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Acute Toxicity Studies with Earthworms, *Lumbricus terrestris*

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There have been reports on the effects of pesticides on non-target terrestrial invertebrates. Earthworms were reported to be insensitive to the acute effects of these compounds at levels higher than those likely to be found in the environment. For example, DDT has little or no effect on earthworms at dose levels likely to be encountered in the field; hence worms were unaffected by 2000mg/kg soil (Goffart,1949). Cook et al.,(1980) examined the effects of cultivation and DDT treatment on earthworm activity and populations in Nigeria following the application of DDT(1kg/ha) as a foliar spray on cow pea plots. The number of casts on the surface was reduced by DDT application, but there was no effect on the number of worms in the soil. However, cocoon production, hatchability and hence number of juveniles produced per worm were affected when earthworms- Eisenia andrei were exposed to paraquat, fentin, benomyl, phenmedipham or parathion (Van-Gestel et al.,1992).

In the event of chemical assault to the environment, emphasis had been placed on damage to aquatic and terrestrial biota of economic importance without any consideration of other non target organisms of lower or no economic importance. Previous studies had been carried out using P africanus, D trispimosa, and Gobius sp. to test the toxicity of some of these chemical pollutants (Ebere and Akintonwa,1992). These organisms were found to be difficult and expensive to maintain in the laboratory. Lumbricus terrestris is one of the many species of earthworm which are widely distributed throughout Nigeria. Also, this organism is a good representative of the terrestrial soil organisms which are affected by the use of this category of chemicals.

This study investigates the suitability of L terrestris, which is cheap and readily available, as a toxicity test organism.

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A correlation is also made of results obtained in this study and those obtained with P africanus (in our earlier study).

MATERIALS AND METHODS

Crude oil- Escravos light, Bonny light, Bonny medium, Forcados were obtained from oil exploration companies with kind permission of the Nigerian National Petroleum Corporation (NNPC); gasoline, kerosene, and diesel were purchased from a commercial petrol vending station. Pesticides- Igran combi (Terbutryn and Metolachlor), Diazinon, Gesapax, Avirosan (Dimethametryn + Piperophos) were obtained from Swiss-Nigerian Chemical Company PLC, Lagos; Lindane and Cypermethrin were obtained from Chemical and Allied Products Limited (CAPL) Lagos. Earthworms are widely distributed in Nigeria with varying habitats. These include soil, in which species such as Lumbricus sp., Nereis sp. can be found; aquatic species include Tubifex limnodrilux which are mainly found in shallow water and margins of lakes. For this study, L terrestris were sourced from the soil at the bank of a slow moving stream in the College of Medicine compound, Idi Araba. Fresh well water was used as the dilution water.

The stock population of earthworms were acclimatized in glass tanks totally submerged in water in the laboratory (25±2°C) for at least five days. Measured amounts of crude oil, refined products, or pesticide were shaken vigorously respectively with fresh water in a flask and then emptied into test chambers, and the concentrations were expressed as μL/Litre. The tests were begun by adding 20 earthworms into each test tank containing respective test agent concentrations; mortalities and other toxic responses were recorded at intervals, including 24,48,72 and 96 hours. Test solutions were renewed each day to discard accumulated metabolites and also to replace lost active ingredients due to evaporation. A control tank was also set up in which no test agent was added, and if more than 10% mortality occurred in it, the whole experiment was discarded. From the results, median lethal concentrations -LC(1)50s (Lloyd and Tooby,1979) were calculated after transforming percentage response to probit values, and concentrations into logarithm (Litchfield and Wilcoxon,1949). Correlation coefficients between 96hr LC(1)50s obtained with earthworms and P africanus were also performed by simple regression using Statgraphics programme.

RESULTS AND DISCUSSION

Behavioural responses played an important role in this study, and might be suitable for making ecological

predictions. When the worms were exposed to high concentrations of test chemicals, they were totally distressed and died within 15min. At the experimental concentrations used in this study, their bodies were distorted and blood aggregation was noted at some areas of the body especially, the clitellium and prostomium. These later ruptured leading to large lesions which may have contributed to death.

Table 1 shows the results obtained with different blends of crude oil and its refined products. At 96hr exposure, gasoline and Escravos light were the most toxic of the refined products and crude oil respectively. Bonny light is the least toxic with an LC(1)50 value of 68.5μL/L. At 24hr, Bonny light, Bonny medium, and Forcados exhibited very low toxicity, but as exposure period increased, the cumulative toxicity increased tremendously. At 96hr, the toxicity ranking is: Gasoline > Diesel > Escravos light > Forcados > Bonny Medium > Bonny light > Kerosene.

Table 1. Median lethal concentrations - LC(1)50s of crude oils and refined products obtained with Lumbricus terrestris.

Test agent	LC(1)50 (μL/L)			
	24hr	48hr	72hr	96hr
Bonny light	$2.6x10^3$	249.7	128.8	68.5
Bonny medium	$1.2x10^3$	389.0	219.0	66.8
Forcados	744.9	173.8	92.6	59.9
Escravos light	91.6	72.4	49.5	49.5
Diesel	99.5	60.4	60.4	44.5
Kerosene	124.3	94.0	94.0	94.0
Gasoline	59.6	37.0	37.0	37.0

The use of pesticides in Nigeria for agricultural or vector control purposes is relatively new. The remarkable thing about these pesticides is that most of them are hydrocarbon based and hence water solubility and biodegradation are difficult therefore they persist long in the soil. Table 2 shows the median lethal toxicities of the pesticides as tested on the earthworms. The insecticides are relatively more toxic than the herbicides, with 96hr LC(1)50s of 2.5, 6.5, 7.7, and 17.7μL/L for Aldrex 40, Lindane, Cypermethrin, and Diazinon respectively. The herbicides also had LC(1)50s of 17.8, 11.8, and 25.2 for Igran combi, Gesapax, and Avirosan respectively at 96hr. Earthworms used in this

Table 2 Median lethal concentrations - LC(1)50s of pesticides obtained with Lumbricus terrestris.

Pesticide	LC(1)50 (µL/L)			
	24hr	48hr	72hr	96hr
Igran combi*	37.4	27.4	20.5	17.8
Lindane	11.5	6.5	6.5	6.5
Diazinon	31.9	25.8	21.1	17.7
Cypermethrin	19.4	16.2	11.8	7.7
Gesapax*	57.6	34.7	16.6	11.8
Aldrex 40	9.4	6.0	4.1	2.5
Avirosan*	54.1	42.1	32.3	5.2

* Herbicides

study are of agricultural importance. They also represent the terrestrial organisms that are affected when there is a spill on the shore or when pesticides are used in the farmlands. The results show a good sensitivity of this organism to environmental assault. At 96hr exposure, there was a correlation coefficient of 0.9161 when the LC(1)50s of pesticides obtained with P africanus (Ebere and Akintonwa,1992) and L terrestris were compared. Also a correlation coefficient of 0.8493 was obtained when the LC(1)50s of both pesticides and crude oils obtained from same organisms (earthworms and shrimps) were compared. This result shows that such cheap, easily obtained and maintained terrestrial representative as earthworms could be very useful in acute toxicity tests especially in laboratories that lack equipment for the upkeep and maintenance of usually recommended test organisms. Moreover sea foods including shrimps are a veritable source of proteins. The constant use of these organisms for routine laboratory toxicity test would lead to depletion of adult population stock. This will in turn reduce the supply of the much needed protein in developing nations like Nigeria.

REFERENCES

Cook AG, Critchley BR, Perfect JJ, Yeadon R (1980) Effects of cultivation and DDT on earthworm activity in a forest soil in the sub- humid tropics. J Appl Ecol 17:21-29.

Ebere AG, Akintonwa A (1992) Acute toxicity of pesticides to Gobius sp, Palaemonetes africanus and Desmocaris trispimosa. Bull Environ Contam Toxicol

49:588-592.
Goffart H (1949) [The effect of new kind of insecticides on earthworms.] Anz Schadlingskd (Berlin).
Litchfield JT, Wilcoxon F (1949) A simplified method for evaluating dose-effect experiments. J Pharmacol Exp Therap 96:99-113.
Lloyd R, Tooby TE (1979) New terminology required for short term static fish bioassays; LC(1)50. Bull Environ Contam Toxicol 22:1-3.
Van-Gestel CA, Dirven-van-Breemen EM, Baerselman R, Emans HJ, Jassen JA, Postuma R, Van-Vliet PJ (1992) Comparison of lethal and sublethal criteria for nine differnt chemicals in standardised toxicity tests using the earthworm Eisenia andrei. Ecotoxicol Environ Safety 23:206-220.