Applied Soil Ecology 44 (2010) 56–60

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm *Perionyx excavatus*

P. Mangala C.S. De Silva [a,b,*], Asoka Pathiratne [c], Cornelis A.M. van Gestel [a]

[a] Department of Animal Ecology, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands
[b] Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka
[c] Department of Zoology, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka

ARTICLE INFO

Article history:
Received 16 June 2009
Received in revised form 23 September 2009
Accepted 23 September 2009

Keywords:
Compost worms
Pesticide formulations
Pesticide additives
Pesticide active ingredients

ABSTRACT

Effects of chlorpyrifos, carbofuran, mancozeb and their formulated products on survival, growth and reproduction of the tropical earthworm *Perionyx excavatus* were investigated in standard artificial soil. The toxicity of the three chemicals decreased in the order carbofuran > chlorpyrifos > mancozeb. In general, formulations were more toxic than the active ingredients, but differences in LC_{50} and EC_x values were significant only in two cases and not more than a factor of 2.0. This could mainly be due to masking of the effects of additives in the soil. Comparison with available survival data revealed that *P. excavatus* is more sensitive than the standard test species *Eisenia andrei* or *E. fetida*. The use of tropical species in the risk assessment of pesticides in tropical regions should therefore be encouraged.

1. Introduction

Although tropical ecosystems cover a relatively small fraction (26%) of the land area of the world, these systems are of great importance as their primary productivity is as high as 60% of the total (Deshmukh, 1986). Increasing productivity in the tropics and resulting human activities have caused serious damage to tropical ecosystems. The degradation of terrestrial and aquatic ecosystems due to xenobiotics is a major concern and is a direct result of the increased use of synthetic substances such as pesticides to boost productivity. Nevertheless, risk assessment of pesticides in the tropics is rarely performed due to scarcity of data and most of the limited number of tropical risk assessments currently available rely mainly on data generated in temperate regions. But species sensitivity, fate of xenobiotics and complex interactions within the system could be different in the tropical environment. As a consequence, risks may be over or under estimated when using additional extrapolation factors.

Sensitivity comparisons of tropical and temperate species have so far shown contradictory results. Maltby et al. (2005) and Kwok et al. (2007) have reported no large differences in the sensitivity of species belonging to the same taxonomic groups in aquatic ecosystems, except for a few pesticides like chlorpyrifos. But studies by Garcia (2004) and De Silva et al. (2009) have shown that the situation in soil ecosystems could be different with more factors such as the nature of the pesticide, temperature, soil type, endpoints measured and their interactions causing different sensitivities. Species diversity and abundance of soil invertebrates such as earthworms could also vary under tropical conditions; hence prediction of field effects will be rather difficult. Toxicity assessment of pesticides to earthworms is mainly based on *Eisenia andrei* or *E. fetida* as the standard test species (OECD, 1984, 2004). As compost worms, *Eisenia* species have a limited ecological role compared to local mineral-dwelling species. In addition, they mainly occur in temperate regions. Therefore the use of tropical species in toxicity tests could contribute to a more relevant and reliable risk assessment of chemicals in these areas.

Perionyx excavatus (Perrier, 1872) (Oligochaeta, Megascolecidae) is mainly found in tropical regions, especially in Asia (Blakemore, 2006) and is also present in Europe and North America (Edwards et al., 1998). Although primarily a compost worm, it is commonly found in the top soil layer (0–15 cm) at temperatures ranging between 20 °C and 28 °C and pH of 6.4–7.4 (Bhattacharjee and Chaudhuri, 2002). The life cycle and biology of this species have been extensively studied (Reinecke and Hallatt, 1989; Hallatt et al., 1990; Edwards et al., 1998; Bhattacharjee and Chaudhuri, 2002; Chaudhuri and Bhattacharjee, 2002; Joshi and Dabral, 2008). It has previously been used in ecotoxicity studies on metals (Maboeta et al., 1999; Reinecke et al., 2001), pesticides (Callahan et al., 1994; Reinecke et al., 2002) and gasoline products (An, 2005; An and Lee, 2008).

* Corresponding author at: Department of Animal Ecology, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands. Tel.: +31 20 5987085; fax: +31 20 5987123.
E-mail address: msilva@falw.vu.nl (P. Mangala C.S. De Silva).

0929-1393/$ – see front matter.
doi:10.1016/j.apsoil.2009.09.005

Pesticide testing has usually been carried out on the active ingredients. However farmers apply formulated products, which can be a combination of active ingredients and additives. Some additives are added to enhance the toxicity of the pesticide e.g. by affecting uptake or metabolism of the active ingredient. Specific assessment of additives of the formulated products has been hampered due to trade secrecy (Cox and Surgan, 2006). Alternatively, the toxicity of such ingredients could be assessed by studying formulated products and their active ingredients separately. Comparative studies of formulated and pure pesticides in birds (Arias, 2003), frogs (Swann et al., 1996; Mann and Bidwell, 1999) and fish (Kiparissis et al., 2003) have reported higher toxicities of formulations compared to their respective active ingredients. Only few studies have so far been performed to investigate the comparative toxicity of formulations and pure chemicals in the soil ecosystem (Salminen et al., 1996; Marques et al., 2009; Pereira et al., 2009).

The aim of this study was to determine the toxicity of three commonly used pesticides namely chlorpyrifos, carbofuran and mancozeb together with their formulated products to the tropical earthworm species *P. excavatus* and to compare the relative toxicities of pure and formulated products. In addition, the generated toxicity data for the tropical species were compared with the data available in the literature for the standard test species *E. andrei* and *E. fetida*.

2. Materials and methods

Age-synchronized adult earthworms (250–275 mg, wet weight) of the tropical earthworm species *P. excavatus* were obtained from cultures at the Department of Zoology, University of Ruhuna, Matara, Sri Lanka. These cultures were maintained at a temperature representative of tropical conditions (26 ± 2 °C) in a substrate with 30% cow dung and 70% composted coco peat with 12:12 h photoperiod. The selected worms were transferred to untreated test substrate 24 h before the experiment to acclimatize. The standard OECD artificial soil for toxicity studies (OECD, 1984) was used as the test soil substrate. The OECD soil was composed of 70% fine sand, 20% kaolin and 10% sphagnum peat with a small amount of $CaCO_3$ added for pH adjustment. This soil had a pH (0.01 M $CaCl_2$) of 6.5–6.8 and a maximum water holding capacity (WHC_{max}) of 80%.

Toxicity tests were performed with chlorpyrifos (98%, Cheminova Ltd, Denmark), carbofuran (97%, Sigma–Aldrich, The Netherlands) and mancozeb (85% Limin Chemical Co Ltd, China) together with their commercial formulations Judo 40 EC (40% a.i. EC, Lankem Ceylon Ltd, Sri Lanka), Curater (3% a.i. G, Hayleys Agro Products Ltd, Sri Lanka) and Dithane M 45 (80% a.i. WP, Chemical Industries Colombo Ltd, Sri Lanka), respectively. The concentrations tested were 1, 3, 10, 30, 100, 300 and 900 mg a.i. kg^{-1} dry soil of chlorpyrifos, 0.5, 1, 2, 4, 8, 16 and 32 mg a.i. kg^{-1} dry soil of carbofuran and for mancozeb 1, 3, 10, 30, 100, 300, 900 and 1200 mg a.i. kg^{-1} dry soil. For the pure compounds (active ingredients), solutions of the test compounds in acetone were added to 50 g soil. These soil samples were kept overnight ensuring evaporation of the solvent and the remainder of the soil (450 g) for each treatment was added and mixed intensively with the spiked soil. Control soil samples were treated with acetone in a similar way. Pesticide formulations of chlorpyrifos and mancozeb were directly added to the substrate as aqueous solutions, whereas carbofuran 3% G was added as finely ground powder. Soil moisture content was adjusted to 50% WHC_{max}. The control soils were treated with water only.

Earthworm toxicity tests were performed according to OECD (2004). Glass bottles (750 ml) were filled with approximately 710 g (wet weight) of the test soils. Earthworms (n = 10, four replicates) were introduced into the soil, and the lids were not closed tightly to facilitate free exchange of air. Before introduction, the worms were rinsed with water, blotted dry on filter paper, and mass was determined (per ten worms and individually for two replicates in each treatment). The test containers were kept at 26 ± 2 °C and 12:12 h photoperiod. Five grams (dry weight) of food (finely ground cow manure) moistened to 50% (w/w) was added in a hole made in the middle of the soil. Additional food was given when all the food added was consumed. Moisture loss from the test soils was checked by weighing the test containers at weekly intervals and replenished as needed to maintain moisture content of 50% WHC_{max}. After 28 days of exposure, adults were removed by hand sorting, and mortality and biomass were determined. The soil was returned to the test containers and incubated for another 28 days for cocoon development. After 56 days, juveniles were extracted from the test soil using a water bath kept at 60 °C and counted. The final endpoints studied were adult mortality, change of biomass after 28 days, and number of juveniles produced after 56 days.

Levene's test and the Kolmogorov–Smirnov test were performed to assess homogeneity of variance and normal distribution of the data, respectively. LC_{50} values, the concentrations causing 50% mortality of the earthworms and corresponding 95% confidence limits were calculated by the Trimmed Spearman Karber method (Hamilton et al., 1977). The Haanstra et al. (1985) model for logistic response was used for the calculation of concentrations affecting reproduction by 50% (EC_{50}) and 10% (EC_{10}) and the corresponding 95% confidence limits. Lowest Observed Effect Concentrations (LOEC) and No Observed Effect Concentrations (NOEC) values were identified using ANOVA followed by Dunnett's post hoc test. Differences in EC_{50} values between pure compounds and the formulations were tested for significance using a generalized likelihood ratio test (Sokal and Rohlf, 1995). These analyses were run in SPSS version 14.0.

3. Results

No mortality was observed in the controls except for a few individuals (one each in three replicates) missing in the mancozeb series. The LC_{50} values for the effects of chlorpyrifos, carbofuran, mancozeb and their formulations on survival of *P. excavatus* are given in Table 1. The toxicity decreased in the order of carbofuran > chlorpyrifos > mancozeb for both the pure compounds and the formulations. The highest LC_{50} values were recorded for pure mancozeb and its formulation and the lowest ones for carbofuran. The toxicities of the pure and formulated pesticides were similar with overlapping 95% confidence intervals and differences in LC_{50} values being less than a factor of 1.2.

Mean number of juveniles produced in the controls varied for the different tests and ranged between 80 and 92 per test container. Juvenile production in solvent-treated controls (112–130 juveniles per replicate) was significantly higher ($p < 0.05$) than in their respective water-treated controls and therefore solvent controls were used in further data analysis for the pure compounds. The dose–response relationships for the effects on reproduction of *P. excavatus* are given in Fig. 1 and the estimated values for EC_{50}, EC_{10} and NOEC for reproduction are presented in Table 1. Formulated carbofuran depressed reproduction of *P. excavatus* more than its pure compound and EC_{50} values were significantly different (χ_1^2 = 37.2, $p < 0.001$) from each other. The EC_{50} value for pure chlorpyrifos was 1.3 times higher than for its formulation but the difference was not significant (χ_1^2 = 0.63, $p > 0.05$). No significant difference of EC_{50} values was found for mancozeb (χ_1^2 = 1.96, $p > 0.05$) although the EC_{50} was 1.7 times higher for the formulation than for the pure compound. Similar trends for the pure and formulated products were found for EC_{10} values (Table 1). However EC_{10} values for chlorpyrifos were lower than those for carbofuran even though carbofuran was the most toxic pesticide in terms of LC_{50}

Table 1
Toxicity of pure and formulated products of chlorpyrifos, carbofuran and mancozeb to the earthworm *Perionyx excavatus* in OECD artificial soil. LC_{50} values (28 days) for the effect on survival and EC_x values for the effect on reproduction are given with corresponding 95% CI. Also included are NOEC values for effects on reproduction (Rp) and growth (Gr). All values expressed are in mg a.i. kg^{-1} dry soil. * indicates significant difference from the respective value for the pure chemical.

Pesticide	Type	LC_{50}	EC_{50}	EC_{10}	NOEC (Rp/Gr)
Chlorpyrifos	Pure	122 (104–145)	4.0 (2.2–5.7)	0.27 (0.02–0.54)	<1
	Formulation	100 (84–120)	3.0 (2.4–3.7)	0.16 (0.08–0.24)	<1
Carbofuran	Pure	9 (8–10)	1.7 (1.6–2.0)	0.60 (0.47–0.72)	<0.5
	Formulation	8 (7–9)	1.1* (1.0–1.2)	0.30 (0.24–0.35)	<0.5
Mancozeb	Pure	541 (497–590)	37.4 (18.6–56.2)	0.80 (0.17–1.7)	<1
	Formulation	500 (460–544)	22.0 (11.6–32.0)	0.68 (0.25–1.38)	<1

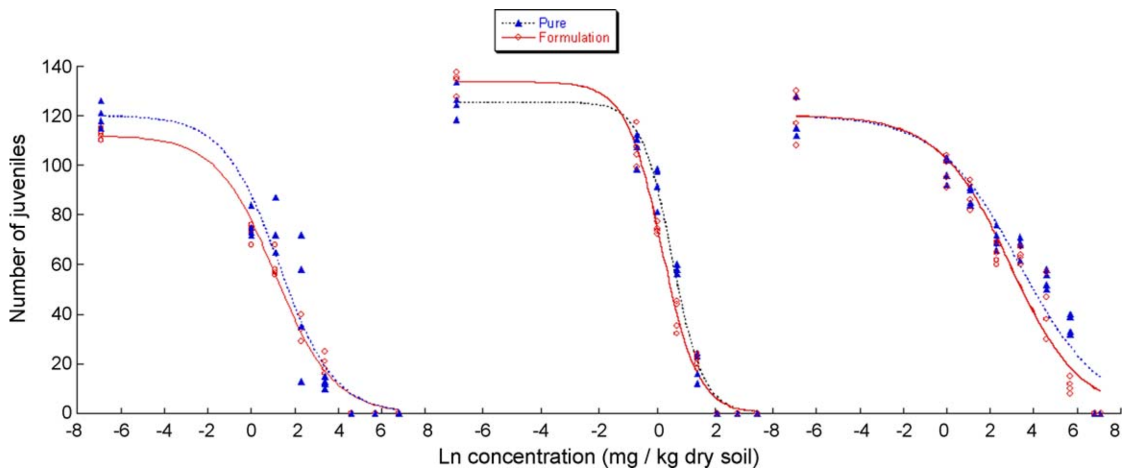

Fig. 1. Dose–response relationships for the effects of chlorpyrifos (left), carbofuran (middle) and mancozeb (right) and their formulated products on the reproduction of *Perionyx excavatus* in OECD artificial soil.

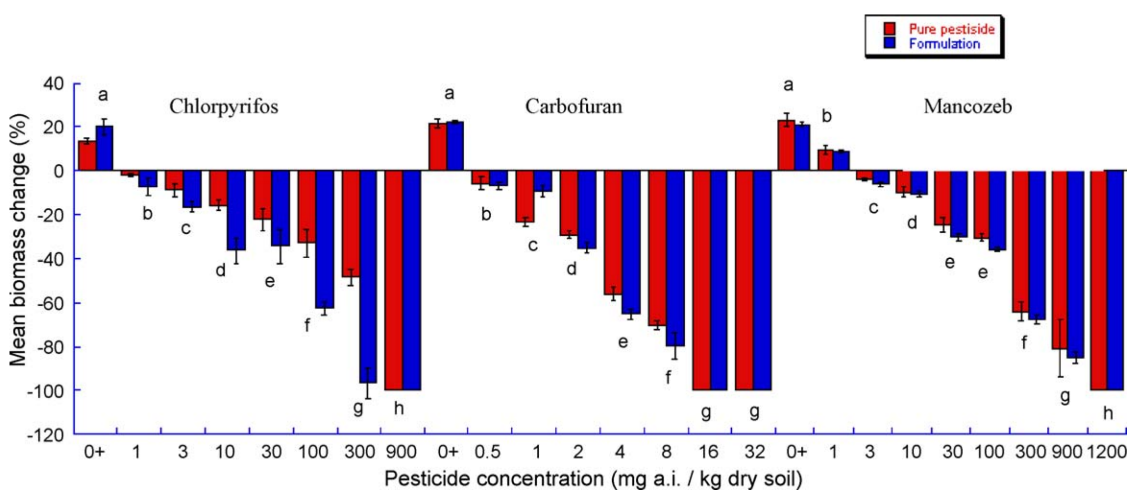

Fig. 2. Mean biomass change (%) of *Perionyx excavatus* after 28-day exposure to chlorpyrifos (left), carbofuran (middle) and mancozeb (right) and their formulated products in OECD artificial soil under tropical conditions. Letters denote significant difference ($p < 0.05$, ANOVA, Dunnett's test).

and EC_{50} values. The NOEC was always lower than the lowest concentration tested for all pure and formulated pesticides (Table 1). The adult *P. excavatus* in the controls gained 16–22% of weight during the 4-week test period. In general an increasing and significant weight loss was recorded with increasing concentrations for all pesticides and their formulations (Fig. 2) and NOEC values for biomass change were similar to those for reproduction (Table 1).

4. Discussion

Mortality in the controls ($\leq$10%), loss of weight ($\leq$20%), juvenile production per replicate ($\geq$30) and coefficient of variation of reproduction ($\leq$30%) reported in the tests indicate the validity of the tests performed with *P. excavatus*. These validity criteria were mainly set for *E. fetida* and *E. andrei* in standard guidelines by OECD and ISO. Nevertheless these criteria can also be applied to the tropical species *P. excavatus*. In this study, pH of the OECD artificial soil was slightly higher than the range described by the test guidelines (pH-$CaCl_2$ 6.5–6.8 instead of 6.0 ± 0.5). Considering the ecological preferences of *P. excavatus* (abundant in soils with pH values up to 7.4) and the nature of the pesticides tested (non-dissociating), this small deviation of soil pH is not expected to have any effects on the outcome of the tests performed.

For all three pesticides, formulations were more toxic than the active ingredients although the differences were small. Chlorpyrifos was moderately toxic with 4-week LC_{50} values of 100 and 122 mg a.i. kg^{-1} dry soil. The toxicity of chlorpyrifos to *P. excavatus* was 8.8 times higher than for *Eisenia fetida* (14-day LC_{50} = 1077 mg a.i. kg^{-1} dry soil artificial soil; Ma and Bodt, 1993) and only 1.2 times higher than for *E. andrei* under similar conditions (28-day LC_{50} = 154 mg a.i. kg^{-1} dry soil artificial soil; De Silva et al., 2009). Toxicity of chlorpyrifos to earthworm species varies greatly as reported, for example, by Ma and Bodt (1993), with 14-day LC_{50} values ranging between 129 and 1174 mg a.i. kg^{-1} dry soil and ecologically relevant species like *Lumbricus rubellus* and *L. terrestris* being more sensitive than *Eisenia* sp. This is in accordance with this study reporting higher sensitivity of *P. excavatus* at 26 °C than *Eisenia* species. For carbofuran, LC_{50} values found in this study are within the range of values reported for *E. andrei* (5–10 mg a.i. kg^{-1} dry soil; Heimbach, 1985) and toxicity is 1.6 times higher for *P. excavatus* than for *E. andrei* under similar conditions (De Silva et al., 2009). Vermeulen et al. (2001) found relatively low toxicity of mancozeb to *E. andrei* with a 14-day LC_{50} of 1262 mg a.i. kg^{-1} dry soil. Our study reports a 2.3 times higher toxicity of mancozeb to *P. excavatus* than *E. andrei*. Reinecke et al. (2002) showed that *P. excavatus* could avoid low concentrations of mancozeb (8 mg a.i. kg^{-1} dry soil), which also confirms that *P. excavatus* is more sensitive to mancozeb than *Eisenia* species. Higher sensitivity of *P. excavatus* has also been reported with methyl tert-butyl ether (MTBE) in tests on filter paper and in two different soils (An, 2005). In contrast to the survival data, available EC_{50} values for chlorpyrifos and carbofuran under similar conditions show that effects on reproduction were slightly higher for *E. andrei* than *P. excavatus* (De Silva et al., 2009).

Additives used in pesticide formulations may themselves be chemically and biologically active and can be toxic. This is confirmed by the fact that more than 500 additives are currently also being used as active ingredients (Cox and Surgan, 2006). Results obtained in the present study for survival, reproduction and growth of *P. excavatus* indicated no toxicity attributable to additives, except in two cases. This observation contrasts with the findings of most of the aquatic studies that compared toxicity of formulations and their active ingredient alone (Swann et al., 1996; Mann and Bidwell,1999; Kiparissis et al., 2003). In aquatic environments increased toxicity of formulations could be linked with higher solubility of the active ingredients in the medium. Aquatic organisms could be exposed to chemicals through the body surface or gills and additives might affect the uptake sites e.g. due to their effects on the surface tension or structure of membranes. Such an effect might also occur in the case of soil organisms like earthworms that also take up chemicals from pore water via their skin (Belfroid et al., 1994). In soil, additives may however, easily be bound to soil particles, such as clay and organic matter, reducing their availability. This masking of additive effects through sorption probably explains the small differences in the toxicity of formulations and pure compounds. Recent studies by Marques et al. (2009) and Pereira et al. (2009) with herbicide formulations reported either underestimated or overestimated toxicity of the active ingredients. The lack of studies on additives and their interactions with soil hampers our understanding of their behaviour and the way in which they can affect toxicity. Additional research in soils with different pesticides and their additives is needed to enhance further interpretation.

Species sensitivity is a critical factor in the risk assessment of pesticides in soil, and particularly so for tropical soils with their higher diversity and abundance of earthworm species compared to temperate soils. Nevertheless, current tropical risk assessment mainly uses data generated in tests with the temperate compost worms *Eisenia* spp., which are less ecologically relevant because they are not true soil-dwelling species. While *P. excavatus*, is mainly known as a compost worm, it has also been found in natural soils in tropical regions such as India, Sri Lanka and other countries in South East Asia (Edwards et al., 1998; Bhattacharjee and Chaudhuri, 2002; Suthar and Singh, 2008) and therefore seems more representative of natural habitats than *Eisenia* spp. This study as well as literature data (Maboeta et al., 1999; Reinecke et al., 2001; An and Lee, 2008) show that *P. excavatus* may be used in earthworm toxicity tests as a standard test species for tropical soils. As in the case of *Eisenia* spp., which were adopted as temperate standard test species about 25 years ago, the biology and ecology of *P. excavatus* is well known and it can easily be cultured under laboratory conditions. Although the life cycle of *P. excavatus* is shorter than that of *Eisenia* spp., current test guidelines can easily be applied. The preference of *P. excavatus* for optimum temperatures around 25 °C makes it more suitable for tropical regions than *Eisenia* spp.

5. Conclusions

Chlorpyrifos, carbofuran and mancozeb are more toxic to *P. excavatus* than to the standard test species *E. andrei* at temperatures representative of tropical conditions. Tropical risk assessment will therefore be more realistic when conducted on ecologically relevant earthworm species. The toxicity of formulations and their respective active ingredients was more or less similar, probably due to masking of the effects of additives by sorption to the soil. Additional research on both aspects is needed to arrive at more definite general conclusions. *P. excavatus* may be used as an alternative test species under tropical conditions. Future studies with this species, including different endpoints, temperature regimes and soil types, are required.

Acknowledgements

This work was supported by the Netherlands Foundation for the Advancement of Tropical Research (Project number—WB 84-609) residing under the Netherlands Organization for Scientific Research (NWO). We thank H.G. Hector and C.H. Priyantha for technical assistance.

References

An, Y.J., 2005. Assessing soil ecotoxicity of methyl *tert*-butyl ether using earthworm bioassay; closed soil microcosms test for volatile organic compounds. Environ. Pollut. 134, 181–186.

An, Y.J., Lee, W.M., 2008. Comparative and combined toxicities of toluene and methyl tert-butyl ether to an Asian earthworm *Perionyx excavatus*. Chemosphere 71, 407–411.

Arias, E., 2003. Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. Ecotoxicol. Environ. Saf. 55, 338–343.

Belfroid, A., Meiling, J., Sijm, D., Hermens, J., Seinen, W., Van Gestel, K., 1994. Uptake of hydrophobic halogenated aromatic hydrocarbons from food by earthworms (*Eisenia andrei*). Arch. Environ. Contam. Toxicol. 27, 260–265.

Bhattacharjee, G., Chaudhuri, P.S., 2002. Cocoon production, morphology, hatching pattern and fecundity in seven tropical earthworm species—a laboratory-bases investigation. J. Biosci. 27, 283–294.

Blakemore, R.J., 2006. In: Kaneko, N., Ito, M.T. (Eds.), A Series of Searchable Texts on Earthworm Biodiversity, Ecology and Systematics from Various Regions of the World. COE Soil Ecology Research Group, Yokohama National University, Japan CD-ROM. http://bio-eco.eis.ynu.ac.jp/eng/database/earthworm/

Callahan, C.A., Shirazi, M.A., Neuhauser, E.F., 1994. Comparative toxicity of chemicals to earthworms. Environ. Toxicol. Chem. 13, 291–298.

Chaudhuri, P.S., Bhattacharjee, G., 2002. Capacity of various experimental diets to support biomass and reproduction of *Perionyx excavatus*. Bioresour. Technol. 82, 147–150.

Cox, C., Surgan, M., 2006. Unidentified inert ingredients in pesticides: implications for human and environmental health. Environ. Health. Perspect. 114, 1803–1806.

Deshmukh, I., 1986. Ecology and Tropical Biology. Blackwell, Palo Alto, CA, USA.

De Silva, P.M.C.S., Pathiratne, A., Van Gestel, C.A.M., 2009. Influence of temperature and soil type on the toxicity of three pesticides to *Eisenia andrei*. Chemosphere 76, 1410–1415.

Edwards, C.A., Dominguez, J., Neuhauser, E.F., 1998. Growth and reproduction of *Perionyx excavatus* (Perr) (Megascolecidae) as factors in organic waste management. Biol. Fertil. Soils 27, 155–161.

Garcia, M.V.B., 2004. Effects of Pesticides on Soil Fauna: Development of Ecotoxicological Test Methods for Tropical Regions. Ecology and Development Series, vol. 19. University of Bonn, Germany.

Haanstra, L., Doelman, P., Oude Voshaar, J.H., 1985. The use of sigmoidal dose response curves in soil ecotoxicological research. Plant Soil. 84, 293–297.

Hallatt, L., Reinecke, A.J., Viljoen, S.A., 1990. Life-cycle of the oriental compost worm *Perionyx excavatus* (Oligochaeta). S. Afr. J. Zool. 25, 41–45.

Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11, 714–719.

Heimbach, F., 1985. Comparison of laboratory methods using *Eisenia fetida* and *Lumbricus terrestris* for the assessment of hazard of chemicals to earthworms. Z. Pflanzenkrank Pflazenschutz 92, 186–193.

Joshi, N., Dabral, M., 2008. Life cycle of earthworms *Drawida nepalensis*, *Metaphire houlleti* and *Perionyx excavatus* under laboratory controlled conditions. Life Sci. J. 5, 83–86.

Kiparissis, Y., Metcalfe, T.L., Balch, G.C., Metcalfe, C.D., 2003. Effects of the anti-androgens, vinclozolin and cyproterone acetate on gonadal development in the Japanese medaka (*Oryzias latipes*). Aquat. Toxicol. 63, 391–403.

Kwok, K.W.H., Leung, K.M.Y., Lui, G.C.S., Chu, V.K.H., Lam, P.K.S., Morritt, D., Maltby, L., Brock, T.C.M., Van Den Brink, P.J., Warne, M.St.J., Crane, M., 2007. Comparison of tropical and temperate freshwater animal species' acute sensitivity to chemicals: implication for deriving safe extrapolation factor. Integr. Environ. Assess. Manag. 3, 49–67.

Ma, W.C., Bodt, J., 1993. Differences in toxicity of the insecticide Chlorpyrifos to six species of earthworms (Oligochaeta, Lumbricidae) in standardized soil tests. Bull. Environ. Contam. Toxicol. 50, 864–870.

Maboeta, M.S., Reinecke, A.J., Reinecke, S.A., 1999. Effects of low levels on growth and reproduction of Asian earthworm *Perionyx excavatus* (Oligochaeta). Ecotoxicol. Environ. Saf. 44, 236–240.

Maltby, L., Blake, N.N., Brock, T.C.M., Van den Brink, P.J., 2005. Insecticide species sensitivity distributions: the importance of test species selections and relevance to aquatic ecosystems. Environ. Toxicol. Chem. 24, 379–388.

Mann, R.M., Bidwell, J.R., 1999. The toxicity of glyphosate and several glyphosate formulations to four species of Southwestern Australian frogs. Arch. Environ. Contam. Toxicol. 36, 193–199.

Marques, C., Pereira, R., Goncalves, F., 2009. Using earthworm avoidance behaviour to assess the toxicity of formulated herbicides and their active ingredients on natural soils. J. Soils Sediments 9, 137–147.

OECD, 1984. Guideline for testing of chemicals No. 207, Earthworm Acute Toxicity Test. Organization for Economic Co-Operation and Development. Paris, France.

OECD, 2004. Guideline for testing of chemicals No. 222, Earthworm Reproduction Test (*Eisenia fetida/andrei*). Organization for Economic Co-Operation and Development Paris, France.

Pereira, J.L., Antunes, S.C., Castro, B.B., Marques, C.R., Goncalves, A.M.M., Goncalves, F., Pereira, R., 2009. Toxicity evaluvation of three pesticides on non-target aquatic and soil organisms: commercial formulation versus active ingredient. Ecotoxicology 18, 455–463.

Reinecke, A.J., Hallatt, L., 1989. Growth and cocoon production of *Perionyx excavatus* (Oligochaeta). Biol. Fertil. Soils 8, 303–306.

Reinecke, A.J., Reinecke, S.A., Maboeta, M.S., 2001. Cocoon production and viability as endpoints in toxicity testing of heavy metals with three earthworms species. Pedobiologia 45, 61–68.

Reinecke, A.J., Maboeta, M.S., Vermeulen, L.A., Reinecke, S.A., 2002. Assessment of lead nitrate and mancozeb toxicity in earthworms using the avoidance response. Bull. Environ. Contam. Toxicol. 68, 779–786.

Salminen, J., Eriksson, I., Haimi, J., 1996. Effects of terbuthylazine on soil fauna and decomposition processes. Ecotoxicol. Environ. Saf. 34, 184–189.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H. Freeman and Company, New York.

Suthar, S., Singh, S., 2008. Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*). Int. J. Environ. Sci. Tech. 5, 99–106.

Swann, J.M., Schultz, T.W., Kennedy, J.R., 1996. The effects of the organophosphorus insecticides Dursban and Lorsban on the ciliated epithelium of the frog palate *in vitro*. Arch. Environ. Contam. Toxicol. 30, 188–194.

Vermeulen, L.A., Reinecke, A.J., Reinecke, S.A., 2001. Evaluation of the fungicide manganese-zinc ethylene bis(dithiocarbamate) (mancozeb) for sublethal and acute toxicity to *Eisenia fetida* (Oligochaeta). Ecotoxicol. Environ. Saf. 48, 183–189.