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Pesticide potential dermal exposure during the manipulation of concentrated mixtures at small horticultural and floricultural production units in Argentina: The formulation effect

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HIGHLIGHTS

- A horticultural and floricultural worker's pesticide exposure study was done.
- A correlation between the pesticide formulation type and the exposure was found.
- Granulated formulations were the safest for the mix and load stage.
- The opening of the pesticide container is a risk operation for liquid formulations.
- External pesticide contamination of the containers could contribute to the exposure mechanism.

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ABSTRACT

Potential dermal exposure measurements of horticultural and floricultural field operators that handled concentrated pesticides showed a correlation with the types of formulations used (liquid or solid) during the mix and load stage. For liquid formulations, hand exposure was 22–62 times greater than that for solid ones. The dermal exposure mechanism was studied for this formulation under laboratory conditions, finding that the rupture of the aluminum seal of the pesticide container and the color of the liquid formulation are important factors. Additionally, significant external surface contamination of pesticide containers collected at horticultural farms was found. This could partially account for the differences between the exposure levels of field and laboratory experiments for liquid formulations.

1. Introduction

Crop productivity has experienced an important increase since the middle of last century (Dyson, 2000), as a result of better soil and water management, improved plant varieties, application of fertilizers and the use of pesticides (Cooper and Dobson, 2007). In the particular case of pesticides, the main beneficial effects of these products have been associated with the control of agricultural pests, having other indirect positive consequences such as the reduction of fungal toxins, and control of invasive species. Besides these positive aspects some negative characteristics have been observed, being the impact on the environment and human health the ones with more conspicuous repercussions. Despite these negative aspects, farmers continue to apply these products, in some countries in increasing quantities. This fact could be partially explained by the short term economic profit that derives from their use (Wilson and Tisdell, 2001), although at present concerns about sustainable use of pesticides are a matter of discussion between producers and regulatory authorities.

As mentioned above, one of the main negative effects of pesticide use is their effect on human health. In this respect it is well known that farm operators in particular are some of the most exposed subjects, especially when the pesticide application is done without the proper protection (Lesmes-Fabian et al., 2012). In an investigation done on 6300 cases with manual sprayers in 24 different countries, the effect of pesticide use on human health was studied (Tomenson and Matthews, 2009), finding that 1.2% of the operators experienced serious agrochemical related incidents (with hospitalization), while 5.2% of the total users had a moderate incident which required medical intervention. In another

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international study analyzing 8500 cases of operator's attitude and behavior regarding the use of pesticides (Matthews, 2008), in 26 different countries, it has been established that only 50% of the operators used gloves during the mixing stage of the concentrated pesticide. This occurred despite the recommendations of using personal protective equipment during this operation (International Labour Organization-WHO, 1991), perhaps because of climate stress factors such as high humidity and temperature (Park et al., 2009). In the same sense, in a study done among farmers in the Philippines, Lu (2009) has pointed out that 31.8% of the operators interviewed have experienced spillage on their hands.

The pesticide exposure risk is particularly important in small horticultural (Ramos et al., 2010) and floricultural (Flores et al., 2011) production units surrounding Buenos Aires city. There, working conditions are unfavorable, associated with lack of education, low technology and highly manpower-dependent tasks. In these cases we have previously shown that the mixing and loading operations could constitute a considerably risky stage. It has been pointed out that the type of pesticide formulation can modulate the toxicological effects on non-target systems and affect the pesticide's environmental fate (Cox and Surgan, 2006). In particular, for human exposure, the influence of the formulation type on dermal absorption has been studied from experimental (Aust et al., 2007) and modeling (Krüse and Verbek, 2008) perspectives. Moreover, it has been established that insecticides with the same active ingredient but different formulation have different biocide actions (Moreno et al., 2008).

Despite being recognized that the type of pesticide formulation may affect the operator's exposure (Damalas and Eleftherohorinos, 2011), to our best knowledge no quantitative analysis has been made studying the factors that modulate the exposure risk from this point of view. In this sense, the survey described in this report studied the exposure to pesticides under real working conditions in horticultural and floricultural production units and under controlled laboratory situations, analyzing the effect that the pesticide formulation has on the operator's exposure.

2. Methodology

2.1. Reagents and materials

For the preparation of each reference material, technical grade pesticides used in field trials were purified by recrystallization (95% pure by GC–FID), and the identity and purity of the active principles were confirmed by ^{1}H- and ^{13}C NMR. A primary solution of 300–1000 ppm w/w was prepared in acetone or cyclohexane, and all other working solutions were made by dilution as needed. Acetone and cyclohexane (Aberkon p.a. grade) used for all solutions and extracts, were previously distilled and chromatographically checked as suitable for GC–ECD use.

Commercial products used in the field trials were as follows:

- Captan ((3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione), CASRN [133-06-2]): Captan® (WP, 85% w/w) (Tomen-Chemiplant).
- Deltamethrin ((*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, CASRN [52918-63-5]): Decis Forte® (EC, 10% w/v) (Bayer CropScience Argentina).
- Procymidone (3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo [3.1.0]hexane-2,4-dione, CASRN [32809-16-8]) liquid: Sumilex® (CS, 50% w/v) (Summit Agro Argentina); and solid: Sumilex® (WP, 50% w/w) (S. Ando Argentina).
- Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide), CASRN [115-29-7]): Thionex ® (EC, 35% w/v, Magan).

For the preparation of pesticide surrogates Brilliant Blue #1, CI Nº 42090 (Sensient — Ardennes S.A.), phenolphthalein (Sigma-Aldrich) and glycerine pharmaceutical grade (Química Wisconsin, Argentina) were used as provided.

2.2. Study sites

All field experiments were carried out by local operators following their usual practices using commercial pesticides, during normal activities in greenhouses or open field plantations in the following locations, in the province of Buenos Aires (Argentina):

H_1–H_3: maize field in Moreno district;
H_4–H_8: mixed vegetable fields in Moreno district;
H_9–H_{16}: tomato greenhouses in San Pedro district;
F_1–F_7: flower greenhouses in Moreno district

2.3. Measuring devices

In the case of liquid formulations, the amount of product measured out by the operators was defined by volume (e.g. 10 mL) and checked by weighing on a portable scale. In the case of solid formulations, the amount handled was measured by weight difference of the pesticide or surrogate original vessel. Measuring devices used in commercial plantations were varied: spoons, cups, measuring cylinders, Falcon-type centrifuge tubes, etc. In laboratory experiences, various devices were tested: i) 15 mL graduated plastic cup, similar to those provided with medicines; ii) Falcon tube, 50 mL graduated plastic centrifuge tube with screw-top; iii) 2 mL piston bottle pumps, with screw-top, used for soap dispensers; and iv) plastic disposable teaspoons.

2.4. Field procedures

All field trials were carried out by the operators that usually perform the pesticide application in each plantation, following their habitual measuring and dilution methods, without any indication about procedure or dose. All products used were dispensed from commercial containers, some with intact seals, while others were in use so their seals were already opened. Prior to starting, operators were equipped with clean cotton gloves (used as samplers), and asked to open the container and prepare the mixture needed in a 20 L backpack sprayer. In experiences H4–H8, after opening the container, the operator measured out the dose with a plastic spoon into a tank but did not actually add water. The amount measured out was weighed in a portable scale before transferring it to the tank. After closing the tank and container, the gloves were taken off and placed in separate bags (right or left hand) and then taken to the lab for analysis. The spoons used in experiences H4–H8 (solid formulations) were collected individually and placed in extraction flasks for analysis.

In the cases where the pesticide content on the outside of the commercial containers was measured, the external surface of the container was rubbed with a piece of tissue paper soaked in acetone or cyclohexane. This was repeated with a fresh tissue. Both tissues were placed in a 100 ml bottle, taken to the laboratory and frozen.

2.5. Laboratory sampling methods with pesticide surrogates

Laboratory procedures were done using formulations of a food dye (Brilliant Blue) or phenolphthalein as pesticide surrogates under controlled laboratory working conditions.

2.6. Surrogates preparation

2.6.1. Solid formulations

For the preparation of 100 g of water soluble granules, 20 g of Brilliant Blue was mixed with 70 g of a soluble carrier (ammonium sulfate), 5 g of wetting agent (sodium lauryl sulfate), 4.5 g of dispersing agent (sodium naphtalensulfonate) and 0.5 g of powdered antifoam. The mixture was homogenized by milling and 5.0 to 8.0 mL of water was added to form a wet paste. Then, it was transferred to a LCI Benchtop

Granulator equipment, and small granules (3–4 mm) were obtained. The granules were dried applying dry heat in an oven and stored until use.

Surrogate containers were prepared by placing 30–40 g of the granules in 120 mL wide-orifice containers, with a disk of aluminum–polyethylene thermoseal composite heat-bonded over the mouth and a plastic screw-top over it.

Powder formulations were prepared by mortaring the granules, and bottled as previously described.

2.6.2. Liquid formulations

In order to obtain comparable results a liquid pesticide surrogate was required with flow characteristics similar to a real pesticide formulation; Sumilex CS was chosen as the model as it is a popular fungicide as well as very viscous. Brilliant Blue, a harmless dye used as food coloring was selected and glycerine used as a thickening agent. The viscosity was estimated comparing with Sumilex CS by the time needed for 5 mL of the liquid to flow out of a 25 mL burette. The mixture adopted finally was a 0.01 M solution of Brilliant Blue dye in 8% v/v water/glycerine, with a density of 1.27 g mL^{-1}.

In the case of phenolphthalein solutions a 0.01 M indicator concentration was prepared in a 33:77 v/v mixture of ethanol/glycerine, with viscosity similar to Sumilex CS.

Surrogate containers were prepared by placing 50–100 mL of the Brilliant Blue or phenolphthalein solutions in 250 mL and 1000 mL wide-opening (35 mm diameter) containers, with a disk of aluminum–polyethylene thermoseal composite heat-bonded over the mouth and a plastic screw-top over it.

2.7. Sampling procedures

Surrogate experiments were done by volunteer students, from an initial chemistry course for non-chemistry majors. Each student was provided with a set of clean cotton gloves, a measuring instrument (cup, falcon tube, spoon, pumping device), a container of colored pesticide surrogate (liquid or solid), beakers, wash-containers, etc. Instructions to the students were kept to a minimum, for example: put the gloves on and open the container (this could require breaking the aluminum seal), measure out a specified volume (e.g. 10 mL) and weigh it, transfer it into a beaker, rinse out the measuring device; take off the gloves and place them in separate extraction flasks.

2.8. Laboratory analysis

2.8.1. Samples with commercial pesticides

In the laboratory, cotton gloves used for field pesticide trials were placed in separate polyethylene flasks and extracted with 100 mL of cyclohexane (20 min, overhead shaker) not later than 8 h after the trial. Extracts were kept frozen until analyzed by GC–ECD. Quantification was done with external standards for each pesticide.

Tissue papers with pesticides were thawed, air-dried and extracted with 50 ml cyclohexane or acetone in an overhead shaker and analyzed by GC–ECD.

2.8.1.1. Chromatographic conditions. All chromatographic analyses were performed on a Perkin-Elmer (Norwalk CT, USA) AutoSystem XL Gas Chromatograph with an Autosampler automatic injector, equipped with an electron capture detector (ECD), and a fused silica capillary column (PE-5: 5% diphenylpolysiloxane–95% dimethylpolysiloxane stationary phase, 30 m in length, 0.25 mm i.d. and 0.25 μm film thickness). The GC–ECD operating conditions were: injector temperature: 280 °C; ECD temperature: 375 °C; oven temperature: 190 °C for 1.5 min, 45 °C min^{-1} to 300 °C then 10 °C min^{-1} to 320 °C and hold 2 min; injection volume 1 μL, splitless; carrier gas: N_2, 30 psi; ECD auxiliary flow 30 mL min^{-1}.

2.8.1.2. Pesticide stability on the sampler. Experiments were performed in order to investigate if pesticides were stable or suffered decomposition or were otherwise lost on the cotton cloth used for sampling (Hughes et al., 2006). No loss was observed for storage periods of up to 24 h.

2.8.2. Samples with Brilliant Blue as pesticide surrogate

Gloves used in the dye experiments were treated similarly as in 2.8.1 using 100 mL of distilled water. Brilliant Blue recovery was in the range 90–110%. The absorbance of the extracts was measured at 629 nm and quantified by comparison with a Brilliant Blue calibration curve in a Lambda 20 spectrophotometer (Perkin-Elmer, Norwalk, NJ) with a 10 mm cell.

2.8.3. Samples with phenolphthalein as pesticide surrogate

Gloves used in the phenolphthalein experiments were extracted using 100 mL of water at pH = 10 (with two drops of NaOH 1 M). Phenolphthalein recovery was in the range 90–110%. The absorbance of the extracts was measured at 562 nm and quantified by comparison with a calibration curve of phenolphthalein at pH = 10 in a Lambda 20 spectrophotometer (Perkin-Elmer, Norwalk, NJ) with a 10 mm cell.

2.9. Potential Manual Exposure and Margin of Safety calculations

Potential Manual Exposure (PME) is defined in this work as the amount of pesticide that can reach the hands (either skin or protective gloves) of the operator at the mix and load step, expressed in mg of the active ingredient. The percentual PME (% PME) is defined as the PME divided by the amount of active ingredient manipulated in the mix and load step, expressed in mg, and multiplied by 100.

The Margin of Safety (MOS, Flores et al., 2011), a risk indicator, was calculated according to the following expression:

$$MOS = (AOEL \times BW)/(PME \times 0.11)$$

where:

AOEL: is the acceptable operator exposure level (expressed in mg kg^{-1} d^{-1}).
BW: is the body mass (considered as 70 kg).
PME: is the total Pesticide Manual Exposure expressed in mg.

The coefficient 0.11 is accepted as the relative amount of the manipulated pesticide that could be absorbed through the operator's skin (Flores et al., 2011).

Taking into account that the use of protective gloves was not a usual practice between the horticultural and floricultural workers, for the MOS calculation we are assuming a “worst case scenario”. This is the reason why we are not using any additional coefficient for considering personal protection equipment.

To obtain the total mass of analyte in each glove, concentrations resulting from the chromatographic or photometric analysis were combined with the volume of the extraction solvent used in each case.

3. Results and discussion

3.1. PME of horticultural and floricultural workers

A set of PME determinations was made on floricultural and horticultural operators that manipulated different pesticides in single mix and load operations. The measured PME was transformed to percentual PME (% PME) by normalizing the PME value with the total amount of the manipulated active ingredient, and expressing it as a percentage. This conversion was done in order to allow comparisons between different trials, where different active ingredients and consequently dissimilar pesticide amounts were used.

Table 1 shows the results for the operators' manual exposure, expressed as % PME, classified by activity (horticulture or floriculture)

Table 1
Floricultural and horticultural workers PME during the mix/load stage.

	% PME for horticulture (solid formulations)								
	H_1[a]	H_2	H_3	H_4	H_5	H_6	H_7	H_8	Mean ± SD
Left hand	0.004	0.003	0.005	0.0007	0.0005	0.0002	0.0003	0.0002	0.002 ± 0.002
Right hand	0.057	0.017	0.018	0.0025	0.0077	0.0046	0.0030	0.0044	0.014 ± 0.017
Total	0.061	0.020	0.023	0.0032	0.0082	0.0048	0.0033	0.0046	0.02 ± 0.02
	% PME for horticulture (liquid formulations)								
	H_9[b]	H_{10}	H_{11}	H_{12}	H_{13}	H_{14}	H_{15}	H_{16}	Mean ± SD
Left hand	0.065	0.075	0.052	0.202	0.055	0.111	0.064	0.807	0.18 ± 0.24
Right hand	0.044	0.110	0.173	0.150	0.047	0.091	1.035	0.453	0.26 ± 0.32
Total	0.109	0.185	0.225	0.352	0.102	0.202	1.099	1.260	0.44 ± 0.43
	% PME for floriculture (liquid formulations)								
	F_1[c]	F_2	F_3	F_4	F_5	F_6	F_7	–	Mean ± SD
Left hand	1.45	2.21	0.34	0.05	1.28	0.05	0.55	–	0.85 ± 0.82
Right hand	0.25	0.21	0.22	1.37	0.25	0.02	0.55		0.41 ± 0.45
Total	1.70	2.42	0.56	1.42	1.53	0.07	1.10		1.25 ± 0.77

[a] Total masses of manipulated pesticide. H_1: captan, 8755 mg; H_2: captan, 8594 mg; H_3: captan, 6715 mg; H_4: procymidone, 36300 mg; H_5: procymidone 19300 mg; H_6: procymidone, 18.000 mg; H_7: procymidone, 10800 mg; H_8: procymidone, 19100 mg.
[b] H_9: deltamethrin, 573 mg; H_{10}: deltamethrin, 562 mg; H_{11}: procymidone, 3592 mg; H_{12}: procymidone, 4085 mg; H_{13}: deltamethrin, 488 mg; H_{14}: deltamethrin, 467 mg; H_{15}: procymidone, 3909 mg; H_{16}: procymidone, 4331 mg.
[c] F_1: endosulfan, 3500 mg; F_2: endosulfan, 9410 mg; F_3: endosulfan, 8288 mg; F_4: procymidone, 1901 mg; F_5: endosulfan, 3665 mg; F_6: endosulfan, 4226 mg; F_7: endosulfan, 6280 mg.

and formulation type (solid or liquid). The mean % PME value for the manipulation of solid formulations by horticultural operators was 0.02 ± 0.02%, while the mean % PME found for these workers when they manipulated liquid formulations was practically twenty times higher (0.44 ± 0.43%, Table 1). In the case of the floricultural operators manipulating liquid formulations, the mean % PME value was even higher: 1.25 ± 0.77% (Table 1).

For comparison purposes Fig. 1 presents the logarithmic value of the % PME for all the cases studied. It can be observed that three different groups associated with the activity and type of formulation can be distinguished: horticultural operators that have manipulated solid formulations (HSF, Fig. 1), horticultural operators that have manipulated liquid formulations (HLF, Fig. 1), and floricultural operators that have manipulated liquid formulations (FLF, Fig. 1). The dashed lines represent the mean values of log (% PME) for each of the aforementioned groups. These results seem to indicate that under the same conditions, solid formulations were safer than liquid ones, during the mix and load step. Additionally, the significant differences found for the log (% PME) between different types of formulations (HSF vs HLF, Fig. 1) for the same activity could be an indication that the type of formulation has a more important effect on the exposure than the kind of agricultural activity (HLF vs. FLF, Fig. 1), when the mix and load operation is considered.

The Margin of Safety (MOS) was calculated for each case in order to determine if a single operation was safe or not. The values were represented as logarithm of the MOS in Fig. 1B, which means that unsafe operations (with MOS < 1) have logMOS < 0 (Fig. 1B). As expected, it can be observed that the risk estimation is strongly affected by the toxicological properties (AOEL) of each active ingredient. In any case, 11 of the 23 cases studied were unsafe for a single mix and load operation, emphasizing the risk associated with the manipulation of concentrated pesticide mixtures.

Fig. 2 shows the values of the MOS of the mix and load stage, expressed as its logarithm, for each type of activity, formulation (HSF, HLF, FLF, Fig. 2) and pesticide (captan, procymidone, deltamethrin, endosulfan, Fig. 2). Calculations were done considering one, two and four backpack sprayer preparations, using the mean values of % PME and mean mass manipulated for each activity and pesticide. It can be observed that while the preparation of one backpack was a safe operation for procymidone and deltamethrin, when two backpacks were considered, deltamethrin resulted unsafe, and for the mix and load of four

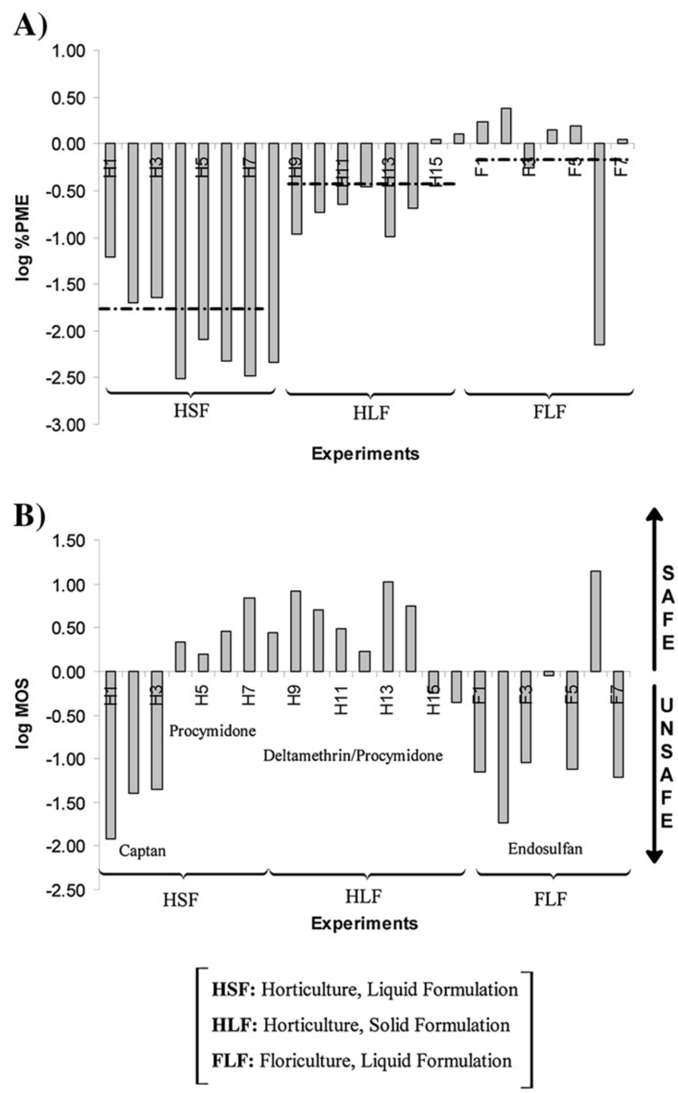

Fig. 1. A) % PME of horticultural and floricultural workers in the mix and load stage. B) MOS of horticultural and floricultural workers in the mix and load stage for a unique mix and load operation.

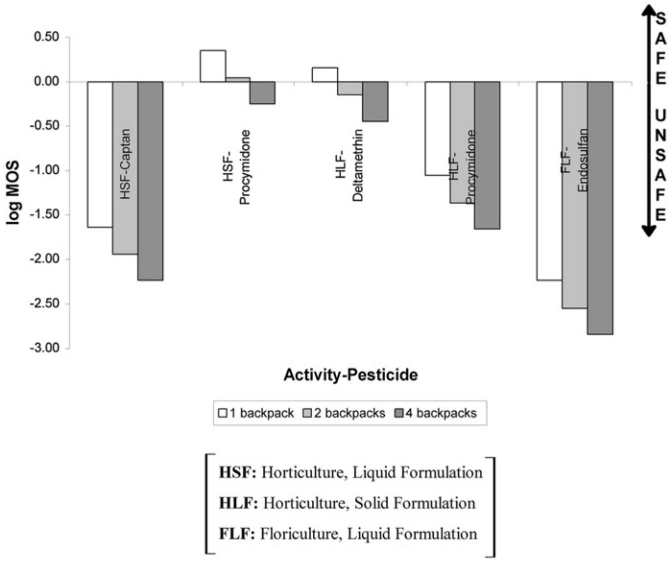

Fig. 2. MOS for the mix and load stage for one, two and four backpack sprayer preparations.

backpack sprayers, which is a common labor duty for a journey (Ribeiro et al., 2012), all the considered pesticides resulted in unsafe operations.

An interesting consideration derived from the field trials results, is that there seems to be a lateralization effect for the solid formulations (right hands 0.014 ± 0.017%, left hands 0.002 ± 0.002%, Table 1). This fact could be partially explained by the observation that most operators hold the pesticide container with their left hand while approximating a spoon to the mouth of the pesticide container, frequently reaching with their hands into the top of the container, which usually had pesticide deposited all round the inside.

Although none of the operators involved in the field determinations normally used protective gloves when they mix and load the pesticide, these results would be considered as a worst case scenario, because we cannot state that this is the situation for all horticultural and floricultural operators in Argentina.

3.2. PME determinations under laboratory conditions

3.2.1. PME for solid formulations

Having observed differentiated tendencies for field operators' PME when the formulation type was considered, a set of laboratory experiments was done, in order to understand some of the factors that could be affecting the exposure mechanism. For this purpose, first year chemistry undergraduate students (non-chemistry majors without laboratory experience) were selected as volunteers. In order to assure maximum safety conditions a set of pesticide surrogates was prepared in order to replace the phytosanitary active ingredient by an innocuous food dye (Brilliant Blue, see Materials and Methods Section). Two different solid formulations containing 20% w/w of the dye were prepared: a granulated one, and the powder that resulted from grinding the granulated formulation. The dye concentration was selected as 20% because this was the low end of the range of commercial solid pesticide formulations. PME experiments were performed with volunteers manipulating the same mass of powder or granulated surrogate (Table 2). After simulating a mix and load activity (see Methodology section), the amount of dye remaining on the gloves was spectrophotometrically determined. For the powder manipulation a mean value of 0.013 ± 0.011% of the total dye handled (Table 2) was found on the gloves, which is in the order of what we detected in the case of the horticultural operators who handled solid formulations (0.02 ± 0.02%, Table 1). For the granulated preparation, all values were below the analytical detection limit, which indicates that the granulated formulation was safer than the powdered one.

For this set of experiments, the residues of dye in the spoons used to transfer the formulation from its original flask to a specified beaker were determined. Table 2 presents the results for the residual amounts found for the powder manipulation (0.29 ± 0.15%, Table 2) and the granulated one (0.029 ± 0.035%, Table 2). The granulated formulation left much less residue of active ingredient on the spoons (practically one order of magnitude less). This could be of importance in reducing field-operators' exposure, as the reutilization of these implements without proper cleaning is commonly observed in the field.

It is interesting to remark that the mix and load operations performed at the laboratory, yielded similar total % PME values to the field experiments (Tables 1 and 2). Unlike the field values, the lateralization effect was less evident, due to the fact that the spoons used under laboratory conditions were in all cases clean. On the other hand, the kind of solid formulation (powder or granulated) had an important effect on the % PME and on the amounts found in the spoons used to manipulated it.

3.2.2. PME for liquid formulations

Taking into account the differences found in the level of exposure of the horticultural and floricultural operators when the formulation type was considered, experiments using liquid pesticide surrogate formulations were made (see Methodology section). In an attempt to understand the exposure mechanism, four different variables were taken into consideration: the presence (or not) of an aluminum seal on the pesticide container, the container size (250 mL or 1000 mL), the measuring device (cup, Falcon tube, soap dispenser-type delivery pump) and the formulation color (blue or colorless).

3.2.2.1. The effect of the aluminum seal on the % PME. This factor was evaluated because all new unopened pesticide containers are factory-sealed with a disk of polyethylene-laminated aluminum foil, in addition to the screw-cap, with no seal removal method provided. We had previously speculated that the opening of this seal could be a potentially risky operation although it is done only once for each new container.

When 250 mL bottles filled with Brilliant Blue solution and a measuring cup were used with an intact (Table 3, entry 1, 0.144 ± 0.130%), broken (Table 3, entry 2, 0.07 ± 0.08%) or absent (Table 3, entry 3, ND %) aluminum seal, a clear tendency was observed, showing that the rupture of the seal produced an increase in the % PME. The same effect was observed independently of the measuring device

Table 2
%PME for the mix and load stage of surrogate powder and granulated solid formulations.

Entry	Exper.	N[b]	Pesticide % on spoon ± SD	% PME Left hand ± SD	% PME Righ hand ± SD	% PME Total ± SD
1	P_{1-8} (SI-TI)[a]powder	8	0.29 ± 0.15	0.01 ± 0.01	0.003 ± 0.001	0.013 ± 0.011
2	P_{9-17} (SI-TII) granu-lated	8	0.029 ± 0.035	ND[c]	ND	ND

[a] SI-TI: Supplementary Material-Table number.
[b] Number of replicates.
[c] ND: not detected.

Table 3
%PME for the mix and load stage of liquid formulations with different bottle sizes, surrogate colors, measuring devices and bottle seals.

Entry	Experiment	N[b]	Bottle size (mL)	Color	Measuring device	Seal	% PME		
							Left hand ± SD	Righ hand ± SD	Total ± SD
1	$P_{17–31}$ (SI-TIII)[a]	15	250	BB[c]	Cup	I[d]	0.040 ± 0.055	0.103 ± 0.098	0.144 ± 0.130
2	$P_{32–45}$ (SI-TIV)	14	250	BB	Cup	B[e]	0.03 ± 0.04	0.04 ± 0.07	0.07 ± 0.08
3	$P_{47–65}$ (SI-TV)	19	250	BB	Cup	W[f]	ND[g]	ND	ND
4	$P_{66–69}$ (SI-TVI)	4	250	BB	Falcon	I	0.045 ± 0.005	0.12 ± 0.009	0.16 ± 0.09
5	$P_{70–74}$ (SI-TVII)	5	250	BB	Falcon	B	ND	ND	ND
6	$P_{75–84}$ (SI-TVIII)	10	250	BB	Pump	I	0.08 ± 0.03	0.13 ± 0.03	0.20 ± 0.18
7	$P_{85–94}$ (SI-TIX)	10	250	BB	Pump	B	ND	ND	ND
8	$P_{95–103}$ (SI-TVX)	9	1000	BB	Cup	I	0.029 ± 0.077	0.048 ± 0.067	0.077 ± 0.094
9	$P_{104–118}$ (SI-TXI)	15	1000	BB	Cup	W	ND	ND	ND
10	$P_{118–128}$ (SI-TXII)	10	250	Ph[h]	Cup	I	0.259 ± 0.245	0.466 ± 0.325	0.725 ± 0.327
11	$P_{129–138}$ (SI-TXIII)	10	250	Ph	Cup	W	0.050 ± 0.087	0.102 ± 0.209	0.152 ± 0.244
12	P_{139}–P_{148}(SI-TXIV)	10	1000	Ph	Cup	I	0.090 ± 0.122	0.228 ± 0.224	0.318 ± 0.299
13	$P_{149–158}$ (SI-TXV)	10	1000	Ph	Cup	B	0.141 ± 0.125	0.229 ± 0.284	0.371 ± 0.358
14	$P_{159–168}$ (SI-TXVI)	10	1000	Ph	Cup	W	0.092 ± 0.211	0.175 ± 0.343	0.267 ± 0.517

[a] SI-TI: Supplementary Material-Table number.
[b] Number of replicates.
[c] BB: Brilliant Blue.
[d] I: intact aluminum seal.
[e] B: broken aluminum seal.
[f] W: without aluminum seal.
[g] ND: not detected.
[h] Ph: uncolored phenolphthalein.

(Table 3, entries 4 vs 5 and 6 vs 7) or the container size (Table 3, entries 8 vs 9 and 10 vs 11). The only case where no clear seal effect was observed was for 1000 mL containers filled with phenolphthalein solution (Table 3, entries 12, 13 and 14); a possible explanation could be that the "colorless" effect could be masking the intact/broken seal influence.

In any case, the breaking of the container seal seems to be an important step in the exposure mechanism when new containers are handled.

3.2.2.2. The effect of container size on the % PME. A set of experiments were done comparing the % PME when handling 250 mL and 1000 mL surrogate pesticide containers. When the total % PME for liquid Brilliant Blue formulations with intact aluminum seal was measured with a small cup (Table 3, entry 1, mean of 0.144 ± 0.130%), the values were not very different when 1000 mL containers were manipulated under the same conditions (Table 3, entry 8, mean of 0.077 ± 0.094%). The same tendency was observed when 250 mL and 1000 mL containers with a colorless phenolphthalein formulation were manipulated (Table 3, entries 10 and 12).

These results could be indicating that the container size is not a determinant factor affecting the operator % PME. The higher values found for the 250 mL could probably be explained by the hand's proximity to the container orifice in comparison to the 1000 mL containers.

3.2.2.3. The effect of the measuring device on the % PME. In order to evaluate the influence of the devices used to measure the pesticide dose, a set of experiments was done using 250 mL Brilliant Blue surrogate containers and different measurement devices (a cup, a Falcon tube or a small soap-dispenser pump attached to the top of a pesticide container). The total % PME for the three different set of experiments was not very different when the containers had an aluminum seal (Table 3, entries 1, 4 and 6). When the experiments were repeated with containers without the aluminum cap (Table 3, entries 2, 5 and 7), although smaller % PME values were observed, they were quite similar, independently of the measuring device, indicating that the type of vessel used was not a critical variable associated to the exposure mechanism.

3.2.2.4. The effect of the formulated surrogate color on the % PME. Two different pesticide surrogates were used, a Brilliant Blue solution and a colorless acid phenolphthalein formulation. Using 250 mL containers with intact aluminum seals and cups as vessels, the total % PME for the uncoloured phenolphthalein solution was significantly larger (Table 3, entry 10, 0.725 ± 0.27%) than for Brilliant Blue formulation (Table 3, entry 1, 0.144 ± 0.130%). The same tendency was observed when 250 mL containers without the aluminum seal were used (Table 3, entries 3 and 11).

In order to study if this could be a general behavior, the same set of experiments was repeated using 1000 mL containers. When intact (Table 3, entries 8 and 12) or broken (Table 3, entries 9 and 14) aluminum seals, filled with Brilliant Blue or phenolphthalein formulations were used, an analogous tendency was observed, finding significant total % PME values for the uncoloured formulations. A possible explanation to this behavior could be associated to the fact that liquids with high color contrast (as happens with Brilliant Blue) could be more precisely transferred from one container to another, because they are easily observed. Another possible explanation could be related to the operators' subconscious precaution against touching colored liquids.

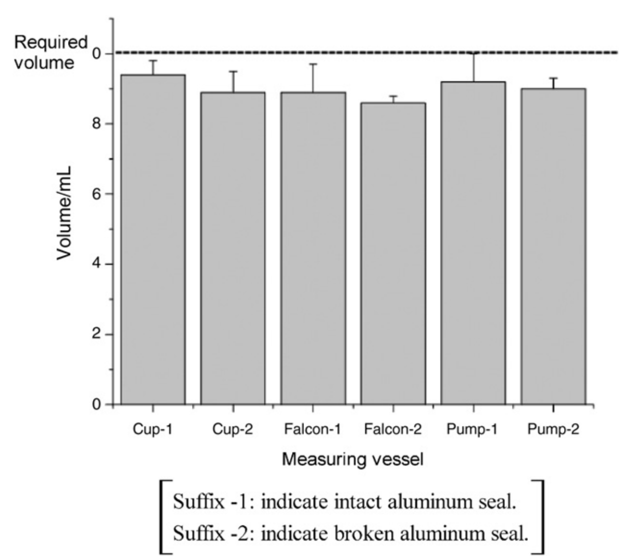

Fig. 3. Accuracy and precision of a liquid formulation measurement using different devices.

Table 4
Pesticide mass (mg) found on the exterior surface of commercial pesticide bottles.

M_i	Commercial name, active ingredient, condition of bottle	Pesticide mass (mg)				
		Chlorothalonil	Chlorpyrifos	Procymidone	Endosulfan	Deltamethrin
M_1	Starfos, chlorpyrifos 48%, empty/discarded	0.0005	0.31	ND[a]	0.0012	0.057
M_2	Shooter, chlorpyrifos 48%, empty/discarded	0.0002	0.054	ND	0.0010	0.021
M_3	Talone, chlorothalonil 50%, in use	0.735	0.021	ND	10.0	0.18
M_4	Endoglex, endosulfan 35%, in use	6.4	0.045	ND	0.10	0.19
M_5	Sumilex, procymidone 50%, in use	0.14	0.037	0.25	0.81	0.061
M_6	Decis Forte, deltamethrine 10%, in use	0.074	0.0086	0.011	0.37	24.2
M_7	Trigermin, trifluraline 48%, empty/discarded	ND	ND	NM[b]	ND	ND
M_8	Daconil, chlorothalonil 75%, empty/discarded	4.5	0.010	NM	0.012	0.0042
M_9	Thionex, endosulfan 35%, in use	ND	0.0078	NM	7.1	0.30
M_{10}	Daconil, chlorothalonil 75%, in use	0.34	0.059	NM	0.091	11.8

[a] ND = not detected.
[b] NM = not measured.

3.3. Precision and accuracy of the measuring vessels for liquid formulations

Another factor studied was the accuracy and precision of the measuring procedure (device plus volunteer) in the case of liquid formulations for a required volume of 10 mL. The portion actually measured was weighed and divided by its density to obtain the real volume. Fig. 3 presents the results for cups, Falcon tubes and delivery pumps for two different sets of experiments for each kind of device. The suffix on each device (cup, Falcon, pump), indicates intact seal (suffix = 1, Fig. 3) or broken seal (suffix = 2). As the maximum deviation from the required volume was about 10%, all cups, pumps and falcon tubes were considered equally adequate for measuring pesticides under field conditions (Fig. 3).

3.4. Differences between field and laboratory experiments

Having found that for liquid formulations, the total % PME seemed to be greater in field experiments (Table 1) than under laboratory conditions (Table 3), a potential missing factor in the exposure mechanism was suggested.

A possible explanation could be related to the "history of container-use"; this is that in field trials, operators used pesticide containers that had been repeatedly employed (e.g. for Decis, 2–5 mL doses taken from a 250 mL container), while in laboratory experiences, volunteers always used new and clean flasks. Therefore, we measured the pesticide amounts found in the exterior surfaces of a set of used containers collected from horticultural farms, some of which were still in use while others had been discarded. Table 4 shows the pesticide mass found (in mg) on the exterior surface of each container. It should be noted that the real values are probably higher, as the residues were sometimes dried and encrusted, in which case swabbing was not quantitative (see Methodology section for details). The container set included different formulated products (Starfos: chlorpyrifos, Shooter: chlorpyrifos, Talone: chlorotalonyl, Endoglex: endosulfan, Sumilex: procymidone, Decis forte: deltamethrine, Trigermin: trifluralin, Daconil: chlorotalonyl, Thionex: endosulfan, Table 4), all of which were analyzed on the exterior of each container.

It is interesting to note that not only the expected ingredient of each container was found on the exterior surface, but practically in all cases (with the exception of M_7, Table 4) the rest of the studied pesticides were also found. In most cases (M_1–M_3, M_6–M_9, Table 4) the main product corresponded with the contents, but in some cases (M_4, M_5, M_{10}, Table 4) the main pesticide found was different. This fact could be explained by the general unsafe storage conditions of these products, which could have contributed to pesticide transference from the exterior of one container to another.

The second interesting feature is that the pesticide amount found in certain cases (M_6, M_8, M_{10}, Table 4) was really important (above several milligrams) raising the possibility that an important transfer of pesticide from the exterior surface of the container to the worker's hands could occur just by handling the container.

4. Conclusions

Field and laboratory experiments indicated that handling liquid formulations during the mix and load stage causes higher % PME than solid formulations for horticultural and floricultural operators. For solid formulations in particular, granulated ones implied lower exposure levels than powders. The contamination level of the spoons used to take the solid pesticide from the container was also lower for granulated than powdered formulations, emphasizing the benefits of this kind of preparation (from a risk-control point of view).

When the total % PME associated to the handling of liquid formulations was studied, the breaking of the aluminum seal of the containers, and the color or the formulation solutions appeared as important steps in the exposure of the operator's hands, possibly indicating that the operators' risk assessment can include behavioral components that could be mitigated with simple measures. The measuring vessel and the container size seem to play a less important role in the exposure mechanism.

The difference between the % PME in field and laboratory experiments for liquid formulations may be partially explained by the significant amount of pesticide found in the exterior surface of used containers that could imply a pesticide transfer from the container's exterior to the operator's hands.

These results indicate that although formulations are optimized for the best physicochemical performance when being applied, the mixer's potential exposure should be taken into account when a product, and its container, are designed and approved for use.

Conflict of interest

We declare that there is no conflict of interest in the authors associated to this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.11.071.

References

Aust GF, Wilkinson S, Dunn M, Glass R, Williams FM. Influence of formulation on the dermal absorption of pesticide actives at spray dilution concentrations. Toxicology 2007;231:112–3.

Cooper J, Dobson H. The benefits of pesticides to mankind and the environment. Crop Prot 2007;26:1337–48.

Cox C, Surgan M. Unidentified inert ingredients in pesticides: implications for humans and environmental health. Environ Health Perspect 2006;114:1803–6.
Damalas CA, Eleftherohorinos IG. Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 2011;8:1402–19.
Dyson T. World food trends and prospects to 2025. Proc Natl Acad Sci U S A 2000;96: 5929–36.
Flores AP, Berenstein G, Hughes EA, Zalts A, Montserrat JM. Pesticide risk assessment in flower greenhouses in Argentina: the importance of manipulating concentrated products. J Hazard Mater 2011;189:222–8.
Hughes EA, Zalts A, Ojeda J, Flores AP, Glass R, Montserrat JM. Analytical method for assessing potential dermal exposure to captan, using whole body dosimetry, in small vegetable production units in Argentina. Pest Manag Sci 2006;62:811–8.
International Labour Organization-WHO. Safety and health in the use of agrochemicals: a guide. International programme on chemical safety; 1991 [Accessible at http://www.ilo.org/wcmsp5/groups/public/—ed_protect/—protrav/—safework/documents/instructionalmaterial/wcms_110196.pdf, April 20].
Krüse J, Verberk CWE. Modeling of systemic uptake of agrochemicals after dermal exposure; effects of formulation, application and exposure scenarios. Environmentalist 2008;28: 57–65.
Lesmes-Fabian C, García-Santos G, Leuenberger F, Nuyttens D, Binder CR. Dermal exposure assessment of pesticide use: the case of sprayers in potato farms in the Colombian highlands. Sci Total Environ 2012;430:202–8.
Lu JL. Total pesticide exposure calculations among vegetable farmers in Benguet, Philippines. J Environ Public Health 2009:5. http://dx.doi.org/10.1155/2009/412054. [article ID 412054].
Matthews GA. Attitudes and behaviours regarding use of crop protection products. A survey of 8500 smallholders in 26 countries. Crop Prot 2008;27:834–46.
Moreno J, Falcó JV, Oltra MT, Jimenez R. The requirement for the inclusion of formulation efficacy trials in pesticide preregistration evaluations. Pest Manag Sci 2008;64: 527–35.
Park EK, Hannaford-Turner K, Lee HJ. Use of personal protective equipment in agricultural workers under hot and humid conditions. Ind Health 2009;47:200–1.
Ramos LM, Querejeta GA, Flores AP, Hughes EA, Zalts A, Montserrat JM. Potential Dermal Exposure in greenhouses for manual sprayers: analysis of the mix/load, application and re-entry stages. Sci Total Environ 2010;408:4062–8.
Ribeiro MG, Colasso CG, Monteiro PP, Pedreira Filho WR, Yonamine M. Occupational safety and health practices among flower greenhouses workers from Alto Tietê (Brazil). Sci Total Environ 2012;416:121–6.
Tomenson JA, Matthews GA. Causes and types of health effects during the use of crop protection chemicals: data from a survey over 6,300 smallholder applicators in 24 different countries. Int Arch Occup Environ Health 2009;82: 935–49.
Wilson C, Tisdell C. Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecol Econ 2001;39:449–62.