Science of the Total Environment 586 (2017) 1254–1262

Contents lists available at ScienceDirect

Science of the Total Environment

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

Human and soil exposure during mechanical chlorpyrifos, myclobutanil and copper oxychloride application in a peach orchard in Argentina

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HIGHLIGHTS

- Mean workers (tractor drivers) Potential Dermal Exposure was $30.8\ \mathrm{mL \cdot h^{-1}} \pm 16.4\ \mathrm{mL \cdot h^{-1}}$.
- Pesticide on the orchard soil ranged between 4.7% and 9.3% of the total applied pesticide.
- Total drift values varied from 2.4% to 11.2% of the total applied pesticide.
- Bystander, resident and earthworm Risk Indicators were below 1 in all cases.
- Earthworm Risk Indicators had good correlation with *Eisenia andrei* ecotoxicological assays.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 2 December 2016
Received in revised form 14 February 2017
Accepted 16 February 2017
Available online 22 February 2017

Editor: D. Barcelo

Keywords:
Chlorpyrifos
Myclobutanil
Copper oxychloride
Soil
Exposure

ABSTRACT

The objective of this study was to measure the impact of the mechanized chlorpyrifos, copper oxychloride and myclobutanil application in a small peach orchard, on humans (operators, bystanders and residents) and on the productive soil. The mean Potential Dermal Exposure (PDE) of the workers (tractor drivers) was $30.8\ \mathrm{mL \cdot h^{-1}} \pm 16.4\ \mathrm{mL \cdot h^{-1}}$, with no specific pesticide distribution on the laborers body. Although the Margin of Safety (MOS) factor for the application stage were above 1 (safe condition) for myclobutanil and cooper oxycloride it was below 1 for chlorpyrifos. The mix and load stage remained as the riskier operation.

Pesticide found on the orchard soil ranged from 5.5% to 14.8% of the total chlorpyrifos, copper oxychloride and myclobutanil applied. Pesticide drift was experimentally measured, finding values in the range of 2.4% to 11.2% of the total pesticide applied.

Using experimental drift values, bystander (for one application), resident (for 20 applications) and earthworm (for one application) risk indicators (RIs) were calculated for the chlorpyrifos plus copper oxychloride and for myclobutanil treatments for different distances to the orchard border. Earthworm RI was correlated with experimental *Eisenia andrei* ecotoxicological assays (enzymatic activities: cholinesterases, carboxylesterases and glutathione S-transferases; behavioral: avoidance and bait-lamina tests) with good correlation.

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http://dx.doi.org/10.1016/j.scitotenv.2017.02.129

1. Introduction

Argentinean agriculture ranges from extensive and technologically developed crops (like soybean, wheat and maize) to vegetable and fruit production in greenhouses and orchards with low technology and manpower dependence (Cabrini and Calcaterra, 2016; Zelaya et al., 2016). Both situations have in common an intensive use of pesticides, mainly herbicides in the first case and fungicides and insecticides in the last one (Barni et al., 2016), with potential adverse effects on the environment. In spite of the improvements in the Argentinean phytosanitary registration process, no advances have been achieved regarding the development of human and environmental risk scenarios involving the use of pesticides under different working conditions. This issue is particularly relevant in small production units where risk to pesticide exposure is vaguely perceived by the workers (Damalas and Abdollahzdeh, 2016).

Focusing on the human exposure in orchards and their surroundings, the general study of the occupational health hazards associated to pesticide use (Sumon et al., 2016), the determination of the factors that affect operator's exposure (Berenstein et al., 2014), the analysis of the best working conditions that maximizes laborer's safety (Tsakirakis et al., 2014) and the development of exposure models for bystanders and neighbour residents (van der Berg et al., 2016), are all important issues in the pesticide impact agenda.

While the highest human exposure usually occurs for the operator during mixing-loading and application stages (Ramos et al., 2010), the off-field adverse health effects on humans by occasional (bystanders) or continuous (residents) exposure due to proximity to fields treated with pesticides are caused by the pesticide drift. Measurement of the pesticide drift is a complex task, since it is influenced by different factors, including equipment and application techniques, spray characteristics, operator training and environmental and meteorological conditions (Gil et al., 2014). Alternatively, new standard procedures for drift measurements based on bench tests are under development (Gil et al., 2014). Despite these difficulties, the experimental drift determination is an important task for establishing dedicated buffer zones adjacent to the production areas that can diminish the off-field pesticide effects. In the particular case of the European Community, the framework for Community action to achieve the sustainable use of pesticides is established in Directive 2009/128/EC of the European Parliament and of the Council, known as the Sustainable Use Directive (SUD). According to the SUD, EU Member-States must establish appropriately-sized buffer zones, with permanent vegetation, next to areas where pesticides are in use. Naturally, the buffer zone width will be dependent upon the pesticide application technique and its corresponding drift (Gil et al., 2015).

Considering this background, the general objective of this work was to study the related risks on humans and soil caused by pesticide use in small peach orchards in Argentina. The specific objectives were:

a) To determine the operators Potential Dermal Exposure (PDE) and Margin of Safety (MOS) for the mechanized application of pesticides (chlorpyrifos, copper oxychloride, myclobutanil),
b) To measure the pesticide spray drift and the amount of pesticide that fell on the orchard's soil,
c) To calculate bystander, resident and earthworm Risk Indicators (RI) associated to the spray drift for different distances to the orchard's border,
d) To correlate the earthworm RI obtained with enzymatic and behavioral experimental data using *Eisenia andrei* as bioindicator.

2. Materials and methods

2.1. Pesticides

Chlorpyrifos, myclobutanil and copper oxychloride were chosen for this study due to their use in peach production. Chlorpyrifos and myclobutanil were used as tracers for PDE and drift measurements.

Commercial products used in the field experiments were as follows:

- ➢ Chlorpyrifos (0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate, CASRN [2921-88-2]), Clorfox® (EC, 48% w/v, Gleba).
- ➢ Myclobutanil (2-(4-chlorophenyl)-2-(1,2,4-triazol-1-ylmethyl) hexanenitrile), CASRN [88671-89-0], Systhane E® (EC, 25% w/v, Dow Agrosciences).
- ➢ Copper oxychloride ($Cu_2(OH)_3Cl$), CASRN [1332-65-6]): Coura® (WP, 84% w/w, Tort Valls S.A).

2.2. Chemicals and solvents

Chemicals and solvents were of the best analytical grade. Acetylthiocholine iodide (ATCh), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH); phenylthioacetate (PTA) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich.

To prepare reference material for chlorpyrifos and myclobutanil, technical grade pesticides were purified by recrystallization (>95% pure by GC-FID). The identity and purity of the active principles were confirmed by ^1H and ^{13}C NMR. A primary solution of 100 ppm w/w was prepared in acetone or cyclohexane, and the working solutions were obtained by dilution as needed. Acetone, cyclohexane and n-hexane (Anedra p.a. grade) used for all solutions and extracts were previously distilled and chromatographically checked as suitable for GC-ECD use.

2.3. Chromatographic conditions

All chromatographic analyses were performed on a Perkin-Elmer (Norwalk CT, USA) AutoSystem XL Gas Chromatograph with 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 chlorpyrifos determinations were as follows: injector temperature: 280 °C; ECD temperature: 375 °C; oven temperature: 190 °C for 1.5 min, 45 $°C \cdot min^{-1}$ to 300 °C then 10 $°C \cdot min^{-1}$ to 320 °C and hold 2 min; injection volume 1 μL, splitless; carrier gas: N_2, 30 psi; ECD auxiliary flow 30 $mL \cdot min^{-1}$.The GC-ECD operating conditions for myclobutanil determinations were as follows: injector temperature: 280 °C; ECD temperature: 375 °C; oven temperature: 80 °C for 0.5 min, 45 $°C \cdot min^{-1}$ to 300 °C then 10 $°C \cdot min^{-1}$ to 320 °C and hold 2 min; injection volume 1 μL, splitless; carrier gas: N_2, 45 psi; ECD auxiliary flow 30 $mL \cdot min^{-1}$.

2.4. Method validation

2.4.1. Linear ranges

Linear GC-ECD calibration curves for chlorpyrifos and myclobutanil were obtained using cotton fabric as matrix. Samples of cotton fabric were spiked with known amounts of each pesticide as a pure compound dissolved in acetone and extracted as described in Section 2.6. The calibration curve was constructed between 0.12 and 1.64 $mg \cdot kg^{-1}$ for chlorpyrifos and 0.7–5.3 $mg \cdot kg^{-1}$ for myclobutanil. The ECD response for both pesticides was linear in both cases with $R^2 > 0.99$.

2.4.2. Limit of detection (LD) and limit of quantification (LQ)

The LD and LQ for each pesticide were obtained using the signal to noise rate. The LD was estimated at a signal to noise ratio of 3 and the LQ was determined at a signal to noise ratio of 10. Both limits were obtained for individual pesticides on cotton fabric by CG-ECD. In this way the LD for chlorpyrifos was 0.02 $mg \cdot kg^{-1}$ and the LQ was 0.12 $mg \cdot kg^{-1}$. The LD for myclobutanil was 0.3 $mg \cdot kg^{-1}$ and the LQ was 0.7 $mg \cdot kg^{-1}$.

2.4.3. Recoveries

In order to study the recovery of the pesticides from the cotton fabric, the slope of the calibration curve prepared spiking cotton fabric (SC) was compared with a calibration curve of each pesticide in cyclohexane or a mixture of acetone:hexane 15:85 (SS). The recovery was calculated as follows:

$$\%\text{Recovery} = (SC/SS) \times 100$$

Recoveries of 103.5% for chlorpyrifos and 123% for myclobutanil were obtained.

2.4.4. Intermediate precision

The intermediate precision of the analytical method was determined by performing the analysis of two field samples: one containing chlorpyrifos and the other containing myclobutanil. Both samples were injected three times with the corresponding calibration curve with an automatic injector. This procedure was repeated six times in different days, switching off the gas chromatograph between each repetition. The mean relative standard deviation (RSD) values obtained for the concentration of each pesticide were 3.0% for chlorpyrifos and 16.8% for myclobutanil.

2.5. Field trials

All field experiments were carried out in a peach orchard in San Pedro district (Buenos Aires, Argentina), in winter 2014 (10th July and 20th August) and 2015 (14th July). Peach trees (3.0 m to 3.5 m height) were aligned in rows separated by 5 m. Each tree in a row was separated from the next one by 3 m.

Pesticides were applied with a John Deere 5403 tractor coupled with a Jacto Arbus 2000 mechanical sprayer (Supplementary material, Fig. I-SM). Pesticide applications of 1085 $L \cdot ha^{-1}$ were done using Albuz ATR 80 Lilac yellow and brown, and NCH80-040 nozzles. For each application the tractor engine speed was 2100 rpm 3°A. The application speed was of 3.7 $km \cdot h^{-1}$ and the pressure setting was 980 kPa (9.8 bar). The Tree Row Volume (TRV) model was used for determining spray volume in the peach orchard. Meteorological conditions of field experiments are reported in Table II-SM.

Date, application duration, application volume, pesticide concentrations and sprayed area of each experiment were as follows:

Experiment C1: July 2014, 10.2 min; 100 L; 600 mg Chlorpyrifos L^{-1} (+$Cu_2(OH)_3Cl$: 5000 mg Cu°/L); 1000 m^2.

Experiment C2: July 2014, 10.2 min; 100 L; 600 mg Chlorpyrifos L^{-1} (+$Cu_2(OH)_3Cl$; 5000 mg Cu°/L; 1000 m^2.

Experiment C3: July 2015, 10.8 min; 150 L; 600 mg Chlorpyrifos L^{-1} (+$Cu_2(OH)_3Cl$; 4000 mg Cu°/L; 1000 m^2.

Experiment C4: July 2015, 9.6 min; 150 L; 600 mg Chlorpyrifos L^{-1} (+$Cu_2(OH)_3Cl$; 4000 mg Cu°/L; 1000 m^2.

Experiment M1: 10.2 min; 150 L; 37.5 mg Myclobutanil L^{-1}; 1000 m^2.

Experiment M2: 10.2 min; 150 L; 37.5 mg Myclobutanil L^{-1}; 1000 m^2.

2.5.1. PDE measurements

The potential dermal exposure was measured using the whole body dosimetry technique as previously reported (Hughes et al., 2008). Further details are provided in the Supplementary material.

2.5.2. Drift and soil measurements

The amount of chlorpyrifos and myclobutanil on the peach orchard soil was determined using square (20 cm × 20 cm) cloth samplers lined on one side by a polyethylene film to avoid external contamination. Ten samplers were randomly located on the soil of the peach orchard for the different experiments (Supplementary material, Figs. II, III-SM). Pesticide drift was measured using the same square (20 cm × 20 cm) cloth samplers as previously described. To define drift sampling location, the samplers were located downwind (wind direction was determined using a meteorological portable station Geos n°9, JDC Electronic). Inside the drift sampling area (96 m long × 30 m width) three rows of samplers separated 15 m each were located on the soil (with the polyethylene film down face) at 16 m (S_1–S_3), 48 m (S_4–S_6) and 80 m (S_7–S_9) from the orchard border (Supplementary material, Figs. II, III-SM). Once the pesticide application was finished, 15 min were waited before collecting the samplers in order to capture as much drift as possible. The cotton samplers were stored and tagged in individual bags for laboratory determinations.

Drift was measured in all field trials with wind conditions below 3 $m \cdot s^{-1}$. Based on a set of experimental results of drift measurement under different wind conditions, Gil et al. (2015) determined that drift quantization was not significantly affected when field trials were performed under low wind velocities.

2.6. Laboratory analysis

Laboratory analyses were done no later than 20 h after the field trial. Both pesticides were stable on the cotton samplers at least up the first 30 h (data not shown). In the laboratory, the cotton suit was cut into pieces as indicated in Fig. 1 (Hughes et al., 2008) and each piece was placed separately in polypropylene containers and extracted using suitable volumes of cyclohexane for chlorpyrifos and acetone:*n*-hexane 15:85 mixture for myclobutanil. 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 drift and soil measurements were analyzed in the same way as described above.

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

2.6.1. PDE calculations

The concentration 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 are expressed as volume of spray-mixture to which the operator would be exposed if he continued spraying for 1 h (in $mL \cdot h^{-1}$). 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 is given as the amount of pesticide (in mg) found on each body section or in an equivalent form.

In this study the same person (which was the tractor driver defined also as "operator") performed the mix/load and the application stages.

2.6.2. MOS calculation

The MOS was measured as previously reported (Hughes et al., 2008). 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). Further details are provided in the Supplementary Material.

2.6.3. Pesticides on the orchard soil

The mean chlorpyrifos or myclobutanil amount found in the samplers located on the orchard's soil was divided by the area of the 20 cm × 20 cm sampler (400 cm^2) and multiplied by the orchard's surface in cm^2.

2.6.4. Drift calculation

The drift sampling zone was divided in four sections: 0 m–8 m (section 1), 8 m–32 m (section 3), 32 m–64 m (section 3) and 64 m–96 m (section 4) from the orchard's border (Supplementary material, Figs. II, III-SM). To calculate the mean pesticide amount in each section the following criteria was used:

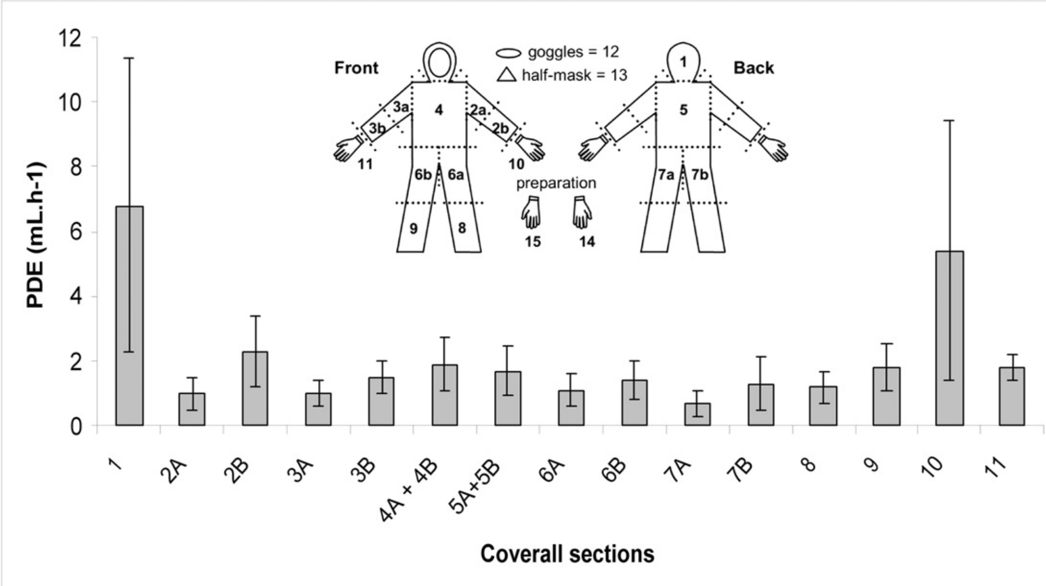

Fig. 1. Mean percentage of PDE on the worker's cotton sampler.

Section 1: the mean pesticide mass/cm^2 found for the orchard soil was considered. The total pesticide mass of this section was calculated multiplying by the section area in cm^2.

Section 2, 3, 4: the total mass of pesticide in section/cm^2 (mean pesticide mass (S_i, S_{i+1}, S_{i+2}) / 400 cm^2)) was multiplied by the area section $_i$ (cm^2).

To calculate the relative amount of pesticide in soil, the total mass of pesticide in section i was divided by the total applied pesticide and multiplied by 100.

2.6.5. Risk indicators calculation (RI bystander, resident, earthworm)

We considered bystanders as adult people located within or directly adjacent to the area where pesticide application is in progress. Residents are defined as people living in nearby fields (Cunha et al., 2012).

Considering the bystander, resident and earthworm risk indicators definitions introduced by Cunha et al. (2012), the RIs as a function of the distance from the orchards border (x) were calculated as follows:

$$RI_{bystander}(x) = \frac{([pest.] \cdot V_{appl}/A_{appl}) \cdot 0.3261\ m^2 \cdot 1.01 \cdot N \cdot (\%D(x)/100)}{BW \cdot AOEL}$$

$$RI_{resident}(x) = \frac{([pest.] \cdot V_{appl}/A_{appl}) \cdot 0.3261\ m^2 \cdot 1.01 \cdot 0.246 \cdot FA \cdot (\%D(x)/100)}{BW \cdot AOEL}$$

$$RI_{earthworm}(x) = \frac{([pest.] \cdot V_{appl}/A_{appl} \cdot 0.05\ m.d_{soil}) \cdot (\%D(x)/100)}{LD50_{earthworm}/10}$$

where:

A_{appl}: orchard area were pesticide was applied (m^2)

AOEL: Acceptable Operator Exposure Level ($mg \cdot kg^{-1} \cdot d^{-1}$)

BW: Body Weight (70 kg)

FA: Frequency of application (number of pesticide applications in a year, in this case FA = 20)

LD_{50}: Lethal dose for 50% (*Eisenia andrei*)

N: Number of applications (in this case $N = 1$)

[pest.]: pesticide concentration ($mg \cdot L^{-1}$)

V_{appl}: pesticide volume applied (L)

% D(x): drift percentage respect to the total pesticide applied as a function of the orchards border distance (x)

δ_{soil}: Soil density ($kg \cdot m^{-3}$, 1500 $kg \cdot m^{-3}$, Querejeta et al., 2014)

0.3261 m^2: exposed bystander or resident body surface, considering EFSA surface body values and 100% pesticide absorption for hands and head and 10% absorption for trunk, legs and arms

1.01: inhalation factor (1% inhalation with 100 absorption, Ramos et al., 2010)

0.246: = 90/365 (corresponding to an equivalent of 90 days of residence in the house per year; Cunha et al., 2012)

0.05 m: soil depth considered for pesticide retention (Cunha et al., 2012).

In the case of the simultaneous application of chlorpyrifos and copper oxychloride, the RIs of the mixture were calculated considering chlorpyrifos and copper oxychloride RIs effects independent and additive (Fig. 3). Individual chlorpyrifos and copper oxycloride RI for bystander and residents are shown in Tables IV-VII-SM (Supplementary material).

For RIs calculation the following LD50 and AOEL were took into account:

LD50 chlorpyrifos = 313 $mg \cdot kg^{-1}$, (WHO, 2008). LD50 copper oxychloride = 489.6 mg/kg (Pesticide Properties Database, Hertfordshire University). LD50 myclobutanil = 10.3 $mg \cdot kg^{-1}$ (Pesticide Properties Database, Hertfordshire University).

AOEL chlorpyrifos = 0.001 $mg \cdot kg^{-1} \cdot d^{-1}$ (EFSA, 2014); AOEL myclobutanil = 0.03 $mg \cdot kg^{-1} \cdot d^{-1}$ (EFSA, 2010); AOEL copper oxychloride = 0.25 $mg \cdot kg^{-1} \cdot d^{-1}$ (Pesticide Properties Database, 2017).

2.6.6. Ecotoxicological determinations

2.6.6.1. Soil. For laboratory bioassays, soil samples (12–15 kg) of the treated peach orchard and of a non-exposed control area (reference) soil of 0–10 cm depth were collected and kept refrigerated until analysis and bioassays were done. Physicochemical analyses of soil samples were performed by SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria) Quality Service, Buenos Aires, Argentina. The results are shown in Table III-SM.

2.6.6.2. Soil preparation. Soil samples were 2-mm sieved and adjusted to 50–60% of maximum water holding capacity before running the bioassays.

2.6.6.3. Earthworms. Eisenia andrei earthworms 0.30–0.60 g fresh weighted and maintained in our laboratory, were exposed to soils prepared as described above with a 16 h/8 h 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.

2.6.7. Toxicological analyses

2.6.7.1. Enzymatic determinations. To each of four replicate containers, 300 g soil and six adult earthworms were added. Humidity of soils was maintained constant during the entire experiment. After 7 days of exposure, earthworms were removed from soil and were homogenized using a Potter–Elvehjem homogenizer fitted with a teflon pestle in Tris-HCl buffer 100 mM pH 7.5 in 1:3 ratio (tissue weight: buffer volume). The homogenate was centrifuged at 9000 x g at 4 °C, and the pellet was discarded. The supernatant fraction was used for the enzymatic studies. Enzyme activities were measured on a Perkin Elmer Lambda 25 UV-VIS dual- beam spectrophotometer.

Cholinesterase activities (ChE) were assayed by the method of Ellman et al. (1961). Carboxylesterase activities (CaE) were determined by an adaptation of Ellman method at 412 nm using PTA as substrate (Ferrero et al., 1991). Gluthatione S-Transferase activities (GST) were determined using CDNB as substrate, and monitoring absorbance changes at 340 nm (Habig et al., 1974). Bradford's (1976) method was used for quantitative determination of proteins.

2.6.7.2. Avoidance behavior. ISO N 281 (2004) was followed. Further details are provided in the Supplementary material.

2.6.7.3. Bait-lamina test. Bait-lamina consist of plastic strips about 12 cm long, 1 cm broad, and 1 mm thick. The lamina was perforated at 5 mm distances by 16 small (1 mm) holes, filled with a bait substance (a mixture of cellulose, wheat bran and activated carbon). In the laboratory, an adaptation of Helling et al. (1998) method was performed. Containers with 400 g soil, 4 bait-lamina and 6 earthworms each were used (6 replicates/treatment). After 3 days exposure, the number of empty or perforated holes in each lamina was counted and the percentage of feeding activity was calculated.

2.6.7.4. Cu bioaccumulation analyses. In order to measure the metal uptake from the soil by the test organisms, the metal concentrations in wet tissue and in soil samples were analyzed using a Shimadzu AA-6701F atomic absorption spectrophotometer. Detailed procedure is described in the Supplementary material.

2.7. 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 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

PDEs for the preparation (mix and load) and application stages were measured for two pesticides mixtures: chlorpyrifos plus copper oxychloride and myclobutanil, separately, in a peach orchard localized in San Pedro district, Buenos Aires province, Argentina. PDE was determined measuring chlorpyrifos and myclobutanil as tracers. The applications were done using a tractor mounted turbine sprayer with a 2000 L tank in six independent measurements (four for chlorpyrifos plus copper oxychloride and two for myclobutanil). Table 1 shows the PDE results, expressed in $mL \cdot h^{-1}$, for the different body sections for chlorpyrifos plus copper oxychloride (C1–C4) and myclobutanil (M1–M2) determinations. The total average PDE for the six applications was 30.8 $mL \cdot h^{-1}$ ± 16.4 $mL \cdot h^{-1}$.

When the pesticide distribution was analyzed taking into account the average PDE of each body section (sections 1–11, Fig. 1) no significant difference could be found between the coverall parts.

The PDE for copper oxychloride (data not shown) was calculated using the PDE values of the chlorpyrifos application (simultaneous chlorpyrifos and copper oxychloride applications). Considering the PDE expressed as pesticide mass (mg) of chlorpyrifos, copper oxychloride or myclobutanil for the mix and load operations and the exposure associated to 1 h of pesticide application, the Margin of Safety (MOS) was calculated for both stages and for the sum of them (Table 2). For chlorpyrifos cases the pesticide application was unsafe

Table 1
PDE expressed in $mL\ h^{-1}$ for application of chlorpyrifos and myclobutanil to peach crops using a tractor mounted turbine sprayer.

Potential dermal exposure ($mL\ h^{-1}$)								
Active ingredient	Chlorpyrifos				Myclobutanil			
	Field trials[b]							
C. section[a]	C_1	C_2	C_3	C_4	M_1	M_2	Av.[c]	SD
1	1.53	0.84	4.79	5.36	25.07	3.24	6.8	9.1
2A	1.47	0.34	1.86	2.27	ND[d]	ND	1.0	1.0
2B	1.49	0.61	1.19	1.21	6.46	2.64	2.3	2.2
3A	1.58	1.49	1.48	1.69	ND	ND	1.0	0.8
3B	1.52	1.00	1.70	2.25	2.81	ND	1.5	1.0
4A + 4B	3.49	1.34	2.90	3.54	ND	ND	1.9	1.7
5A + 5B	2.00	1.54	3.33	3.55	ND	ND	1.7	1.5
6A	2.34	0.84	1.41	2.22	ND	ND	1.1	1.0
6B	2.82	1.25	2.12	2.50	ND	ND	1.4	1.2
7A	0.65	0.35	1.92	1.30	ND	ND	0.7	0.8
7B	0.90	0.41	0.65	1.30	4.55	ND	1.3	1.7
8	1.93	1.27	1.98	2.27	ND	ND	1.2	1.0
9	3.33	1.64	2.49	3.40	ND	ND	1.8	1.5
10	1.34	1.88	3.31	1.63	21.57	2.41	5.4	8.0
11	3.27	0.96	1.76	1.74	0.99	1.96	1.8	0.8
Total[e]	29.7	15.8	32.9	36.2	61.5	10.3	30.8	16.4

[a] C. section = Coverall section (see Fig. 1).
[b] M_i and C_i denotes field experiment number i.
[c] Av. = average.
[d] ND = Not Detected.
[e] For comparison with other published results, this Total does not include sections 12–15, i.e. facial protection and preparation gloves.

Table 2
MOS of the mix and load and the application stages.

Experiment	MOS[b]		
	Mix and load	Application	Total
C_1 (chlorpyrifos)	0.00054	0.043	0.00053
C_2 (chlorpyrifos)	NM[a]	0.084	–
C_3 (chlorpyrifos)	0.025	0.037	0.015
C_4 (chlorpyrifos)	NM	0.011	–
C_1 ($Cu_2(OH)_3Cl$)	NM	1.3	–
C_2 ($Cu_2(OH)_3Cl$)	NM	2.5	–
C_3 ($Cu_2(OH)_3Cl$)	NM	1.4	–
C_4 ($Cu_2(OH)_3Cl$)	NM	1.2	–
M_1	5.1	9.7	3.3
M_2	NM	14.4	–

[a] NM: Not Measured
[b] AOEL chlorpyrifos = 0.001 $mg \cdot kg^{-1} \cdot d^{-1}$; AOEL myclobutanil = 0.03 $mg \cdot kg^{-1} \cdot d^{-1}$; AOEL ($Cu_2(OH)_3Cl$) = 0.25 $mg \cdot kg^{-1} \cdot d^{-1}$

(MOS < 1). In the three cases were the mix and load and application operations were measured the mix and load stages were riskier than the application steps (Table 2).

3.2. Soil exposure and drift results

Another matrix impacted during pesticide application is soil. Two different situations can be distinguished: the pesticide impact on the soil of the orchard production itself, and the neighbouring soil reached by pesticide drift. To evaluate both situations, a set of samplers were located both on the orchard's soil and on the neighbouring soils. Fig. 2 shows the percentages of pesticide found on both soils. Table I-SM show the mean values for the pesticide percentages (referred to the total pesticide applied) found for the orchard's soil and for each of the drift pesticide sections. Figs. II and III-SM show the sampling scheme followed for the different field trials.

The mean pesticide percentage found on the orchard soil was 8.6% ± 2.0%, while the total drift was 4.9% ± 1.7%. In all cases detectable pesticide amounts were found up to 80 m from the orchard's border.

For an easier comparison to drift bibliographic data, Fig. V-SM shows C_1–C_4 mean drift measurements express as $\mu L_{pesticide\ solution} \cdot cm^{-2}$ as a function of the distance to the orchard's border.

3.3. Risk indicators results

Taking into account the pesticide drift measurements from the orchard's border, a set of RIs for bystanders (for one application), residents (for 20 applications in a year) and earthworms (for one application) were calculated as indicated in Section 2.6.5. Briefly, the RIs correlate the amount of pesticide that was found in a certain point of the production unit with the available toxicological data (AOEL) for humans, or ecotoxicological values for earthworm (LC50). Contrary to MOS, in these cases where the RIs < 1, the condition is safe.

Fig. 3 shows the bystander, resident and earthworm RIs measured at different distances of the orchards border, for the case of the simultaneous application of chlorpyrifos and copper oxychloride, and for the application of myclobutanil.

3.4. Soil ecotoxicological impact results

In an attempt to experimentally verify that the calculated risk indicators correlates with direct ecotoxicological experimental evidence, enzymatic and behavioral parameters for *Eisenia andrei* in orchards soil were determined in the worst scenario conditions (0 m from the orchard border).

Fig. 4 shows the enzymatic activities of organisms exposed to reference and treated (with chlorpyrifos + copper oxychloride) soils expressed as a percentage of control activities (100% ChE, CaE and GST activities correspond to 65 ± 12 nmol ATCh $min^{-1} \cdot$ mg $protein^{-1}$; 312 ± 21 nmol PTA $min^{-1} \cdot$ mg $protein^{-1}$ and 161 ± 25 nmol$\cdot min^{-1} \cdot$mg $protein^{-1}$, respectively). ChE and CaE activity were not modified by exposure to peach orchard soil in laboratory conditions, although GST induction in *Eisenia andrei* was observed (68 ± 17% increase compared to controls, $p < 0.05$). No mortality occurred in earthworms experimentally exposed to the tested soils in the bioassays.

Regarding the behavior assays, Fig. 4c shows the results of the avoidance test after earthworm exposure to soil samples of each collected block. In dual control tests, no significant differences ($p > 0.05$) were found in the distribution of the worms between both chambers of the containers. In the same way, when the earthworms were offered the choice of reference soil versus peach orchard soil, they did not make a specific first choice for either side of the profile. The response of bait-lamina consumption after 3 days of earthworm exposure to soils is shown in Fig. 4C. Substrate consumption rates, measured as the percentage of open holes in the bait-lamina sticks, showed no significant reduction in treated soils (Fig. 4C).

The mean copper concentration in soil (reference and peach orchard) and in worms (exposed and not exposed) were also determined. While the mean copper concentration in the reference soil was 112 ± 2 mg$\cdot kg^{-1}$, in the orchard soil was 286 ± 3 mg$\cdot kg^{-1}$. This copper concentration difference was also reflected in the worm's copper content after the exposure period (Fig. 4B). Copper concentration in worms exposed to reference soil was 2.2 ± 0.2 mg$\cdot kg^{-1}$ and it was 8.5 ± 0.5 mg kg^{-1} in organisms exposed to treated soil.

4. Discussion

4.1. PDE and MOS discussion

Operator's PDE comparison with reported values is a difficult task as consequence of the variety of different expressions available for this property (mass of different pesticides, volume of sprayed solution per time unit, percentage of the total pesticide applied). When comparing mean PDE (30.8 mL$\cdot h^{-1}$ ± 16.4 mL$\cdot h^{-1}$) found for the mechanized

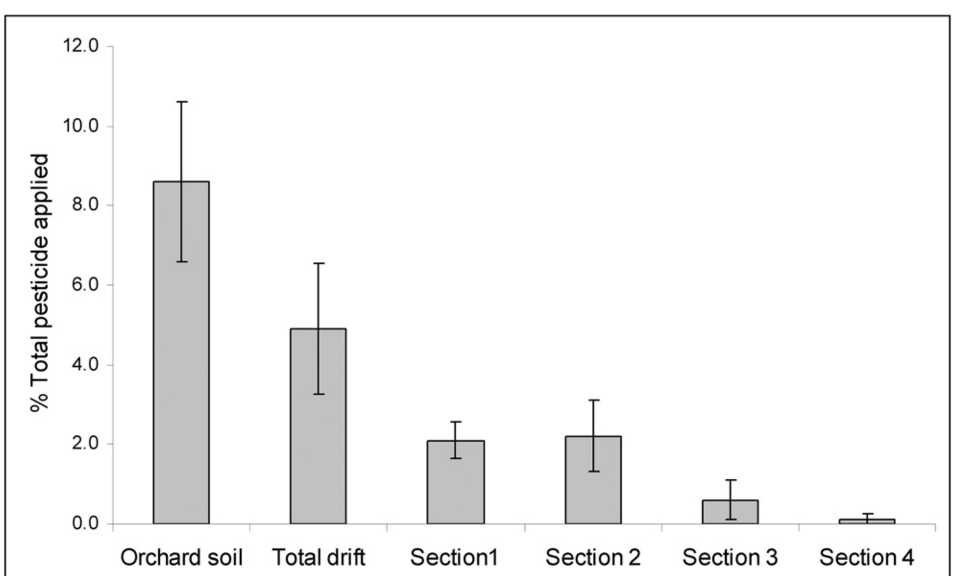

Fig. 2. Pesticide drift for the application of chlorpyrifos (C_1, C_2, C_3, C_4) and myclobutanil (M_1, M_2) in a peach orchard measured as percentage of the total applied product for sections 1, 2, 3 and 4.

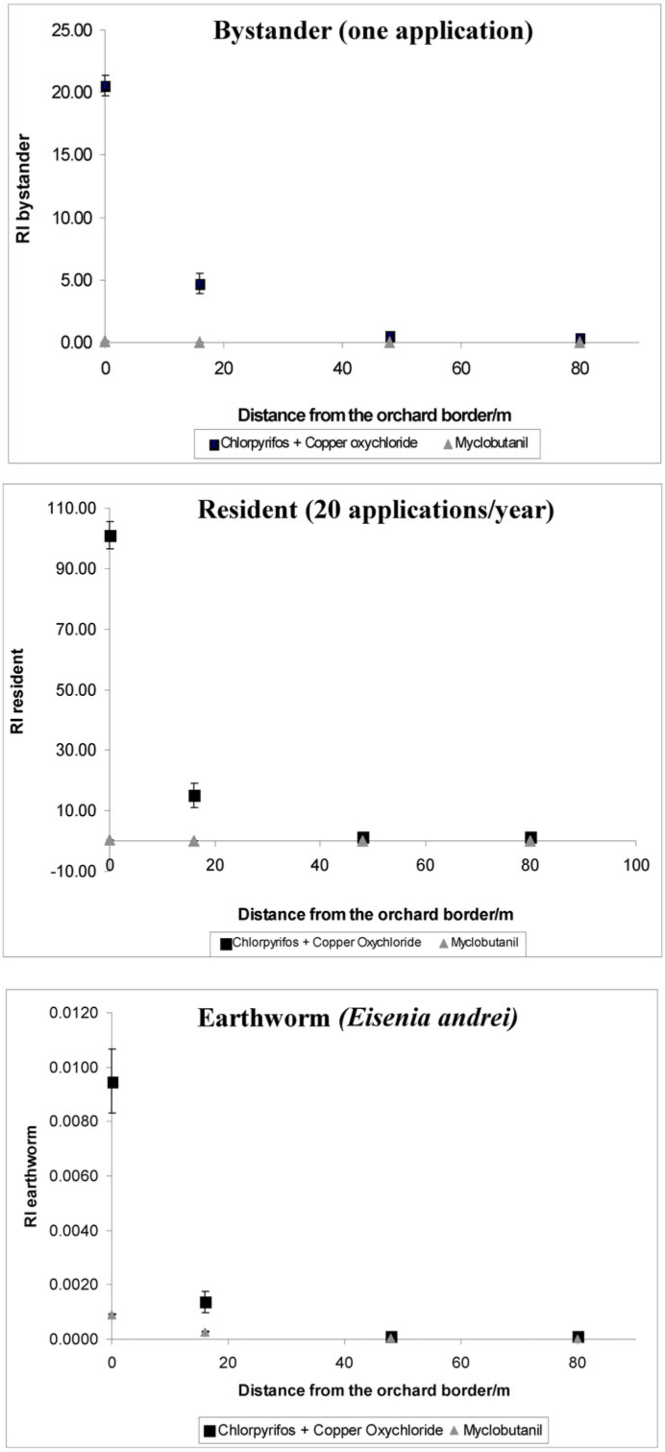

Fig. 3. Bystander, resident and earthworm Risk Indicators (RIs) for chlorpyrifos + copper oxychloride and myclobutanil applications.

application of chlorpyrifos plus copper oxychloride or myclobutanil in the peach orchards with previous PDE data (expressed in $mL \cdot h^{-1}$), obtained by our group for manual knapsack pesticide applications in horticultural (maize, broccoli, tomato) or floricultural greenhouses and open fields, the PDE of peach orchards workers was the lowest one (Fig. IV-SM). The same trend was observed by Baldi et al. (2006), who determined that the median of the dermal contamination on a whole day of work was 40.5 mg of active ingredient for tractor operators and 68.8 mg for backpack sprayers. For tractor operators, the median of the dermal contamination during a single operation was 2.85 mg for mixing, 6.13 mg for spraying and 4.20 mg for cleaning stages. In the same sense, Graham (2002) determined that the exposure of the body of a manual knapsack applicator could be up to five times higher compared to the exposure of a tractor driver for the same concentration and volume of pesticide.

Regarding the pesticide distribution in the operator's body, we have found no prevalent distribution pattern (Fig. 1). Machado-Neto et al. (2000) reported that when tractor powered sprayers were used for glyphosate application in Eucalyptus plantations, the most exposed driver's body parts were the front of thighs, legs, arms and forearms. In the same sense, Vitali et al. (2009) found that in 10 of 14 cases related to pesticide application using tractor, hand contamination was the major contributor to dermal exposure considering a complete working cycle (mix, load and application). This is an interesting issue, because we have also found for the cases where the PDE and MOS were measured, that pesticide preparation was the riskier operation (Table 2). This was also observed by others authors (Lonsway et al., 1997; Leibally et al., 2008). All our measurements were done with a small open-seat tractor. In this sense, it has been reported (Rubino et al., 2012) that the tractor's drivers exposure using an air-conditioning or a simple closed cab were not significantly lower than operator's exposure using an open-seat tractor.

4.2. Soil exposure and drift discussion

The mean amount of pesticide found on the peach orchard's soil was 8.6% ± 2.0% of the total applied pesticide (Fig. 2). These amounts were lower than the relative pesticide amount found by Holland et al. (1997) during an air blast spraying in a kiwi orchard in New Zealand where 16% of the total applied pesticide was left on the orchard's ground. Glotfelty et al. (1990) reported that after diazinon application using an air blast sprayer in a peach orchard, the orchard's soil received two to three times more insecticide than the peach trees, which was not the case in our measurements (Fig. 2). When the relative amounts of chlorpyrifos, copper oxychloride and myclobutanil on the peach orchards are compared with relative pesticide amounts found in vegetable open field and greenhouses with manual applications (Querejeta et al., 2012), it is interesting to observe that relative pesticides amounts found on soil in the first case were lower than in the manual application scenario.

Regarding the relative amounts of pesticides that drifted outside the orchard's soil, we found a mean value of 4.9% ± 1.7% of the total applied pesticide. Holland et al. (1997) reported a total drift of 2% for a kiwi orchard application, with a mean distance of 50% of decline (which is the distance to the orchard border where 50% of the drift value is found) of 26 m. Although in our case the estimated mean distance of 50% of decline was circa 10 m (Fig. V-SM), detectable amounts of chlorpyrifos were found up to 80 m of the orchard's border (Table I-SM).

4.3. Risk indicators discussion

The development of RIs is an important task because of the necessity of establishing buffer zones with acceptable widths in order to diminish general pesticide exposure. Taking into account bystander, earthworm and resident RI descriptions introduced by Cunha et al. (2012), we have calculated, using our experimental drift values, the RIs as distance function to the orchard's border (Fig. 3). Our worst case scenario was at a distance from border = 0 m. Using this condition the bystander RI was 20.5 ± 0.8, considering the combined effects of chlorpyrifos and copper oxychloride (both effects were considered independent and consequently additive), and 0.105 ± 0.005 for myclobutanil. Results shown in Fig. 3 indicates that a distance of at least 48 m in necessary for having safe conditions for bystanders when chlorpyrifos was applied. Cunha et al. (2012) reported bystander RI values at 8 m from a citrus production border of 1.1482 for chlorpyrifos and 0.0785 for copper oxychloride, using the German drift values model.

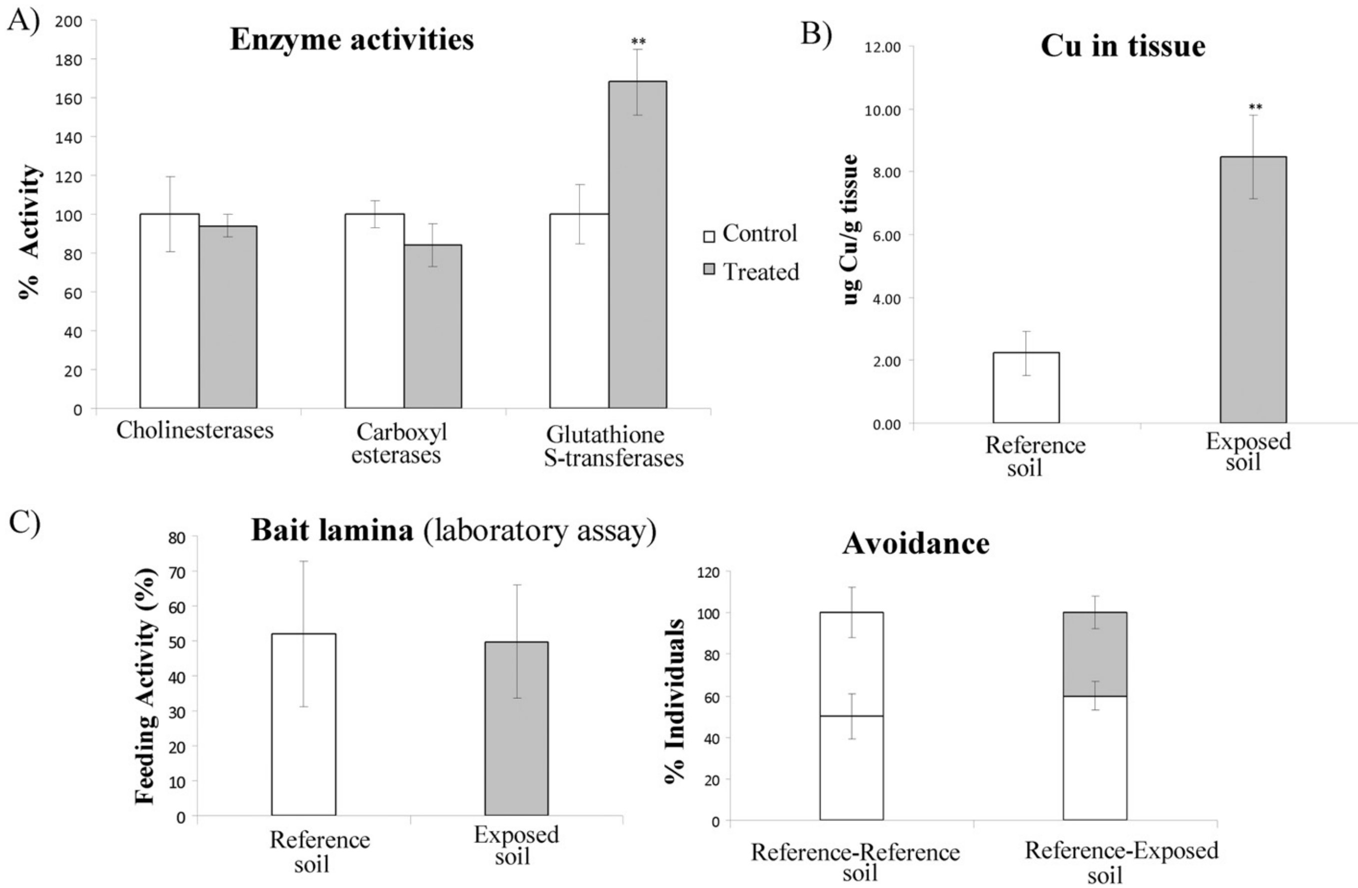

Fig. 4. Earthworm enzyme activities, copper in tissue and behavior tests in exposed soils.

Regarding resident RI, we have used as hypothesis that the resident received 20 pesticide applications in one year (FA = 20, section, obtaining a RI of 100.0 ± 4.6 for chlorpyrifos plus copper oxychloride at a distance = 0 m and 1.4 ± 2.2 at 80 m, indicating that safe conditions for residents required even longer distances (for individual chlorpyrifos and copper oxycloride RI of bystanders and residents, please see Tables IV–VII-SM, Supplementary Material).

Finally, we have calculated earthworms RIs of 0.095 ± 0.001 for chlorpyrifos plus copper oxychloride and 0.00904 ± 0.00002 for myclobutanil. Cunha et al. (2012) estimated values of 0.072 for chlorpyrifos and 0.009 for copper oxychloride at 3 m from the citrus production border.

It must be emphasized that the calculation or these RIs represent the risk estimation of bystanders, residents or earthworms in a peach orchard for chlorpyrifos, copper oxychloride and myclobutanil use. Further RIs exercises, using the reported experimental drift values should be done for different pesticides.

4.4. Soil ecotoxicological discussion

Eisenia andrei are commonly used organisms for testing soil toxicity produced by xenobiotics (Paoletti et al., 1998; Hund-Rinke et al., 2003; Park et al., 2015). This impact can be studied using a "multibiomarker" approach (for example including: ChE, CaE and GST) complemented with short behavior test (for example feeding test and bait lamina).

ChE and CaE in invertebrates have been reported as sensitive biomarkers for organophosphorous contaminations (Oneto et al., 2005; Reinecke and Reinecke, 2007; Sanchez-Hernandez et al., 2009; Bednarska et al., 2016). In our case, although chlorpyrifos was used, no statistically significant effect was observed on both enzymes (Fig. 4A) when compared with control soils, probably due to the low concentration found in soil (ca. 0.4 μg chlorpiyrifos·g^{-1} dried soil, in the moment of the application). Although GST original function in earthworms is still unclear, its inducible character has been reported for different pesticides like chlorpyrifos, diazinon and endosulfan (Booth et al., 1998; Velki and Hackenberger, 2013). El-Gendy et al. (2009) reported that GST activities of treated snails were significantly higher than those of untreated controls when exposed to copper based pesticides and they indicated that the relative activation power of these compounds followed the order: copper sulphate > copper hydroxide > copper oxychloride. These effects could explain the statistically significant activation of GST in exposed soils compared to control soils (Fig. 4A).

Considering the behavior tests, in our short term experiments, the earthworms did not develop visible damage, since they did not try to avoid the substrate and have no significant effect on the feeding activity. Bait lamina data were, in general, in good agreement with the *E. andrei* avoidance responses (van Gestel et al., 2003; Casabé et al., 2007).

5. Conclusions

It can be concluded that the mean operator's PDE for the mechanized chlorpyrifos, copper oxychloride and myclobutanil application in a peach orchard was lower than previously measured PDEs for manual knapsack applications of other pesticides in vegetable and flower greenhouses and open field production units in Argentina (Hughes et al., 2006; Hughes et al., 2008; Ramos et al., 2010; Flores et al., 2011). No evident particular pesticide distribution pattern was detected on the operator's body.

The relative pesticide amounts that reached the orchard's soil were lower than in previously reported studies for mechanized (Holland et al., 1997; Glotfelty et al., 1990) and manual (Querejeta et al., 2012) applications. When compared to manual pesticide application drift, the percentage of pesticide carried out of the sprayed area for the mechanized application were similar but reached much larger distances (up to 80 m).

Bystander and resident RIs for chlorpyrifos plus copper oxychloride and myclobutanil drifts were calculated. Riskier scenarios for chlorpyrifos were observed compared to myclobutanil use. In chlorpyrifos applications at least 48 m for bystanders and longer distances than 80 m for residents were necessary for achieving safe conditions. Earthworm RI values were in accordance with enzymatic and behavioral ecotoxicological data experimentally obtained with *Eisenia andrei*.

Acknowledgements

This work has been financially supported by the Universidad Nacional de General Sarmiento and CONICET (PIO-UNGS 2015-2016 14420140100012CO). G. B. and P. F. thanks CONICET for a doctoral fellowship to CONICET. S. N. thanks UNGS for a postgraduate fellowship. We thank Lic. Julio Fuchs for *Eisenia andrei* provision. J.M.M. is a CONICET member.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.02.129.

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