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Variability of organophosphorus insecticide residues in large size crops grown in commercial farms in Brazil

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Abstract
Field trials were conducted in commercial agricultural areas in Brazil to determine the variability of residues of parathion methyl, diazinon and methidathion in individual units of large crops treated twice with a mixture of the three pesticides. Over 120 random samples were collected, extracted with ethyl acetate and residues determined by GC/FPD. The recoveries and their coefficient of variation were, in general, within the acceptable levels during sample analyses. Residues in papaya and mango were not affected by the position of the fruits in the plant, apparently more or less exposed to the pesticides. Variability factor υ, defined as the 97.5th percentile divided by the mean of residues in all samples taken from a field ranged from 2.0–2.6. The variability of residues within the plant contributed to about 34–61% of the field variability. The results found in this study support the variability factor of 3 adopted by the FAO/WHO for the deterministic estimation of dietary acute intake of pesticides.

Keywords: *Organophosphorus, variability factor, pesticides, food*

Introduction

The significance of the dietary acute exposure to pesticide was recognized in the beginning of the 1990s after reports of poisonings from consumption of contaminated food resulting from misuse of pesticides (Goldman et al. 1990; MAFF 1993). Much higher pesticide residues were found in a small proportion of individual units of crops treated according to good agricultural practices than what would be expected based on the residues detected in composite samples (Ambrus 1979; Andersson 2000; Carter et al. 2000; Harris 2000). These findings draw attention to the potential risk of exposure of consumers to pesticides when eating such food. Particularly, the organophosphorus and carbamate pesticides are of concern, due to their anticholinesterase activity in the nervous system, with potential to alter neurological development and cause subtle and long-lasting neurobehavioral impairments in infants and children (Ahlbom et al. 1995; Marrs 2000).

A procedure to calculate the acute dietary intake for pesticide residues was proposed in 1997 by a Joint FAO/WHO Consultation (WHO 1997). In this methodology, for crops of unit weight higher than 25 g, the residue concentration to be used in the exposure estimation is calculated by multiplying the residue found in a composite sample by a 'variability factor' to take into account the different concentrations of residues in individual units of a composite sample. During the Consultation, the variability factor was defined as ''the ratio of a highest level of residues in the individual commodity unit to the corresponding residue level seen in the composite sample''. The definition was later refined by an international conference sponsored by the UK in 1999 to represent ''the 97.5th percentile of the residues present in crop units divided by the mean of the residue population of the sampled lot'' (Harris et al. 2000).

Due to the lack of extensive residue data to reflect the variability factor at the time of the Consultation,

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default values of 5 and 10 were recommended, assuming the worst case that all residues were present in one fruit of the composite sample containing 5 or 10 crop units. These were based on the Codex sampling protocol, which specifies that for crops with units >250 g a composite sample should contain at least 5 units, and for crops with unit weight between 25 and 250 g, a minimum of 10 units should be taken (Codex 1988).

Since the Consultation, many studies have been conducted to determine the variability of residues in various commodity pesticides combinations. Hamilton et al. (2004) summarized many of these studies, and found that the majority of the variability factors fall into the 2.0–3.0 range. In general, variability factors were found not be influenced by the physicochemical characteristics of the pesticides, crop unit size, environmental conditions and mode of application. The authors recommended that a variability factor of 3 should replace the default values established earlier. In 2003 the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) agreed that a variability factor of 3 would be suitable to be used in the acute intake estimations at international level (FAO 2004).

The vast majority of the available unit crop residue data, however, were obtained with medium size crops, and data on large crops (>250 g unit weight) are missing. Furthermore, questions still persists on whether the sole variability factor of 3 would be appropriate to assess the acute exposure of consumers to pesticide residues and to guarantee the protection of consumers.

This paper describes the findings of four field studies conducted in Brazilian commercial farms, designed to determine the variability of organophosphorus residues in individual units of large size crops grown according to normal commercial farming practice under tropical conditions.

Material and methods

Field studies and sampling

Two field trials were conducted with two varieties of papaya (*Carica papaya* L.), named papaya formosa and papaya havai, in the state of Bahia, in the northeast region of Brazil (Lat: 11°56′–14°05′ S; Long: 45°43′–46°22′ W). The plantations were pivots of 516–614 m of diameter with rows, on average, 1.6–1.8 m apart, and the plants 1.5 m apart within the row. The studies in mango (*Mangifera indica*) and summer squash (*Cucurbita pepomelo* pepo) were conducted in the Federal District area, in the central region of the country (Lat: 15°30′–16°03′ S; Long: 47°25′–48° 12′ W). Mango trees (about 5 m high with canopy of 3–4 m diameter) were planted at 7×5 m square. The summer squash plants were 1×0.5 m apart. During the experiments, minimum and maximum temperatures ranged from 12–17°C and 29–30°C, respectively. Rainfall was <10 mm. The pesticides, purchased in the local market, were mixed in the tank and applied to the plants by farm employees according to normal commercial practices. The application rates were the highest authorized in Brazil for the pesticide/crop at the time of the study. The number of applications, the application interval and the PHI were chosen to assure that residues would be at a detectable concentration in the crop units. For all crops, a pre-test with 2–3 plants was performed, using the selected application protocol, to confirm that detectable residues in the units would be found in the trial. Details of the plots and application rates are shown in Table I.

The study design required a minimum of 120 treated individual crop units from each field, however additional units were taken to allow for any loss during transportation and analysis. The units were taken according to random sampling plans prepared taking into account the size and arrangement of the treated area. For papaya and mango, fruits from different positions within the plants were harvested. Three positions were distinguished according to the apparent exposition to spray: Fruits being on the surface of the canopy (exposed, E); fruits which were partially covered by leaves, by other fruits or in the side part of the plant, (partially exposed, ME); and fruits in the internal part of the plant (non-exposed, NE).

In the papaya formosa experiment, a sampling area of 6 rows (258 plants) was selected within the treated plot (60 rows; 2210 plants). In all the other experiments, the sampling area was the whole treated plot, excluding the border area, which contained 1 (squash) to 3 rows. Units were collected at growing stage of crops ready to be commercialized. Each individual crop unit was placed directly in a polyethylene bag, tested for interferences and put in another bag previously labelled with the sample code. Information on position of the unit in the plant was added to the label at the time of sampling. The crop unit code also identified, along with the row from which the unit was taken, the number of the plant in the row and the number of the unit sampled in the plant. Twenty control crop units were collected from an untreated area located at appropriate distance (minimum of 30 rows apart) from the treated plot, in the same commercial field. Crop units were placed in paper or plastic boxes, with care to prevent crop damage, and transported on the same day to the Central Laboratory of Public Health of the Federal District (LACEN-DF), in Brasilia, DF, to be processed and analysed. The approximate

Table I. Information on field experiments where 2 applications were made to the plants.

Crop	Time of the study	Field characteristics[a]	Treated plot	Application technique	Compounds applied[b]	Application rate			Sampling after last treatment
						kg a.i/ hl	kg a.i/ha	Interval	
Papaya formosa	July 2003	3 years 109 ha/516 m ϕ 1100 plants/ha	0.2 ha 258 plants 6 rows	2000 l tank truck, manual spray	Parathion methyl Diazinon	0.06 0.09	0.6 0.9	7 days	16 h
Papaya havai	July 2004	4 years 210 ha/614 m ϕ 1000 plants/ha	0.3 ha 559 plants 20 rows	Arbus 2000 20 × nozzle #16 spray	Parathion methyl Methidathion	0.06 0.04	1.0 0.7	7 days	16 h
Mango	February 2004	2 years 12 ha 3480 plants	0.6 ha 148 plants 6 rows	400 l tank truck, manual spray	Parathion methyl Methidathion	0.075 0.05	1.2 0.8	3 days	18 h
Summer squash	September 2004	40 days 3 ha 6000 plants	0.02 ha 310 plants 12 rows	20 l costal, manual spray	Parathion methyl Methidathion	0.06 0.04	0.6 0.4	1 day	2 h

[a]Age of the plants, area (hectare, ha), diameter ϕ (papaya) and number of plants; [b]EC formulation; containing 600 g active ingredient (a.i.)/l for parathion methyl and diazinon and 400 g/l of methidathion.

transportation time from the field to the Laboratory ranged from 30 min for mango and summer squash to 6 h for papaya formosa.

Sample processing and extraction

The weight of each individual crop unit was recorded. The papaya formosa fruits were sliced longitudinally (corresponding to the vertical position on the trees) and two opposite sides were taken for processing (the other two sides were discarded); mango fruits were de-stoned before homogenization, and the weights of the stones were recorded. Residues in mango were expressed as mg residue/kg whole fruit. The whole unit of the other crops were processed and analysed. Units were cut in small peaces ($\sim$2 cm^2), mixed and portions placed in a blender to be homogenized (1–2 mm particles size). This procedure was performed in 2–3 steps, as the size of the blenders did not allow processing the whole unit. All processed portions were transferred to the original polyethylene bag and thoroughly mixed until reaching homogeneity. The processing and extraction procedures started on the same day when the samples arrived in the laboratory, and lasted for a maximum of 4 days. Units were kept at 4°C until processed and at −20°C between processing and extraction.

Portions (15 ± 2 g) of all homogenized units were extracted with 40 ml ethyl acetate (pesticide residue grade) in the presence of 30 g of anhydrous sodium sulphate in an ultra sonicator (Elma, GER) for 15 min. 5 ml of the extracts were evaporated under nitrogen at 40°C, dissolved with ethyl acetate and analysed by gas chromatography with a flame photometric detector (GC/FPD). The dried extracts were kept at −20°C until analysed, within 15 days after extraction.

Gas chromatographic analysis

The unit extracts were analysed using either a GC HP 6890 with a HP 7683 auto sampler or a GC Finnigan-9001 with an AS-2000 auto sampler. For each study, a new OV 5% phenyl methyl syloxane column (15.0 m, 250 μm diam., film thickness 0.25 μm) was used. Temperature program was: 70°C/1 min, 30°C/min up to 250°C, hold for 1 min. Hydrogen was used as carrier gas and nitrogen as make-up gas. Total run time was 8 min and 1 μl of the extracts was injected. Certified analytical standards of parathion methyl and fenthion were donated by Bayer Crop Sciences, methidathion and diazinon by Syngenta Analytical Development & Product Chemistry, and dimethoate by Cheminova. Working solutions were prepared in ethyl acetate after sequential dilution of primary stock solutions of 100 μg/ml.

Testing the performance of the method

The recovery of the residues was tested prior to the field trials for each crop/pesticide combination at levels of 0.00 mg/kg ($n = 4$), 0.01 mg/kg ($n = 5$); 0.1 mg/kg ($n = 4$) and 1.0 mg/kg ($n = 4$). The analyte concentration was determined with weighted linear regression calibration carried out with matrix-matched solutions of calibration standards at 50, 200, 500 and 1500 pg/μl levels, prepared on the day of the analysis. The calibration standards were injected in two replicates alternately with the unit extracts. In all cases, the untreated control units (0.00 mg/kg) gave no detectable residues. The stability of the chromatographic system was tested by analysing a mixture of 2 organophosphorous pesticides injected at 100 or 200 pg/μl level (dimethoate and methidathion in the papaya formosa experiment; diazinon and fenthion in papaya havai and summer squash; diazinon and dimethoate in mango) during the injection sequence. No significant change was seen in the retention time of the compounds neither in their peak area and width within one analytical batch. Each batch consisted of 20–30 unit extracts. The performance of the method during each batch was tested by analysing control units fortified at 2 or 3 concentration levels (2 or 3 replicates at each level). The repeatability of the procedure was tested by analysing within the same batch 4 or 5 portions of one treated unit of papaya formosa, papaya havai and summer squash. In addition, a second portion of each 5 treated mango and summer squash units were reanalysed in subsequent batches.

Results and discussion

The results of method validation conducted before the trials are presented in Table II. Mean recoveries were between 74 and 108%, with the exception of mango at 0.01 mg/kg level, where the recovery of parathion methyl and methidathion ($\sim$146%) were higher than the acceptable recovery for this concentration level (120%) (Codex 2003). These high recoveries indicated that the determination of the residues in mango at 0.01 mg/kg level could not be carried out accurately. Coefficients of variation (CV) in all cases were not significantly different from those specified by the Codex Alimentarius Commission (Codex 2003). No significant differences were found among recoveries at various levels and a mean recovery for each crop/pesticide was estimated (Table II). In addition, the CV at the different concentration levels could also be pooled to obtain a mean coefficient of variation, CV_{ave},

Table II. Mean recoveries (%) and coefficient of variations (CV%) obtained during method validation at 3 fortification levels.

Crop	Compound	Mean recovery (CV), %			Total mean recovery (CV_{ave})[a], %
		0.01 mg/kg, $n=5$	0.1 mg/kg, $n=4$	1.0 mg/kg, $n=4$	
Papaya formosa	Parathion methyl	108 (12.3)	90.2 (9.8)	88.4 (3.9)	100 (8.7)
	Diazinon	99.5 (16.2)	90.9 (13.9)	86.2 (5.4)	92.2 (11.8)
Papaya havai	Parathion methyl	85.2 (8.0)	88.7 (5.5)	82,3 (5.3)	85.4 (6.2)
	Methidathion	88.5 (9.8)	86.7 (7.4)	80.5 (7.1)	85.2 (8.1)
Mango	Parathion methyl	147 (12.9)	88.3 (23.9)	105 (24.6)	113 (20.3)
	Methidathion	145 (8.6)	91.4 (12.6)	99.8 (18.6)	112 (13.3)
Summer squash	Parathion methyl	79.5 (8.1)	86.8 (3.9)	89.5 (3.6)	85.3 (5.2)
	Methidathion	74.8 (12.2)	82.4 (4.9)	89.5 (1.8)	82.2 (6.3)

[a]Total mean recovery: average of all recovery data obtained at 3 spike level; CV_{ave}: average coefficient of variation at all fortification levels; n is the number of fortified samples at each level.

Table III. Method performance during the experiments.

Crop	Pesticide	Fortified samples	Replicate of treated samples	
		Mean % recovery (CV, %)	Same batch mean residue, mg/kg (CV_A, %)	Subsequent batches, CV_L, %
Papaya formosa	Parathion methyl	58.1 (20.2)[a]	0.15 (28.2)[b]	–
	Diazinon	75.2 (17.6)[a]	0.09 (14.5)[b]	
Papaya havai	Parathion methyl	86.1 (12.5)[c]	0.05 (16.2)[d]	–
	Methidathion	85.7 (14.2)[c]	0.08 (17.2)[d]	
Mango	Parathion methyl	79.2 (13.4)[c]	–	14.3
	Methidathion	83.7 (6.2)[c]		18.2
Summer squash	Parathion methyl	82.0 (19.7)[c]	0.03 (16.6)[d]	7.6
	Methidathion	83.7 (16.5)[c]	0.09 (18.1)[d]	13.8

[a]0.01, 0.05, 0.5 mg/kg, 1 single batch, 3 replicates at each level; [b]4 replicate samples, same batch; [c]0.01, 0.1 mg/kg, 6 batches, 2 or 3 replicates at each level at each batch; [d]5 replicate samples, same batch; CV_L = reproducibility coefficient of variation calculated from the relative differences in residues found in replicate test portions analysed in 5 sets of subsequent batches.

which indicates a typical reproducibility value for the method.

The method performance verification during the analyses of the individual units also did not show concentration dependency, and the recovery data from various concentration levels were pooled for each crop/pesticide combination (Table III). For papaya formosa, the procedure control was done in a single batch at 3 concentration levels (0.01, 0.05 and 0.5 mg/kg, 3 replicates samples at each level). For this crop, low mean recovery of parathion methyl was found, which indicated some gross errors, but the source of it could not be identified. The mean recoveries and their CVs for the other crops were not significantly different from the limits specified by the Codex document on Good Laboratory Practice. As the majority of the residues were above 0.06 and 0.1 mg/kg, where the performance of the method was acceptable, it was concluded that the analytical uncertainty did not have significant effect on the conclusions drawn from the trials.

The analyses of replicate test portions of the treated samples within a single batch and in subsequent batches are also shown in Table III. The within batch repeatability CV_A, calculated from the residues found in replicate test portions of the same individual unit sample, were less than 20% in all cases, with the exception of parathion methyl in papaya formosa (28.2%), and showed no relation to the incurred residue levels or type of matrix. The CV_L, calculated for the relative differences between residues found in 2 subsequent bathes (5 sets of replicate analysis), of mango and summer squash ranged from 7.6–18.2%.

As the individual units were stored at 4°C before extraction (maximum 4 days), we studied the effect of this storage time on the stability of residues. From 30–60 units were extracted each day. The ANOVA test showed that the mean residues found in mango and papaya units processed and extracted in different days were not significantly different (Table IV). In case of summer squash, one unit analysed on day 2 contained much higher residues

Table IV. Mean residues (mg/kg) found in individual units processed and extracted after up to 4 days of storage at 4°C.

Crop	Compound	Day of extraction, after storage at 4°C			
		2	3	4	*p*
Mango	Parathion methyl	0.215	0.173	0.216	0.130
	Methidathion	0.194	0.166	0.217	0.048
Papaya	Parathion methyl	0.053	0.048	0.042	0.081
	Methidathion	0.080	0.064	0.068	0.110
Summer squash	Parathion methyl	0.089	0.048	0.062	0.001
	Methidathion	0.180	0.112	0.147	0.002

than the rest of the units (0.30 mg/kg and 0.66 mg/kg of parathion methyl and methidathion, respectively), which increased the mean residue of units analysed on that day. This led to a significant difference between the mean residues found on units analysed on days 2 and 4. This difference was of 30% for parathion methyl and 18% for methidathion, which is within the acceptability criteria for storage stability (30%) set by the FAO/WHO JMPR (FAO 2000). These results show that there is no correlation between extraction time and residue level, and that the storage at 4°C did not affect the validity of the results. The tendencies of residue levels in mango and summer squash units extracted after each other are shown in Figure 1. This figure also indicates that the residues of both pesticides varied in the same direction, confirming that the random variation of the GC analysis did not affect significantly the results either.

Table V shows the crop unit weights, the level of residues found in the crops and the variability of the residues. In all experiments, residues in control units (10 or 20 units were analysed) were <LOQ. The crop mean unit weights ranged from 446–1491 g, which characterize the crops as large size (Codex 1988). As the residues in the treated crops did not resemble any parametric distribution, non-parametric method was used to calculate the required number of individual crop units (sample size) which should taken from a field for analysis. Taking into consideration of the basic requirements for estimating the variability factor (97.5th percentile of the sampled population at 95% confidence level), a minimum of 119 units had to be taken randomly to obtain at least one value above the 97.5th percentile (P97.5) with 95% probability. In the present study, 128 treated units of papaya havai and summer squash and 139 units of mango were analysed, which enabled the estimation of the P97.5 with >95% confidence. In the papaya formosa trial, due to technical problems detected afterwards, the second application was not performed in half of the treated field. For this trial, only the 66 fruits were collected from the area which received the two applications. That enabled the estimation of the P97.5 with 81% confidence.

In some studies, variability of residues in individual units have been estimated from very limited number of data (down to 10 units) (Lentza-Rizos and Balokas 2001; Lentza-Rizos and Tsioumplekou 2001; Fernandez-Cruz et al. 2004; Boulard et al. 2005). As the probability of finding a crop unit containing residues at or above the 97.5th percentile of the residues in the treated crops with small number of samples is very low (e.g. 50% with 27 samples), the results from these studies should be interpreted with caution, and should not be used for estimating the variability factor as defined by the FAO/WHO.

For all crop/pesticide combinations, more than 90% of the treated units had detected residues (>0.01 mg/kg), and for the calculation of the mean, residues <LOQ were replaced with ½ LOQ. Some authors have also used this approach (Hill and Reynolds 2002; Earl et al. 2000), while others assumed the values at <LOQ as zero (Hamilton et al. 2004). Ambrus (2000) has tested the effect of non-detectable residues and their LOQ on the calculation of the variability factor. The author found that the effect of replacing the LOQ with its half value on the mean and variability factor depends on the ratio of the LOQ and the mean residue. Generally, where the residues below the LOQ are not more than 20% of the data points the replacement can be done without significantly affecting (<10%) the estimated mean values.

Pesticide residues in treated samples had a maximum of 0.66 mg/kg (methidathion in summer squash), and the mean residues ranged from 0.05–0.18 mg/kg (Table V). The observed variability of residues was in the range of 49.6% and 73.0%. Subtracting the analytical errors (Table III) according to the error propagation law resulted in true variability in the range of 46.5–67.3, indicating that the relatively large analytical errors did not affect significantly the estimation of the true variability of residues.

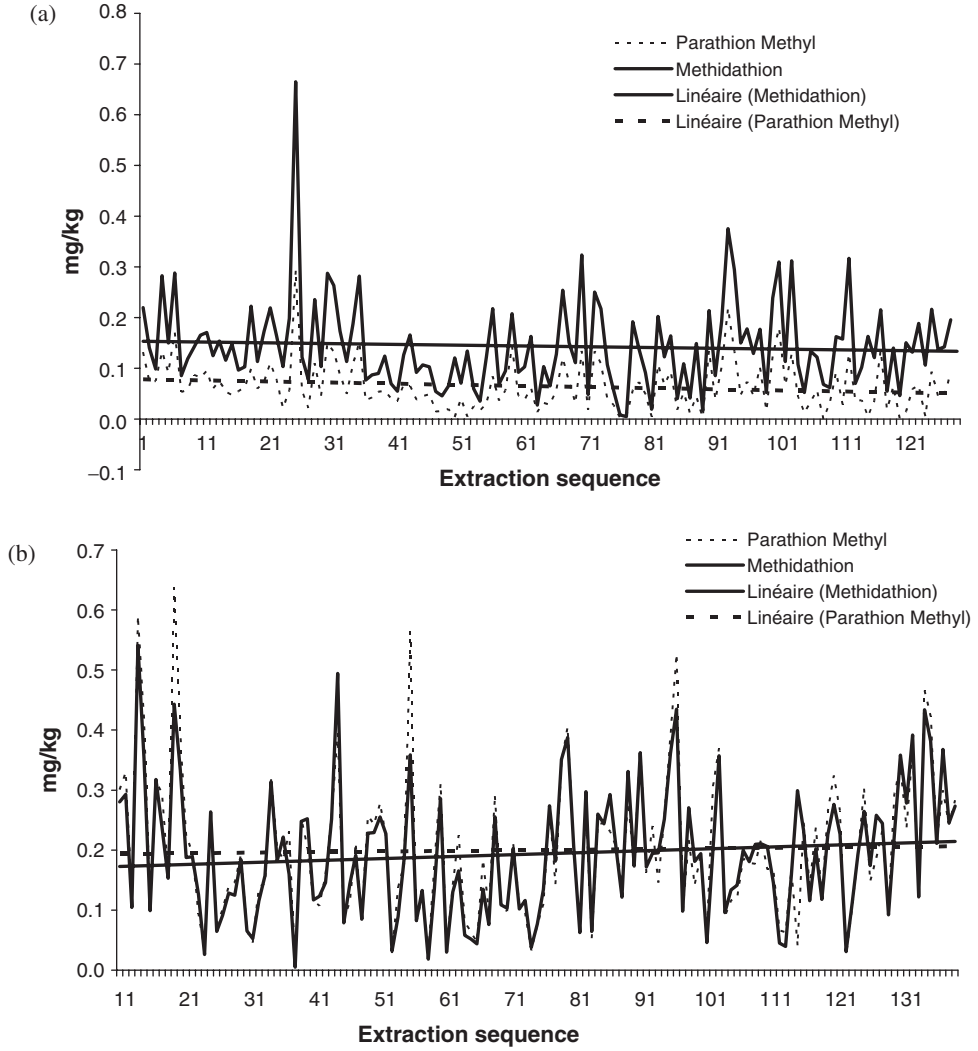

Figure 1. Residues of parathion methyl and methidathion on individual units of summer squash [a] and mango [b] processed and extracted up to 4 days stored at 4°C.

The $R_{max/mean}$ ranged from 2.1–4.6. Residue levels at P97.5 estimated from the sampled data set ranged from 0.10–0.45 mg/kg. It should be pointed out, however, that the residue data used here represents only a sample taken from the unknown population of the residues in the treated area. Hamilton et al. (2004) have emphasized that the P97.5 of residues in the sample may not be the same as the P97.5 percentile of residues in the sampled commodity, and recommended a methodology for estimation of the 97.5th percentile of the residues in the sampled population (P97.5*). Table V shows for each crop/pesticide the P97.5* calculated according to the methodology described by Hamilton et al. With the exception of parathion methyl in papaya formosa, where the number of samples was only 66, and the two values were equal, P97.5* was always higher than P97.5. This was expected, in view of the skewed distribution of the residues (see Figure 2).

The variability factors calculated either as the ratio between P97.5 and the mean (υ) or the P97.5* and the mean (υ^*) are also shown on Table V. The values, between 2.0 and 3.1, were within the same range found in other studies with different pesticides and medium size crops (Ambrus 2000; Roberts et al. 2002; Hamilton et al. 2004).

In this study, the units were collected after a maximum of 12 h after the last application, to ensure detectable residues. The shortest PHI for the pesticides in crops used in Brazil was 14 days, furthermore, significantly longer than the actual sampling interval. Although deposition concentrations, pesticide metabolism, or residue dilution by crop growth might affect residue levels at a certain PHI, previous studies indicated that the variability of residues was not significantly influenced by the average residue and the time interval between

Table V. Residue levels and variability of organophosphorus insecticide residues in the crops.

Crop	Unit weight mean, g CV, %	Compound	Samples analysed/>LOQ	Min–max, mg/kg	Mean[a], mg/kg	CV, %	R_{max}/R_{mean}	P97.5, mg/kg	P97.5⋆, mg/kg	Variability factor, υ	Variability factor, $\upsilon^{\star}$
Papaya formosa	1491.1	P. methyl	6/66	0.01–0.42	0.15	73.0	2.9	0.38	0.38	2.6	2.6
	24.1	Diazinon	6/66	0.02–0.31	0.14	53.0	2.1	0.29	0.30	2.0	2.1
Papaya Havai	658.3	P. methyl	128/126	<0.01–0.12	0.05	51.0	2.6	0.10	0.11	2.1	2.4
	32.0	Methidathion	128/128	0.01–0.16	0.07	49.6	2.3	0.14	0.15	2.0	2.1
Mango	853.3	P. methyl	139/138	<0.01–0.64	0.18	59.3	3.2	0.45	0.49	2.4	2.7
	27.8	Methidathion	139/138	<0.01–0.54	0.18	55.2	2.8	0.38	0.41	2.2	2.3
Summer squash	446.5	P. methyl	128/118	<0.01–0.29	0.06	72.7	4.5	0.17	0.20	2.6	3.1
	27.7	Methidathion	128/127	<0.01–0.66	0.14	61.2	4.6	0.32	0.39	2.2	2.7

[a]Residues <LOQ were considered at ½ LOQ; P97.5 = 97.5th percentile in the data set; P97.5⋆ = 97.5th percentile in the population from which the data was sampled (Hamilton et al. 2004); R_{max}/R_{mean} = ratio between the highest residue in the data set and the mean; υ = ratio between the P97.5 and the mean; $\upsilon^{\star}$ = ratio between the P97.5⋆ and the mean.

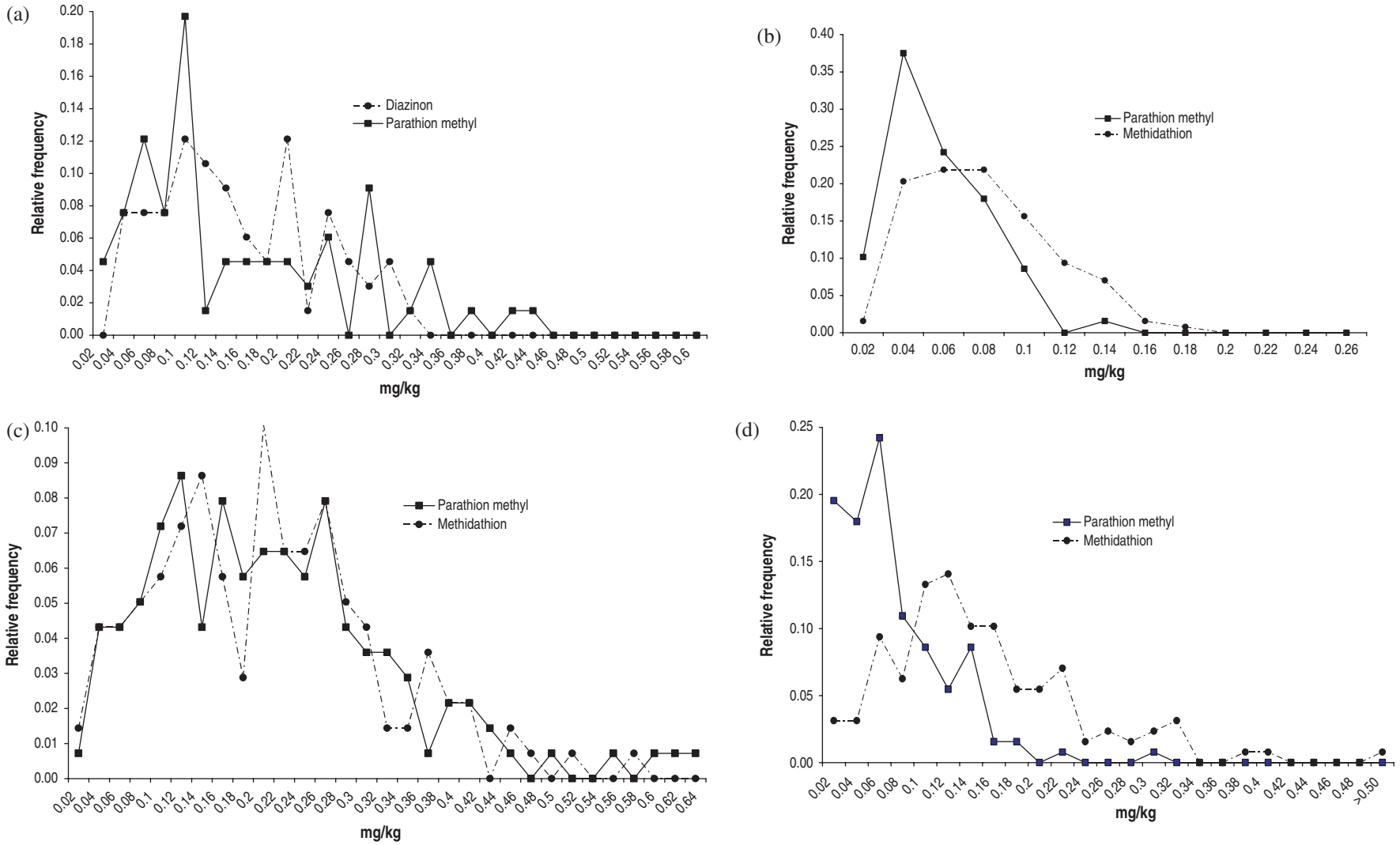

Figure 2. Distribution of residues in [a] papaya formosa ($n = 66$); [b] papaya havai ($n = 128$); [c] mango ($n = 139$) and [d] summer squash ($n = 128$).

Table VI. Residues in units according to position in the plant and variability of residues within a single plant.

Crop	Compound	Exposed	Partly exposed	Non-exposed	R_{max}/R_{mean} [b]
Papaya/formosa	*N*	35	–	31	
	Parathion methyl[a]	0.15 ± 0.10	–	0.14 ± 0.10	–
	Diazinon[a]	0.14 ± 0.07	–	0.15 ± 0.08	–
Papaya/havai	*N*	66	24	38	
	Parathion methyl[a]	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	1.3/1.6 (n = 5/4)
	Methidathion[a]	0.08 ± 0.03	0.06 ± 0.03	0.06 ± 0.03	1.2/1.4 (n = 5/4)
Mango	*N*	54	49	36	
	Parathion methyl[a]	0.21 ± 0.11	0.20 ± 0.12	0.19 ± 0.13	1.8 (n = 10)
	Methidathion[a]	0.22 ± 0.10	0.20 ± 0.11	0.17 ± 0.11	1.7 (n = 10)
Summer squash	Parathion methyl	–	–	–	1.8/1.3/2.0 (n = 3)
	Methidathion	–	–	–	1.2/1.7/1.8 (n = 3)

N is the total number of individual units taken from each position of all sampled plants; [a]mean of residues ± standard deviation, in mg/kg; [b]each entry corresponds to the data from one plant; (n) is the number of individual units analysed from a single plant.

last application and sampling (Ambrus 2000; Harris et al. 2000; Kaethner 2001).

The distributions of residue levels in the treated crops shown in Figure 2 were positively skewed, with the asymmetric tail extending towards large values. The coefficient of variation (CV) of the residues ranged from 49–73%, with the highest variation for parathion methyl in papaya formosa and summer squash (Table V). The same kind of distribution and CV has been observed by other authors for describing residue levels in many crops (Ambrus 2000; Hill 2000).

Table VI shows the distribution of residues in papaya and mango collected from different parts of the plants, being apparently more or less exposed to the pesticide spray, depending on the degree of coverage of fruits by leaves or other fruits. Due to the large overall variation of residues (Table V), no significant difference could be observed in the residues in fruits being in different the positions within the plants. In Hungary, no significant difference was found between the average residue levels of the chlorpyrifos methyl in fruits collected from different positions in the apple tree (n = 320), although apparently higher residues were found in the apples collected from the bottom and middle third of the tree compared with residues in the top (Ambrus 2000). Similar distribution was reported from Switzerland (n = 126) (Dieterle RM et al. personal communication in Hamilton et al. 2004).

For papaya havai, mango and summer squash, the variability of the residues within the plants were also estimated (Table VI). The ratio R_{max}/R_{mean} ranged from 1.2–2.0 (n = 3–10). The contribution of the within plant variability for the total variability of residues in these crops, calculated by dividing the mean R_{max}/R_{mean} for each pesticide/crop combination shown in Table VI by the R_{max}/R_{mean} found in the corresponding sample set (Table V), ranged from 55.7–61% in papaya havai and mango and from 34.7–37.8% in summer squash.

This study presents for the first time residue data in large tropical fruits treated according to commercial agriculture practice. Thus it fills the gap in the database required for estimating acute intake to pesticides. The results support the variability factor of 3 currently used by the FAO/WHO JMPR in the deterministic methodology for the estimation of dietary acute intake at international level. It is emphasized that the variability factor as it is currently defined is only applicable to data set from a single lot. In case of residue data from market place, where it is likely that samples from different lots are mixed, the concept is not applicable.

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