Terrestrial and Aquatic Environmental Toxicology ©2011 Global Science Books

Toxicity Testing of Paraquat and 2,4-Dimethylamine on *Lumbricus terristris*, *Palaemonetes africanus* and *Tilapia guineensis*

Awodele Olufunsho[1] • Jaime A. Teixeira da Silva[2] • Alade Akintonwa[1*]

[1] Department of Pharmacology College of Medicine, University of Lagos, Nigeria
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan

Corresponding author: * aladeakintonwa@yahoo.com

ABSTRACT

Agricultural practices, especially in developing countries, have currently been given high priority among government programmes to match feeding of an ever-increasing population. The use of herbicides such as paraquat (Para) and 2,4-dimethylamine (2,4-D) have been greatly employed to control both aquatic and terrestrial weeds and may affect agricultural products. The ecotoxicity of Para and 2,4-D was determined in *Lumbricus terrestris* (earthworm), *Palamonetes africanus* (shrimp) and *Tilapia guineensis* (fish) using a probit analysis. This method was used to determine the LC_{50} over 24, 48 and 96 hrs of herbicide contact with the three organisms. Twenty *L. terrestris* individuals were kept in 5 groups in a glass tank containing loamy soil and sprayed with different concentrations of either herbicide. In addition, 5 groups of 20 individuals each of *T. guineensis* and *P. africanus* were introduced into the glass tank containing sample water of different concentrations of either herbicide. The behavioral patterns of the organisms were observed and documented. The LC_{50} for Para was 162.1, 83.10 and 66.80 mg/L for *T. guineensis*, 42.80, 28.6 and 20.4 mg/L for *P. africanus* and 64.60, 50.10 and 39.80 mg/L for *L. terrestris* at 24, 48 and 96 hrs, respectively. The LC_{5O} for 2,4-D was 328.8, 175.6 and 140.60 mg/L for *T. guineensis*, 105.8, 62.8 and 42.50 mg/L for *P. africanus* and 1122.0, 630.90 and 562.3 mg/L for *L. terrestris* over 24, 48 and 96 hrs contact of the herbicides with the aquatic organisms, respectively. The results showed increasing toxicity of the herbicides on test organisms. As the concentration of the herbicides increased, the toxicity also increased in the aquatic organisms. This could be due to bioaccumulation of these herbicides

Keywords: agricultural products, aquatic organisms, ecotoxicity

INTRODUCTION

Agricultural practices, particularly in government programmes of developing countries, have been prioritized so as to be able to feed an ever-increasing population. These could also boost the economy in terms of foreign exchange of agricultural products among other sources of revenue. This philosophy has led to improved crop and fishing production strategies within which a control strategy for aquatic and terrestrial weeds is paramount (Weed Control Recommendation 1994; Weed Science Society 1994).

Some organisms such as *Lumbricus terrestris*, an earthworm, contribute enormously to the earth's physical, chemical, geological and biological environment (EPA 1994). They incorporate crop residue, improve soil structure, increase water holding capacity of the soil and increase water infiltration rates. Thus, they contribute to the overall constitution of the soil (EPA 1994) and consequently affect crop production. Some aquatic organisms such as *Tilapia guineensis* (a fish) and *Palamonetes africanus* (a shrimp) are sources of food and revenue and are of high ecological importance to the aquatic environment.

Paraquat (Para) and 2,4-dimethylamine (2,4-D) are both herbicides that are used to control noxious, poisonous and aquatic weeds and also used for wildlife habitat improvement (Pesticide Background Statement 1994). However, the work of Birdsall *et al*. (2001) demonstrated that polycyclic aromatic hydrocarbon (PHAs) and herbicides are important contaminants of world water systems with an effect on aquatic organisms. Para is persistent in the soil environment with a reported field half-life of > 1000 days (Pesticide Background Statement 1984; Ngenpaa *et al*. 2003). Although Para is toxic to fish it is often used as an aquatic herbicide where it is absorbed by plant matter and soil. Their toxicity to fish varies with the species, size of fish and the softness or hardness of the water (Bauer and Dial 1995). Para generally acts by destroying plant cell membranes and thus inhibits photosynthesis, growth and metabolic activity of chlorophytes (Ezzat 1990; Steven and Summer 1991). On the other hand, 2,4-D is used for selective control of broadleaf weeds (US EPA 2005). It has been documented by Wong (2000) to completely inhibit algal growth, photosynthesis and chlorophyll synthesis. Thus, it is used in aquatic weed control.

The toxicity of Para and 2,4-D on aquatic organisms stimulated interest to investigate their toxicity on *L. terrestris*, *P. africanus* and *T. guineensis*.

MATERIALS AND METHODS

Para (Gramaxone®) and 2,4-D (Select®) were obtained from Zeneca Agrochemicals (C. Zard and Co., Ltd, Lagos, Nigeria). The chemicals were of analytical grade, undiluted and uncontami-nated. *T. guineensis* and *P. africanus* were obtained from a fresh water body at Itedo Zion Village in the Lekki area of Lagos State, Nigeria. *L. terrestris* was obtained from loamy soil (natural habitat) around Itedo Zion fresh water at Lekki, Lagos state, Nigeria.

T. guineensis and *P. africanus* were acclimatized in natural habitat water at 28°C and fed on a normal fish meal diet obtained from a local fisherman for 10 days. Twenty *L. terrestris* were also acclimatized in the wet loamy soil sample in a 2-l glass cage for 10 days at 28°C and they were fed with decayed plant materials.

The method of the American Public Health Association (1975) was used to determine the physicochemical parameters of water samples. A pH meter (portable PH/35032, PHEP) was used to measure the pH, Winkler titration was used to determine DO_2 and

Received: 5 January, 2010. Accepted: 21 May, 2010.

Research Note

BOD_5 was determined on day 5 from the DO_2 measurement. A conductivity meter and thermometer (TH/35002) were used to determine the conductivity and temperature, respectively.

Several concentrations of Para (10.0, 20.0 40.0 80.0 and 160.0 mg/L) and 2,4-D (100, 200, 400, 800 and 1,600 mg/L) were prepared from stock solutions of 200 and 20 g/L, respectively. 20 organisms each in 5 groups of *L. terrestris* were kept in a 2-L glass tank containing loamy soil and the organisms were sprayed with equal volume (100 ml) of each of Para and 2,4-D. In a separate experiment, 20 organisms each in 5 groups of *T. guineensis* and *P. africanus* were also introduced into glass tanks containing sample water (3 L each) of different concentrations of Para and 2,4-D.

The experiments were monitored for organism death and behavioral abnormalities over a period of 24, 48 and 96 hrs. The LC_{50} of each herbicide was then determined over these periods using a probit analysis method as described by Miller and Tainter (1944) and Awodele *et al.* (2007). Statistical significance between the groups was analyzed by means of a student's *t*-test and ANOVA. $P < 0.05$ was considered to be significant.

RESULTS

The result in **Table 1** shows the toxicity of Para on *T. guineensis*, *P. africanus* and *L. terrestris.* There were significant decreases in Para LC_{50} in *T. guineensis* from 24 to 48 hrs and also from 48 to 96 hrs and in Para LC_{50} on *P. africanus* and *L. terrestris* from 48 to 96 hrs.

Table 2 shows the effect of 2,4-D on *T. guineensis*, *P. africanus* and *L. terrestris*. There was a significant decrease in the LC_{50} of 2,4-D from 48 to 96 hrs in all three organisms tested.

Table 3 shows the physiochemical parameters of water samples at the beginning and end of the experiment. There were no significant differences in all parameters for both *P. africanus* and *T. guineensis* at the beginning and end of the experiment.

Table 1 Effect of paraquat on *Tilapia guineensis*, *Palaemonetes africanus*, and *Lubricus terrestris*.

Test organism	Calculated LC_{50} (mg/L)		
	24 hrs	**48 hrs**	**96 hrs**
Tilapia guineensis	162.1 ± 2.30	83.10 ± 3.05 a	66.80 ± 1.05 b
Palaemonetes africanus	42.80 ± 3.20	28.60 ± 2.40	20.40 ± 1.75 b
Lumbricus terrestris	64.60 ± 2.70	50.10 ± 2.15	39.80 ± 1.80 b

a $P < 0.05$ between 48 and 24 hrs
b $P < 0.05$ between 96 and 24 hrs

Table 2 Effect of 2,4-dimethylamine on *Tilapia guineensis*, *Palaemonetes africanus* and *Lumbricus terrestris*.

Test organism	Calculated LC_{50} (mg/L)		
	24 hrs	**48 hrs**	**96 hrs**
Tilapia guineensis	328.8 ± 4.20	175.6 ± 2.30 a	140.60 ± 1.05 b
Palaemonetes africanus	105.8 ± 1.40	63.8 ± 1.40 a	42.50 ± 1.55 b
Lumbricus terrestris	1122.0 ± 6.75	630.9 ± 1.55 a	562.3 ± 2.40 b

a $P < 0.05$ between 48 and 24 hrs
b $P < 0.05$ between 96 and 24 hrs

Table 3 Physico-chemical parameter of water samples at the beginning and end of the experiment.

Parameter	Sample A		Sample B	
	Initial	**End**	**Initial**	**End**
pH	7.1	6.9	7.2	6.8
Conductivity (5 cm^{-1})	2.05×10^2	2.10×10^2	$3.15v^{102}$	$3.20v^{102}$
Total solid (ppm)	524	530	510	510
Nitration (ppm)	0.50	0.5	0.4	0.3
Sodium (ppm)	240.0	242.0	230.5	2320
Potassium (ppm)	14.3	4.2	136	140
COD (mg/L)	23.5	22.8	23.2	23.6
BOD_5 (mg/L)	12.5	8.6	132	17.2
Dissolved oxygen (mg/L)	6.8	6.2	5.6	5.1
Temperature (°C)	27.2	27.0	27.0	27.2

A. Habitat water from which *Palaemonetes africanus* was obtained
B. Habitat water from which *Tilapia guineensis* was obtained
No significant difference between water samples at the initial and the end of the experiment for both sample A and B.

DISCUSSION

Weeds are a major problem in agriculture. Inappropriate weed control may affect both the crop yield and fish farming. Thus, herbicides are important to control both aquatic and terrestrial weeds.

The results of this study demonstrate the toxic effect of two commonly used herbicides, Para and 2,4-D, on three ecologically important organisms, *L. terrestris*, *T. guineensis* and *P. africanus.*

Para was toxic to all three test organisms. This corroborates the reports of the Weed Science Society of America (1994), the US National Library of Medicine (1995) and Alexander *et al.* (1985). After 96 hrs, the LC_{50} of Para decreased significantly [$P < 0.05$] from 162.1 to 66.80 mg/L in *T. guineensis*, from 42.80 to 20.4 mg/L in *P. africanus* and from 64.60 to 39.80 mg/L in *L. terrestris*, indicating the increased toxicity of Para on all three test organisms, possibly as a result of the bioaccumulation of the herbicides. Ngenpaa *et al*. (2003) and Wauchope *et al*. (1992) earlier demonstrated that soil microorganisms bioaccumulate herbicides. Bauer and Dio (1995) showed that Para is toxic to aquatic organisms, as we showed in this study.

2,4-D was toxic to all three test organisms, decreasing the LC_{50} over the 96 hrs period of study. This increasing toxicity is in agreement with the report of US EPA (2005) and US National Library of Medicine (1995), which showed 2,4-D is highly toxic to fish and other aquatic organisms.

We caution the use of 2,4-D and Para to control aquatic and terrestrial weeds.

REFERENCES

Alexander HC, Gersich FM, Mayer MA (1985) Acute toxicity of four phenoxy herbicides to aquatic organism. *Bulletin of Environmental Contamination and Toxicology* **35**, 314-321

American Public Health Association (1975) *Standard Methods for the Exanimation of Water and Waste Water* (14th Edn), American Public Health Association, Inc. N.Y., 1193 pp

Awodele O, Emeka PM, Akintonwa A, Aina OO (2007) Antagonistic effect of vitamin E on the efficacy of artesunate against *Plasmodium berghei* infection in mice. *African Journal of Biomedical Research* **10**, 51-57

Bauer-Dial CA, Dial NA (1995) Lethal effects of the consumption of field levels of paraquat contaminated plants on frog tadpoles. *Bulletin of Environmental Contamination and Toxicology* **55**, 870-877

Birdsall K, Kukor J, Cheney M (2001) Uptake of polycyclic aromatic hydrocarbon compounds by the gills of the bivalve mollusk *Elliptio complamata*. *Environmental Toxicology and Chemistry* **20**, 309-316

Evironmental Protection Agency (1994) Effect of organism on soil quality. www.epa.gov/R5Super/ecology/html/benchmemo.htm

Ezzat AI (1990) The influence of the herbicide paraquat "Gramaxon" on growth and metabolic activity of three chlorphytes. *Water, Air and Soil Pol-lution* **5**, 89-93

Hugh M (1994) Sustainable farming. In: Gliessman SR (Ed) *Ecological Agricultural Projects. A Guide for Earthworm Experts*, UK, 1-3 pp

Ngenpaa KA, Sormunen AJ, Kukkonen JV (2003) Bioaccumulation and toxicity of sediment associated herbicides (lox nil, Pendimethalin and bentazine) in *Lumbricus variegation* and *Chironomus*. *Ecotoxicology and Environmental Safety* **56**, 398-410

Pesticide Background Statements (1984) Herbicides Forest Service, US Department of Agriculture, Agriculture Handbook 633

Stevens JT, Summer DD (1991) Herbicides. In: Hayes WJ Jr, Laws ER Jr (Eds) *Handbook of Pesticide Toxicology*, Academic Press, New York, NY, pp 10-88

US Environmental Protection Agency (2005) Pesticides. 2,4-D Red facts. www.epa.gov/oppsrrd1/REDs/factsheets/24d_fs.htm

US National Library of Medicine (1995) Hazardous substances Databank. Bethesda MD, pp 10-19. www.pesticideinfo.org/references/67-97-0hsdb.doc

Wauchope RD, Buttler TM, Hornsby AG, Augustin B, Burt JP (1992) SCS/ARS/CES Pesticides properties database for environmental decision making. *Review of Environmental Contamination and Toxicology* **123**, 1-157

Weed control Recommendation for Nigeria (1994) Weed Control Recommendations: Technical Version Series No 3, 996 pp

Weed Science Society of America (1994) Herbicide Handbook (7th Edn), Champaign, 1L, pp 10-59

Wong PK (2000) Effect of 2,4-D, glyphosphate and paraquat on growth, photosynthesis and chlorophyll syntheses of *Scenedesmus quadricanda* Berb 614. *Chemosphere* **41**, 177-182