Science of the Total Environment 527–528 (2015) 262–269

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The influence of tomato processing on residues of organochlorine and organophosphate insecticides and their associated dietary risk

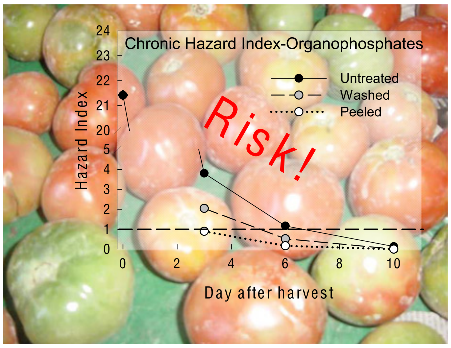
Emilie Reiler [a], Erik Jørs [b], Jesper Bælum [b], Omar Huici [c], Maria Mercedes Alvarez Caero [d], Nina Cedergreen [a,*]

[a] Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark
[b] Department of Occupational and Environmental Medicine, Odense University Hospital, University of Southern Denmark, Sdr. Boulevard 29, 5000 Odense C, Denmark
[c] Fundacion Plagbol, Calle Fernando Guachalla 705, Sopocachi, La paz, Bolivia
[d] Center of Water and Environmental Sanitation, Faculty of Science and Technology, University Major of San Simón, Sucre street in front of Park La Torre, Cochabamba, Bolivia

HIGHLIGHTS

- Six organochlorines and five organophosphates were analyzed in 54 tomato samples.
- All insecticides were detected in the µg/kg range (OC) and mg/kg range (OP).
- Storage, washing and peeling reduced the concentrations.
- A cumulative risk assessment showed elevated risk for up to 6 days of the OPs.
- Farmer education and introduction of less hazardous pesticides are urgently needed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 19 April 2015
Accepted 21 April 2015
Available online 14 May 2015

Editor: D. Barcelo

Keywords:
Dietary risk assessment
Consumer health
Pesticides
Organochlorines
Organophosphates
Hazard index

ABSTRACT

Due to the increasing food demand, the use of pesticides in agriculture is increasing. Particularly in low income countries poor training among farmers, combined with the use of obsolete pesticides may result in a high risk for the consumers. In this study six organochlorines and five organophosphates were analyzed in 54 samples of tomatoes from small scale farmers in Bolivia. The analyses were done on unprocessed, stored, washed and peeled tomatoes. The cumulated risk associated with consumption of the tomatoes after different storage times and processing treatments was evaluated using the Hazard Index (HI) for acute risk assessment. All 11 pesticides were detected in the analyses although several of them are obsolete and included in the Stockholm convention ratified by Bolivia. The organochlorines were found in the µg pesticide/kg tomato range and below the HI, while the organophosphates were present in the mg pesticide/kg tomato range and most often above the HI. The low organochlorine concentrations were not significantly affected by time or treatment, but storage significantly decreased the concentrations of organophosphates. Washing decreased the initial concentrations to between 53% (malathion) down to 2% (ethyl parathion), while peeling had a larger effect reducing the initial concentrations to between 33% (malathion) and 0.7% (chlorpyriphos). Both the acute and chronic cumulative risk assessment of organophosphates showed a dietary risk for unprocessed tomatoes three days after harvest. For children, also the consumption of washed tomatoes constituted a dietary risk. To reduce the dietary risk of pesticide residues in Bolivia, there is an urgent need of farmer education and introduction of less hazardous pesticides as well as resources for surveillance and enforcement of legislation in order to ensure public health.

---

* Corresponding author.

http://dx.doi.org/10.1016/j.scitotenv.2015.04.081

1. Introduction

There is an ever-increasing degree of public awareness and concern surrounding the issue of pesticides. Although the most commonly reported cases of adverse pesticide effects on human health are due to self-harm and occupational exposure, pesticide residues in food may add to exposures and/or in themselves cause a potential risk in the general population.

The use of pesticides in crops and the levels of residues in food are regulated in high income countries. Thus pesticide residues in food are generally low and not considered to cause severe adverse effects in the consumers (Jensen et al., 2003, 2009; Lozowicke, 2015; Nougadère et al., 2012). A lack of regulation and knowledge in low income countries, however, results in the farmer's practices and perceptions of pesticide risk being very different compared to standards in high income countries (Ecobichon, 2001). In low income countries pesticides are usually bought from agricultural supply stores, general shops, pharmacies or markets and are often dispensed in smaller quantities and in unlabelled containers, thus making it impossible for users to get information of optimal use practice, toxicity and safety measures needed (Dasgupta et al., 2007; Ngowi et al., 2007; Snelder et al., 2008). Use of instructions provided on the pesticide packaging is low, even when available, and the majority of farmers are functional illiterates and do seldom read the instructions for use, although it is an important factor for prevention of poisoning (Jørs et al., 2006). Instead the learning methods of spraying and tank filling are obtained by imitating relatives or neighbors (Ecobichon, 2001).

In Bolivia tomatoes are a major economic crop for small scale farmers living close to the big markets in the cities (Alverez et al., 2010). In most areas the tomato is grown conventionally implying a high use of external inputs such as chemical fertilizers and pesticides, but without the knowledge of how to use these inputs optimally and safely (Jørs et al., 2006). In addition the prescribed pre-harvest interval is generally not respected (Jørs et al., 2006). Using a higher dose and not respecting the prescribed time between the last spray and harvest make the probability of accumulation of residues in crops greater, thus potentially jeopardizing the health of the consumer.

With today's complexity in the mixture of chemicals present in our environment, their possible combination effects have attracted increasing amounts of attention (Reffstrup et al., 2010). Particular emphasis has been put on the shortcomings in the current mainstay of risk assessment. No matter how precautionary it may seem for the separate pesticides, risk assessment today widely disregards how different chemicals may add up or even act together to produce an effect (Kortenkamp, 2007; Kortenkamp et al., 2009; Reffstrup et al., 2010) and only certain chemicals sharing a common mode of action are subject to cumulative risk assessment (US EPA, 1999). Mixtures of chemicals in concentrations below their respective No Observable Adverse Effect Concentration (NOAEL) for a particular endpoint have been shown to be able to produce adverse effects simply by adding them up (Kortenkamp et al., 2007).

The aim of this study was to investigate whether dietary exposure of pesticides through a diet rich in tomatoes could pose a risk to human health using a cumulative risk assessment approach. In addition we wished to quantify the degree of risk reduction obtained by storage and treatment of the tomatoes. Therefore, Bolivian tomatoes were analyzed for two large groups of insecticides; the organochlorines and the organophosphates, both known for having relatively high human toxicities. The organochlorines are of special interest due to their remarkable persistence in the environment and bioaccumulation in organisms, which makes many of them listed in the Stockholm Convention of banned or restricted persistent organic pollutants (Stockholm Convention on Persistent Organic Pollutants, 2001). Organophosphates, on the other hand, are less persistent but acutely toxic and widely used (Boobis et al., 2008; Buratti et al., 2007; Marrs, 1993).

To make a cumulative risk assessment of pesticide residues in food we used the approach described by Jensen et al. (2003). For the risk assessment the acute reference dose (ARfD) and the acceptable daily intake (ADI) were used as predicted no effect levels for acute and chronic exposures, respectively. Both measures define the maximum dose which, according to all known facts at the time, will result in no harm to human health. The ARfD is the limit for consumption in one meal or in one day (acute toxicity), and the ADI is the amount that can be consumed every day for a lifetime (chronic toxicity) (IPCS, 2009). In the case of oral exposure, exposure assessment equals intake assessment. This can be made using different models, ranging from worst-case scenarios to more probabilistic models based on surveillance data (Boobis et al., 2008). In this study we use the country based estimations of intake of tomatoes for children and adults determined by the World Health Organisation (EFSA/FAO/WHO, 2011). Risk is then defined by the ratio of pesticide intake to ARfD or ADI yielding a hazard quotient (HQ) (see Eq. (1a) and (1b)). If the HQ has a value higher than 1.0 (>100% of ARfD or ADI), this indicates that the intake exceeds the value believed to be safe, hence, there is a risk (Boobis et al., 2008; Wilkinson et al., 2000).

$$HQ_{Chronic} = \frac{\text{Estimated Daily Intake}}{\text{ADI}} \tag{1a}$$

$$HQ_{Acute} = \frac{\text{Estimated Daily Intake}}{\text{ARfD}} \tag{1b}$$

In order to assess the cumulative effect of chemicals with the same mode of action, individual Hazard Quotients were summed to yield a hazard index (HI) (Eq. (2)) representing cumulative toxicity for chemicals with a common mode of action. The method is frequently applied to organophosphates (Jensen et al., 2003; Wilkinson et al., 2000).

$$HI = \sum_{\mathrm{i}}^{n} \mathrm{HQ_i} \tag{2}$$

More sophisticated ways of assessing cumulative risk exist (Boobis et al., 2008), however, in this study, we will use the HI based on cumulative groups as described in Jensen et al. (2003).

2. Materials and methods

2.1. Study area

The study was conducted in 2008 in 17 villages in the Municipalities of Omereque and Rio Chico placed relatively close north and south of Bolivia's Capital Sucre. In both regions the tomato is of growing economic interest for export to the markets in the big cities, such as Sucre. The municipality of Omereque has a population of 5148 inhabitants and is situated at an altitude of 1550 m above sea level having an annual average temperature of 23.0 °C and an average precipitation of 641 mm. Rio Chico has 10,630 inhabitants, is situated at an altitude of 1860 m and has an average annual temperature and precipitation of 22.8 °C and 506 mm, respectively.

2.2. Sampling

Samples of tomatoes were collected at harvest time by simple random sampling at 18 producers in 17 villages in the two municipalities. Tomatoes from the 18 producers were pooled in six groups representing well defined areas in the two municipalities. From each group three 2 kg sub-samples were selected and stored in paper bags for later treatments and pesticide analyses at day 3, day 6, and day 10 after harvest (Fig. 1).

Each sub sample was then either: left untreated (1), one was washed by cleaning with a soft brush under running water for approximately 1 min per tomato (2) and one was peeled with a knife (3). A total of 54 samples were prepared for analysis (18 samples per sampling time). All safety precautions, including use of special gloves to protect from organic compounds, were taken in the laboratory.

2.3. Materials

The following chemicals were used for the analyses: Sodium chloride, acetone (Merck, min. 99.5% purity), anhydrous sodium sulfate (Vel s.a., min. 99% purity), PSA Silica Bonded (Supelco 98% purity), acetonitrile, citrate sodium sesquihydrate, ethylacetate (Sigma-Aldrich 99% purity), dichloromethane, n-hexane (Scharlau, purity 99.9%), florisil (Supelco, PR 60/100 mesh), pentachloronitrobenzene, triphenylphosphate (Restek, 99%), Aldrin, dieldrin (ChemService, 98.7%), endrin (ChemService, 98.2%), endosulfan, heptachlor, methoxychlor, chlorpyriphos, dimethoate, malathion, ethyl- and methyl-parathion (Pestanal, 99.6%), 8141B Pesticide Calibration Mix B (Restek, 200 mg/l for individual pesticides), and a standard substitute dibutylchlorendate 1000 mg/l (Restek, 99%).

2.4. Analysis

The tomato samples were analyzed for pesticide residues in the laboratory of Centro de Aguas y Saneamiento Ambiental at Universidad Mayor de San Simon, Cochabamba, Bolivia.

The pre-treatment for the samples was conducted according to QuEChERS — Multiresidue Method for the Analysis of Pesticides. The quantification of residual levels was performed by gas chromatography following an adapted method of US Environmental Protection Agency (USEPA), 1996. The pesticides analyzed were: Aldrin, dieldrin, endosulfan, endrin, heptachlor, methoxychlor, chlorpyriphos, dimethoate, malathion, ethyl-parathion, and methyl-parathion. The limits of detection, limits of quantification and recoveries are given in Table 1.

For extraction, the tomatoes were blended and 10 g of sample was added to a falcon tube. Acetonitrile (10 mL) was added and the sample was mixed for a minute on a vortex shaker. Sodium chloride (1 g) and magnesium sulfate (4 g) were added and samples were again shaken for 1 min. The sample was transferred to a volumetric flask and the volume was adjusted with acetonitrile to 25 ml. Four milliliters of the extract was transferred to a falcon tube and the cleaning agent, consisting of Primary-secondary Amine (PSA) (0.1 g), magnesium sulfate (0.6 g) and activated carbon (0.03 g), was added. The solution was mixed for 1 min and left to precipitate. The acetonitrile fraction was removed and evaporated to dryness in a rotary evaporator at 40 °C and 410 mm Hg pressure. The resulting residue was re-dissolved in 1 mL acetone and concentrated under nitrogen gas flow with SUPELCO MINIVAP to a volume of 1 ml, and 80 μl of the internal standard was added. For the organochlorine pesticide, pentachlornitrobenzene in n-hexane (20 mg/l) was used as an internal standard and dibutylchlorendate (20 mg/l) was used as external standard. For the detection of organophosphates, tributylphosphate in acetone (40 mg/l) was used as internal standard and triphenyl phosphate (4 mg/l) was used as external standard.

The quantification of the pesticide residues on the final extract was performed using a SHIMADZU 17A gas chromatograph and

Fig. 1. Tomatoes were collected at the sample sites in 25 kg crates (A). Tomatoes from the 18 producers were pooled in six groups. From each group three subsamples of approximately 2 kg each were selected by random sampling in a frame subdivided in 16 squares (B). Residues from recent spray applications could be visually detected on several samples (C).

Table 1
Limit of detection (LOD), the Limit of Quantification (LOQ) and the recovery percentages of the pesticide residues measured in tomatoes together with the Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) as given by the British Crop Protection Council (2014). The ADI for aldrin and dieldrin represent the sum of the two. ARfD values could not be found for all organochlorines.

Pesticide	LOD (μg pesticide/kg of tomato)	LOQ (μg pesticide/kg of tomato)	R%	ADI (μg pesticide/kg body weight/day)	ARfD (μg pesticide/kg body weight/day)
Organochlorines					
Aldrin	0.75	0.88	79.5	0.1	3*
Dieldrin	0.68	0.79	80.2	0.1	3*
Endosulfan	2.70	3.15	79.2	6	20
Endrin	3.00	3.50	76.8	0.2	n/a
Heptachlor	0.81	0.95	80.5	0.1	n/a
Methoxychlor	0.78	0.91	77.3	100	n/a
Organophosphates					
Chlorpyriphos	0.98	1.14	78.5	10	100
Dimethoate	2.48	2.89	84.6	2	20
Malathion	0.65	0.76	72.9	300	2000
Ethyl-parathion	0.45	0.53	78,5	4	10
Methyl-parathion	0.45	0.53	91.4	3	30

* The ARfD is for the sum of Aldrin and dieldrin (EU Pesticide Database: http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=homepage&language=EN).

the software SHIMADZU CLASS VPTM 4.3. For organochlorines an Electron Capture Detector (ECD) was used. Organophosphates were detected using Flame Thermionic Detector (FTD). The carrier gas was helium, unless otherwise stated. A Cpsil19cb (Varian) column (50 m length, 0.25 mm internal diameter and 0.2 μm film thickness) was used. Split less injector temperature was 220 °C. The temperature program was the following: 100 °C for 2 min, 100–150 °C at 15 °C/min, 150 °C for 5 min, 150–240 °C at 10 °C/min, 240 °C for 11 min, 240–245 °C at 10 °C/min, and 245 °C for 19.5 min. The detector parameters were the following: ECD detector at 300 °C, carrier gas He (inlet pressure 60 KPa, split ratio 1:10 make up gas (N_2), 80 Kpa 15 ml/min). FTD detector at temperature 290 °C, carrier gas He (inlet pressure 60 KPa, split ratio 1:10, make up gas (He), 80 KPa 30 ml/min, hydrogen 50 KPa 3 ml/min, air 30 KPa 80 ml/min).

In order to quantify samples and evaluate the method, the areas of the chromatograms were integrated and the concentrations were calculated by comparison to the standards.

2.5. Risk calculations

ADI and ARfD defined by FAO/WHO Joint Meeting on Pesticide Residues (JMPR) were used to evaluate the pesticide residue levels found in the Bolivian tomatoes. ADI and ARfD values are presented in Table 1and are mainly retrieved from British Crop Protection Council (2014). As surveillance data of local Bolivian tomato consumption was not available, the values estimated by WHO were used (EFSA/FAO/WHO, 2011). For chronic intake of tomatoes an average of 25 g of tomato per person per day is estimated for most South American countries, including Bolivia. The adult body weight is estimated to be 52.2 kg, while for children it is 18.9 kg. For chronic risk assessment 25 g of tomato/day (app. 1/4 tomato) is the suggested intake value, while for acute risk one large portion of either 385 g of tomatoes for an adult or 215 g of tomatoes for a child is the suggested intake value (EFSA/FAO/WHO, 2011). For pesticides with the same mode of action, dose addition was assumed (Boobis et al., 2008). Thus the Hazard Index was used as described in Eq. (2) for organochlorines and organophosphates separately.

2.6. Statistical analysis

The pesticide concentrations of each of the 11 pesticides in the 54 measurements were analyzed for the effect of time (days 3, 6 and 10) and treatment (untreated, washed and pealed) using an Analysis of Covariance (ANCOVA) on the log transformed data. This was done assuming first order decay, giving a linear regression when the concentrations are log transformed:

$$C_t = C_{harvest} \cdot e^{-kt} \Leftrightarrow ln(C_t) = ln(C_{harvest}) - k \cdot t \tag{3}$$

where C_t is the pesticide concentration in the tomato at the time t, $C_{harvest}$ is the concentration at the time of harvest and k is the degradation constant. As there were observations below the limit of detection (LOD), and zero values cannot be log transformed, the value of ½ LOD was added to all data before the analyses were done. The estimated pesticide concentrations at harvest ($C_{harvest}$) for the untreated tomatoes are given together with the rate of decay for all pesticides where a significant time effect was detected in the ANCOVA. All analyses were done using the software program *R* (R Development Core Team).

3. Results

3.1. Pesticide residues in tomatoes

All pesticides analyzed for were detected in the tomatoes. Each of the organochlorines aldrin, dieldrin, endosulfan, endrin and methoxychlor were only detected in four or less samples of the 54 analyzed samples. For heptachlor, however, concentrations above the LOD and up to 63 μg of pesticide/kg tomato were detected in 46 samples. The geometric mean of all samples and the 95% confidence intervals were 3.30 (1.81–4.79) μg of heptachlor/kg of tomato (Fig. 2). There was no significant effect on residue level of either time or treatment for any of the organochlorines (Table 2). Dieldrin showed a slight statistical dependence on time, as all four positive samples were measured at day three.

The organophosphate residues were found in much higher amounts than the organochlorines, with dimethoate, methyl parathion and malathion being present in concentrations above 1.000 μg/kg (Table 3). All organophosphate concentrations decreased significantly with time presenting decay rate constants ranging from 0.42 to 0.82 per day, corresponding to half times (DT_{50}) of 0.85 to 1.65 days (Tables 2 and 3, Fig. 2). Washing or peeling also reduced organophosphate concentrations significantly, except for malathion where processing lowered the concentrations, although not significantly (Tables 2 and 3). Washing decreased the concentrations to an average of 54 ± 0.4% (mean ± st. dev.) in day three for dimethoate, malathion, and methyl-parathion, while chlorpyriphos and ethyl-parathion almost disappeared with washing (Table 3, Fig. 2). Peeling decreased the concentrations to an average of 28 ± 7% in day three for dimethoate, malathion and methyl-

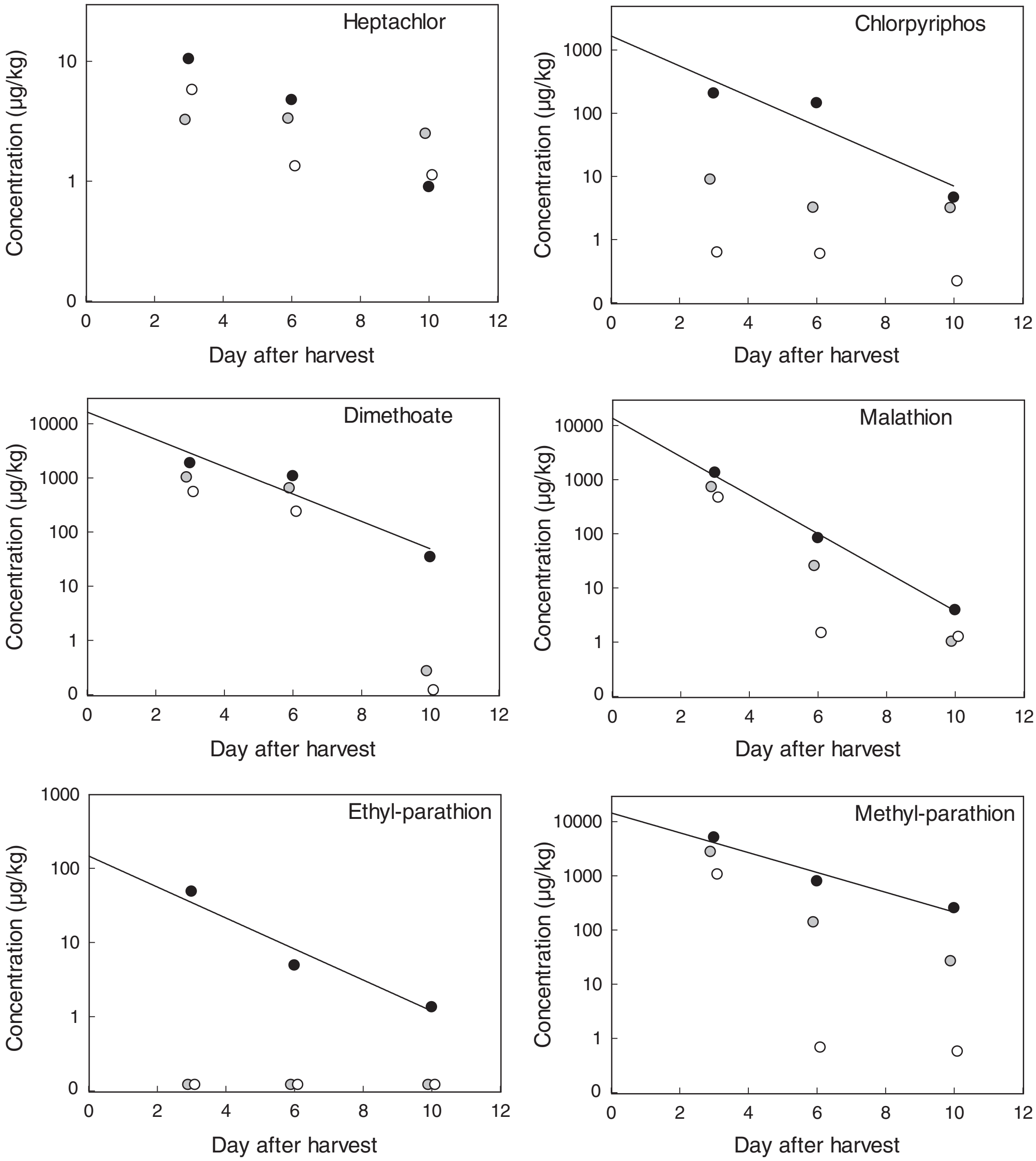


Fig. 2. The geometric mean of the concentrations of the organochlorine heptachlor and all the organophosphorous pesticides in untreated (black symbol), washed (gray symbol) and peeled (white symbol) tomatoes ($n = 6$). The organophosphate concentrations in the untreated tomatoes are described with a first order exponential decay model (black line). The R^2 for the regressions are: 0.33, 0.52, 0.42, 0.33 and 0.67 for chlorpyriphos, dimethoate, malathion, ethyl-parathion, and methyl-parathion. The 95% confidence intervals of the geometric means are given in Table 3. Note the different scales on the y-axis.

Table 2
The results of the Analyses of Covariance (ANCOVA) given as the p-values for significant effect, given in bold, of time after harvest (time), tomato treatment (treatment) and their interactions.

Pesticide	Time	Treatment	Interactions
Organochlorines			
Aldrin	0.53	0.51	0.09
Dieldrin	**0.04**	0.28	0.18
Endosulfan	0.11	0.14	0.08
Endrin	0.87	0.15	0.97
Heptachlor	0.08	0.80	0.60
Methoxychlor	0.17	0.38	0.27
Organophosphates			
Chlorpyriphos	<**0.01**	<**0.001**	0.06
Dimethoate	<**0.001**	<**0.001**	**0.04**
Malathion	<**0.001**	0.10	0.92
Ethyl-parathion	<**0.01**	<**0.001**	<**0.01**
Methyl-parathion	<**0.001**	<**0.001**	0.06

parathion, whereas chlorpyriphos and ethyl-parathion were entirely removed by peeling (Table 3, Fig. 2).

The geometric means of each organophosphate and heptachlor is depicted in Fig. 2 together with the exponential fit of the concentrations in the untreated tomatoes for the pesticides, where the ANCOVA showed significant effects of time.

3.2. Dietary risk assessment

The organochlorines posed neither an acute nor a chronic dietary risk as the Hazard Index values were all below the value of one (data not shown).

The cumulative risk of organophosphates, however, showed both an acute and a chronic risk for unprocessed tomatoes at day three, and for children there was also an acute and a chronic risk from eating washed tomatoes at day three after harvest (Fig. 3). There was no risk eating

Table 3
Geometric means of organophosphate residues found in tomatoes at each treatment and time ($n = 6$). Data are given in μg/kg with 95% Confidence Intervals (CI) in brackets, assuming log normally distributed data. Also an estimated value of the level of pesticide residue on the day of harvest, including 95% CI, as well as the degradation rate constant *k*, both calculated from the exponential decay model for untreated tomatoes (Eq. (3)) are given. ND is given for analyses with values below the limit of detection.

		Days after harvest				k (d^{-1})
Pesticide	Treatment	3	6	10	Estimated day zero	
Chlorpyriphos	None	204 (44–939)	144 (44–463)	4.60 (0–64)	1680 (97–29,122)	0.55
	Washed	8.86 (3.93–19.3)	3.19 (−0.03–29.1)	3.15 (0.26–17.2)		
	Peeled	0.62 (−0.01–2.12)	0.59 (0.03–1.73)	0.22 (−0.04–0.64)		
Dimethoate	None	1869 (1311–2666)	1074 (931–1238)	34.5 (2.43–345)	16,512 (2087–130,641)	0.58
	Washed	1021 (627–1664)	641 (447–919)	ND		
	Peeled	547 (251–1189)	237 (118–474)	ND		
Malathion	None	1344 (908–1990)	83.0 (3.61–1766)	3.90 (−0.06–68.0)	13,785 (395–481,511)	0.82
	Washed	725 (553–951)	25.4 (1.24–421)	1.01 (0.01–5.06)		
	Peeled	463 (300–716)	1.47 (−0.11–15.0)	1.24 (−0.16–15.4)		
Ethyl-parathion	None	48.4 (15.7–148)	4.90 (0.58–32.6)	1.34 (0.00–10.6)	146 (12–1860)	0.48
	Washed	ND	ND	ND		
	Peeled	ND	ND	ND		
Methyl-parathion	None	5080 (2695–9577)	791 (744–842)	252 (90.1–704)	14,494 (4920–42,702)	0.42
	Washed	2750 (1249–6056)	139 (64.0–303)	26.5 (3.71–181)		
	Peeled	1059 (295–3794)	0.67 (−0.06–4.83)	0.57 (−0.10–5.03)		

peeled tomatoes under the assumption used in the present study. The extrapolated concentrations at harvest are associated with great uncertainty (Table 3), and will not be further discussed. If, however, they are higher than at day three after harvest, the risk of eating freshly picked tomatoes will consequently be larger.

For unprocessed tomatoes dimethoate and methyl parathion were the two organophosphates contributing most to the chronic Hazard Index. At day three, dimethoate contributed with 64% of the risk, decreasing to 17% after 10 days of storage, due to its relatively fast disappearance rate, whereas the contribution of methyl parathion increased from contributing 36% to the risk at the day of harvest to 82% after 10 days due to its slower disappearance (Fig. 4).

4. Discussion

Results from this case study revealed a risk associated with the consumption of Bolivian tomatoes, particularly for children. This risk was mostly associated with the organophosphate residues while the risk linked to organochlorines was minor.

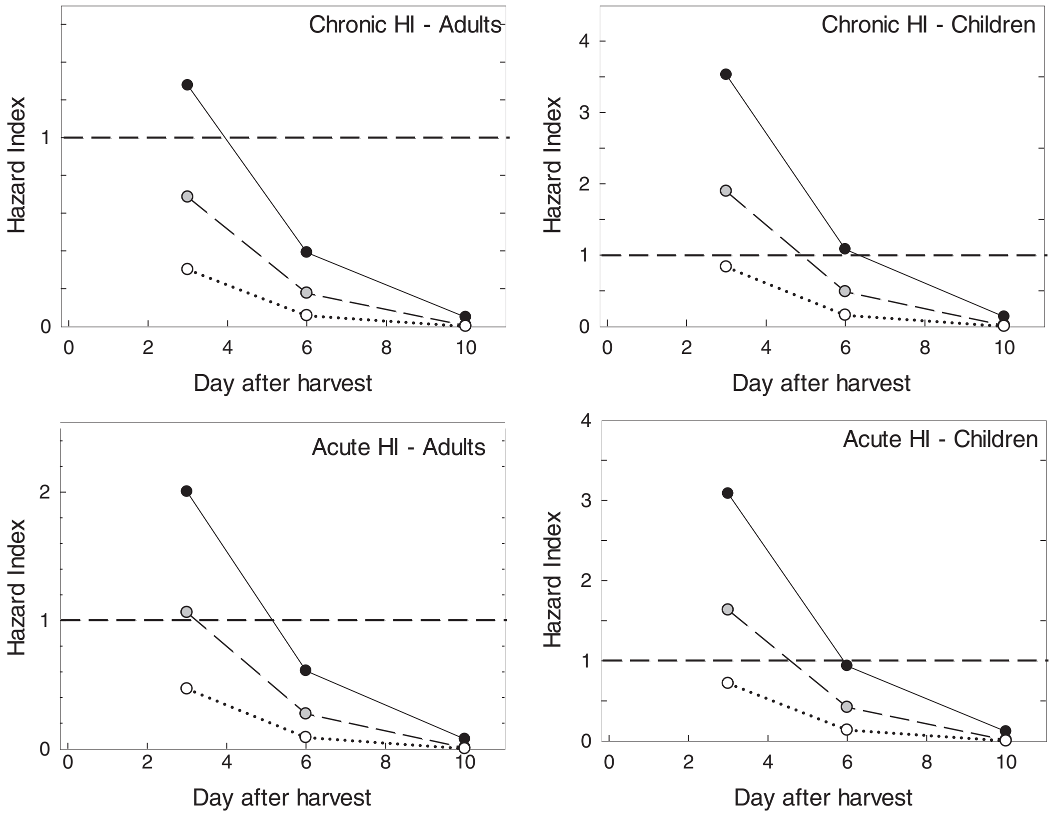


Fig. 3. Chronic and acute Hazard Index for adults and children based on summarized hazard quotient (geometric means) for organophosphate residues (Eq. (2)) as a function of time for the three treatments: Untreated (black symbols), washed (gray symbols) and peeled (white symbols). A Hazard Index based on the estimated concentration at the time of harvest for untreated tomatoes is included (Square). A Hazard Index above one (broken horizontal line) indicates a potential risk for an adult or child eating either 25 g of tomato a day (app. 1/4 tomato) in average (Chronic Risk), or one large portion of either 385 g of tomatoes for an adult or 215 g tomatoes for a child (Acute Risk) (EFSA/FAO/WHO, 2011).

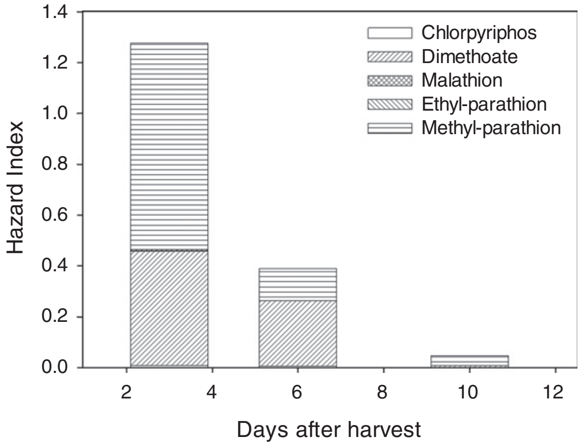


Fig. 4. Stacked Hazard Quotient (based on geometric means) summing up to the Hazard Index for organophosphate residues (Eq. (2)) in untreated tomatoes at each time, including the Hazard Quotient based on the estimated concentration at the time of harvest (Eq. (3)).

As expected, the concentration of pesticide residues and the associated risk were especially high in unprocessed tomatoes stored for only three days, whereas storage and processing reduced the risk of adverse effects considerably. The relatively rapid decay, with DT_{50} values of 0.85–1.65 days, was most likely partly due to a combination of hydrolysis and microbial degradation, as none of the organophosphates are known to be particularly volatile (British Crop Protection Council, 2014). Chlorpyriphos, dimethoate and malathion all have documented hydrolysis rates in the range of 0.5–6 days depending on pH (British Crop Protection Council, 2014), indicating that hydrolysis might be an important degradation pathway under the humid storage conditions. In addition, all five organophosphates have reported half-lives in soils of a few days, indicating that microbial degradation on the surface of the unwashed tomatoes could also be of importance (British Crop Protection Council, 2014). The organophosphate half-lives in this study are, however, in the very short end of literature values for environmental degradation, particularly considering the cool storage conditions, though degradation half-lives of chlorpyriphos residues in apples of <1 day have been reported (Knight and Hull, 1992). Hence, it cannot be excluded that physical removal of the pesticides to the paper bags that the tomato sub samples were kept in during storage have played a role in the decrease of the pesticides over time.

Washing and peeling the tomatoes efficiently removed almost all chlorpyriphos and ethyl-parathion. This was expected, as these two organophosphates have log K_{ow} values of 4.7 and 3.84, respectively, making them immobile in plant tissue and therefore located on the outer surface of the peel (British Crop Protection Council, 2014; Devine et al., 1993). Dimethoate, malathion and methyl-parathion, however, have log K_{ow} values of 0.7, 2.75 and 3.0, making them xylem mobile. Dimethoate has, with its pK_a of 2, also phloem-mobile properties (British Crop Protection Council, 2014; Devine et al., 1993). These three organophosphates might therefore also end up in the tomato-fruits via xylem or phloem transport from other parts of the plant, thereby being present in the tomato pulp in addition to the peel. This is most likely the main reason why washing and peeling removes these three organophosphates less efficiently compared to chlorpyriphos and ethyl-parathion. A meta study investigating the reduction of pesticide residue levels in fruit and vegetables found that washing on average reduced pesticide residues to 68% (CI95: 52–0.82) whereas peeling on average reduced pesticide residues to 41% (CI95 :30–54%) (Keikotlhaile et al., 2010). The reductions found in the present study for dimethoate, malathion and methyl-parathion where washing and peeling reduced the residues on day 3 to approximately 50% and 30%, fall well within the ranges found by Keikotlhaile et al. (2010).

The cumulated chronic and acute risk of eating unprocessed tomatoes on day three was significant for both adults and children. This is despite using the relatively low estimated daily intake of tomatoes in Bolivia of 25 g/person/day, corresponding to 1/4 of a tomato per day (EFSA/FAO/WHO, 2011). As this number might well be a Bolivian average, it is our impression that the tomato consumption in the tomato growing areas of Bolivia is considerably higher, more likely resembling the 100–200 g/person/day typical for Caribbean countries and eastern Mediterranean countries (EFSA/FAO/WHO, 2011). Increasing the chronic consumption to more than 1/4 tomato per person per day, will also increase the chronic risk. In addition, it is important to emphasize the fact that this study is confined to a small selection of pesticides in only one food commodity. Other pesticides are also present in various quantities in all cultivated commodities constituting a Bolivian diet. A Venezuelan study of organophosphate pesticide residues in six different vegetables, though, found tomatoes to have the highest detection frequency (62.5%) (Quintero et al., 2008), making tomatoes an important crop to investigate. In addition to the pesticides measured as part of this study, tomato farmers reported the use of various other pesticides, of which the concentrations are unknown (Table A.1). A recent review, however, confirms the finding of for example pyrethroids, carbamates and various fungicides in fruits and vegetables produced in low income countries (Syed et al., 2014). Some of these pesticides may give rise to additional dose addition or synergistic or antagonistic effects. Carbamates, for example, have been reported on the list of pesticides used in tomato crops in Bolivia (Table A.1). Since carbamates have the same mode of action as organophosphate pesticides, and in addition have shown to synergize these (Laetz et al., 2009; Walker, 2009), these could contribute to a higher HI, thus yielding a higher risk. In Brazil, studies have shown that the cumulative intake of organophosphorus and carbamate pesticides by high consumers of fruits and vegetables may represent a health concern (up to 169% of the ARfD) (Caldas and Jardim, 2012), hence, the problem seems to occur also outside Bolivia.

Illegal pesticides were also found in Brazilian fruits and vegetables (Jardim and Caldas, 2012). In a total of 13,556 samples of 22 fruit and vegetable crops, rice, and beans analyzed within two Brazilian pesticide residue monitoring programs between 2001 and 2010, 13.2% of the samples revealed non-authorized active ingredient use (Jardim and Caldas, 2012). Most of the organophosphates found in the Bolivian tomatoes are all legal; however, ethyl-parathion is illegal in Bolivia and should therefore not be found. In the present study, other illegal pesticides found included organochlorines. Even though the organochlorines in general did not indicate a risk for adults, aldrin, dieldrin, endrin, and heptachlor are listed in Annex A of the Stockholm convention, meaning that most countries in the world consider these chemicals so dangerous to humans and the environment that the chemicals should not be used (Stockholm Convention on Persistent Organic Pollutants, 2001). Heptachlor, which is classified as a possible carcinogen (WHO IARC, 2001), was detected in 85.2% of the Bolivian tomato samples, which is a very high amount considering that Bolivia ratified the Stockholm convention in 2003 and therefore has agreed not to produce or use these pesticides for any purpose since 2004. And though the organochlorines are persistent in the environment and the detected aldrin, dieldrin, endosulfan, endrin and metoxychlor therefore might be old residues circulating in the environment, the high frequency of heptachlor could indicate that it is still being used. A recent study showed considerable amounts of obsolete pesticides in the stocks in small holder farm houses in Bolivia (Haj-Younes et al., 2015).

A study of pesticide intoxications among Bolivian farmers from 2002 also showed that several banned and restricted pesticides such as aldrin and ethyl parathion were in use by more than 75% of farmers, and self reported symptoms of pesticide intoxications by organophosphates were common after pesticide handling (Jørs et al., 2006). Farmers used very few protective measures and had poor knowledge of the dangers related to pesticides and the benefit of protective measures when handling them (Jørs et al., 2006). Many low income countries have insufficient resources for enforcing limits and bans, and farmers may lack incentives to comply with the law (Ecobichon, 2001). These factors, along with poor literacy and a lack of training among farmers, make low income countries vulnerable to accidents and contamination of food, water, and environment (Dasgupta et al., 2007; Jørs et al., 2006; Ngowi et al., 2007; Snelder et al., 2008).

The task to reduce the population dietary exposure to toxic chemicals is a challenge for government authorities in all countries. Since pesticide exposure through tomato consumption showed to cause a real risk, measures should be taken in order to eliminate this risk. Though pesticide residues could be minimized by washing and peeling, applying the recommended dose and respecting pre-harvest intervals would be the best way to ensure that pesticide residue levels do not cause adverse effects on human health.

5. Conclusion

The findings of this Bolivian case study revealed a risk associated with the consumption of tomatoes, particularly for children. This risk was associated with the organophosphate residues while there was no risk linked to the detected organochlorines. The risk could be reduced by approximately 50% by washing the tomatoes and by approximately 70% by peeling the tomatoes. To reduce the dietary risk of pesticide residues in Bolivia, there is an urgent need of farmer education and introduction of less hazardous pesticides as well as resources for surveillance and enforcement of legislation in order to ensure public health.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.04.081.

Acknowledgment

The authors wish to thank the Bolivian NGO Plagbol and the two Danish NGO's Dialogos and ICOEPH running the Plagbol project for providing the data for the article. We also wish to thank the DANIDA (Danish Foreign Aid) (Nr. 104.N.366.b.4.) for funding activities to prevent pesticide poisonings through the project “Pesticides in Bolivia”.

References

Alverez, O., Rodrigo, G., Aramayo, A., 2010. Assessment of the contamination in the tomato by waste pesticides. The Municipalities of Omereque — Campero Province and Chico River, Municipality of Sucre — Chuquisaca (2010 November Cochabamba Bolivia).

Boobis, A.R., Ossendorp, B.C., Banasiak, U., Hamey, P.Y., Sebestyen, I., Moretto, A., 2008. Cumulative risk assessment of pesticide residues in food. Toxicol. Lett. 180 (2), 137–150.

British Crop Protection Council, 2014. Pesticide Manual—Online. http://bcpcdata.com.

Buratti, F.M., Leoni, C., Testai, E., 2007. The human metabolism of organophosphorothionate pesticides: consequences for toxicological risk assessment. J. Verbr. Lebensm. 2, 37–44.

Caldas, E.D., Jardim, A.N.O., 2012. Exposure to toxic chemicals in the diet: is the Brazilian population at risk? J. Expo. Sci. Environ. Epidemiol. 22, 1–15.

Dasgupta, S., Meisner, C., Wheeler, D., Xuyen, K., Thi, Lam N., 2007. Pesticide poisoning of farm workers—implications of blood test results from Vietnam. Int. J. Hyg. Environ. Health 210 (2), 121–132.

Devine, M., Duke, S.O., Fedke, C., 1993. Physiology of Herbicide Action. Prentice-Hall, Englewood Cliffs, NJ, p. 441.

Ecobichon, D.J., 2001. Pesticide use in developing countries. Toxicology 160 (1–3), 27–33.

European Food Safety Authority/FAO/WHO, 2011. Towards a harmonised total diet study approach: a guidance document. Including the Associated Spreadsheets: “Template for the Evaluation of Chronic Exposure (IEDI)” and “Template for the Evaluation of Acute Exposure (IESTI)”p. 71 (Available at: http://www.who.int/foodsafety/areas_work/chemical-risks/gems-food/en/).

Haj-Younes, J., Huici, O., Sale, Jørs E., 2015. Storage and use of legal, illegal and obsolete pesticides in Bolivia. Cogent Food Agric. 1, 1008860. http://dx.doi.org/10.1080/23311932.2015.1008860.

International Program on Chemical Safety (IPCS), 2009. Principles and methods for the risk assessment of chemicals in food. Environ. Health Criteria 240 (Available from http://www.inchem.org/documents/ehc/ehc/ehc240_index.htm).

Jardim, A.N.O., Caldas, E., 2012. Brazilian monitoring programs for pesticide residues in food — results from 2001 to 2010. Food Control 25, 607–616.

Jensen, A.F., Petersen, A., Granby, K., 2003. Cumulative risk assessment of the intake of organophosphorus and carbamate pesticides in the Danish diet. Food Addit. Contam. 20 (8), 776–785.

Jensen, B.H., Petersen, A., Christensen, T., 2009. Probabilistic assessment of the cumulative dietary acute exposure of the population of Denmark to organophosphorus and carbamate pesticides. Food Addit. Contam. 26 (7), 1038–1048.

Jørs, E., Morant, R.C., Aguilar, G.C., Huici, O., Lander, F., Bælum, J., Konradsen, F., 2006. Occupational pesticide intoxications among farmers in Bolivia: a cross-sectional study. Environ. Health 5, 10.

Keikotlhaile, B.M., Spanoghe, P., Steurbaut, W., 2010. Review: effects of food processing on pesticide residues in fruits and vegetables: a meta-analysis approach. Food Chem. Toxicol. 48, 1–6.

Knight, A.L., Hull, L.A., 1992. Linking insecticide bioassays with residue analyses to evaluate control of platynota-ideaeusalis (*Lepidoptera*, tortricidae) neonates on apple — seasonal spray program. J. Econ. Entomol. 85 (3), 932–938.

Kortenkamp, A., 2007. Ten years of mixing cocktails: a review of combination effects of endocrine disrupting chemicals. Environ. Health Perspect. 115 (1), 98–105.

Kortenkamp, A., Faust, M., Scholze, M., Backhaus, T., 2007. Low-level exposure to multiple chemicals: reason for human health concerns? Environ. Health Perspect. 115, 106–114.

Kortenkamp, A., Backhaus, T., Faust, M., 2009. State of the art report on mixture toxicity. European Commision Reportp. 31 (Available from http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf).

Laetz, C.A., Baldwin, D.H., Collier, T.K., Hebert, V., Stark, J.D., Scholz, N.L., 2009. The synergistic toxicity of pesticide mixtures: implications for risk assessment and the conservation of endangered pacific salmon. Environ. Health Perspect. 117, 348–353.

Lozowicke, D., 2015. Health risk for children and adults consuming apples with pesticide residues. Sci. Total Environ. 502, 184–198.

Marrs, T.C., 1993. Organophosphate poisoning. Pharmacol. Ther. 58 (1), 51–66.

Ngowi, A.V.F., Mbise, T.J., Ijani, A.S.M., London, L., Ajayi, O.C., 2007. Pesticides use by smallholder farmers in vegetable production in Northern Tanzania. Crop. Prot. 26 (11), 1617–1624.

Nougadère, A., Sirot, V., Kadar, A., Fastier, A., Truchot, E., Vergnet, C., Hommet, F., Baylé, J., Gros, P., Leblanc, J.-C., 2012. Total diet study on pesticide residues in France: levels in food as consumed and chronic dietary risk to consumers. Environ. Int. 45 (1), 135–150.

Quintero, A., Caselles, M.J., Ettiene, G., de Colmenares, N.G., Ramírez, T., Medina, D., 2008. Monitoring of organophosphorus pesticide residues in vegetables of agricultural area in Venezuela. Bull. Environ. Contam. Toxicol. 81 (4), 393–396. http://dx.doi.org/10.1007/s00128-008-9511-9.

Reffstrup, T.K., Larsen, J.C., Meyer, O., 2010. Risk assessment of mixtures of pesticides. Current approaches and future strategies. Regul. Toxicol. Pharmacol. 56, 174–192.

Snelder, D.J., Masipiqueña, M.D., de Snoo, G.R., 2008. Risk assessment of pesticide usage by smallholder farmers in the Cagayan Valley (Philippines). Crop. Prot. 27, 747–762.

Stockholm Convention on Persistent Organic Pollutants, 2001. English. PDFp. 43 pp. (Available at: http://chm.pops.int/Portals/0/Repository/convention_text/UNEP-POPS-COP-CONVTEXT-FULL).

Syed, J.H., Alamdar, A., Mohammad, A., Ahad, A., Shabir, Z., Ahmed, H., Ali, A.M., Sani, S.G.A.S., Bokhar, H., Gallegher, K.D., Amad, I., Eqani, S.A.M.A., 2014. Pesticide residues in fruits and vegetables from Pakistan: a review of the occurrence and associated human health risk. Environ. Sci. Pollut. Res. 21, 13367–13393.

US Environmental Protection Agency (USEPA), 1996. Organophosphorous Pesticides, SW-846 Method 8141A. Available from http://www.epa.gov/region9/qa/pdfs/8141.pdf.

US Environmental Protection Agency (USEPA), 1999. Guidance for Identifying Pesticide Chemicals and Other Substances That Have a Common Mechanism of Toxicity. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC (January 29, 1999).

Walker, C.H., 2009. Factors determining the toxicity of organic pollutants to animals and plants. Organic Pollutants. CRC Press, London, pp. 17–66.

Wilkinson, C.F., Christophn, G.R., Julien, E., Kelley, J.M., Kronenberg, J., McCarthy, J., Reis, R., 2000. Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: how to cumulate? Regul. Toxicol. Pharmacol. 31, 30–43.

World Health Organization International Agency for Research on Cancer (WHO IARC), 2001. Monographs on the Evaluation of Carcinogenic Risk to Humans. vol. 79.