



Research article

Human exposure and mass balance distribution during procymidone application in horticultural greenhouses

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ABSTRACT

The purpose of this study was to analyze the impact of procymidone application in periurban horticultural greenhouses, especially on workers (applicators and assistants) and soil and plastic mulching, when mechanically pressurized application systems were employed. The mean Potential Dermal Exposure (PDE) was measured using the Whole Body Dosimetry technique. The PDE for the applicators was $188 \text{ mL h}^{-1} \pm 103 \text{ mL h}^{-1}$, and $14.7 \text{ mL h}^{-1} \pm 6.3 \text{ mL h}^{-1}$ for the assistants. In the first case, the most exposed body sections were the upper right and left ($46.8 \text{ mL h}^{-1} \pm 23.4 \text{ mL h}^{-1}$; $47.0 \text{ mL h}^{-1} \pm 23.5 \text{ mL h}^{-1}$) and lower ($20.8 \text{ mL h}^{-1} \pm 10.4 \text{ mL h}^{-1}$; $17.3 \text{ mL h}^{-1} \pm 8.7 \text{ mL h}^{-1}$) legs, while in the case of assistants, hands and legs were the most impacted limbs. Regarding the Margin of Safety (MOS) during the mix and load stage, two of three pesticide preparations resulted unsafe, while for the applicators, six of six spraying operations were unsafe. For the assistants, five of five operations were safe, but three of them were close to the safety limit.

Procymidone distribution between drift ($0.03\% \pm 0.07\%$), applicator ($0.20\% \pm 0.15\%$), polyethylene mulching ($8.5\% \pm 4.5\%$) and soil ($3.0\% \pm 1.1\%$) was determined with respect to the total pesticide applied. Procymidone soil impact was also evaluated using *Eisenia andrei* behavioral tests, finding positive correlations between procymidone application and avoidance and reproduction tests.

1. Introduction

Periurban horticulture plays an important socio-economic role in supplying fresh fruit and vegetables to nearby urban areas (Midmore and Jansen, 2003). Its production was increased by the adoption of technologies like plastic sheeting (Espí et al., 2012), and pesticide application (Hillocks, 2012). In this sense, it has been pointed out that negative undesired consequences of the application of these technologies are: plastic fragments incorporation (Ramos et al., 2015) and pesticide dispersion, in horticultural soils (Hilber et al., 2008) and nearby water-courses (Mac Loughlin et al., 2017).

Regarding major concerns about pesticide use, horticultural workers' safety during the mix and load, application and reentry stages is of critical importance. This is the reason why national (EPA, 2018) and supranational (European Commission, 1991) pesticide regulations require occupational risk evaluation for those applying agrochemicals. In these working scenarios, it is well established that transdermal absorption through the skin can be the most important

pathway for pesticides uptake under typical field working conditions (Drexler, 2003).

In order to evaluate occupational risk, the first step is to experimentally determine the worker's Potential Dermal Exposure (PDE). It has been stated that the PDE is deeply affected by a set of critical factors like: the pesticide formulation (Berenstein et al., 2014), the crop geometry (Hughes et al., 2008), the drop size of the spray (Garrido Frenich et al., 2002) and the workers experience (Hughes et al., 2006), among others. As consequence, the experimental evaluation of PDE under different working conditions is necessary in order to develop accurate occupational predictive scenarios.

Related to the PDE evaluation in horticultural greenhouses, we have previously reported the PDE for deltamethrin and procymidone applications to tomato plants using lever-operated backpacks ($38 \text{ mL h}^{-1} \pm 17 \text{ mL h}^{-1}$; Ramos et al., 2010). These PDE values were of the same order as previously reported data for the case of malathion application in tomato greenhouses using low-pressure knapsacks (25.4 mL h^{-1} - 35.8 mL h^{-1} , Machera et al., 2003), and for the application of fenitrothion,

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metidathion, metamidophos and malathion ($46.9 \text{ mL h}^{-1} \pm 19.7 \text{ mL h}^{-1}$; Garrido Frenich et al., 2002).

The horticultural worker's safety conditions are usually aggravated by the lack of use of personal protective equipment (PPE). According to Bondori et al. (2018), the PPE is not always used as consequence of uncomfortable climatic conditions. These authors reported that farmer's attitude, knowledge, source of information and past experience of poisoning with pesticides explained 85% of variance in PPE use. Another factor that conspires against workers' safety is the use of manual systems in pesticide applications. Sánchez-Hermosilla et al. (2013) reported that in south-eastern Spanish greenhouses, pesticides were applied by manual systems involving spray guns or lances in 91.7% of the cases. In Italy, Cerruto et al. (2008) described that the application of pesticides in greenhouses was accomplished in 71% of the cases by means of hand-held high-pressure devices. Although there is not official information, the pesticide application techniques used in Argentinean horticultural greenhouses are quite similar.

Taking into account the application equipment and technique, Machera et al. (2003) have pointed out that when pesticide application was done in greenhouses employing high pressure, the PDE was high, indicating that this variable deeply affects the workers' exposure. Rincón et al. (2018) stated that when using a pressurized spray gun walking backwards the PDE was ca. 100 mL h^{-1} , while when the equipment was changed to a pressurized hand-lance the PDE increased to ca. 180 mL h^{-1} . In the same sense it has been reported that the crop stage and the operator's walking direction are two critical variables modifying workers' PDE (Ceruto et al., 2018).

Beside horticultural workers, pesticide impact on soil and agricultural covers is another aspect that must be considered. Querejeta et al. (2012) have reported that when pesticides were applied in tomato and lettuce greenhouses ca. 30% of the product reached the bare soil. Regarding pesticide drift during greenhouse applications with manual equipments, authors (Querejeta et al., 2012; Ramos et al., 2010) agreed that this phenomenon was not very significant (less than 0.03 %–0.05 % of the total pesticide applied). Concerning the amount of pesticide that could reach plastic mulching inside the greenhouses during the application stage, as far as we know, no values are known.

Considering this background, the general objective of this work was to study the potential exposure of humans, soil and film covers caused by procymidone applications in periurban horticultural greenhouses in Argentina. The specific objectives were:

- Determine the operator's PDE and MOS for the pressurized applications of procymidone and to compare it to manually-pressurized applications;
- Measure the pesticide spray drift and the amount of pesticide that falls on the greenhouse's soil and plastic mulching;
- Evaluate procymidone's impact on greenhouse soil using *Eisenia andrei* as bioindicator.

2. Materials and methods

2.1. Site description and field trials

All field trials were done in greenhouses of Melchor Romero district (La Plata, Buenos Aires Province, Argentina) between April and June of 2017. The greenhouses were 60 m long and 15 m wide (900 m^2), with a window in each long side and a front doorway. Each greenhouse had eight rows of tomato plants of 2.0 m height, ready for harvesting, separated 2 m from each other (the aisles in between were 0.8 m wide). A black polyethylene film was used to cover the plant mulch. The total ground-surface inside the greenhouse covered with plastic film was 642 m^2 , while the surface of the exposed soil was 258 m^2 .

All procymidone sprayings were manually done using a lance with a double nozzle (Yamaha D3), connected via a hose to a 200 L external

tank pressurized with a mechanical pump (Yamaha) where the pesticide was loaded. Table 1 summarizes the main features of E1 to E6 field trials.

2.2. Chemicals

The procymidone commercial formulation used was Sumilex® (CS, 50% w/v) (Summit Agro Argentina). For the preparation of reference material for analytical purposes, procymidone (3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo [3.1.0]hexane-2,4-dione, CASRN [32809-16-8]) technical grade, was recrystallized (95 % pure by GC-FID), and confirmed by ^1H - and ^{13}C -NMR. A primary solution of 1020 ppm w/w was prepared in cyclohexane: acetone (60 : 40), and all other working solutions were made by dilution as needed. Cyclohexane (Aberkon pa grade) and acetone (Sintorgan) in mixtures 60: 40 were used for all solutions and extracts, distilled prior to use and chromatographically checked as suitable for use under GC-ECD conditions.

2.3. Chromatographic conditions

All chromatographic analysis were performed on a Perkin-Elmer (Norwalk CT, USA) Clarus 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 length, 0.25 mm i.d. and 0.25 μm film thickness). The GC-ECD operating conditions for procymidone determinations 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} .

2.4. Method validation

Experiments were done in order to investigate if procymidone was stable or suffered decomposition or was otherwise lost on the cotton cloth used for sampling. No loss was observed for storage periods of up to 48 h (data not shown).

Chromatographic linear ranges were studied for procymidone calibration curves using cotton fabric as matrix, finding linear responses between 0.05 and 1.00 mg L^{-1} ($R^2 > 0.998$). The lowest points of the calibration curve were considered as the limit of quantitation. The intermediate precision was studied by injection of a complete calibration curve for procymidone by duplicate on six consecutive days and calculating the percentual standard deviation of the slope of the calibration curves, observing a maximum variation of 10%.

2.5. PDE measurements

The potential dermal exposure was measured using the Whole Body Dosimetry technique as previously reported (Hughes et al., 2008). The operators (applicator and assistant) were dressed with protective

Table 1. Procymidone applicator's PDE in six field trials (E1-E6) in a tomato greenhouse.

	Assay					
	E1	E2	E3	E4	E5	E6
T/°C	24	24	16	16	13	13
Wind: direction/intensity (Km.h ⁻¹)	SW/5	SW/5	NE/3	NE/3	WNW/7	WNW/7
Atmospheric pressure (bar)	1018	1018	1018	1018	1021	1021
% Humidity	59	59	-	-	97	97
Application time (min)	24	22	36	33	29	21
Application volume (L)	52	48	44	41	32	23
Applied procymidone (mg)	26087	23913	22174	20326	15950	11550
Working pressure (bar)	11.0	11.0	10.3	10.3	12.4	12.4

equipment (30 cm high rubber boots, a Tyvek® coverall, and latex gloves) over which the absorbent media was worn: cotton coverall with hood, cotton gloves and a half-face respiratory mask; goggles were also used for eye protection.

Then, the applicator performed the mix and load stage. He poured into the tank the procymidone concentrate (100 mL–120 mL) and diluted it up to the final volume (100 L–120 L). When the mix and load stage was finished, the applicator's gloves were changed and replaced by a new set. Pressure was raised to 10.3–12.4 bar; the applicator sprayed the pesticide on the tomato plants, and the assistant helped unwinding and rewinding the feed-hose. Both applicator and assistant followed their habitual spray-practice for procymidone applications. Application time was typical for greenhouse treatments, usually between 21 and 36 min, with applied volumes between 23 L–52 L (Table 1). When the procymidone application was finished, the cotton coverall was taken off and hung up to dry in the shade. The Tyvek coverall was checked for stains that could indicate penetration of the cotton outer suit. All gloves, mask, cotton-wool, etc. were placed in individual polyethylene bags for later processing.

2.6. Drift and soil measurements

The amount of procymidone on the greenhouse: soil, mulching and on the soil outside the greenhouse (drift), was determined using square (10 cm × 10 cm) cloth samplers (Berenstein et al., 2017), lined on one side by a polyethylene film to avoid external contamination. For each field trial (E1 to E6), sixteen samplers were randomly located in the greenhouse, eight on the bare soil and the rest on the mulching cover. For drift measurements, four lines of samplers separated 20 m each were located at 0.5 m, 1.5 m, 4.5 m and 10 m from the greenhouse lateral window (Figure 1). Once the pesticide application was finished, a further 15 min were allowed before collecting the samplers, in order to capture as much

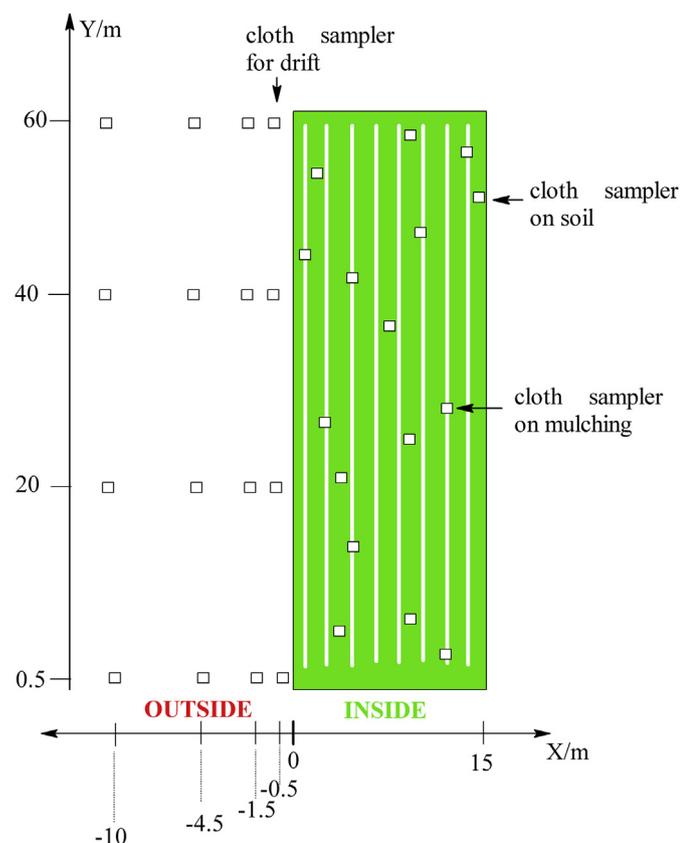


Figure 1. Schematic representation of the pesticide cotton samplers location inside and outside each individual field trial.

pesticide as possible. The cotton samplers were stored and tagged in individual bags for laboratory determinations.

2.7. Laboratory analysis

Laboratory analyses were done no later than 24 h after the field trial. In the laboratory, the cotton suit was cut into pieces as indicated in Figure 3 (Hughes et al., 2008) and each piece was placed separately in polypropylene containers and extracted using suitable volumes of cyclohexane. The containers were shaken for 20 min in a rotary shaker at room temperature. A fraction of each extract was sealed into a GC vial and stored in a freezer until analysis. The cotton samplers used for soil, mulching and drift measurements were analyzed in the same way as described above using glass containers. Goggles and hose were swabbed with a clean piece of cotton cloth with cyclohexane, which was extracted as mentioned above.

All extracts were analyzed by GC-ECD, under the previously described conditions.

2.7.1. PDE calculations

The PDE was calculated as previously reported (Hughes et al., 2008). Briefly, the concentrations of the sprayed mixtures were calculated knowing the weight and concentration of the used commercial pesticides and the water volume loaded into the tank. PDE results were expressed as volume of spray-mixture to which the operator would be exposed if he continued spraying for 1 h (in mL.h⁻¹). This, was obtained by extrapolation of the respective application times, using the extraction volumes for each cotton section and the pesticide concentration chromatographically determined. PDE was given as the amount of pesticide (in mg) found on each body section or in an equivalent form.

In this work, the applicator performed the mix/load and the application stages while the assistant moved the hose line helping the applicator.

2.7.2. MOS calculation

The MOS was measured as previously reported (Berenstein et al., 2017). We considered an absorption factor of 0.11, which includes an effective dermal absorption of 10%, with an additional 1 % added to include the inhaled fraction (Machado-Neto et al., 2000). An AOEL = 0.012 mg kg⁻¹.d⁻¹ was used for procymidone (EU Pesticide Database). Briefly, the MOS was calculated as follows:

$$\text{MOS} = \text{AE} / (\text{DE} \times \text{AF})$$

Where: AE = acceptable exposure; DE = dermal exposure; AF = absorption factor. AE values were calculated based on appropriate toxicological end-points according to the following expression: AE = AOEL × average body weight (Procymidone AOEL = 0.012 mg kg⁻¹.d⁻¹, EU Pesticide Database); and average body weight of 70 kg.

We considered DE = PDE (as mg of procymidone). For the mix and load stage only the pesticide mass found in the preparation gloves was considered. For the application stage all the body parts including gloves but not considering goggles and mask were taken into account. The MOS was evaluated considering the amount of pesticide that reached the worker in 1 h of application, not just the subplot directly measured.

For calculation, we considered AF = 0.11, which includes an effective dermal absorption of 10%, with an additional 1 % added to include the inhaled fraction.

Thus, the actual formula used was: MOS = AOEL × 70 / (PDE × 0.11)

2.7.3. Pesticides on the greenhouse soil

The mean procymidone amount found in the samplers located on the bare greenhouse soil was divided by the sampler surface (100 cm²) and multiplied by the greenhouse's bare soil in cm² (2.58 × 10⁶ cm²). The

Table 2. Procymidone applicator's PDE in six field trials (E1-E6) in a tomato greenhouse.

Coverall Section	PDE Applicator mL.h ⁻¹						Mean	SD
	E1	E2	E3	E4	E5	E6		
1.0	23.4	13.9	0.0	0.0	0.0	0.0	6.2	10.1
2a	13.2	13.1	4.1	8.6	6.3	7.3	8.8	3.7
2b	20.4	12.6	2.6	5.0	3.7	3.4	7.9	7.1
3a	13.8	12.8	2.1	3.5	3.6	5.4	6.9	5.1
3b	14.4	16.7	1.1	1.6	1.4	1.1	6.0	7.4
4a	14.9	16.5	1.1	1.4	1.6	0.0	5.9	7.6
4b	17.8	11.9	0.8	0.0	0.0	0.0	5.1	7.8
5a	7.9	9.9	0.8	0.0	0.0	0.0	3.1	4.6
5b	8.5	8.0	0.0	0.8	0.0	0.0	2.9	4.2
6a	118.1	56.7	3.4	4.6	6.0	5.7	32.4	46.8
6b	119.4	39.8	2.6	2.8	2.9	2.6	28.3	47.0
7a	15.9	14.2	1.1	1.9	0.0	0.0	5.5	7.4
7b	7.7	6.9	1.4	1.5	0.0	0.0	2.9	3.5
8.0	57.8	51.8	10.0	13.1	17.8	19.8	28.4	20.8
9.0	47.2	42.4	6.7	12.7	10.8	18.5	23.0	17.3
10	17.4	14.3	9.1	0.0	4.2	4.4	8.2	6.6
11	19.1	8.9	1.7	0.0	4.6	9.2	7.2	6.9
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	536.7	350.2	48.5	57.4	62.9	77.3	188.8	206.1
Time (min)	24.0	22.0	36.0	33.0	29.0	21.0	-	-

Table 3. Procymidone assistant's PDE in five field trials (E2-E6) in a tomato greenhouse.

Coverall Section	PDE Assistant mL.h ⁻¹						Mean	SD
	E1	E2	E3	E4	E5	E6		
1.00	NM ¹	<LD ²	<LD	<LD	<LD	<LD	<LD	<LD
2a	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
2b	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
3a	NM	1.53	<LD	0.48	<LD	<LD	0.3	0.6
3b	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
4a	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
4b	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
5a	NM	2.49	<LD	<LD	<LD	<LD	0.4	1.0
5b	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
6a	NM	3.76	1.69	<LD	<LD	<LD	0.9	1.6
6b	NM	1.41	1.71	<LD	<LD	<LD	0.5	0.1
7a	NM	6.59	<LD	<LD	<LD	<LD	1.1	2.7
7b	NM	3.36	2.44	<LD	<LD	<LD	1.0	1.5
8.00	NM	2.52	<LD	1.89	<LD	<LD	0.7	1.2
9.00	NM	1.91	<LD	1.60	8.46	<LD	2.0	3.3
10	NM	7.79	6.70	5.80	3.44	3.08	4.5	2.9
11	NM	4.26	3.16	3.28	7.75	1.15	3.3	2.7
12	NM	<LD	<LD	<LD	<LD	<LD	<LD	<LD
Total	NM	35.62	15.69	13.05	19.65	4.23	14.7	12.6
Time (min)	-	22.00	36.00	33.00	29.00	21.00	-	-

¹ NM: not measured.² <LD: below the limit of detection.

same procedure was used for calculating the amount of procymidone on the greenhouse mulching. In this case the surface of mulching was $6.42 \times 10^6 \text{ cm}^2$.

2.7.4. Drift calculation

For each drift-section, the mean procymidone amount found in the samplers located on the ground outside the greenhouse was divided by the sampler surface (100 cm^2), and multiplied by the section's area in cm^2 ($3.00 \times 10^5 \text{ cm}^2$, $6.00 \times 10^5 \text{ cm}^2$, $1.80 \times 10^6 \text{ cm}^2$ and 3.30×10^6

cm^2 for the first, second, third and fourth line of samplers, respectively). Total drift was evaluated as the total amount found on all four sections.

2.8. Ecotoxicological determinations

2.8.1. Soil

For laboratory bioassays, soil samples (12–15 kg) of the treated greenhouse and of a non-exposed control area (reference), 0–10 cm

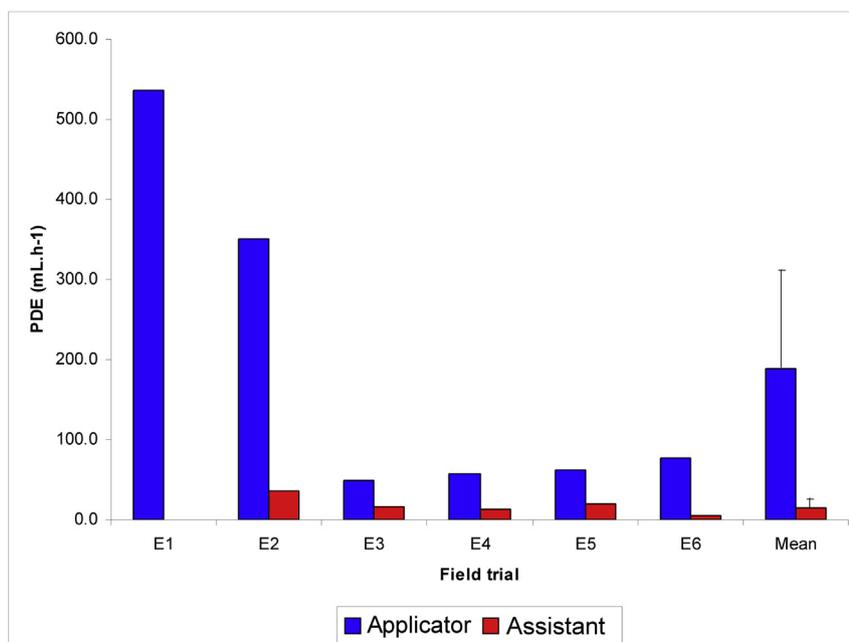


Figure 2. PDE (mL.h⁻¹) for the applicator and assistant of each individual field trial.

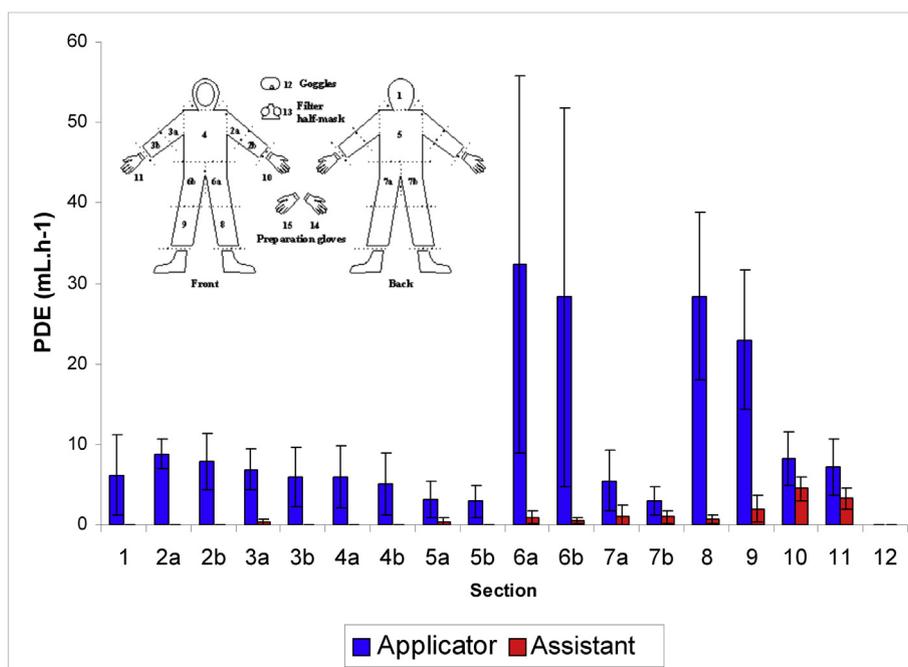


Figure 3. Mean PDE (six field trials) for each section of the cotton sampler.

depth, were collected and kept refrigerated until analysis and bioassays were done.

2.8.2. Soil preparation

Soil samples were sieved (2 mm) and adjusted to 50–60% of maximum water holding capacity (WHC) before running the bioassays. WHC was measured by placing soil samples in cylindrical glass tubes closed at the bottom with Whatman N° 1 filter paper fastened with elastic bands. Excess water was added and the test ended when liquid stopped draining through the bottom. WHC was determined by weighing each soil sample obtained, and then drying it at 105 °C and weighing it again

(Querejeta et al., 2014). The soil samples WHC expressed as a percentage of dry mass were: E1 (71 ± 2%) and E2 (66 ± 5%).

2.8.3. Earthworms

Eisenia andrei earthworms, 0.30–0.60 g fresh weighed and maintained in our laboratory, were exposed to soils prepared as described above with a 16h/8h day/night photoperiod and at (20 ± 2) °C. Before starting the bioassays, earthworms were washed with dechlorinated tap water and placed on moist filter paper for a minimum of 3 h, in order to let them empty their guts. OECD/ISO guidelines were used for the tests (ISO, 2004).

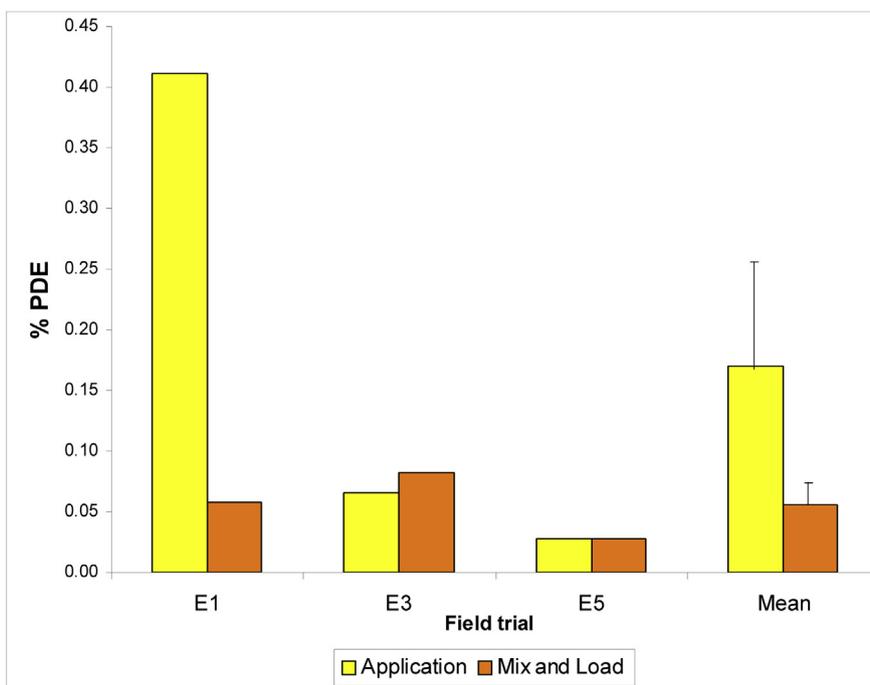


Figure 4. Percent PDE (% PDE) for application and mix and load stages in E1, E3 and E5 field trials.

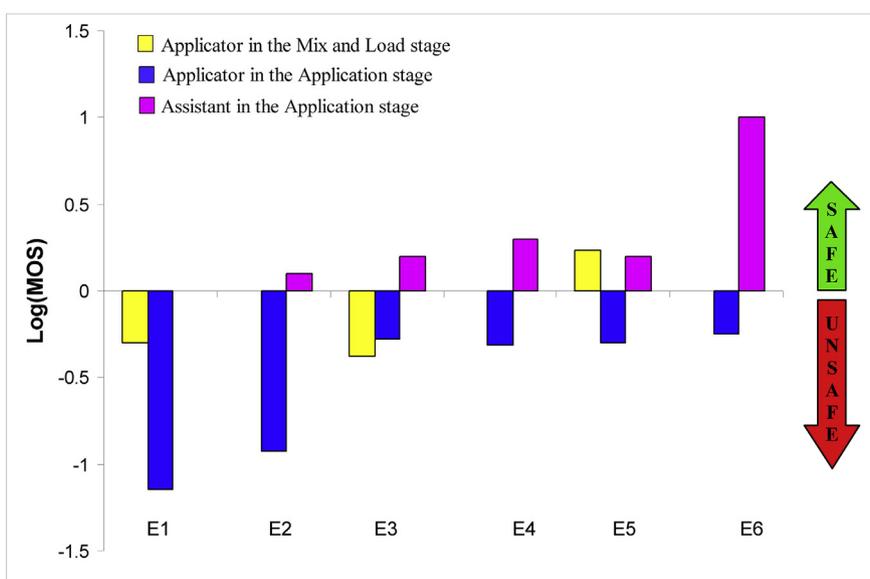


Figure 5. Margin of Safety logarithm (log (MOS)) for the applicator and assistant in E1-E6 field trials (application stage). White bars: Applicators in the Mix and Load stage; black bars: Applicators in the Application stage; grey bars: Assistants in the Mix and Load stage.

2.8.4. Avoidance behavior

ISO N 281 (2004) was followed.

2.8.5. Reproduction test

The test was performed according to ISO 11268-2 (1998), with minimal modifications. Soil (350 g of E1) and six adult earthworms were added to each of four replicate containers. Dry baby cereal mixture (2g) was used as food and added at the beginning of the experiment, and once weekly. Humidity of soils was maintained during the entire experiment. After 28 days of exposure, earthworms were removed from soil and survival rates were recorded.

Each portion of soil (containing cocoons) was returned to its previous container and incubated for another 28 days to continue the exposure of cocoons to the test soil. At the end of the test (56 days), the number of hatched and non-hatched cocoons was recorded.

2.9. Statistical analysis

Statistical analyses were performed with GraphPad InStat 3 (GraphPad Software, San Diego, USA). Data were first tested for normality (Kolmogorov-Smirnov's test) and for homogeneity of variances (Bartlett's test). Depending on these results, means were compared by one-way

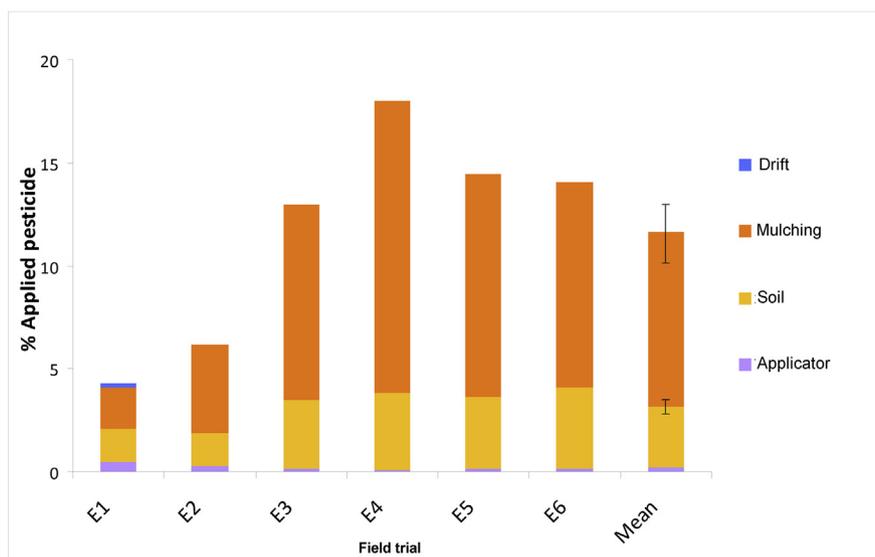


Figure 6. Procymidone distribution between drift, mulching, soil and applicator.

ANOVA (parametric) or non-parametric Kruskal-Wallis tests. When significance was demonstrated ($p < 0.05$), Tukey-Kramer or the non-parametric Dunn's tests were applied for post-hoc comparison of means. For avoidance experiments, Student t-test was used (one-tailed test for control-treated experiments; two-tailed test for the dual control tests) (ISO N 281 2004, Natal Da Luz et al., 2004).

3. Results

3.1. PDE and MOS results

The workers' PDE for procymidone spraying in tomato greenhouses was measured for applicators and assistants (who help to manipulate the hose) in a periurban horticultural production unit located in Gran La Plata, Buenos Aires Argentina, using the Whole Body Dosimetry technique (Table 2 and Table 3) Figure 2 shows the total mean PDE for applicators (blue bars, Figure 2) and assistants (red bars, Figure 1) expressed in mL of the sprayed mixture in 1 h ($\text{mL}\cdot\text{h}^{-1}$) for six field trials. In these cases, the pesticide applications were done using a mechanically pressurized 200 L tank with a hose that reached all points inside the greenhouse. The mean PDE for the applicators was $188 \text{ mL}\cdot\text{h}^{-1} \pm 103 \text{ mL}\cdot\text{h}^{-1}$ while for the assistants it was $14.7 \text{ mL}\cdot\text{h}^{-1} \pm 6.3 \text{ mL}\cdot\text{h}^{-1}$.

The pesticide distribution was analyzed taking into account the average PDE of each body section (Figure 3). In the case of the applicators the highest mean PDE was observed for upper (6a: $46.8 \text{ mL}\cdot\text{h}^{-1} \pm 23.4 \text{ mL}\cdot\text{h}^{-1}$, 6b: $47.0 \text{ mL}\cdot\text{h}^{-1} \pm 23.5 \text{ mL}\cdot\text{h}^{-1}$, Figure 3) and lower (8: $20.8 \text{ mL}\cdot\text{h}^{-1} \pm 10.4 \text{ mL}\cdot\text{h}^{-1}$, 9: $17.3 \text{ mL}\cdot\text{h}^{-1} \pm 8.7 \text{ mL}\cdot\text{h}^{-1}$, Figure 3) leg sections. The other body parts had a more homogeneous PDE distribution.

In the case of the assistants, the most exposed sections were left (10: $4.5 \text{ mL}\cdot\text{h}^{-1} \pm 2.9 \text{ mL}\cdot\text{h}^{-1}$, Figure 3) and right (11: $3.3 \text{ mL}\cdot\text{h}^{-1} \pm 2.7 \text{ mL}\cdot\text{h}^{-1}$, Figure 3) hands. Additionally, the lower body parts (6a, 6b, 7a, 7b, 8 and 9, Figure 3) were more exposed than the upper body sections (Figure 3).

The worker's exposure during the mix and load stage was measured in three field trials (E1, E3 and E5) and compared with the exposure in the application step for the same field trials. For comparison purposes the PDE was expressed as %PDE, which is the PDE expressed as a percentage relative to the total pesticide applied (Figure 4). The mean % PDE was $0.170\% \pm 0.074\%$ for the application stage while for the mix and load step was $0.056\% \pm 0.014\%$ (Figure 4).

Figure 5 shows the logarithm of the MOS for the mix and load, and application stages for the applicator and the assistant. The MOS values correspond to the preparation of one 200 L tank of procymidone (mix and load stage), and to the pesticide application in one greenhouse (application stage). Field trials with $\log(\text{MOS}) > 0$ indicate safe operations. Two of the three mix and load operations resulted unsafe (Figure 4), and the safe one had a $\log(\text{MOS})$ value of 0.24, quite close to the security limit. In the case of the application stage, all the operations were unsafe (six of six, Figure 5). Regarding the assistant during the application stage all the operations were safe but with $\log(\text{MOS})$ values (except in E6) close to the safety limit (Figure 5).

3.2. Procymidone amounts found in soil, plastic and drift

The relative procymidone amount that reached the soil and the polyethylene mulching inside the greenhouse and the portion that drifted outside the greenhouse were determined for E1 to E6 field trials. Figure 6 shows the relative procymidone percentage respect to the total pesticide applied, for drift, mulching, soil and the applicator, respectively. Plastic mulching was the most exposed system ($8.5\% \pm 4.5\%$ of the total procymidone applied, Figure 6), followed by the greenhouse soil ($3.0\% \pm 1.1\%$, Figure 6), the applicator's protective equipment ($0.20\% \pm 0.15\%$, Figure 6) and drift ($0.03\% \pm 0.07\%$, Figure 6).

3.3. Avoidance and reproduction test using *Eisenia andrei*

Considering that the greenhouse soil was the second most exposed system, the procymidone application impact was evaluated using *Eisenia andrei* ecotoxicological tests (avoidance and reproduction, Figure 7).

No mortality occurred in controls and exposed earthworms after exposure to the soils tested. The avoidance test, which is a rapid technique (compared to the 56 days of the reproduction test), showed that earthworms avoided soils after procymidone application (Figure 7A). Regarding the reproduction test, significant differences in cocoon production were observed between earthworms exposed to procymidone treated soils (Figure 7B) and those exposed to soils without this pesticide (Figure 7B), finding that procymidone caused a lower production of cocoons in E1 soils. The ratio 'hatched cocoons/total cocoons' was not significantly different in E1 soils before and after procymidone application (Figure 7C).

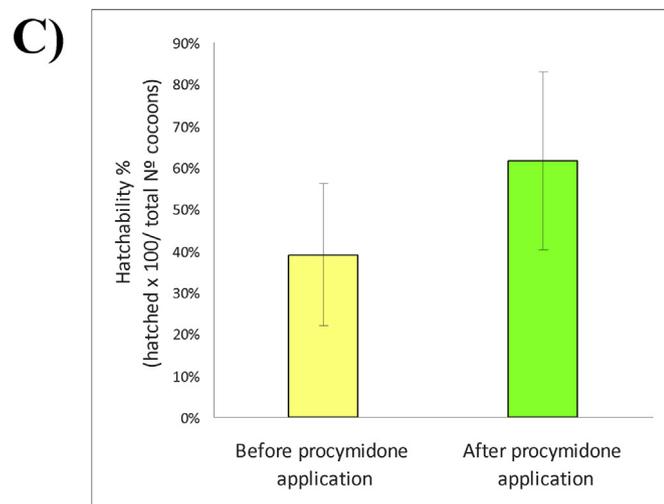
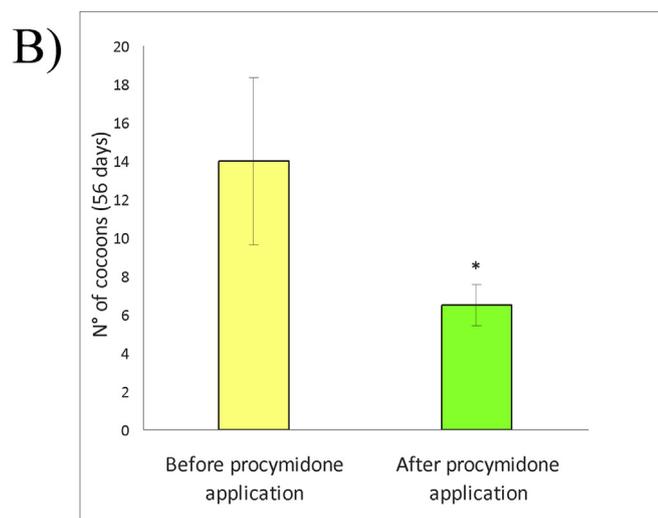
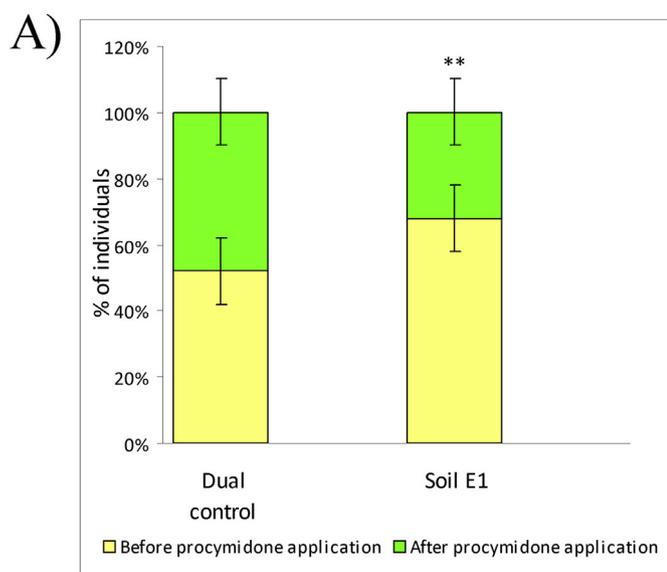


Figure 7. Procymidone impact on soil evaluation using *Eisenia andrei* in E1 field trial: A) Avoidance test; B) Reproduction test: Number of cocoons; C) % Hatchability.

Table 4. Procymidone found on the assistant's hose.

Field trial	mg procymidone/m hose
E1	0.12
E2	<LD
E3	<LD
E4	0.36
E5	<LD
E6	0.18
Mean \pm SD	0.11 \pm 0.13

Table 5. Mean MOS values and SD for the mix/load and application stages using low and high pressure application equipments.

Pressure	MOS	
	Mix/load	Application
Low ^a : 3–4 bar	0.7 \pm 0.5	13 \pm 11
High ^b : 10.3–12.4 bar	0.9 \pm 0.4	0.3 \pm 0.1

^a Manually pressurized knapsacks (Ramos et al., 2010).

^b Range values of this work.

Table 6. Procymidone found outside the greenhouse as a consequence of drift.

Distance (m)	Row ^a	Field trial		
		E1	E3	E5
Procymidone (mg/m ²)				
0.5 m	1	0.611	<LD	<LD
1.5 m	1	0.250	<LD	<LD
4.5 m	1	<LD	<LD	<LD
10 m	1	<LD	<LD	<LD
0.5 m	2	0.985	<LD	<LD
1.5 m	2	0.582	<LD	<LD
4.5 m	2	<LD	<LD	<LD
10 m	2	<LD	<LD	<LD
0.5 m	3	0.728	<LD	<LD
1.5 m	3	0.336	<LD	<LD
4.5 m	3	<LD	<LD	<LD
10 m	3	<LD	<LD	<LD
0.5 m	4	<LD	<LD	<LD
1.5 m	4	<LD	<LD	<LD
4.5 m	4	<LD	<LD	<LD
10 m	4	<LD	<LD	<LD

^a Row: direction where a set of samplers (at 0.5 m, 1.5 m, 4.5 m and 10 m) are lined up perpendicular to the greenhouse border.

4. Discussion

4.1. PDE and MOS discussion

One of the objectives of this work was to compare worker's PDE under similar conditions (tomato greenhouse, procymidone application, comparable weather conditions) with the exception of the application method (a mechanically pressurized hose, considered a high-pressure condition, and a lever-operated backpack sprayer treated as low-pressure application). The PDE measured in this work under high-pressure conditions ($188 \text{ mL h}^{-1} \pm 103 \text{ mL h}^{-1}$, section 3.1) was five times higher than the PDE when low-pressure application methods were used ($38 \text{ mL h}^{-1} \pm 17 \text{ mL h}^{-1}$, Ramos et al., 2010). The increase in the worker's PDE when the application pressure is raised was previously observed for the application of malathion in tomato greenhouses

(Machera et al., 2003). The authors reported an increase of six to eight times in the PDE when the application system was changed from low-pressure knapsacks to high-pressure hand-lance.

In relation to assistant exposure, although the employment of long high-pressure hoses usually demands the help of an additional worker (defined as assistant), the risk assessments of these laborers has not been systematically studied. Castro Cano et al. (2000) reported that when chlorpyrifos methyl and fenitrothion were applied in green beans greenhouses using a pressurized spray gun, the applicators PDE was in the range 60 mL h^{-1} to 63 mL h^{-1} , while in the case of the assistants the PDE was between 13 mL h^{-1} to 20 mL h^{-1} . This former range is in accordance with our results for the assistant PDE ($14.7 \text{ mL h}^{-1} \pm 6.3 \text{ mL h}^{-1}$, section 3.1). In any case, the pesticide application experience of the horticultural laborers that participated in this work (not certified trainee, similar amount of years) was similar to the laborers at Ramos et al. (2010). Therefore, it can be concluded that the use of high pressure application systems is riskier than the use of low pressure ones. Although these findings and as consequence of the high influence of the applicators experience on PDE (Hughes et al., 2006), it is possible that ensuring and improving applicators training, high pressure systems could be used with low PDEs.

Regarding the pesticide distribution pattern, we found that when using high-pressure spray guns the most exposed sections for the applicator were leg-fronts (Figure 3). In the case of using low-pressure manual-operated knapsacks, the distribution pattern includes arms, torso and back sections (Ramos et al., 2010), probably as consequence of knapsack liquid leaks. In the case of procymidone application the most exposed sections for the assistants were the hands. This finding could be explained by hose contamination with procymidone ($0.11 \text{ mg} \cdot \text{m}^{-1}$ hose $\pm 0.13 \text{ mg} \cdot \text{m}^{-1}$ hose, Table 4), with the consequent assistant's exposure.

Another interesting issue is the comparison of the exposure during the mix/load and application stages. As these operations can not be compared using PDE in $\text{mL} \cdot \text{h}^{-1}$, we contrasted them expressing the exposure as % PDE (mg of procymidone in worker's outer clothing relative to the total mg of procymidone applied). We found in this work that the mean % PDE for the application stage was $0.170\% \pm 0.074\%$, which was higher than the value found for the mix/load stage ($0.056\% \pm 0.014\%$). In any case, the mean mix/load exposure represents ca. 25 % of the mean total exposure, considering this as the combination of the mix/load plus the application steps. A different situation was found when the application was done using low-pressure manual lever-operated knapsacks (Ramos et al., 2010). In this case the %PDE for the mix/load stage ($2.2\% \pm 1.2\%$) was considerably higher than the exposure during the application stage ($0.43\% \pm 0.58\%$). This difference could be related to the five times increase of workers exposure in the application stage as consequence of the high pressure employed.

Although the PDE is the basic experimental data for analyzing workers' exposure, it gives no indication about risk. For this purpose, the MOS, which is a quantitative risk indicator applicable to any specific stage, can be used. When the MOS of the mix/load and application stages are compared for low- and high-pressure spraying conditions (Table 5), it is interesting to observe that while the MOS of the mix/load stage is comparable for both cases (low-pressure: 0.7 ± 0.5 , high-pressure: 0.9 ± 0.4 , Table 5), an interesting difference was found for the MOS of the application stage (low-pressure: 13 ± 11 , high-pressure: 0.3 ± 0.1 , Table 5), showing that pesticide spraying with pressurized hand-held nozzles was riskier than manually-operated lever-backpacks, at least for procymidone spraying in tomato greenhouses.

4.2. Procymidone amounts found in soil, plastic and drift

We have previously showed that the fraction of pesticide that moves away from the greenhouse by drift mechanisms was 0.03 % of the total pesticide applied, for the case of procymidone spraying in tomato

greenhouses using low-pressure application systems (Ramos et al., 2010). This value is similar to the drift value found in this study ($0.03\% \pm 0.07\%$, section 3.1), where high-pressure application systems were employed. Additionally, procymidone amounts on soil rapidly decreased with respect to the greenhouse border (no pesticide was observed more than 10 m away from the greenhouse border, Table 6).

Regarding the environmental pesticide distribution in different horticultural subsystems, we have previously showed that for tomato greenhouses 30% of the pesticide reached the soil when no mulching technique was used (Querejeta et al., 2012). In our case, $8.5\% \pm 4.5\%$ of the total pesticide applied was found on the polyethylene mulching, while $3.0\% \pm 1.1\%$ (section 3.2) reached the bare soil. The amount of procymidone that reached the plastic mulching is important especially considering that pieces of polyethylene film can be incorporated into the greenhouse soil (Ramos et al., 2015).

4.3. Avoidance and reproduction test using *Eisenia andrei*

Many authors (Hund-Rinke et al., 2005; Loureiro et al., 2005; Casabé et al., 2007) concluded that the avoidance assay is useful as a first screening test to evaluate soil quality. Avoidance behavior may be of crucial importance for the species populations and can significantly contribute to their exposure and survival in field conditions (Lukkari and Haimi, 2005). As earthworms have chemoreceptors and sensory tubercles, they present a high sensitivity to chemicals in soils (Reinecke et al., 2002). In our case, avoidance test could discriminate soil exposure before and after procymidone application. The amount of pesticide applied was 415 mg of procymidone in 258 m^2 of soil (E1 field trial). Considering a soil density as 1.5 g mL^{-1} (Querejeta et al., 2014) and that only the first 10 cm of soil were sampled, the procymidone concentration that could be discriminated by *Eisenia andrei* in this assay was $10.7 \mu\text{g procymidone} \cdot \text{kg}^{-1}$ of soil.

Regarding the reproduction test, procymidone application affected the production of cocoons but not their fertility. As reported by Yasmin and D'Souza (2010), cocoon production was found to be the most sensitive parameter for various xenobiotics such as fentin, benomyl, phenmedipham, carbaryl, copper oxychloride, dieldrin in earthworms.

Both reproduction and avoidance tests resulted sensitive to procymidone treatments and it seems that it has potential as a screening test for evaluation of contaminated soils. Although the field population density of earthworms could be affected by multiple factors, the effects observed on the reproduction and avoidance tests caused by procymidone could contribute to its decrease, with the subsequent loss of the earthworms' beneficial functions.

5. Conclusions

The PDE for the spraying of procymidone in tomato greenhouses using mechanically pressurized lines was $188 \text{ mL h}^{-1} \pm 103 \text{ mL h}^{-1}$ for the applicators and $14.7 \text{ mL h}^{-1} \pm 6.3 \text{ mL h}^{-1}$ for the assistants. The MOS values showed that two of the three mix and load operations measured resulted unsafe, while all the procymidone application stages resulted below the security limit. Although for the assistants the operations were in all cases secure, their MOS values were close to the security limit. When compared to the procymidone application in tomato greenhouses using low pressure knapsacks, the application using a high-pressure method was clearly riskier.

When considering a mass balance distribution of procymidone after the application stage, $0.03\% \pm 0.07\%$ of the total pesticide applied corresponds to drift, $0.20\% \pm 0.15\%$ is found on the applicator's body, $8.5\% \pm 4.5\%$ is the procymidone found on the polyethylene mulching under the tomato plants, and $3.0\% \pm 1.1\%$ is the pesticide that reached the ground. In particular, greenhouse soil was clearly affected by pesticide application as can be observed by the avoidance and reproduction tests using *Eisenia andrei*.

Declarations

Author contribution statement

Gretel Fitó Friedrichs: Performed the experiments; Analyzed and interpreted the data.

Giselle Berenstein, Soledad Nasello, Yohana Y. Dutra Alcoba: Performed the experiments.

Enrique Hughes, Silvana Basack: Analyzed and interpreted the data.

Javier M. Montserrat: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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