire study were 0.7 µg/kg (1.7 µg/kg), 1.8 (2.1), and 0.2 (0.6), respectively. Mean cycle-specific concentrations of malathion ranged from 1.4 to 2.4 µg/kg among cycles (Table 4), and the intercycle variation was significant ($p = 0.0198$) according to the mixed GLM analysis. For the K-W analyses, median concentrations varied significantly among cycles for chlorpyrifos ($p = 0.0326$), malathion ($p = 0.0045$), and *p,p´*-DDE ($p = 0.0087$).

Summary statistics for the mass of duplicate solid food samples and dietary exposure (micrograms per day) to chlorpyrifos, malathion, and *p,p´*-DDE for each cycle are shown in Table 5. Because the weight of food samples was not measured in cycle 1 and was also not measured for approximately 8% of food samples in other cycles, we obtained only 279 measures of dietary exposure. Median exposure to chlorpyrifos ($p = 0.0106$) and *p,p´*-DDE ($p = 0.0188$) varied significantly among cycles when assessed using the K-W procedure. For malathion, exposures did not vary among cycles according to the K-W analysis ($p = 0.2055$), but did vary significantly ($p = 0.0182$) in the mixed-model analysis that controlled for the effect of interindividual variability.

Interindividual variation for pesticides in solid food samples. Chlorpyrifos, malathion, and *p,p´*-DDE were detected in at least one duplicate solid food sample obtained from

Table 3. Population-weighted detection rates and quantiles of pesticide concentrations (µg/kg) in duplicate solid food samples ($n = 379$) collected from 75 individuals in Maryland, September 1995–September 1996.

Pesticide	% Detected	50%	75%	90%	95%	99%	Maximum
Atrazine	0.0	0.0	0.0	0.0	0.0	0.0	0.0
cis-Chlordane	1.6	0.0	0.0	0.0	0.0	0.3	0.6
trans-Chlordane	2.1	0.0	0.0	0.0	0.0	0.5	1.5
Chlorpyrifos	38.3	0.0	0.8	1.8	2.9	7.7	24.3
Dieldrin	6.9	0.0	0.0	0.0	0.3	0.7	1.7
Heptachlor	4.5	0.0	0.0	0.0	0.0	1.7	6.6
Malathion	75.2	1.3	2.3	4.4	5.9	12.4	16.5
p,p´-DDD	0.3	0.0	0.0	0.0	0.0	0.0	1.0
p,p´-DDE	21.4	0.0	0.0	0.7	1.0	2.5	5.8
p,p´-DDT	0.3	0.0	0.0	0.0	0.0	0.0	1.0

Table 4. Population-weighted descriptive statistics for pesticide concentrations (µg/kg) in duplicate solid food samples for each cycle.

		Cycle						Prolonged
		1	2	3	4	5	6	average
Pesticide	Measure	($n = 75$)	($n = 69$)	($n = 68$)	($n = 61$)	($n = 47$)	($n = 59$)	($n = 75$)[a]
Chlorpyrifos	% Detected	34.7	27.5	47.1	57.4	36.2	27.1	79.7
($p < 0.0001$)	Median	0.0	0.0	0.0	0.4	0.0	0.0	0.4
	Mean	0.9	0.5	0.4	0.9	0.9	0.5	0.8
	SD	2.1	1.8	0.7	1.5	2.5	1.2	1.0
Malathion	% Detected	61.3	68.1	85.3	93.4	74.5	71.2	98.7
($p < 0.0001$)	Median	1.0	1.3	1.6	2.0	1.3	1.3	1.6
	Mean	1.5	1.7	2.0	2.4	1.9	1.4	1.9
	SD	2.4	1.8	2.2	2.4	2.1	1.5	1.3
p,p´-DDE	% Detected	22.7	17.4	35.3	11.5	10.6	27.1	64.0
($p = 0.0017$)	Median	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	Mean	0.2	0.2	0.4	0.1	0.2	0.2	0.2
	SD	0.4	0.3	0.8	0.6	0.8	0.4	0.3

n, number of observations. Result (*p*-value) of the mixed model test of intercycle variability of occurrence is shown below the label for each pesticide.
[a]Values in this column refer to average values for each individual in the study; % detected in this column refers to the fraction of individuals with at least one measurable residue concentration.

Table 5. Population-weighted descriptive statistics for food weight (kg) and pesticide exposure (µg/day) in duplicate solid food samples for each cycle.

		Cycle					Prolonged
		2	3	4	5	6	average
Analyte	Measure	($n = 64$)	($n = 59$)	($n = 57$)	($n = 40$)	($n = 59$)	($n = 74$)[a]
Food Weight	Median	0.72	0.74	0.68	0.63	0.63	0.67
	Mean	0.75	0.72	0.68	0.66	0.63	0.72
	SD	0.32	0.29	0.26	0.28	0.26	0.24
Chlorpyrifos	Median	0.0	0.0	0.2	0.0	0.0	0.3
	Mean	0.5	0.4	0.6	0.5	0.3	0.5
	SD	1.7	0.7	1.1	1.5	0.6	0.9
Malathion	Median	1.1	1.0	1.2	0.9	0.9	1.1
	Mean	1.2	1.4	1.4	1.3	0.9	1.3
	SD	1.2	1.6	1.1	1.7	1.4	1.0
p,p´-DDE	Median	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	0.1	0.4	0.1	0.2	0.1	0.2
	SD	0.3	1.2	0.2	0.8	0.3	0.3

n, number of observations. For cycle 1, $n = 0$.
[a]Values in this column refer to average values for each individual in the study.

most of the 75 study participants (Figure 1). Four individuals had measurable concentrations of chlorpyrifos in all five duplicate solid food samples, and 18 individuals had measurable quantities of malathion for all five duplicate plate samples. No individual had measurable quantities of *p,p´*-DDE in all five samples. According to the K-W procedure, pesticide occurrence in solid food samples varied significantly among individuals for chlorpyrifos ($p < 0.0001$), marginally significantly for *p,p´*-DDE ($p = 0.0848$), and did not vary significantly ($p = 0.2428$) for malathion. In contrast, mean and median malathion concentrations varied significantly among individuals according to the two-way GLM ($p = 0.0158$) and the K-W analysis ($p = 0.0375$).

Cumulative exposure. The frequency of joint occurrence of chlorpyrifos, malathion, and *p´p´*-DDE in duplicate solid food samples is summarized in Table 6. The combination of chlorpyrifos and malathion occurred the most frequently (134/379 samples) overall. The frequency of chlorpyrifos and malathion co-occurrence varied significantly among cycles according to the mixed GLM analysis, with the greatest frequency in cycle 4. Concentrations of chlorpyrifos and malathion in a sample were weakly correlated when examined overall, and exhibited little correlation when examined by cycle (Table 4). We obtained similar results for combinations of chlorpyrifos and malathion with *p,p´*-DDE.

Discussion

Several investigations have been conducted of exposure to pesticides via solid food ingestion. Based on a food consumption survey of the adult population (age 25–60 years) in Basque Country (Spain), total diet samples were obtained and analyzed for the presence of different contaminants and nutrients (*9*). Among organochlorine pesticides *p,p´*-DDE was detected most frequently, being found in 20.65% of the food samples, with estimated mean intake of 0.9 µg/day (nondetects set to 0 µg/day) and maximum intake 3.5 µg/day. In a similar study, food samples representing the major dietary foods were collected randomly from 3 markets in Hsinchu, Taiwan (*13*), and *p,p´*-DDE was detected in 18% of food samples. The average *p,p´*-DDE concentration was found to be 0.71 µg/kg food (SD = 0.21 µg/kg). In the NHEXAS–Maryland study, *p,p´*-DDE was detected in 21.4% of the duplicate plate samples, which is comparable to the detection rate in Basque Country and Hsinchu. However, the mean pesticide intake (0.2 µg/day) and mean pesticide concentration (0.2 µg/kg) were lower than those found in the other studies. Caution should be exercised when comparing values across studies because of possible differences in application rates, dates of deregistration, food intakes, degree of food preparation, analytical methods, and other study protocols (*9*). Measurements of organophosphate pesticides may be biased low in these food measurements due to the potential hydrolysis of the phosphate ester either through chemical or biochemical processes.

The Total Diet Study (TDS) is a national market-basket survey carried out annually by the FDA (*12,20*). The survey is used to assess the population's intake of pesticides, radionuclides, and various chemicals and nutrients. Based on the 1986–1991 TDS, the estimated pesticide intakes for a typical U.S. adult were 0.3, 5.5, and 1 µg/day, for chlorpyrifos, malathion, and *p,p´*-DDE, respectively (*12*). The estimated pesticide intakes in the NHEXAS study (0.4, 1.3, and 0.2 µg/day for chlorpyrifos, malathion, and *p,p´*-DDE, respectively) are lower than those observed in the TDS, except for chlorpyrifos. Differences in the values observed may be due to the differences in the market-basket and duplicate-plate approaches, number of foods analyzed, timing of the studies, and analytical methods (*21*).

Daily intakes of these pesticides may also be compared to published levels of acceptable or safe exposure. Acceptable daily

Table 6. Relative frequency of joint pesticide occurrence and Spearman correlation (*r*) between pesticide concentrations (µg/kg) in duplicate solid food samples for each cycle and overall.

		Cycle						
Pesticide 1	Pesticide 2	1 ($n = 75$)	2 ($n = 69$)	3 ($n = 68$)	4 ($n = 61$)	5 ($n = 47$)	6 ($n = 59$)	Overall ($n = 379$)
Chlorpyrifos	Malathion	27.6%	26.8%	41.6%	61.4%	39.3%	18.5%	35.4%
($p < 0.0001$)	*r*	0.27**	0.21*	–0.04	0.10	0.20	0.12	0.17***
Chlorpyrifos	*p,p´*-DDE	8.4%	7.9%	17.7%	6.6%	1.5%	9.1%	8.9%
($p < 0.0004$)	*r*	0.03	–0.03	0.02	0.08	0.05	0.15	0.04
Malathion	*p,p´*-DDE	14.9%	19.2%	39.6%	10.0%	6.4%	18.4%	18.8%
($p < 0.0016$)	*r*	0.12	0.16	0.14	0.11	–0.02	0.28**	0.13***

n, number of observations. Result (*p*-value) for significance test of intercycle variability of joint occurrence is shown below the label for each pesticide.
Asterisks indicate *p*-value from significance test for the hypothesis that $r = 0$. *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$.

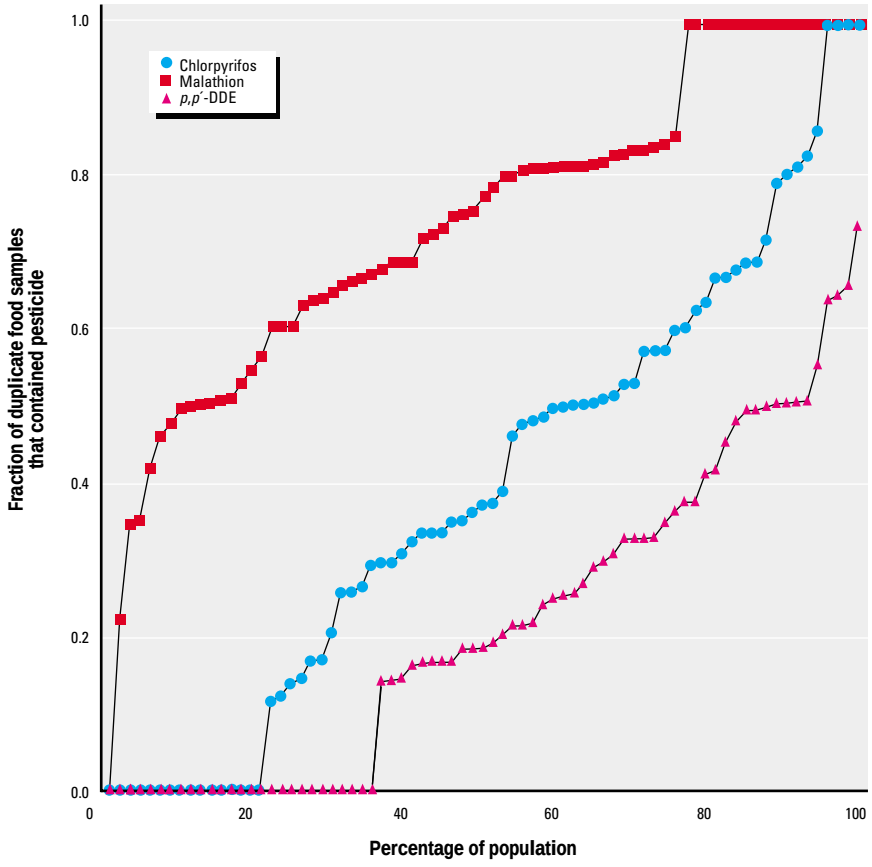

Figure 1. Population-weighted distribution for fraction of duplicate solid food samples per person found to contain a pesticide.

intakes (ADI) are established by experts working with the United Nations Food and Agricultural Organization and the World Health Organization and represent the maximum amount of pesticides and other chemicals that can be ingested daily without causing adverse effects (*22*). The ADI values for chlorpyrifos, malathion, and total DDT (*p,p´*-DDE > 95%) are 10, 20, and 20 µg/kg body weight/day, respectively (*12*). The U.S. Environmental Protection Agency (U.S. EPA) establishes oral reference doses (RfD) that are an estimate of daily oral intake over a lifetime that is unlikely to increase the risk of adverse effects in the human population, including those in sensitive subgroups (*23*). The RfDs for chlorpyrifos and malathion are 3 and 20 µg/kg/day, respectively. Based on measured weights of the duplicate solid food samples and body weight self-reported by NHEXAS–Maryland participants, mean (maximum) body-weight adjusted exposures were 6.8×10^{-3} (0.2), 1.8×10^{-2} (0.2), and 2.0×10^{-3} (7.2×10^{-2}) µg/kg/day for chlorpyrifos, malathion, and *p,p´*-DDE, respectively. Thus, exposures were below the corresponding ADI and RfD values.

The goal of this portion of the NHEXAS–Maryland study was to investigate temporal variation in dietary exposure to pesticides. We observed significant variation in the frequency of detection, concentrations, and exposures to chlorpyrifos and malathion among sampling cycles. Detection frequency, concentration, and exposure were greatest in cycles 3 (27 February–20 April) and 4 (22 April–15 June). Seasonal variation of pesticide occurrence in environmental media was observed in other studies and may reflect increased pesticide application during spring and summer months in response to increased activity of pests and vulnerability of agricultural commodities (*24–26*). Other factors that could explain temporal variation in dietary exposure to pesticides include periodic changes in food consumption and sources of food by season.

As described elsewhere (*15*), levels of 3,5,6-trichloro-2-pyridinol (TCP) in urine obtained from the NHEXAS–Maryland study participants, the major biological metabolite of chlorpyrifos found in urine, also varied across cycles. Geometric mean urinary TCP concentrations were significantly ($p < 0.0001$) greater in the spring and summer than in the fall or winter (*15*). This finding was consistent with other studies where concentrations of metabolites of nonpersistent pesticides were hypothesized to be greatest in summer months due to a higher rate of pesticide use (*26*). Additional research is needed to ascertain the relationship between biological markers of chlorpyrifos exposure and intake via food and other media.

Detection of *p,p´*-DDE varied by a factor of 3 among cycle—surprisingly, because it is a persistent metabolite of DDT, which is no longer in use in the United States. Occurrence of *p,p´*-DDE did not exhibit an apparent seasonal dependence, as did chlorpyrifos and malathion. Temporal variation in occurrence of this organochlorine compound in duplicate plate samples may reflect changes over time in abundance of imports in the U.S. food supply, food consumption patterns, or a combination of factors yet to be identified.

Different organophosphorus (OP) insecticides exhibit a similar toxicological mechanism in mammals (*27*). They bind with and consequently inhibit the ability of enzymes such as acetylcholinesterase to stop the synaptic transmission of electrical impulses by neurotransmitters such as acetylcholine. Thirty-five percent of the 379 food samples found in this study contained measurable quantities of two OP substances—chlorpyrifos and malathion. In addition, the incidence of such cumulative exposure varied across the year. The detection frequency in spring and summer months was 2–3 times the frequency in winter months (Table 6). Note that the samples analyzed in this study are composites of 4 consecutive days of food consumption. Thus, the results reflect joint exposure within the span of 4 days and provide little information about coincident exposure on a shorter time scale. Nevertheless, the data indicate that cumulative dietary exposure to chlorpyrifos and malathion may occur within a toxicologically relevant period of time given their biological half-lives of less than 3 days (*28–30*). In future analyses of data from this investigation, we will explore cumulative exposure to OP compounds in multiple media including indoor air, settled dust, soil, and drinking water.

Temporal variability of occurrence and concentrations of chlorpyrifos, malathion, and *p,p´*-DDE (single and cumulative) was explored more fully by fitting the models described earlier to the 210 observations obtained from the 35 subjects who participated in all 6 sampling cycles—i.e., a complete, year-long, balanced data set. Descriptive statistics of pesticide occurrence and concentrations in the reduced data set were nearly identical to those in the full, unbalanced data set. Results for tests of significant variability among cycles were consistent with results from the full data set, with one exception. In the restricted data set, *p,p´*-DDE occurrence did not vary significantly ($p = 0.1665$) according to mixed GLM procedure. Results from the reduced data set should be interpreted with caution. The reduced sample size increases the standard error estimates by nearly a factor of 1.5 over those for the full data set. The loss of power due to the reduction in sample size may be reflected in increased *p*-values for effects. This is the most likely explanation for the apparent anomaly, because the cycle-specific point estimates of *p,p´*-DDE occurrence in the full and restricted data sets are nearly equal. In conclusion, we find no indication that analyses of the unbalanced data set influenced the findings regarding temporal variability of pesticide exposure in a meaningful way.

Several efforts are underway to construct reliable models for conducting aggregate and cumulative population-based assessments of pesticide exposure and risk (*31*). The data presented in this paper may be useful for setting parameters for these models or for evaluating model performance, particularly with regard to longitudinal exposure. For example, information is presented that can be used to characterize the fraction of the modeled population that is exposed to chlorpyrifos or malathion in food on one or more occasions over a year (Figure 1). Similarly, the results can be used to establish parameters for cross-sectional frequency and magnitude of dietary exposure as a function of time of year (Table 3). In addition, we found that body-weight adjusted exposure (micrograms per kilogram body weight per day) and unadjusted exposure (micrograms per day) are highly correlated. Spearman and Pearson correlation coefficients between body-weight adjusted and unadjusted exposures were > 0.95 and as high as 0.99 for chlopyrifos, malathion, and *p,p´*-DDE. In this population dividing by body weight introduced little reordering of exposure among individuals and little change in the relationship of exposure level among individuals when compared to exposure expressed without regard to body weight (i.e., as micrograms per day). Specifically, uncertainty about the distribution of body weight or the relationship between body weight and determinants of dietary pesticide exposure for persons between 12 and 84 years old (the age range in our study) is unlikely to be an important source of overall uncertainty for model predictions of dietary exposure to these pesticides.

Limitations of the NHEXAS–Maryland duplicate-plate pesticide results for modeling purposes include the 4-day integration period, the 8–10-week interval between collection of repeated samples from a single participant, and 1-year overall scope. As a result, the data contain little information about exposure on a per-serving, day-to-day, or year-to-year basis that may be important for evaluating pesticide safety and risks. Nevertheless, these data may be used to benchmark or evaluate models with time resolution equal to the temporal frequency and range of this study.

In future work, we will report analyses of correlations between the pesticide intakes described here, food consumption reported on the NHEXAS–Maryland diet questionnaire, and pesticide intake predicted from the diet records and residue levels measured in specific foods as part of national market-basket studies.

Conclusion

The results of this study demonstrate the feasibility, utility, and some of the limitations of duplicate plate methods for assessing dietary exposure to pesticides. Occurrence and concentrations of chlorpyrifos, malathion, and *p,p´*-DDE in 4-day composite solid food samples were shown to vary over time, whereas 4-day composite beverage samples were shown rarely to contain a target pesticide over the analytical detection limit. Co-occurrence of chlorpyrifos and malathion in solid food samples was found relatively frequently and also varied with time. Additional analysis of these and other NHEXAS–Maryland data is required to investigate aggregate or multiple media exposure to pesticides in this study population and the relationship between levels in environmental media and biological tissues. New field and laboratory investigations are required to address questions about short-term (e.g., day-to-day) and chronic (e.g., lifetime) dietary exposure to one or more pesticides and contemporaneous multimedia/multipesticide exposure.

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