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THE VALIDATION OF MODIFIED TROPICAL ARTIFICIAL SOIL BY ECOTOXICOLOGICAL STUDIES ON *EISENIA FOETIDA*

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ABSTRACT

The standard soil invertebrate toxicity tests developed by OECD (Organization of Economical Cooperation and Development) and ISO (International Organization of Standardization) use an artificial soil as the test substrate, which contains sphagnum peat as a component. This type of peat is not widely available in tropical country like India. Investigation of possible alternative substrates using locally available materials therefore is vital for performing such ecotoxicity tests, particularly in the tropics. In the present study, the suitability of commonly available coir pith as a replacement for sphagnum peat was undertaken. Thus a modified Tropical Artificial soil (TAS) was prepared by mixing 70% sand and 20% kaolin clay with 10% coir pith (fermented). The validity of modified TAS was determined by investigating the acute and chronic toxicity of urea (Nitrogen fertilizer) on *Eisenia foetida* using the original OECD artificial soil as control. Though both substrates were valid, TAS was considered more valid, as the biomass loss percentage (Acute toxicity) was <20% (recommended by ISO) in control while it was >20% in OECD soil. In the same way, the number of juveniles (Chronic toxicity) in each replicate must be higher than 30 to valid the soil for toxicity tests. This criterion was conquered in TAS whereas it was lower than 30 in OECD soils (1500mg and 2000mg spiked). Thus it was concluded that coir pith might be a suitable replacement for sphagnum peat in artificial soil for ecotoxicological studies in tropical countries.

INTRODUCTION

Fertilizers are intentionally utilized in agriculture in huge tonnages each year in order to enrich soils artificially for improving the growth of crops and without them soils could not sustain intensive food production. Urea is the most commonly used nitrogen fertilizer worldwide. With more than 46% nitrogen, it has the highest nutrient concentration among the commercially available solid nitrogen fertilizers. Global urea production in 2009 was estimated at 151.7 Mt products, representing a 4% increase over 2008. The main production expansions occurred in China, Oman, India and Russia. IFA (International Fertilizer Industry Association) estimated world urea trade in 2009 at 34 Mt, representing a 2.9% increase over 2008. Entering 2010, the potential demand for urea shows more promising prospects compared with those in early 2009 and this will certainly increase in 2012. India and the United States were the main importing countries, accounting for 65% of global trade (Heffer and Prud'homme, 2009). These facts about urea show the intensive use of it in Indian agricultural lands. The rate of application for all types of nitrogen fertilizers should be based on Good Agricultural Practice, a concept described in EFMA's "Code of Best Agricultural Practice - Nitrogen". But Indian farmers use many times the amount recommended by scientists or according to multiple studies by Indian agricultural experts resulting in throwing off the chemistry of the soil. The impact of urea on soil fauna diversity especially on earthworms has become an issue of great concern. Urea is applied in the environment to fulfil a specific purpose, but at the same time may cause damage to earthworms, decreasing its diversity, growth or reproduction, and consequently organic matter decomposition and soil fertility. Earthworms are considered as important bioindicators of chemical toxicity in the soil ecosystem. Studies on this aspect are important because earthworms are the common prey of many terrestrial vertebrate species such as birds and small mammals, and thus they play a key role in the biomagnification process of several soil pollutants. Therefore, there is an increasing need for appropriate methods to assess the side effects of urea on earthworms. Usually, the effects on soil organisms have been assessed based on the results of single species laboratory tests using artificial soils as a simplified and adjustable tool to disentangle soil processes and test ecological theories on earthworms. Ecotoxicological studies are normally carried out in artificial substrate proposed by OECD and ISO guidelines.

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These guidelines were written focusing on the situation in countries of temperate regions. In these countries, the components of artificial substrate particularly, the sphagnum peat moss is easily obtained. However, in order to allow its use in other regions of the world where the sphagnum peat moss is not readily available, some modifications are necessary. This study aims to determine the suitability of coir pith from coconut peel (*Cocos nucifera* L.) as an alternative organic matter (OM) in the artificial substrate planning to contribute to the construction of such a scientific basis for tropical soil ecotoxicology.

MATERIALS AND METHOD

Test substrates

This study was carried out in laboratory of Zoology Department - DDE Wing, Annamalai University. In this study two different artificial soils had been identified for laboratory tests. One is the standard OECD artificial soil and the other is the modified Tropical Artificial Soil (TAS) incorporating coir pith as organic matter. The preparation of the main test substrate for toxicity studies, artificial soil, was based on an international guideline published by OECD (1984a) and the modified Tropical Artificial Soil (TAS) was prepared by following the same guideline substituting the coir pith instead of sphagnum peat. The components of OECD artificial soil and modified TAS are depicted in Tables 1 & 2. The quartz sand and kaolinite clay (air dried) were purchased from Himedia Chemical Laboratories, Bangalure, Karnataka, India. Sphagnum peat moss, was bought from 5P Ottathycal, Coimbatore, Tamilnadu, India. The material was ground after drying it at room temperature and used as a part of the substrate. The other organic matter, coir pith was procured from the coir mounts closer to the coir industry located in Kullanchavadi village, Cuddalore District, Tamilnadu, India. Coir pith was air dried and finely ground. Before use in soil substrate, the coir pith was wetted and stored for a complete composting process for at least 30 days. After the fermentation activity ceased, the material was air dried and sieved (Paul and Vlek *et al*, 2004).

The usual peat moss (as recommended by OECD), and also the alternative organic matter source, coir pith were chemically analyzed (Table 3). The soil substrates will be herein referred to as OECD soil (the artificial soil standardized by OECD) and TAS soil (the modified artificial soil). All dry constituents were thoroughly mixed. The pH of the soil was measured using the CaCl2 method, according to the ISO guideline (ISO, 1994). The soil pH was adjusted to 6.0 ± 0.5 with calcium carbonate. The moisture content and water holding capacity of the soil were also measured according to test guidelines ISO-11268-2 (ISO 1998a). The new artificial substrates were analyzed with regard to their chemical and physical properties (Table 4).

Test organism and its mass culture

In this work, mass cultures of *E. foetida* were established in laboratory from samples provided from the vermicomposting unit of Annamalai University for temperate and tropical artificial soils in plastic boxes (25 x 36 cm area, 12 cm height) (Figure 2). The temperate culture of *Eisenia foetida* was kept in a mixture of OECD soil at room temperature of 15 - 30 □C (mean: 22 □C). The tropical culture was kept in a mixture of tropical artificial soil (TAS) at room temperature 23 - 30 ºC (mean = 26 ºC), relative air humidity near 90%. The animals were maintained in a shaded place with a natural light cycle (12h light/12h dark). The breeding substrates were maintained with a pH-value set to 6 ± 0.5. The moisture was adjusted to 50 ± 10% dry weight using deionized water. The earthworms were fed according to demand, usually once a week, with finely ground cattle manure free of any chemical contamination.

Test chemical and reagents

The test chemical urea was obtained from the Preliminary Agricultural Co-operative Bank, Annamalai Nagar, Tamilnadu, India. The preparation of the test solutions was done for each concentration by weighing and dissolving an amount of urea in deionized water. Deionized water was obtained using a Millipore Super-Q water purification system and was used throughout this study. All glass wares were washed with phosphate-free detergent, rinsed with acetone, and acid-washed before a final and thorough rinse with deionized water.

Preliminary tests (Range Finding)

Considering that the tests were carried out for the first time with tropical species, and in different conditions (higher temperature) than those usually recommended, the toxicity of the test substances for the organisms was not known. Therefore, a preliminary or range-finding test (RF test) was performed for each combination of test chemical in order to identify the concentrations causing mortality. In this way, the range of concentrations for the definitive test was determined. In these range-finding tests, the organisms were exposed to the following concentrations: 1000; 100; 10; 1; 0.1 mg of urea per kilogram of test substrate (dry weight). Glass vessels (12.5 cm diameter, 16 cm height) with perforated plastic lids were used (Figure 3). One test vessel was used per concentration and one for the untreated control. Usually, no replication is required in these preliminary tests. Ten individuals were put in each test vessel. After seven days, the live animals were counted and weighed, the dead ones removed. The final evaluation was done on day 14, when the live animals were again counted and the mortality in percent of the initial number was calculated. The LC50 values were calculated, thus allowing the definition of the concentration to be used in the definitive acute tests. The final concentrations for acute tests were chosen in a way that two concentrations above and two concentrations below the LC50 as determined in the RF test were chosen.

Acute Toxicity Test

The test system for the determination of the acute toxicity of urea to earthworms was based on the guidelines OECD no. 207 (OECD 1984a) and ISO-11268-1 (ISO 1993a). Usually, only adult earthworms (with clitellum) with a fresh weight between 300 and 600 mg were used. The selected test animals were acclimatized in untreated soil substrates at least 24 hours prior to the start of the urea test. Urea was mixed in different concentrations in the soil substrates and was applied only once at the beginning of the test. Ten earthworms were exposed to each concentration of urea in glass vessels (12.5 cm diameter, 16 cm height) filled with 500g (dw) of the substrate

(Figure 3). Three replicates for each concentration was maintained. The test period (exposure of earthworms to the soil substrate with test substance) was 14 days. Temperature for two artificial soils was maintained constantly throughout the test period. Test parameters (7 and 14 days after application) followed were mortality and biomass development of the earthworms. The relative loss in biomass, i.e., changes in weight of earthworms between first and the fourteenth day, was calculated only in treatments where the mortality was lower than 15% (according to Kula 1998).

Chronic toxicity test

The test system for the determination of the chronic toxicity of urea to earthworms was based on the guidelines OECD no. 222 (OECD 2003) and ISO-11268-2 (ISO 1998a). Only adult earthworms (with clitellum) with a fresh weight between 300 and 600 mg were used. Urea was mixed in different concentrations in the soil substrate and was applied only once at the beginning of the test. The test period was 56 days. Temperature for two artificial soils was maintained constantly throughout the test period. After 28 days, the adult worms, which had been exposed to urea, were removed from the test vessels and monitored for mortality. During the following 28 days, juveniles hatched from cocoons laid by the adults were investigated. Test parameters were mortality and biomass development of the adults 28 days after application and the number of juveniles 56 days after application. All the treatments were replicated in order to reduce experimental error. The data from this experiment were statistically processed using the Microsoft EXCEL 2007, including the calculation of average values, standard deviations and correlation.

Figure 3 &4: Test vessel (left) and earthworm weighing (right)

RESULTS AND DISCUSSION

Validity by preliminary tests

Based on the performance of the earthworms in the modified TAS and OECD soils, the LC_{50} values, LOEC and NOEC values for the effects of urea on the mortality and biomass of *E. foetida* are given in Table 5. The acute effects of urea on *E. foetida* in TAS was valid concerning the mortality, since only few worms died in TAS compared to OECD soil. It was indicated in the experiment that no death of the tested earthworms took place when the concentration of urea in the soil was lower than 500 mg/kg. When the range of urea applied to the soil was 500–1000 mg/kg, the mortality of earthworms increased. Once the concentration of urea was higher than 1500 mg/kg, the mortality approached 60%. According to the calculations based on our results (Table 5), the LC_{50} of the tested earthworms after a 14-day exposure to urea was 1107.59 mg/kg. Similar LC_{50} values were recorded in Maroni et al. work (2002) (i.e. >1000mg). For urea, the LC_{50} value in the modified TAS was more or less similar to the value recorded in the OECD control soil. In the test with urea, effects on the mortality and the biomass development in the tested range of concentrations (10 - 1000 mg/kg) were almost non-significant. Hence, the LC50 value was determined as > 1000 mg/kg. Accordingly, the estimated LOEC and NOEC values for mortality were also higher in OECD soil than in TAS (Table 5). Taking mortality and biomass results together, it seems that the worms clearly reacted most in the TAS, while the effects of OECD soil were less pronounced. Concerning biomass effect of urea, the NOEC determined with TAS was lower than the OECD soil.

Validity by acute tests

The test system for the determination of the acute toxicity of urea to earthworms was based on the guidelines OECD no. 207 (OECD 1984a) and ISO-11268-1 (ISO 1993a). According to these guidelines, the mortality of adult worms in the controls must be ≤ 10 % in order to consider a test as valid. In addition, the ISO guideline requires that the control worms should not loose more than 20 % of their initial biomass. The mortality criterion was met for tests with both standard OECD soils and TAS. According to table 6, after a 7-day exposure to 500 mg/kg, no deaths of the tested earthworms took place, and

Table 1: Components of artificial soil (OECD)

Soil component	Content % dry mass
Sphagnum peat moss (air dried), finely ground	10
Kaolinite clay (air dried, containing not less than 30 % of kaolinite)	20
Industrial quartz sand (air dried), predominantly fine sand with more than 50 % by mass of particle size 0.05-0.2 mm (amount dependent on calcium carbonate required)	70
Calcium carbonate ($CaCO_3$, pulverized, analytical grade) to obtain an initial pH of 6.0 ± 0.5	0.3 -1.0

Table 2: Components of Tropical Artificial Soil (TAS)

Soil component	Content % dry mass
Coir pith (air dried), finely ground	10
Kaolinite clay (air dried, containing not less than 30 % of kaolinite)	20
Industrial quartz sand (air dried), predominantly fine sand with more than 50 % by mass of particle size 0.05-0.2 mm (amount dependent on calcium carbonate required)	70
Calcium carbonate ($CaCO_3$, pulverized, analytical grade) to obtain an	0.3 -1.0

Table 3: Physico-Chemical characteristics of organic matter sources used in artificial soils

Characteristic	Unit	Sphagnum Peat Moss	Coir pith
pH (CaCl2)	g/kg	2.77	6.21
P		0.19	1.05
K		0.1	8.5
Ca		1.13	1.95
Mg		0.94	1.08
S		1.97	0.68
B	mg/kg	6.6	15.5
Cu		2.0	9.0
Fe		514	189
Mn		9.0	10.0
Zn		34.0	14.0
Ash Content	%	1.92	2.95
Organic Carbon		47.2	43.6
N_{Total}		0.72	0.8
CN Ratio		65.7	54.3

Table 4: Physico-chemical characteristics of OECD soil and TAS

Characteristic	Unit	OECD	TAS
pH (CaCl2)		6.1	6.3
P	mg/dm^3	1	35
K		10	660
Na		64	160
Ca	$c.molc/dm^3$	6.29	2.46
Mg		0.55	0.56
N_{Total}	%	0.11	0.07
Organic Carbon		3.59	3.59
Organic Matter		6.17	6.17
CN Ratio		32.6	51.3
Fe	mg/dm^3	9	8
Zn		1.30	2.54
Mn		0.41	0.58
Cu		0.02	0.74
Density	g/cm^3	1.1	1.0
WHCmax	%	56.1	48.5

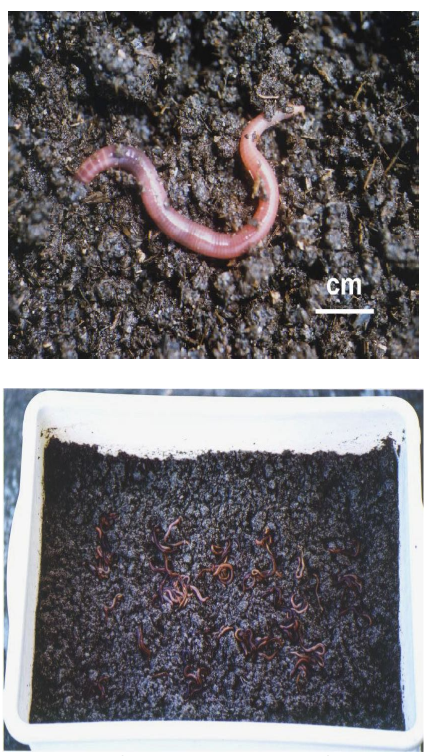

Figure 1 & 2: *Eisenia foetida*: Individual (left) and a culture box (right)

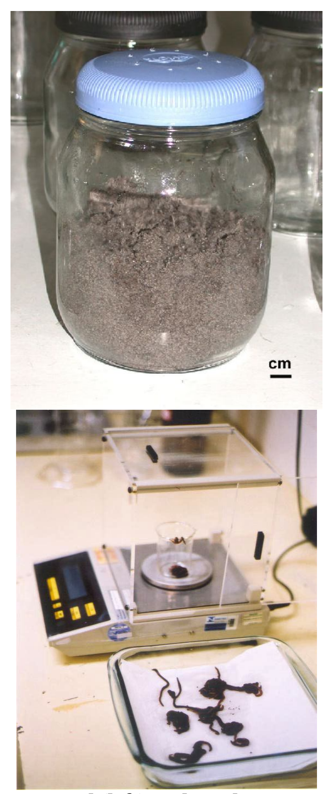

Figure 3 &4: Test vessel (left) and earthworm weighing (right).

Table 5: LC_{50} values, LOEC and NOEC values of *Eisenia foetida* after 14 days corresponding 95% confidence intervals are given in mg/kg dry soil

Substrate	LC_{50}	LOEC		NOEC	
		Mortality	Biomass	Mortality	Biomass
OECD	1080 (960-1200)	1000	540	500	100
TAS	1135 (970-1300)	1061.9	560	495	110

Table 6: Validity of acute tests with *Eisenia foetida* (Mortality values in %)

Substrate		Urea in Different Concentrations				
		Control	500mg	1000mg	1500mg	2000mg
OECD	7th Day	0.0	0.0	3.3	7.5	8.5
	14th Day	2.5	2.5	10.5	12.5	15.5
TAS	7th Day	0.0	0.0	2	2.5	4.5
	14th Day	0.0	1.5	5	5	7.5

TAS - *Eisenia* at 28 °C; OECD - *Eisenia* at 20 °C;

Table 7: Validity of acute tests with *Eisenia foetida* (Biomass values in %)

Substrate		Urea in Different Concentrations				
		Control	500mg	1000mg	1500mg	2000mg
OECD	7th Day	5.1	8.1	11.7	24.5	31.9
	14th Day	11.1	12.5	13.8	25.4	33.7
TAS	7th Day	0.0 [9.9]	0.0 [8.2]	0.0 [7.9]	8.1	14.2
	14th Day	0.0 [16.1]	0.0 [9.9]	0.0 [7.1]	11.4	16.6

TAS - *Eisenia* at 28ºC; OECD - *Eisenia* at 20ºC; Values in square brackets - increase of biomass

Table 8: Validity of chronic tests with *Eisenia foetida* (mortality and Number of Juveniles)

Substrate		Mortality in %				
		Control	500mg	1000mg	1500mg	2000mg
OECD	7th Day	0.0	0.0	1.7	2.1	7.3
Soil	14th Day	2.5	3.1	5.6	7.6	9.0
TAS	7th Day	0.0	0.0	1.3	1.9	4.0
	14th Day	1.5	2.9	4.7	6.7	8.9
		Number of Juveniles				
OECD	28th Day	118.4	97.9	78.0	43.4	28.8
Soil	56th Day	103.1	81.8	69.5	29.4	12.1
TAS	28th Day	122.6	117.3	87.6	54.9	48.3
	56th Day	122.3	101.2	81.2	42.7	31.0

after a 14-day exposure, the mortality was still only 1.5% in TAS and it was 2.5% in OECD. After a 7-day exposure to 1000 mg/kg, the mortality of the tested earthworms was 2% in TAS and 3.3% in OECD, after a 14-day exposure, the mortality raised to 5% in TAS and 10.5% in OECD. After a 7-day exposure to 1500mg/kg, the mortality was 2.5% in TAS and 7.5% in OECD soil. The mortality percentage exceeds 10% in 14-day exposure of urea in OECD soil. Thus, taking into account that acute tests mortality is the most important criterion and that the biology of the *Eisenia foetida* is well known in TAS, the results (Table 6) can be considered as valid for TAS.

Taking into account the acute tests biomass loss, the TAS was considered as valid, as the biomass loss percentage was <20% in tests with control and all other concentrations of urea. But in OECD soil, though the biomass loss percentage was <20% in control, it exceeds 20% in higher concentrations of urea (1500 and 2000mg/kg) (Table 7). In fact a positive effect was seen when the concentration of urea was increased from 0 to 500 mg/kg, (from 8.2 to 9.9%) in TAS. However, when the concentration of urea was increased from 500 to 1000 mg/kg, there was a negative effect, namely, the weight of the earthworms (not loss) decreased from 7.9 to 7.1%. In the tests with urea in OECD soil, the loss of biomass was even higher than 30%. Hence considering all these results TAS is considered more valid than the OECD soil.

Validity by chronic tests

In chronic tests, the following criteria must be met in the controls: (1) adult mortality ≤10%; and (2) number of juveniles per replicate ≥ 30. All test results for urea were considered valid for both soils, but TAS is more appropriate than the OECD. This is because, the number of juveniles in all concentrations was higher than the required numbers of 30 in TAS whereas it was lower than 30 in higher concentrations of urea (1500mg and 2000mg) spiked in OECD soils. The mean number of juveniles produced after 56 days also varied in the different test soils ($p < 0.001$). The highest numbers of juveniles were recorded in TAS control than the control of OECD soil. In all of the experiments carried out in this study, there was a consistent and significant decrease in fecundity (number of surviving juveniles) at sublethal concentrations of urea in the spiked soil samples. It is, however, possible that the urea-induced decrease in the number of surviving juveniles (Days 28–56 of test) is due to a lethal effect on juveniles following hatching. Based on these results, it is also worth noting that juveniles were 5–10 times more sensitive to the lethal effects of urea than adults (Table 8). Urea exposure in artificial soil also caused a concentration-dependent decrease in the fecundity (based on the number of juveniles counted) of *E. foetida.*

Conclusions

Nowadays, the substrate to be used in ecotoxicological soil tests recommended by international agencies is artificial soil (OECD 1984a; ISO 1993a), since the test conditions are closer to those in the natural environment of earthworms. However, in most tropical countries, international guidelines for the environmental assessment of chemicals in the soil are officially not in use. In case they are, these guidelines are neither adapted for their specific conditions nor modified according to the international discussion. However, there are also technical reasons why OECD artificial soil is difficult to use in tropical countries. The main limitation is the unavailability of the type of organic matter (i.e., *Sphagnum* peat moss) described in the guidelines. Originally, these guidelines were written focusing on the situation of countries in temperate regions, where the peat moss is easy to obtain. In order to allow its use in other regions of the world where this component is not readily available, a new source of organic matter had to be identified in order to get a modified OECD artificial soil especially modified for tropical regions: the Tropical Artificial Soil – TAS. In this study, the source of organic matter potentially suited as a replacement for peat moss was investigated. Coir pith was used as an alternative for the composition of tropical artificial soil. It is common in many tropical countries and is also commercially available in temperate regions. The coir pith before using it as a component of TAS was composted by fermentation as; the raw coir pith may allow the higher availability of chemicals leading to higher toxicity. And also the artificial soil prepared with fermented coir pith (TAS) showed similar characteristics features (e.g., water retention capacity) to OECD soil. Hence, fermented coir pith is recommended for the composition of artificial soil. In conclusion, coir pith is a suitable alternative to *Sphagnum* peat moss for the composition of artificial soil modified for tropical regions (TAS).

REFERENCES

35th IFA Enlarged Council Meeting Amman (Jordan), International Fertilizer Industry Association,17-19 November 2009

Abad M, Noguera P, Puchades, R, Maquieira, A and Noguera, V (2002) Physicochemical and chemical properties of some coconut coir dust as a peat substitute for containerised ornamental plants. Biores Tech 82: 241-241.

ISO (1993a) International Organization for Standardization. ISO-11268-1: Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*) – Part 1: Determination of acute toxicity using artificial soil substrate. Geneve, Switzerland.

ISO (1994) International Organization for Standardization. ISO-10390: Soil quality - Determination of pH, Geneve, Switzerland.

ISO (1998a) International Organization for Standardization. ISO-11268-2: Soil quality - Effects of pollutants on earth-worms (*Eisenia fetida*) – Part 2: Method for the determination of effects on reproduction. Geneve, Switzerland.

Kula C (1998). Endpoints in laboratory testing with earthworms: experience with regard to regulatory decisions for plant protection products. In: Sheppard S, Bembridge J, Holmstrup M and Posthuma L (eds) Advances in Earthworm Ecotoxicology, pp 3-14. Pensacola: SETAC Press.

Leung KMY, Chu KH, Lam PKS and Crane M (2003) Aquatic ecological risk assessment: Comparison of tropical and temperate species sensitivity to chemicals. Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) Asia/Pacific and Australasian Society for Ecotoxicology (ASE), on 28 September-1 October 2003, Christchurch, New Zealand.

Maroni M, Auteri D, Grasso P, Alberio P, Redolfi E, Azimonti G, Giarei C, Visentin S, Cavallaro P and Zaghi C (2002) Statistical evaluation of available ecotoxicological data on plant protection products and their metabolites. Final Report for the European Commission No. B1-3330/2001216.

OECD (1984a) Organization for Economic Co-operation and Development: OECD Guideline for Testing of Chemicals No. 207. Earthworm Acute Toxicity Test. Paris.

OECD (2003) Organization of Economic Cooperation and Development OECD Guideline for Testing of Chemicals No. 222. Earthworm Reproduction Test (*Eisenia fetida* / *andrei*). Paris.

Patrick Heffer and Michel Prud'homme, 2009. "Short-Term Fertilizer Outlook 2009-2010", 35th IFA Enlarged Council Meeting, Amman, November 2009.

Paul L.G.Vlek, Manfred Denich, Christopher Martius, Nick van de Giesen, 2004. Effects of pesticides on soil fauna: Development of ecotoxicological test methods for tropical regions. Ecology and Development Series No. 19.

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