JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Organophosphate Levels in Apple Composites and Individual Apples from a Treated Canadian Orchard

DOROTHEA F. K. RAWN,*,† SUE C. QUADE,† J. BRIAN SHIELDS,† GIACOMO CONCA,† WING-FUNG SUN,† GLADYS M. A. LACROIX,† MARK SMITH,‡ ANDRÉ FOUQUET,§ AND ANDRÉ BÉLANGER⊥

Food Research Division, Bureau of Chemical Safety, Health Products and Food Branch, Health Canada, 2203D Tunney's Pasture, Ottawa, ON, Canada K1A 0L2, Division of Statistics and Epidemiology, Bureau of Biostatistics and Computer Applications, Health Products and Food Branch, Health Canada, 2203E Tunney's Pasture, Ottawa, ON, Canada K1A 0L2, Food Directorate Regional Laboratory, Health Products and Food Branch, 1001 St-Laurent Street West, Longueuil, Quebec, Canada J4K 1C7, and Agriculture and Agri-Food Canada, 430 Gouin Boulevard, Saint Jean Sur Richelieu, Quebec, Canada J3B 3E6

Azinphos-methyl, phosalone, and phosmet were applied individually to separate rows of trees within a commercial apple orchard in Quebec, Canada, during the 2003 agricultural season. Apples were collected for residue analysis immediately prior to the harvesting of the remaining apples for market distribution and were prepared for analysis as both individual apples and as composites of eight individuals. Analysis of the three applied compounds, as well as five organophosphate insecticides that were not applied, was performed using gas chromatography−mass spectrometry. Azinphos-methyl, phosalone, and phosmet, which were applied, were detected in all samples analyzed at concentrations ranging from 0.004 ng/g to 2260 ng/g. Methidathion was not observed in any sample. Chlorpyrifos, diazinon, dimethoate, and malathion concentrations ranged from below method detection limits to 0.71 ng/g, and the detection frequency for these compounds ranged from 20% to 100%. Residues measured in this study were all below the Canadian maximum residue limit for apples. Variability factors ranged from 2 to 19 for all compounds observed in this study. Composite samples may not accurately reflect the extremes of exposure from consumption of single servings of apples.

KEYWORDS: Organophosphate; apples; composites; residues

INTRODUCTION

Organophosphate (OP) pesticides are used throughout the world for broad spectrum insect control (*1*). The mode of action of this class of pesticides is acetylcholinesterase (AChE) inhibition (*2*). Exposure to the OP insecticides can result in acute neurological dysfunction (*3*). In addition to target organisms, effects on nontarget species, including humans, have been observed (*4*, *5*).

OP insecticides are routinely applied to fruit crops (e.g., apple, peach, pear) that are subject to consumption as single servings. Consumption of individual fruits containing high OP insecticide residues may result in high exposure over a short period of time. Residue testing for the presence of pesticides, however, is routinely performed on composites of 5 to 10 individual or unit samples rather than samples of individual fruit (*6*−*8*). The use of data derived from composite samples may result in the underestimation of residues present in individual fruit because dilution may occur during compositing and, therefore, may not accurately represent exposure in single serving commodities (*9*).

Dietary exposure assessments for pesticides are performed internationally (*10*). In the past, acceptable daily intakes (ADI) have been used to assess dietary exposure to pesticides. This measure, however, is no longer considered to be the most appropriate for acutely acting compounds because the ADI is estimated for lifetime exposure (*8*). More recently, acute reference dose (ARfD) values have been used to determine short term dietary exposure and are of particular importance for those acutely acting compounds (e.g., OP insecticides). Dietary risk assessments continue to require accurate measurements of the residue levels in food commodities, in addition to the food consumption patterns and toxicity of the pesticide ingested (*11*).

Variability factors have been established to indicate how much residue concentrations measured in individual samples vary from levels observed in composite samples (*8*, *12*, *13*). Variability factors have been calculated by dividing the maximum pesticide concentration observed in an individual or unit

* Corresponding author. Phone: (613) 941-8462. Fax: (613) 941-4775. E-mail: thea_rawn@hc-sc.gc.ca.
† Food Research Division, Health Canada.
‡ Division of Statistics and Epidemiology, Health Canada.
§ Food Directorate Regional Laboratory.
⊥ Agriculture and Agri-Food Canada.

10.1021/jf052413v CCC: $33.50 Published 2006 by the American Chemical Society
Published on Web 02/07/2006

sample by the mean composite level (*13*). In recent years, studies to establish residue levels observed in individual samples relative to composite samples have been performed in a variety of crops (e.g., apples, carrots, oranges, potatoes, and tomatoes) (*14*−*17*). Residue data in unit samples of individual commodities are required to establish variability factors for comparison with default values currently being used (*18*). Recently, variability also has been estimated using the 97.5th percentile concentration data for individual samples, rather than maximum levels, where sufficient data exist (*18*, *19*).

The present study was performed to establish how well OP insecticide residue levels measured in composite samples represent concentrations in individual apples in order to improve exposure estimates of single serving foods. A field trial was performed to ensure that OP insecticides were applied at known rates, times, and following label practices. By sampling from a commercial orchard, where apples were harvested directly from trees, individual apples were not mixed with others during shipment and distribution. Additionally, intra-tree, inter-tree, and inter-row differences in residue levels could be established.

MATERIALS AND METHODS

Pesticide Application. OP insecticides were applied to three rows of 10 apple trees per row in part of an 18 ha commercial orchard in the province of Quebec, Canada, during the 2003 agricultural season. The distance between rows was approximately 9 m. Each row (10 trees) was treated with a different OP insecticide (azinphos-methyl, phosalone, or phosmet). Application was performed using an air blast sprayer following label practices. Azinphos-methyl was applied at a rate of 1.14 kg active ingredient (a.i.)/ha on 4 June using Guthion 50 WP, guaranteed to contain 50% azinphos-methyl. Phosalone, was applied 25 July at a rate of 1.5 kg a.i./ha with Zolone Flo Insecticide, guaranteed to contain 500 g a.i./L. Phosmet was applied at a rate of 2 kg a.i./ha on 18 August using Imidan Instapaks, guaranteed to contain 50% phosmet. Application dates corresponded to the pest control requirements in the orchard.

No additional OP insecticides were applied to the study rows throughout the growing season. In addition to the three study rows that were sprayed, azinphos-methyl also was applied to individual trees within the orchard as required and phosalone was used to treat insect pests on approximately half of the orchard. These applications did not include the study rows.

Sample Collection. Apples were collected after the preharvest interval was completed for all three compounds (**Table 1**). The sample design provided replicates among trees and within compartments. The trees in each row were generally McIntosh (*Malus domestica*, *Malus* 'McIntosh'), although Cortland (*Malus domestica*, *Malus* 'Cortland') trees were present in some rows.

For this study, 8 apples per tree at 2 apples from each of 4 of the 12 possible tree compartments (3 heights, 4 faces/sides) (**Figure 1**) were harvested from 6 apple trees selected at random from the McIntosh trees in an orchard row. The 4 tree compartments were selected following an incomplete block design with partial balancing of harvested apples from tree compartments among trees within an orchard row. Within an orchard row, this provided 2 full replicates of a 3×2^2 factorial arrangement (tree compartments) in 6 blocks (trees) with replicate apples from the same compartment, from a total sample of 48 apples per orchard row. Selection of McIntosh apple trees within an orchard row was randomized; sampling plans were assigned randomly to rows, and tree-by-tree sampling plans were assigned randomly to trees within a row.

Apples were picked on 9 September, placed into paper bags, labeled by tree number and position within tree, and taken to the laboratory for further processing. Samples were stored at 4 °C while being sorted and processed. Once samples had been prepared for extraction and analysis they were stored at − 80 °C. Apples also were collected from an orchard where no OP insecticides were applied throughout the agricultural season, for use in quality assurance testing as blank and spiked apple matrix. These apples were collected and prepared following the same protocol as study apples.

Table 1. Application Timing and Maximum Residue Limit (MRL) (ng/g) Values for the OP Insecticides in Apples

compd	preharvest interval (d)	application timing (days prior to sampling)	MRL (apples)[a] (ng/g)	max obsrvd residue relative to MRL (%)
azinphos-methyl	14–21	96	2000	0.22
chlorpyrifos	NA[b]	NA	1500	0.003
diazinon	NA	NA	750	0.094
dimethoate	NA	NA	2000	0.006
malathion	NA	NA	2000	0.008
methidathion	NA	NA	500	ND[c]
phosalone	30	45	5000	45.1
phosmet	1	21	10 000	14.7

[a] Government of Canada, 1998 (*20*). [b] Not applicable, compound not applied in orchard. [c] Not detected in any sample.

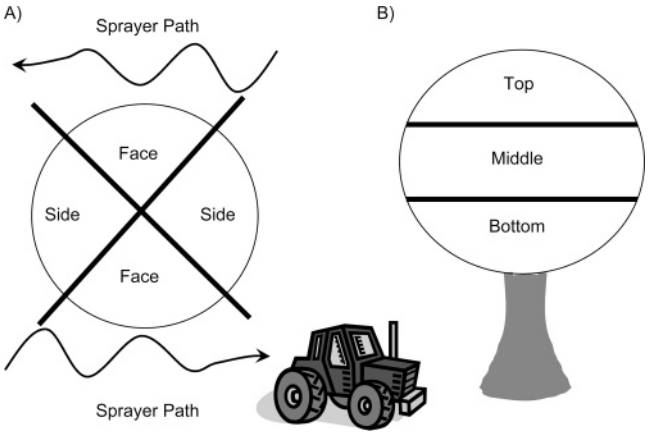

Figure 1. Compartmentalization of apple trees (**A**) around the circumference of the tree and (**B**) along the height of the tree.

Sample Preparation. Individual apples were cored and sliced into 10 equal segments using a corer/slicer retailed for domestic use. The first and alternate slices of each apple were taken, chopped manually using a knife, and placed in a plastic bag for storage at − 80 °C until extraction and analysis. The remaining segments of individual apples were retained in separate bags for preparation of composite samples.

Composites were constructed from the apples harvested from each orchard row by randomly selecting 8 apples for each of 6 composites, without regard to tree location (tree, tree compartment) from which the apple was harvested. Composite samples were prepared by thoroughly mixing the chopped apple pieces from the bags containing the retained portions of the individual apples. Composite samples were frozen until extraction and analysis.

Analytical samples were prepared by weighing 25 g aliquots of individual apples or composite samples into a 500 mL Erlenmeyer flask. Deuterated (d_{10}-) analogues of diazinon and malathion (Cambridge Isotope Laboratories, Andover, MA) were added to each sample as surrogate standards, prior to extraction using homogenization with 250 mL acetone:50 mL hexane. All extracts were filtered through glass wool into a separatory funnel, and 100 mL of saturated NaCl was added. After the mixture was shaken gently, the aqueous layer was removed. The aqueous layer was extracted with an additional 50 mL of hexane. The organic phases were combined, dried with anhydrous Na_2SO_4, and evaporated to near dryness. Sample extracts were dissolved in dichloromethane (DCM):cyclohexane (1:1), filtered through a 0.45 μm poly(tetrafluoroethylene) (PTFE) filter, and cleaned up using gel permeation chromatography (GPC) with 200−400 mesh SX-3 biobeads (O-I Analytical, College Station, TX) to remove pigments and other large molecular weight impurities. Extracts were reduced to 2 mL using a rotary evaporator and were further cleaned up using 6 g of Florisil (2% deactivated) and eluting with 70 mL of 60% DCM:hexane followed by 100 mL 15% acetone:hexane. Extracts were then concentrated with

a rotary evaporator and taken to a final volume of 1 mL in iso-octane. A 100 μL volume of $^{13}C_{12}$ PCB 101 (Cambridge Isotope Laboratories, Andover, MA) was added to each final extract as a performance standard.

Analysis. Analysis was performed using a Micromass Autospec-Ultima (Manchester, U.K.) coupled to an Agilent 6890 gas chromatograph (Mississauga, ON, Canada) equipped with an on-column injection system. A 30 m DB-5 fused silica column with 0.25 mm i.d. and 0.25 μm film thickness was used for gas chromatographic separation (J&W Scientific, Folsom, CA) with a 3 m × 0.53 mm retention gap (Chromatographic Specialties, Brockville, ON, Canada). The injector was set to track the oven temperature, which was initially at 80 °C and ramped at 8 °C/min to 240 °C and then taken to a final temperature of 280 °C at 15 °C/min, where it remained for 5 min. Injection volumes were 1 μL for all samples and standards. Helium was used as the carrier gas with a constant pressure of 150 kPa.

The electron energy was set to 70 eV, with a photomultiplier voltage of 350 V. The trap current was 600 μA, and the source temperature was 250 °C. The re-entrant temperature and capillary line temperature were maintained at 280 °C, and perfluorokerosene-L (PFK) was used as the reference substance for tuning at *m*/*z* 393. The mass resolution was set to between 3000 and 4000 for all analytes.

Quality Assurance/Quality Control. Blank apple matrix was prepared in a manner identical to experimental apples. With each set of samples extracted and analyzed, 25 g of the blank apple matrix and 25 g of the blank apple matrix spiked with analytes of interest (azinphos-methyl [1.9 ng/g], chlorpyrifos [0.83 ng/g], diazinon [0.83 ng/g], dimethoate [1.7 ng/g], malathion [1.6 ng/g], methidation [0.77 ng/g], phosalone [0.90 ng/g], phosmet [0.92 ng/g]) were included and processed as all other samples. Analytical standards of all native compounds were purchased from AccuStandard (New Haven, CT). Background levels of some analytes were detected in the blank matrix samples and were used for background subtraction in the determination of recovery from spiked matrix only. Residue concentrations were not blank corrected. Recoveries of all of the analytes from the spiked samples (n = 30), following blank subtraction, ranged from 84% (azinphos-methyl) to 113% (chlorpyrifos), with the exception of dimethoate, which had very poor recoveries (10%). The average d_{10}-diazinon and d_{10}-malathion recoveries were 85% and 107%, respectively.

Method detection limits (MDL) were established based on a 3:1 signal to baseline noise ratio and are reported as averages of individual chromatograms. MDLs ranged from 0.004 ng/g for chlorpyrifos to 0.022 ng/g for azinphos-methyl.

Statistical Analyses. Statistical analyses were performed to establish if differences between residue levels observed in individual samples and those in composite samples occurred. Additionally, residue levels were examined to establish if differences occurred within a given tree or among trees. Mixed effects models were fit to natural logarithm of residue concentrations (SAS 8.02 *PROC MIXED*, SAS macro *%GLIMMIX*; SAS Institute Inc., Cary, NC) to describe the sizes of fixed effects (orchard row, tree compartments) and random effects (trees within orchard rows, replicate composites, replicate samples within an homogenate, chemical analysis sets).

Variability Factor. Variability factors initially were calculated by dividing the maximum concentration observed in an individual apple by the mean composite level. Variability factors were then determined by using the 97.5th percentile data (Microsoft Excel *PERCENTILE* function) in place of the maximum residue concentration.

RESULTS AND DISCUSSION

Applied OP Insecticides. The three applied pesticides (azinphos-methyl, phosalone, and phosmet) were found to be above the level of detection in all of the samples analyzed in the present study. In general, phosalone was found at elevated levels in composite and individual samples prepared from all treatment rows (492 ng/g ± 443 ng/g) relative to those compounds not applied in the orchard. Concentrations of phosmet, however, were found to be elevated only in samples collected from the row treated directly with this compound (71.7 ng/g ± 187 ng/g). The elevated levels of phosalone detected in all apples from all three treatment rows were consistent with the use of this pesticide to control insect infestations in other parts of the orchard during the 2003 agricultural season. Azinphos-methyl levels were much lower (0.69 ng/g ± 0.69 ng/g) than those of phosalone and phosmet (**Figure 2**), corresponding to its use early in the season.

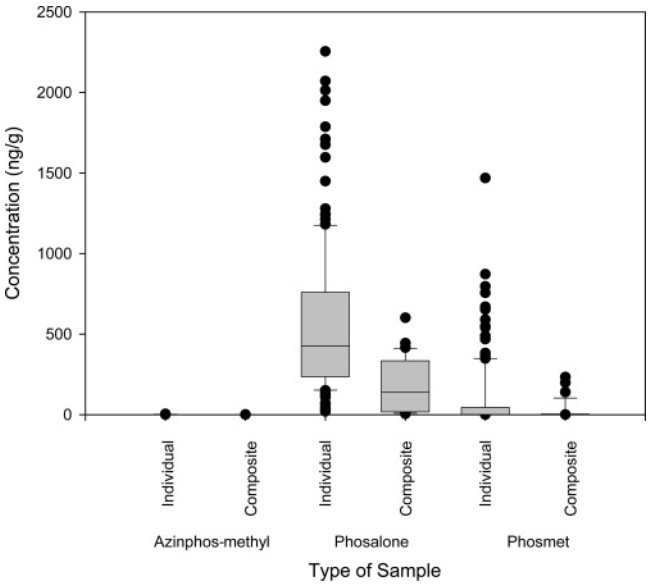

Figure 2. Azinphos-methyl, phosalone, and phosmet concentrations in individual and composite apple samples. Box indicates 25th, 50th, and 75th percentiles. Points indicate data outside of 10th (⊥) or 90th (T) percentiles.

Background Level OP Insecticides. Methidathion was not detected in any of the samples analyzed in this study. Methidathion has not been registered for use on any crop in Canada since 2002. The detection frequency of diazinon, dimethoate, and malathion was 20%, 65%, and 22%, respectively. Although these compounds all are currently registered in Canada, only diazinon and malathion are registered for use on apples. Dimethoate is registered for use on other fruit crops (e.g., pears, peaches), although it is not used locally. Concentrations of these three compounds were found to be low (<0.72 ng/g) in all samples analyzed as expected for pesticides not used in this orchard during 2003. Similar to dimethoate, chlorpyrifos is registered for use on some fruit, although not apples. Chlorpyrifos, however, was observed in all samples analyzed in the present study, including blank apple matrix. Residue levels of chlorpyrifos ranged from the MDL (0.003 ng/g) to 0.042 ng/g, consistent with low level drift and deposit from areas of use. Chlorpyrifos was not detected in any solvent blanks that were processed along with samples, indicating that background levels of this compound were not due to lab contamination.

Maximum Residue Limit. In all samples, residue concentrations were below the maximum residue limit (MRL) established in the Canadian Food and Drug Regulations. The largest observed phosalone concentration (2260 ng/g) was approximately one-half of the MRL value established for apples (5000 ng/g) (*20*). The maximum observed phosmet level (1470 ng/g) was 15% of the MRL value for apples (10 000 ng/g) (*20*). All other compounds were found at concentrations <1% of the MRL values (**Table 1**).

Among Application Rows. Phosmet levels in apples (2.66−1470 ng/g) treated with Imidan (a.i. phosmet) were significantly higher (p < 0.0001) than the levels observed in apples from

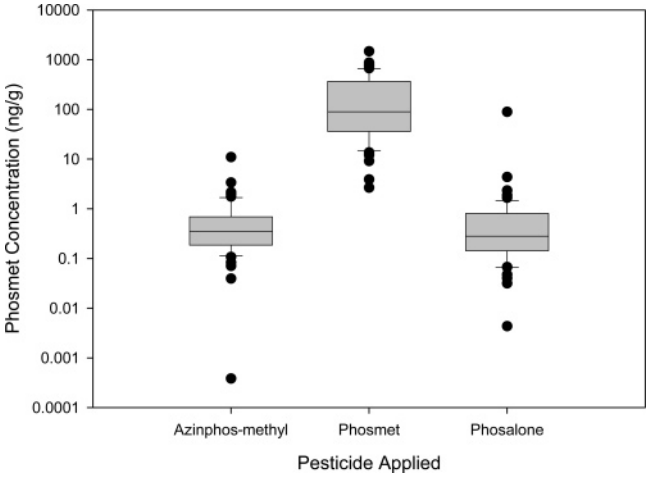

Figure 3. Phosmet concentrations in individual apple samples from each treatment row in the orchard. (See caption to **Figure 2**.)

each of the other treatment rows (<0.004−89.2 ng/g) (**Figure 3**). Phosmet concentrations were significantly higher ($p < 0.0001$) in apple composites prepared from apples harvested from trees sprayed with this OP insecticide (3.81−233 ng/g) than those treated with either phosalone or azinphos-methyl (0.027−1.40 ng/g), similar to the results obtained for individual apples studied.

Although differences in phosalone concentration were observed in individual apples from the row sprayed with this active ingredient, relative to those not treated with Zolone, the difference was only weakly significant ($p = 0.02$). Residue levels in composite samples from the treated row, however, were highly significantly different from composite samples prepared from apples from the rows not treated with phosalone ($p < 0.0001$).

Azinphos-methyl levels in all samples were low relative to the other two applied compounds (**Figure 2**), although individual apples from the Guthion treated row did have the highest concentrations (geometric mean [gm] = 0.686 ng/g; geometric standard deviation [gsd] = 2.84 ng/g) and were significantly higher than residues observed in untreated rows ($p = 0.0003$). Composites prepared using apples from this treatment row also had significantly higher azinphos-methyl levels (gm = 0.739 ng/g; gsd = 1.20 ng/g) than observed in the rows treated with either phosalone (gm = 0.367 ng/g azinphos-methyl; gsd = 1.23 ng/g) or phosmet (gm = 0.536 ng/g azinphos-methyl; gsd = 2.07 ng/g) ($p < 0.0001$).

Residues above the method detection limit were used to characterize the differences among chlorpyrifos, diazinon, dimethoate, and malathion residues. A relationship between pesticide treatment and concentrations in apples was not anticipated to occur for those compounds that were not applied in the orchard because their presence was due to regional or long range transport, rather than use in the orchard. As expected, pesticide treatment was not found to affect the diazinon levels in individual apples ($p = 0.85$); however, treatment was found to be weakly significant in composite samples ($p = 0.03$). Pesticide treatment was weakly significant for malathion in both composite ($p = 0.01$) and individual apple samples ($p = 0.04$). Chlorpyrifos and dimethoate residues in composite samples prepared from apples from each of the OP treatments were found to be significantly different ($p < 0.0001$). Pesticide treatment, however, was not found to be significantly different for chlorpyrifos levels ($p = 0.40$) and only weakly significant for dimethoate ($p = 0.08$) in individual apples. The compositing of individual apples altered the inference of the effect of treatment row for chlorpyrifos, diazinon, and dimethoate, resulting in the appearance that treatment row has influenced residue levels, despite their not being used within the orchard.

Individual vs Composite. Phosmet concentrations in individual apples were found to be higher (gm = 2.37 ng/g; median = 0.70 ng/g) than levels observed in composite samples (gm = 0.71 ng/g; median = 0.20 ng/g). The range in phosmet concentrations observed in individual apples also was much greater than the concentrations observed for composites (**Figure 2**, **Table 2**). Phosalone concentrations observed in individual apples spanned 2 orders of magnitude, similar to the results of the composite samples (**Figure 2**, **Table 2**). The median phosalone concentration in composite samples (138 ng/g) was approximately one-third the concentration observed in individual apples analyzed (424 ng/g), consistent with the expected dilution of high residue levels. Although azinphos-methyl levels were much lower than observed for phosmet and phosalone (**Table 2**), a greater range in concentration was observed in individual samples analyzed relative to the composite samples (**Figure 4**). The median azinphos-methyl concentration in individual apples, however, was lower than observed in the composite samples (0.478 ng/g and 0.586 ng/g, respectively) (**Figure 4**).

The range of residue concentrations for the nonapplied compounds was much smaller than observed for azinphos-methyl, phosalone, and phosmet with mean concentrations closer to levels obtained in composites (**Figure 5**). Statistical evaluations of unit-to-unit and composite samples reported in the literature have shown that the coefficient of variation is lower among composite samples, relative to individual samples (*15*), consistent with the present study.

Variability Factor. The variability factors ranged from 7 to 62 for azinphos-methyl, phosalone, and phosmet, respectively, when the maximum individual concentration was used. Despite not being applied within the orchard, diazinon had a very high variability factor (32), due to one individual sample having a high concentration (0.71 ng/g) relative to the mean composite level (0.005 ng/g). The variability factors for the other nonapplied pesticides detected in this study ranged from 3 (chlorpy-

Table 2. Applied OP Concentrations (ng/g) in Individual and Composite Samples from All Trees Sampled

					concentration (ng/g)			
treatment row	a.i.	sample	N^a	N^b	range	mean ± std dev	gm (gsd)	median
Guthion	azinphos-methyl	individual	142	160	0.009–4.36	0.710 ± 0.744	0.441 (2.91)	0.478
Guthion	azinphos-methyl	composite	18	35	0.122–1.23	0.601 ± 0.284	0.534 (1.66)	0.586
Zolone	phosalone	individual	142	160	20.3–2260	562 ± 455	405 (2.39)	424
Zolone	phosalone	composite	18	35	5.04–602	172 ± 163	82.5 (4.40)	138
Imidan	phosmet	individual	142	160	0.004–1470	82.1 ± 203	2.37 (21.5)	0.701
Imidan	phosmet	composite	18	35	0.027–233	23.9 ± 56.7	0.71 (17.4)	0.200

[a] Number of individual apples or composite samples studied. [b] Total analyses (including replicates).

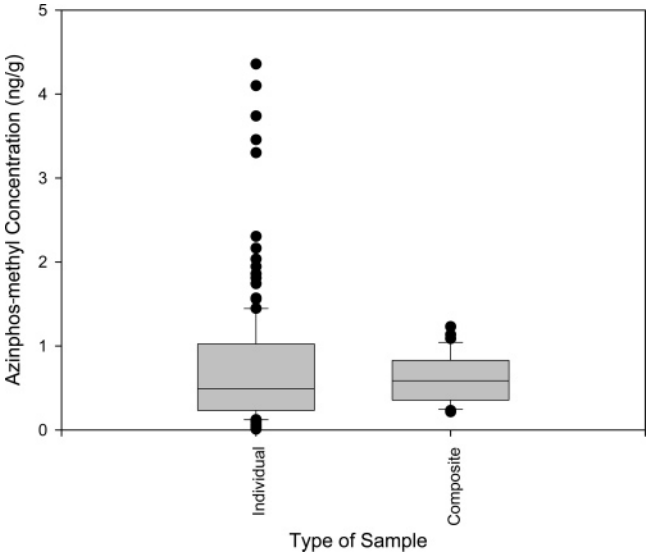

Figure 4. Azinphos-methyl levels in individual apples relative to composite samples. (See caption to **Figure 2**.)

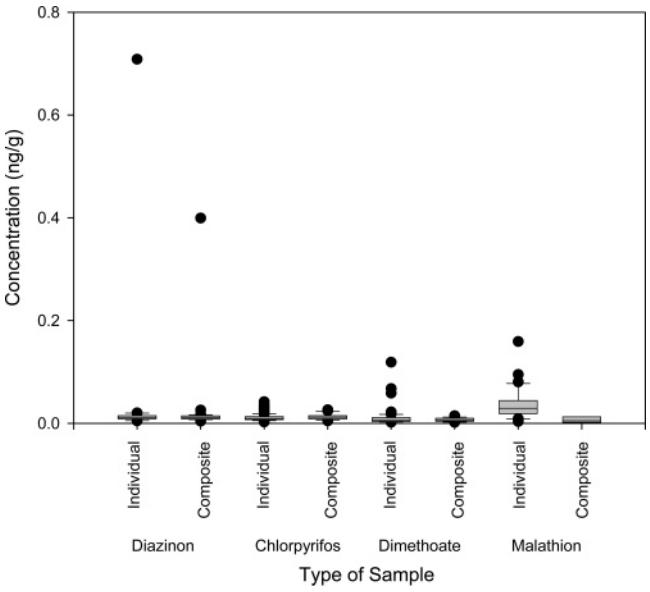

Figure 5. Nonapplied OP residue concentrations in individual apples and composites. (See caption to **Figure 2**.)

rifos) to 20 (dimethoate), respectively, when maximum residue data were considered.

Variability factors calculated using the 97.5th percentile of residual concentrations ranged from 1.5 to 7.8 for all of the pesticides that were detected in apples but were not applied in the orchard (**Table 3**).

Variability factors (5.5−28) were calculated for the three applied OP insecticides using the 97.5th percentile residue levels determined for apples including those that were treated directly and those exposed only via indirect sources (e.g., drift) (**Table 3**). The two populations of apples (treated vs nontreated) also were examined separately. Variability factors for azinphos-methyl, phosalone, and phosmet were 5.3, 11.4, and 11.2, respectively for those apples sprayed directly with these pesticides (**Table 3**). Azinphos-methyl and phosalone variability factors were smaller for apples that were not treated directly (2.5 and 10, respectively); however, the variability factor for phosmet residues in apples without direct application were larger (19) (**Table 3**).

Table 3. Variability Factors for the Seven OP Insecticides Detected

	variability factor[a,b]		
OP insecticide	direct + indirect sources $N_{apples} = 142$	direct spray $N_{apples} = 47$	local drift only $N_{apples} = 95$
azinphos-methyl	5.5	5.3	2.5
chlorpyrifos	2.5[c]	*c*	*c*
diazinon	1.5[c]	*c*	*c*
dimethoate	7.8[c]	*c*	*c*
malathion	7.6[c]	*c*	*c*
phosalone	10.4	11.4	10
phosmet	28	11.2	19

[a] MDL was used for any sample with a concentration less than the MDL value. [b] $[97.5\text{th percentile residue concentration}]_{individual}/[\text{mean concentration}]_{composite}$. [c] Compounds were not applied in orchard.

Variability factors reported previously for chlorpyrifos in apples ranged from 2.9 to 4.8 (*19*), similar to the value obtained in the present study. Phosalone in apples was established to have a variability factor of 3.2 in a study from France (*19*), which is somewhat lower than the value determined in this study. Phosalone was, however, found to have a variability factor of 10.5 for plums (*19*), close to the value determined in the present study. In other studies, OP insecticides were found to have variability factors ranging from 1 to 25 in carrots, kiwi, and kaki fruit, although some factors were determined assuming the maximum concentration, rather than using the 97.5th percentile concentration in individual samples (*8*, *12*).

For acute dietary exposure assessments, the FAO/WHO has now adopted a variability factor of 3 for fruit and vegetables with a unit weight exceeding 25 g, based in part on results from supervised trials (*19*). Variability factors for most compounds measured in the present study exceeded this value, possibly a function of this study having been performed in a commercial orchard where sample collection was designed and executed long after pesticide application (21−96 d, **Table 1**), consistent with the grower's agricultural practices, rather than the 2 d to 14 d post-treatment intervals reported in supervised trials.

Although the measure of central tendency of composite samples and individual samples are similar, composite samples do not accurately reflect the extremes of exposure from consumption of single servings of apples (**Figures 2** and **5**).

Tree Compartment Effects. Diazinon and dimethoate levels were not related to apple position along the height of the tree ($p = 0.24$, 0.47, respectively). Position along the height of the tree, however, did have a weakly significant impact on chlorpyrifos ($p = 0.01$) and malathion residues ($p = 0.05$), with apples from the top of the tree having the lowest residues and the highest residues observed in samples collected from the bottom of the tree, despite their lack of use within the orchard. No significant difference was observed between apples facing the sprayer and those taken from the sides of the tree for chlorpyrifos, diazinon, dimethoate, or malathion, consistent with anticipated results for compounds that were not applied in the orchard.

Azinphos-methyl, phosmet, and phosalone levels all were significantly related to height along the tree ($p < 0.0001$, $p = 0.005$, $p < 0.0001$, respectively). The apples harvested from the tops of the trees were found to have the lowest OP concentrations (gm = 0.247, 28.5, 220 ng/g, respectively) and higher concentrations were observed in apples collected from the bottom of the tree (gm = 0.79, 65.4, 737 ng/g, respectively) for all three compounds.

Apples collected from the compartment of the tree facing the sprayer were found to have higher azinphos-methyl, phosalone, and phosmet residues in each of their respective treatment rows in the present study, whereas apples from the sides of the tree had correspondingly lower concentrations.

At the outset of this study, it was considered most likely that the highest residue levels of the applied compounds would be observed in apples harvested from mid-height along the tree, in addition to those from front/back of the tree relative to the sides. The results of this work have shown that the residues are greatest in apples collected from the bottom third of a treated tree rather than from mid-height, although apples collected from the part of the tree facing direct spray were indeed found to have elevated residue concentrations, consistent with anticipated results.

ABBREVIATIONS USED

a.i., active ingredient; gm, geometric mean; gsd, geometric standard deviation; ha, hectare.

LITERATURE CITED

(1) Jensen, A. F.; Petersen, A.; Granby, K. Cumulative risk assessment of the intake of organophosphorus and carbamate pesticides in the Danish diet. *Food Addit. Contam*. **2003**, *20*, 776−785.

(2) Mol, H. G. J.; van Dam, R. C. J.; Steijger, M. Determination of polar organophosphorus pesticides in vegetables and fruits using liquid chromatography with tandem mass spectrometry: selection of extraction solvent. *J. Chromatogr. A* **2003**, *1015*, 119−127.

(3) CDC (Centers for Disease Control and Prevention, Department of Health and Human Services). Second national report on human exposure to environmental chemicals. 2003. http//www.cdc.gov/exposurereport/.

(4) Souza, M. S.; de Potas, G. M.; de D'Angelo, A. M. P. Organophosphorous and organochlorine pesticides affect human placental phosphoinositides metabolism and PI-4 kinase activity. *J. Biochem. Mol. Toxicol*. **2004**, *18*, 30−36.

(5) Giddings, J. M.; Biever, R. C.; Racke, K. D. Fate of chlorpyrifos in outdoor pond microcosms and effects on growth and survival of bluegill sunfish. *Environ. Toxicol. Chem*. **1997**, *16*, 2353−2362.

(6) USDA (United States Department of Agriculture). United States Department of Agriculture agricultural marketing service, science & technology pesticide data program. PDP-LABOP-10. 2004. http://www.ams.usda.gov/science/pdp/Labop03.pdf.

(7) Andersson, A. Comparison of pesticide residues in composite samples and in individual units: the Swedish approach to sampling. *Food Addit. Contam*. **2000**, *17*, 547−550.

(8) Harris, C. A. How the variability issue was uncovered: the history of the UK residue variability findings. *Food Addit. Contam*. **2000**, *17*, 491−495.

(9) Pennycook, F. R.; Diamand, E. M.; Watterson, A.; Howard, C. V. Modeling of the dietary pesticide exposures of young children. *Int. J. Occup. Environ. Health*. **2004**, *10*, 304−309.

(10) WHO (World Health Organization). Joint FAO/WHO consultation on food consumption and exposure assessment to chemicals in food. Geneva, Switzerland, 10−14 February 1997; http://www.who.int/food safety/publications/chem/exposure_feb1997/en/print.html.

(11) Suhre, F. B. Pesticide residues and acute risk assessment—the US EPA approach. *Food Addit. Contam.* **2000**, *17*, 569−573.

(12) Fernández-Cruz, M. L.; Villarroya, M.; Llanos, S.; Alonso-Prados, J. L.; Garcia-Baudin, J. M. Field-incurred fenitirothion residues in kakis: comparison of individual fruits, composite samples, and peeled and cooked fruits. *J. Agric. Food Chem*. **2004**, *52*, 586−863.

(13) Ambrus, A. Within and between field variability of residue data and sampling implications. *Food Addit. Contam*. **2000**, *17*, 519−537.

(14) Boulaid, M.; Aguilera, A.; Camacho, F.; Soussi, M.; Valverde, A. Effect of household processing and unit-to-unit variability of pyrifenox, pyridaben, and tralomethrin residues in tomatoes. *J. Agric. Food Chem*. **2005**, *53*, 5054−4058.

(15) Ambrus, A.; Soboleva, E. Contribution of sampling to the variability of pesticide residue data. *J. AOAC Int*. **2004**, *87*, 1368−1379.

(16) Lentza-Rizos, C.; Tsioumplekou, M. Residues of aldicarb in oranges: a unit-to-unit variability study. *Food Addit. Contam*. **2001**, *18*, 886−897.

(17) Lentza-Rizos, C.; Balokas, A. Residue levels of chlorpropham in individual tubers and composite samples of postharvest-treated potatoes. *J. Agric. Food Chem*. **2001**, *49*, 710−714.

(18) WHO (World Health Organization). Acute hazard exposure assessment for pesticide residues in food. GEMS/Food data sets used by the Joint FAO/WHO Meeting on Pesticide Residue (JMPR) to assess short-term dietary intake of certain pesticide residues. 2003, accessed 11 July, 2005. http://www/int/foodsafety/chem/acute_data/en/print.html.

(19) EFSA Opinion of the scientific panel on plant health, plant protection products and their residues on a request from Commission related to the appropriate variability factor(s) to be used for acute dietary exposure assessment of pesticide residues in fruit and vegetables. Question No. EFSA-Q-2004-124. *EFSA J.* **2005**, *177*, 1−61. http://www.efsa.eu.int/science/ppr/ppr_opinions/823/opinionresidues2.pdf.

(20) Government of Canada. *Food and Drug Regulations*. Table II Agricultural Chemicals. 1998. http://laws.justice.gc.ca/en/F-27/C.R.C.-c.870/124256.html.

Received for review September 29, 2005. Revised manuscript received November 30, 2005. Accepted December 2, 2005. Support for this work was provided by the Office of the Chief Scientist, Health Canada, and the Pest Management Regulatory Agency, Health Canada.

JF052413V