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Probabilistic assessment of the cumulative acute exposure to organophosphorus and carbamate insecticides in the Brazilian diet

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Abstract

In the present study, the cumulative exposure of 25 acetylcholinesterase (AChE) inhibiting pesticides through the consumption of nine fruits and vegetables by the Brazilian population was assessed. Food consumption data were obtained from a household budget survey conducted in all Brazilian states from July 2002 to June 2003. Residue data from 4001 samples were obtained from the Brazilian national monitoring program on pesticide residues. Relative potency factors (RPF) were calculated with methamidophos or acephate as index compounds (IC), using BMD_{10} or NOAEL for AChE inhibition, mostly in rat brain, obtained from national and international pesticide evaluations. Monocrotophos and triazophos, in addition to aldicarb, had the highest calculated RPF in any scenario. The exposure to AChE inhibiting pesticides for the general population at P99.9, represented 33.6% of the ARfD as methamidophos and 70.2% ARfD as acephate. The exposure calculated as acephate could exceed the ARfD at the upper bound of the 95% confidence interval for this percentile. Exposure for children aged up to 6 years were, on average, 2.4 times higher than the exposure for the general population. Tomato represented about 67% of the total intake of AChE inhibiting pesticides. The highest calculated equivalent residues in tomato, which drove most of the estimated intakes at the high percentiles, were related to the illegal use of monocrotophos and triazophos in this crop.

Keywords: Organophosphorus; Carbamates; Relative potency factor; Acute exposure assessment; Food

1. Introduction

The human intake of pesticides through the consumption of treated foods can be of health concern and dietary exposure assessments are now routinely included in the national registration process of these compounds in some countries (EPA, 2000) and in international evaluations (FAO, 2004). While the assessment conducted for single pesticides is well known, the process to cumulate or combine the potential adverse effects of two or more pesticides acting with different degrees of potency through a common mechanism of toxicity is a relatively complex issue.

Several procedures for the cumulative exposure assessment to chemicals have been considered (Wilkinson et al., 2000). In general, all the methods assume the additivity of the individual effects, although synergism and antagonism among the compounds cannot be totally discarded (Seed et al., 1995). In the method using the toxicity equivalence factor (TEF), also called relative potency factor (RPF), the chemical concentra-

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tion is normalized to yield an equivalent concentration to one of the chemicals, called the index compound (IC). This procedure was initially developed by the US Environmental Protection Agency (EPA) to estimate the potential toxicity of mixtures of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls (EPA, 1986). The method was first applied to pesticides by the US National Research Council to evaluate the dietary exposure of infants and children to organophosphorus insecticides, compounds that inhibit the nervous enzyme acetylcholinesterase (AChE) (NRC, 1993; Mileson et al., 1998).

In addition to the organophosphorus insecticides, the carbamate insecticides are also AChE inhibitors, and have been included in the cumulative assessment of the exposure to compounds with this mechanism of action (Jensen et al., 2003; Boon and Klaveren, 2003). The inhibition of AChE in the nervous system leads to an accumulation of neurotransmitter acetylcholine at the nervous terminal, with the potential to alter neurological development and cause subtle and long-lasting neurobehavioral impairments in human (Ahlbom et al., 1995). These compounds are amongst the most acute toxic pesticides available in the market, and human intoxication from the consumption of contaminated foods have been reported recently (Tsai et al., 2003; Mendes et al., 2005). Symptoms related to acute exposure to AChE inhibitors include abdominal cramps, nausea, diarrhoea, salivation, miosis, dizziness, tremor, anxiety and confusion (Ecobichon, 1996).

In the present work, the acute exposure assessment to AChE inhibiting compounds, including organophosphorus and carbamate insecticides, was conducted with Brazilian food consumption and residue data, using the probabilistic approach. The exposures were estimated using two index compounds—methamidophos and acephate. These two compounds were selected due to the availability of an extensive toxicological database.

2. Materials and methods

2.1. Food consumption data

The food consumption data were obtained from the Household Budget Survey (HBS) conducted by the Brazilian Institute of Geography and Statistic (IBGE, 2005) from July 2002 to June 2003. Data from 45,348 households of the 27 Brazilian states (north, northeast, central west, southeast and south geographical regions), which recorded the amount of food entering the household in a diary over seven consecutive days, were used. Characteristics of household members, including age, sex and weights (for individuals $\geq$20 years) were obtained through questionnaires. The weights of individuals aged less than 20 years were estimated using data from the US National Health and Nutrition Examination Survey (CDC, 2000). For each household, the total week consumption of each food was divided by the household size to generate weekly consumptions per individual. For each individual, the week consumption was decomposed into daily consumption patterns over the 7 days using the weekly consumption frequency (WCF), assuming 10% variation among consumer days. WCF was defined as the number of days within a week that a food is consumed, and it was estimated based on the frequency on which a food was reported in the diaries. For the purpose of this study, the amount of food acquired by each household was considered as food consumed.

2.2. Pesticide residue data and relative potency factors

Residue data were obtained from the Brazilian national monitoring program on pesticide residues (Programa de Análise de Resíduos de Agrotóxicos em Alimentos; PARA). A total of 4001 samples of tomato, potato, carrot, lettuce, orange, apple, banana, papaya and strawberry were analyzed for the content of 96 pesticides, including 38 organophosphorus, carbamates and metabolites of these compounds. The samples were collected at local markets in 13 Brazilian state capitals from June 2001 to December 2004 (PARA, 2005). Pesticides for which registration had been canceled or which were in a phase out process were also included in the monitoring program.

Relative potency factors (RPF), defined as the ratio between the toxicological relevant dose of the index compounds (IC) and of each compound within the group, were calculated using methamidophos and acephate as ICs. The relevant doses were selected from national or international monographs of pesticides. In all cases, brain or red blood cell (RBC) AChE inhibition was chosen as the toxicological end point.

The equivalent residue levels in a sample, expressed as each index compound, were calculated multiplying the level detected per compound, in mg/kg, by its RPF. For samples containing multiple AChE inhibiting residues, the equivalent residue levels were added and one concentration per sample, as IC, was calculated. The limits of quantification (LOQ) of the residues, expressed as the index compound, were set at the lowest equivalent residue level in the samples with detectable residues. The LOQ was 0.00008 mg/kg for methamidophos and 0.001 mg/kg for acephate. For the intake calculations, residue levels <LOQ were considered as 1/2 LOQ. These levels were assigned to samples with non-detectable equivalent levels, i.e., for samples that did not contain detectable levels of any of the compounds addressed.

2.3. Monte Carlo exposure assessment model

The assessments were conducted for each Brazilian region using the Monte Carlo Risk Assessment (MCRA 3.6) system, an internet-based program developed by RIKILT-Institute of Food Safety in The Netherlands (Boer et al., 2005). For the estimation of daily acute exposure, daily consumption patterns selected from the consumption database were multiplied with randomly selected equivalent residue levels from the concentration data base for each IC, summed over foods, and expressed per kg body weight. To account for processing factors, the model applies a logistic-normal distribution, with the processing factors falling around a median processing factor (PF) (Boer et al., 2005). A PF of 0.75 (washing) was applied to residues found in tomato, carrot, apple and strawberry, of 0.05 (peeling) to residues in banana, orange and papaya and of 0.27 (cooking) to residues in potato (Boon and Klaveren, 2003). In this application it was assumed that only 5% of the processing factors would be higher than 0.99, corresponding to a standard deviation for the normal distribution of logistically transformed processing factors of {logit(0.99) − logit(PF)}/1.645.

To determine the exposures at each percentile, 1,000,000 iterations were performed for the general population and 100,000 iterations for children. The outputs from the intake distribution generated after the simulation were specified at percentiles P90, P95, P97.5, P99, P99.9 and P99.99. All estimated intakes were rounded to two or three significant figures. The uncertainties of exposures at each percentile were assessed using bootstrap distributions, which characterizes the uncertainty due to the sampling uncertainty of the original dataset (Efron and Tibshirani, 1993). The principle is to create bootstrap datasets of the same size as the original consumption and pesticide residue databases by sampling with replacement among the original datasets. Then, one uses these bootstrap datasets to do the simulation again and compute the requested output statistics (e.g. the percentiles). Repeating this process B times yields a bootstrap distribution for each percentile that allows building confidence intervals around it. A 95% confidence interval is computed using the P2.5 and P97.5 of the output statistic distribution. As the uncertainty at a very high percentile given by the bootstrap requires a sufficiently large number of data around this percentile, the confidence interval was not reported for P99.99. The number of bootstrap samples for the general population was $B = 200$ in all cases except for the northeast region ($B = 100$), due to the limitation of the MCRA program to process very large databases. To gain computing time, MCRA proposes to reduce the number of iterations in the bootstrap simulations: for children, 10,000 iterations were performed for each of the 200 bootstrap samples; for the general population from 20,000 to 75,000 for each bootstrap sample. The number of iterations performed for the uncertainty calculation was never lower than the region population in order to assure the correct approximation of the percentiles in the initial simulation of exposure or in the bootstrap simulations (Bertail and Tressou, in press).

3. Results

3.1. Food consumption and residue data

The 45,348 households surveyed in the Brazilian HBS during 2002/2003 consisted of 174,378 individuals, being 27,928 individuals from the north region, 72,426 from the northeast region, 26,096 from the central west region, 28,668 from the southeast region and 19,263 from the southern region. About 50.8% of the individuals were female and 11.5% were children aged up to 6 years. Of the surveyed households, 46% did not report acquiring any of the nine commodities relevant for this study during the 7 days of the survey (zero consumption days).

Table 1
Summary of food consumption data for the five geographic Brazilian regions

Crop	Mean ± standard deviation, in g/day of total survey days[a]	% Consumption days[b]
Apple	4.8 ± 2.6	3.4 ± 2.6
Potato	17.4 ± 11.7	15.5 ± 12.3
Tomato	13.1 ± 2.3	20.3 ± 5.8
Strawberry	0.2 ± 0.2	0.1 ± 0.1
Orange	14.4 ± 9.9	5.4 ± 3.7
Papaya	4.4 ± 1.6	1.5 ± 1.0
Lettuce	2.0 ± 0.8	7.1 ± 6.3
Banana	21.5 ± 7.2	16.3 ± 7.2
Carrots	4.4 ± 1.2	3.9 ± 2.0

[a] Included 7 (zero and non-zero) consumption days for all 174,378 individuals.

[b] % of non-zero consumption days for consumers.

Table 1 shows a summary of the food consumption data obtained from the survey. Banana, potato, orange and tomato showed the highest national per capita consumption level (mean of the five Brazilian regions). The percentage of consumption days shown in Table 1 represents the % of non-zero consumption days for each crop in the 7 days food consumption database. This percentage is directly related to the week consumption frequency (WCF). Tomato, which had a WCF of 4.8, meaning that it was consumed, on average, 4.8 days per week, was the most frequent consumed crop considered in this study, having therefore, the highest percentage of consumption days (mean of 20.3%). Banana and potato had the WCF of 4.3 and 3.9, respectively. Strawberry was the least frequently consumed crop in the country (<1 day per week), and had only 0.1% of consumption days in the database.

None of the samples analyzed contained detectable residues of 12 AChE inhibiting compounds analyzed by the PARA program: carbophenothion, chlorfenvinphos, chlorpyrifos methyl, ethoprophos, fenamiphos, malaoxon, mevinphos, omethoate, paraoxon, pirimiphos ethyl, phorate and phosmet.

Fig. 1 shows the pesticides which were detected most in the samples at levels ≥LOQ. Chlorpyrifos, methamidophos and dimethoathe were present in 6.2, 2.3 and 1.8% of the 4001 samples analyzed, respectively. Of all

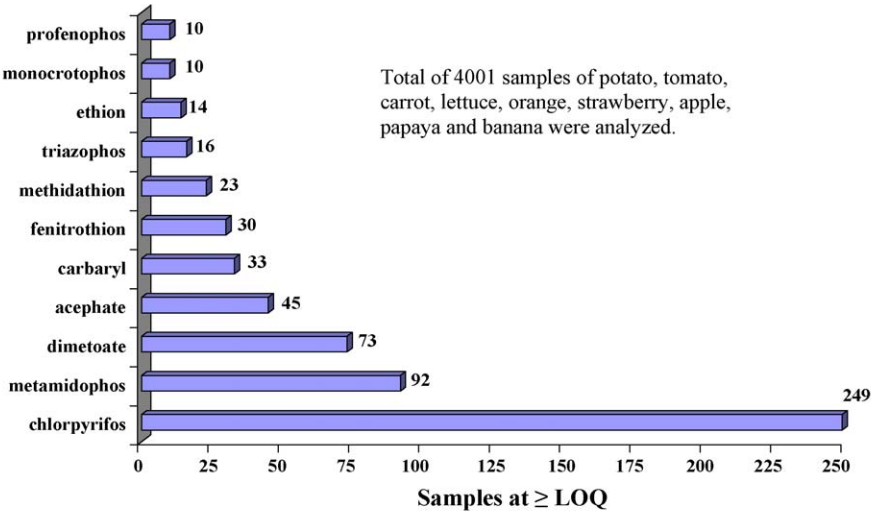

Fig. 1. Ten most frequently found acethylcholinesterase inhibiting pesticides in the 2001–2004 Brazilian monitoring program (PARA).

samples, 547 samples (13.7%) had at least one AChE inhibiting pesticide at levels ≥LOQ and 80 (2.0%) had more than one pesticide present (Table 2). Apple (34.6%), potato (26.0%), tomato (19.8%) and strawberry (19.5%) were the commodities with the highest percentage of samples with levels ≥LOQ, while banana and carrots had only four and three samples with detectable residues, respectively. Apple was also the crop with the highest number of samples with multiple residues (29 samples). The majority of the samples with multiple residues (83.7%) contained two pesticides and only one sample (tomato) contained four pesticides (Table 2).

When investigating the distributions of the pesticide residue levels used in this study, we found that they were highly left skewed, with few high values from the database. Residues detected in the samples (≥LOQ) ranged from 0.0075 mg/kg (chlorpyrifos in tomato) to 10.5 mg/kg (carbofuran in strawberry), with a mean of 0.33 mg/kg ±0.87 and median of 0.10 mg/kg. One papaya sample containing residues of azinphos ethyl (0.2 mg/kg) was not considered in the cumulative study, as no toxicological data was available to generate the RPF for this compound.

3.2. Relative potency factors

Table 3 shows the relevant doses and the calculated relative potency factors (RPF) for the compounds considered in this study, using methamidophos or acephate as index compounds (IC). In both cases, more than one relevant dose was used in the calculations, to meet the requirement of the same time frame and specie of the relevant dose for both the IC and the compound of interest. Whenever available, benchmark dose at 10%

Table 2
Summary of residue data of acetylcholinesterase inhibiting compounds detected on samples analyzed during the 2001–2004 Brazilian monitoring program on pesticide residues

Crop	Samples		Samples with multiple residues	
	Analyzed/detected[a]	Main pesticides	# Samples/# pesticides	Main pesticides
Apple	369/128	CLO	24/2; 5/3	CLO + DIM and/or FEN
Potato	529/138	CLO	3/2	CLO + ACE
Tomato	540/107	MEP	20/2; 3/3; 1/4	MEP + MON; CLO + PRO
Strawberry	435/85	DIM, MEP, ACE	12/2; 3/3	ACE + MEP
Orange	505/44	MET, ETI	6/2; 1/3	MET + ETI or others
Papaya	447/24	MEP	2/2	DIM + MEP
Lettuce	347/14	CLO	–	–
Banana	394/4	ACE	–	–
Carrots	435/3	CLO	–	–

[a] ≥LOQ; CLO: chlorpyrifos; MEP: methamidophos; DIM: dimethoathe; ACE: acephate; MET: methidathion; FEN: fenitrothion; MON: monocrothophos; ETI: ethion; PRO: profenophos.

Table 3
Relevant doses (BMD$_{10}$ or NOAEL) and the relative potency factor (RPF) calculated for the two index compounds

Pesticide	BMD$_{10}$[a] or NOAEL			RPF, as index compound	
	mg/kg bw	Effect	Source	Methamidophos	Acephate
Acephate	0.99 0.58[a] 2.5	Brain/rat Brain/rat Brain/rat	EPA01 JMPR02 JMPR02	0.08	1.0
Aldicarb	0.096	Brain/dog	JMPR92	3.12	26
Azinph. methyl	0.86	Brain/rat	EPA01	0.09	1.15
Carbaryl	1	Brain/rat	JMPR01	0.30	2.5
Carbofuran	0.20	Brain/rat	CalEPA00	1.5	12.5
Chlorpyrifos	1.48	Brain/rat	EPA01	0.05	0.67
Diazinon	6.24	Brain/rat	EPA01	0.01	0.16
Dichlorvos	2.35	Brain/rat	EPA01	0.03	0.42
Dimethoate	0.25	Brain/rat	EPA01	0.32	4.0
Disulfoton	0.07	Brain/rat	EPA01	1.1	14.1
Ethion	0.06[a]	Brain/dog	JMPR90	1.1	9.67
Fenitrothion	2.5[a]	Brain/rat	JMPR00	0.03	0.23
Fenthion	0.24	Brain/rat	EPA01	0.33	4.17
Malathion	313.9	Brain/rat	EPA01	0.0003	0.003
Methamidophos	0.08 0.067[a] 0.3	Brain/rat Brain/rat Brain/rat	EPA01 JMPR02 JMPR02	1.0	12.4
Methidathion	0.25	Brain/rat	EPA01	0.32	4.0
Monocrotophos	0.1	Brain/rat	NL03	3.0	25
Parath. methyl	0.67	Brain/rat	EPA01	0.12	1.49
Parathion	0.5	Brain/rat	EPA99	0.6	5.0
Phenthoate	1.0[a]	Brain/rat	JMPR84	0.067	0.58
Pirimi. methyl	2	Brain/rat	EPA01	0.04	0.5
Profenophos	20.58	Brain/rat	EPA01	0.004	0.05
Pyrazophos	0.21[a]	Brain/rat	JMPR92	0.32	2.76
Terbufos	0.10	Brain/rat	EPA01	0.8	9.9
Triazophos	0.012[a]	RBC/dog	JMPR02	5.58	48.3

[a] Sub-chronic or chronic studies.

acetylcholinesterase (AChE) inhibition (BMD$_{10}$) in rat brain obtained from EPA (2001) was used as the relevant dose in the calculations. In all the other cases, non-observed adverse effect level (NOAELs) obtained from the JMPR (FAO/WHO Joint Meeting on Pesticide Residues), CalEPA (California Environmental Protection Agency) or The Netherlands Health Council monographs were used. In most cases, rat brain NOAELs obtained from single dose acute studies on rat were selected as the relevant dose. For carbaryl, the NOAEL from a 13 weeks study was considered as an acute end point, due to the rapid reversibility of the rat brain AChE activity inhibited by this compound (2001 JMPR; IPCS, 2005). For aldicarb, NOAEL from a 2 weeks study in dog was used, as it was the only acute dose available (1995 JMPR).

No acute data in laboratory animals were available for chlorpyrifos, ethion, fenitrothion, phenthoate, pyrazophos, terbufos and triazophos. In those cases, sub-chronic (90 days) and/or chronic data (1 or 2 years) were used in the calculation of the RPF. For triazophos, NOAEL was only available for RBC AChE inhibition, as this is the most critical effect of this compound in laboratory animals (2002 JMPR).

3.3. Exposure and risk assessment

Table 4 shows the results (mean ± standard deviation among the regions) of the upper tail distributions of the cumulative acute dietary exposure (in μg/kg bw) to AChE inhibiting pesticides for the general population and children for the two index compound scenarios. The lower and upper bounds of the 95% confidence interval (CI) for the estimates are given for the P99.9. For the general population, the mean (national) intakes at the P99.9 were 3.36 μg/kg bw as methamidophos and 35.1 μg/kg

Table 4
Intakes[a] at the highest percentiles of the distribution of the cumulative acute dietary exposure (μg/kg bw) to acetylcholinesterase inhibiting pesticides in the Brazilian general population and children based on different index compounds (mean ± standard deviation among the five Brazilian regions)

Percentile	Index compound	
	Methamidophos ARfD = 10 μg/kg bw[b]	Acephate ARfD = 50 μg/kg bw[b]
General population, 0–110 years old		
90.00	0.000 ± 0.000	0.004 ± 0.004
95.00	0.005 ± 0.006	0.067 ± 0.0.072
97.50	0.086 ± 0.059	0.963 ± 0.654
99.00	0.444 ± 0.152	4.88 ± 1.65
99.90	3.36 ± 0.27	35.1 ± 2.1
CI[c]	1.66 ± 0.22–6.60 ± 0.50	18.7 ± 1.6–70.9 ± 9.0
99.99	13.5 ± 1.15	134 ± 4
Children, 0–6 years old		
90.00	0.001 ± 0.001	0.010 ± 0.010
95.00	0.011 ± 0.013	0.148 ± 0.174
97.50	0.203 ± 0.157	2.25 ± 1.74
99.00	1.14 ± 0.42	12.8 ± 5.3
99.90	8.02 ± 0.97	84.5 ± 12.2
CI[c]	3.59 ± 0.52–18.3 ± 3.8	41.8 ± 5.1–171 ± 19
99.99	30.7 ± 3.3	359 ± 180

[a] Represents the intake given by the Monte Carlo iterations at each percentile.

[b] From 2002 JMPR.

[c] Mean of the lower-upper bounds of the 95% confidence interval around the P99.9 obtained by bootstrap.

bw as acephate. For children, the exposures were, on average, 2.4 times higher than the exposure found for the general population.

When residue levels were calculated with methamidophos as IC, the highest intakes were found in the southern region of the country, 3.66 μg/kg bw (1.81–7.03 μg/kg bw) at P99.9. In the acephate scenario, the central west region had the highest intakes within the country, with 37.6 μg/kg bw (18.1–69.3 μg/kg bw) at P99.9. The north region showed the lowest intakes in both scenarios.

The risk from the cumulative exposure to AChE inhibiting pesticides was evaluated by comparing the calculated intakes as each IC with the respective acute reference dose (ARfD) established by the JMPR (Table 4). The calculated risk having methamidophos as IC, in % ARfD, was about half of the risk calculated as acephate. For the general population at P99.9, the mean (national) exposure did not exceed the ARfD in any scenario, but the exposure exceeded the safe dose in all regions at the upper bound of the 95% CI for acephate (up to 160% of the ARfD). For methamidophos, the upper bound of the 95% CI at P99.9 represented a maximum of 70% of the ARfD (data not shown).

For children aged up to 6 years, the national exposure did not exceed the ARfD at P99.9 in any case. The upper bound of the 95% CI at P99.9 was 183% ARfD for methamidophos and 342% ARfD for acephate (Table 4). In both scenarios, the national exposure at P99.99 exceeded the safe dose for the general population and for children.

3.4. *Crops and pesticides which contributed most to the intake*

Tomato was the crop which contributed most to the total intake of AChE inhibiting pesticides in both IC scenarios (∼67%), followed by lettuce or papaya and apple (Fig. 2). Carrots and banana contributed less than 0.1% to the total intake in both cases. The higher contribution of tomato to the total intake was greatly due to the higher % of consumption days of this crop (Table 1), meaning that less zero-consumption days could be withdrawn from the consumption database during the simulations. The lowest intakes of AChE inhibiting pesticides found in the north region of the country, as shown previously, can be explained by the tomato consumption profile. This region had the lowest % consumption days and the lowest per capita daily consumption of this crop within the country, representing about 52 and 64% of the values in the other regions, respectively (data not shown).

Table 5 shows a summary of the residue data for tomato, papaya, lettuce and apple. The highest

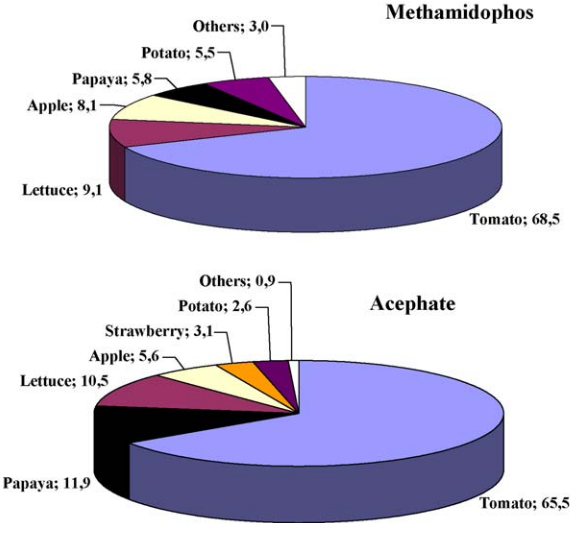

Fig. 2. Mean contribution of the crops to the total intake to acetylcholinesterase inhibiting pesticides at the highest 2.5 percentile of the intake distribution for the general population.

Table 5
Crops which contributed most to the cumulative exposure to acetylcholinesterase inhibiting pesticides and the main pesticides responsible for the top 10 highest concentration, expressed as each index compound (IC)

Crop pesticide	Samples ≥LOQ	Range (mg/kg)	Highest (mean[a] ± S.D.)	
			Methamidophos	Acephate
Tomato				
Methamidophos	51	0.1–2.3	6.2 (0.41 ± 0.74)	56.7 (4.5 ± 7.3)
Monocrotophos	10	0.09–0.6		
Triazophos	13	0.01–0.13; 0.87		
Papaya				
Methamidophos	14	0.01–0.40; 2.7; 7.6	7.6 (0.56 ± 1.6)	95.3 (7.0 ± 20)
Lettuce				
Methamidophos	2	4.3; 5.8	5.8 (0.78 ± 1.77)	72.8 (9.4 ± 22.2)
Apple				
Aldicarb	2	0.40; 0.70	2.18 (0.09 ± 0.23)	18.2 (0.87 ± 2.0)

Mean of residues ≥LOQ for each pesticide.

[a] Mean of residues ≥LOQ for all residues expressed as IC.

equivalent residues were found in papaya (7.6 and 95.3 mg/kg). For tomato, methamidophos, monocrotophos and/or triazophos were present in the samples which yielded the top 10 equivalent residue levels in this crop, expressed as either IC. All these samples had methamidophos, seven contained monocrotophos and nine had multiple residues. The sample with the highest equivalent residue level in tomato had detectable levels of methamidophos, monocrotophos, triazophos and phenthoate, with residues of triazophos representing about 75% of the total equivalent residues. Residues of monocrotophos, expressed as either IC, represented about 58–95% of the total equivalent residues in the samples containing multiple residues.

4. Discussion

The choice of the probabilistic acute intake distribution percentile to assure protection of a certain population should consider the nature of the databases available, their robustness and adequacy (EPA, 2000). As normally a few values from the input data sets seem to "drive" the exposure estimates at the high end of exposure, one should consider whether these values are representative of the real exposure. However, if, on the other hand, low percentiles are chosen, a large number of persons may be exposed to pesticide levels which are estimated to exceed the level of concern. Currently, the US Environmental Protection Agency (EPA) uses the P99.9 of the distribution for calculating a threshold of concern for acute exposure to a single chemical (EPA, 2000). No guidelines exist for the cumulative assessment. In any case, it is essential that all the uncertainties introduced in the assessments are discussed and the choice of the percentile should be made considering these uncertainties. In addition, it is essential to emphasize the limitation of the bootstrap sampling within the probabilistic model to estimate the uncertainties of the exposure at very high percentiles.

4.1. Food consumption and residue data

The source of the food consumption data used in this study was the most updated household budget survey (HBS) conducted in Brazil, and the only food survey available at national level. However, HBS data are not the most adequate source to evaluate food consumption of a certain population, as it reflects the amount of food acquired by the household, and not necessarily what is consumed by its members. The consumption data obtained from HBS data can underestimate real consumption levels by not accounting for outside consumption, or overestimate it by not accounting for wasted food (Serra-Majem et al., 2003). In addition, as the Brazilian HBS included households from the rural area of the country, overestimation can arrive when food self production was reported by the respondents. Indeed, extremely high consumption values were found for all commodities considered in this study (e.g. over 2000 g per person per day of tomato or apple). However, the frequency in which extreme consumption values were selected in the simulations was relatively low at P99.9 or higher. This was due, for the most part, to the large consumption data obtained from Brazilian HBS, which reflects the consumption of, at least, over 19,000 individuals per region.

One limitation of the HBS data is the lack of information on the daily consumption of the individuals. In this study, we assumed that the food acquired during a week will be consumed during this period, and is distributed through the number of days determined by the WCF. Although this might not be totally true for a non-perishable crop like rice, this could approach reality for the fruits and vegetable considered in this work. The most important limitation of the HBS data is, however, the inability to distinguish the consumption pattern among the individuals within a household. As the week consumption is divided equally among the individuals, it is likely that the food consumption for children was overestimated in this study. When the exposure was calculated for the general population of Brazil the impact of high consumption per body weight for children in the intake distribution was probably not major, as children in the HBS represented about 11% of the general population. Even if one considers the consumption of 500 g of potato by a 9 years old child (shown in the detailed description of the top 10 consumers in the simulation) as an overestimation, it can also be an infrequent, but real event. Infrequent events are not relevant for long term food exposure assessment, but are essential to assess acute exposure. When the estimations were performed only for the children population, however, is it likely that the level of exposure was overestimated.

In the current assessment, it was assumed that all residues reported as non-detectable were present at 1/2 LOQ level, as we did not have any evidence of a zero-residue situation in any case, even when arriving from illegal use. The effect of assigning residue levels to samples with levels <LOQ, however, was not expected to impact the outcome of the exposure assessment at the highest percentile. The LOQs assigned in this study were very low and any change in the assumption would be covered by the confidence intervals of the estimates. This hypothesis was confirmed for the south region using both index compounds, when the cumulative exposure assessments were also conducted using the assumption that all non-detects (<LOQ) were equal to 0.

When investigating the exposure for the top nine respondents around the P99.9 (data not shown), we found that highest equivalent residue levels in the crops, including tomato, were selected in the simulation. It is worth to point out that monocrotophos, present in tomato samples with the highest equivalent residues, has no registered use in tomato in Brazil, neither had it at the time the samples were analyzed within the PARA program (ANVISA, 2004). The same is true for methamidophos in papaya and lettuce, and aldicarb in apple. In addition, 19 of the 24 highest residue levels found in tomato were residues of methamidophos present at levels higher than the maximum residue level (MRL) for this compound (0.5 mg/kg). The highest residue level of triazophos in tomato, was also higher than the legal level. Therefore, illegal use of pesticides on tomato, papaya, lettuce and apple were very likely responsible for the acute exposure of pesticides in the Brazilian diet at P99.9 or higher percentiles. Illegal use of pesticides in Brazil, however, is not a frequent event. Data from the PARA program have shown that less than 0.3% of the crop-pesticide analyses (in 12.9% of the samples analyzed) were not according to the current legislation (PARA, 2005), from which about 29% concerned the AChE inhibiting compounds.

In a study on cumulative dietary exposure to AChE inhibiting pesticides in The Netherlands, Boon and Klaveren (2003) concluded that samples with multiple residues did not occur more frequently in the upper percentiles of the cumulative residue level distribution than samples with just one residue. In the present study, although the upper percentile of the cumulative residue distribution in tomato contained a high frequency of samples containing multiple residues, only one pesticide (either monocrotophos, triazophos or methamidophos) within those samples had a major contribution to the total equivalent residues.

4.2. Effect of processing factors and the variability of residues in individual units

Normally, the effects of processing on residues vary according to crop/pesticide combination, as well as with the extent of the processing procedure (FAO, 2002). To account for the uncertainty arriving from the processing factor (PF) values used in this study, which are only an estimation of the real values, a logistic-normal distribution was applied. The approach also includes the probability that unprocessed food was consumed (PF > 0.99). In a real situation, it is unlikely that unpeeled papaya or uncooked potato is consumed; however the consumption of unwashed tomato or/and apple are likely to occur occasionally.

For the purpose of this study, we assumed that residues found in the composite samples analyzed within the PARA program reflect the residues in any given individual unit which composed that sample. However, it is known that higher residues can be found in individual units than what would be expected based on the residues detected in composite samples (Harris and Hamey, 1999). A variability factor value of 3 to account for the variability of residues in units of composite samples has been estimated (Hamilton et al., 2004) and adopted at international level by the JMPR for the deterministic estimation of the acute exposure to pesticides (FAO, 2004). However, the application of one single variability factor is not realistic in the probabilistic model used in the present work. As it considers all crops together for the calculation of the total exposure, it is unlikely that one would eat the unit of every crop containing the highest residue level. However, we should recognize that it is possible that the consumption of highly contaminated units of one or two crops can occur in real life during the period of 1 day. Boon and Klaveren (2003) introduced the variability of the residues in the probabilistic cumulative assessment to AChE inhibiting compounds using the MCRA program. In this approach, the variability was accounted for by defining it as a model parameter, following a normal, beta or bernoulli distribution. The currently available residue data in individual units from market samples, for many pesticides and crops, might allow the introduction of a non-parametric distribution of variability of residues within the MCRA probabilistic model in the future.

4.3. Relative potency factor

In the Dutch study, monocrotophos and parathion, expressed as acephate and phosmet equivalents, were the compounds which contributed most to the upper percentile residue distribution of AChE inhibiting pesticides (Boon and Klaveren, 2003). According to the authors, this was due, for the most part, to the high RPF used for those compounds. In the present study, monocrotophos, triazophos, aldicarb and methamidophos were the main compounds present in the crops which contributed most to the total intake at the highest percentiles. Indeed, the first three compounds had the highest RPFs calculated as any IC in this study and methamidophos was among the six compounds with the highest RPFs.

Ideally, the RPF for a group of compounds with the same mechanism of toxicity should be calculated based on the same toxicological endpoint, time frame of exposure and specie, to provide a uniform measure of relative potency among the cumulative assessment group (Wilkinson et al., 2000). For the organophosphorus and carbamate pesticides, the toxicological endpoint is the ability to inhibit cholinesterase in plasma, RBC and brain, with this last one being the most critical (Carlock et al., 1999; EPA, 2001). A dose causing a particular biological response (e.g. BMD_{10}) is normally preferably than the NOAEL as the relevant dose in cumulative assessment, as the latter normally depends largely on the experimental design (dose selection and spacing) (EPA, 2001; Wilkinson et al., 2000). However, other approaches have been used in the past. The US National Research Council (NRC, 1993) estimated the relative potencies based on the lowest-observed-adverse-effect-levels (LOAEL) and the Environmental Working Group (EWG, 1998) used chronic reference doses. This latter procedure, however, was shown to overestimate the exposure as it incorporates safety factors which are, in most cases, not directly related to the toxicity of the compounds (Barraj et al., 1998).

In their cumulative assessment of organophosphorus insecticides, EPA used BDM_{10} for brain AChE inhibition from studies in female rats lasting 21 days or longer (EPA, 2001). In the present study, we used EPA BMD_{10} for 15 of the 25 compounds considered. In all the other cases, where NOAELs were used as the relevant dose, we opted to calculate the RPF based on single dose studies, to approach the time frame of dietary acute exposure. When data from acute studies were not available, subchronic or chronic studies were used. This was the case of triazophos, among other compounds.

In general, the selected index compound in a cumulative assessment should be the one best studied within the group and the one with the largest body of scientific data of acceptable quality (EPA, 2001; Boon and Klaveren, 2003). EPA has selected methamidophos as IC for their cumulative assessments for the large data for cholinesterase inhibition to support modeling of a BMD_{10} by different routes of exposure (EPA, 2001). Boon and Klaveren (2003) have selected acephate and phosmet for the large database for acute NOAEL available in the literature. In this study, when methamidophos was used as IC the estimated risk from dietary exposure to AChE inhibiting pesticides, in % of the ARfD, was about 50% of the risk calculated when acephate was used. This was due to the relatively low RPFs calculated for methamidophos, the most acute toxic pesticides among the ICs considered, compared with the ones calculated as acephate.

5. Conclusions

In general, the uncertainties of dietary exposure assessments to chemicals, including pesticides, rely mostly on the quality of the contaminant and food consumption data. In the cumulative exposure, the assessment also includes the uncertainties of the method used to cumulate the exposure, and of the toxicological data available for accounting for the differences in toxicity among the compounds with the same mechanism of action. Normally, higher percentiles of exposure might be used to assess the risk of a population to a certain chemical when the expected uncertainties from all the databases used in the estimations are low.

The risk assessment of the Brazilian population to AChE inhibiting pesticides conducted in this study focused mainly on the P99.9 exposure level and the bounds of the associated 95% confidence interval obtained by bootstrap in the probabilistic model. Monitoring residue data, as used here, are relatively refined and are appropriate to be used in exposure assessment to contaminants. However, as only the raw commodity was analyzed, the effect of processing needs to be estimated, which also incorporates uncertainties in the assessment. The possibility of high contaminated units being present within a composite sample analyzed in the monitoring program was not considered in this work, and it is possible that the residue database does not cover high exposure events from consumption of those contaminated food. Additionally, the present work only considered nine crops, which represent about 24% of total amount of fruit, vegetables and cereals consumed by the Brazilian population (IBGE, 2005). The exposure from the consumption of other crops treated with AChE inhibiting pesticides, mainly fruit and vegetables, might be significant.

A close investigation of the HBS data used in this study has shown that, in most cases, the amount of food consumed taken from the database in the simulations probably reflects the Brazilian dietary pattern of the crops considered. For the children, however, it is likely that the risk estimation conducted at P99.9 was conservative, due to the high food consumption levels very likely estimated for this population. However, children exposure to AChE inhibiting pesticides is also of additional concern, as this population might be more sensitive to these compounds than adults (Vidair, 2004). It is therefore very important to have accurate consumption data for children to have a better assessment of the risks that this population is exposed when consuming contaminated food. These data are not currently available for the Brazilian population.

The lower risk found in this study when methamidophos was used as the index compound demonstrates how critical is the choice of the IC in the assessment. The cumulative assessment is a relative new area, and many of the uncertainties arriving in the RPF calculation in this study was related to the lack of appropriate toxicological studies from which the relevant doses could be estimated. With that in mind, it is possible that future toxicological data could incorporate studies designed to generate more appropriate data for conducting cumulative assessment.

Finally, we should point out that assuming the dose additivity among the organophosphorus insecticides (Mileson et al., 1998), which was extrapolated in this work to the carbamates, the difference in pharmacokinetics and pharmacodynamics of these compounds are not considered. Furthermore, the results of the cumulative assessment conducted here need to be looked at also under the limitations of this approach, which does not consider the possible synergism and/or antagonism among the compounds.

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