Bull. Environ. Contam. Toxicol. (2003) 71:248–254

DOI: 10.1007/s00128-003-0157-3

Bulletin of Environmental Contamination and Toxicology

Effect of Gammalin 20 (Lindane) on Differential White Blood Cell Counts of the African Catfish, *Clarias albopunctatus*

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Received: 21 January 2003/Accepted: 21 April 2003

Within the last two decades, there has been increasing awareness and concern among environmentalists regarding the effect of agrochemicals on the status of aquatic health, particularly living resources like fish. The public health implications of eating fish contaminated with poisonous chemicals and heavy metals is worrisome, especially after the well-known Minamata Bay mercury pollution incident (Takeuchi et al. 1962). Several histological, physiological and biochemical alterations have been reported in fish following exposure to agrochemicals (Demael et al. 1980; Van Vuren 1986; Omoregie et al. 1990; Allen 1994; Gimeno et al. 1994; Oluah and Amalu 1998; Santhakumar et al. 1999; Oluah 2001) and drugs (Kreutzmann 1977). Thus, aquatic organisms are continually at risk because the aquatic ecosystem has remained a major recipient and reservoir of large amounts of anthropogenic materials. Pollutants of anthropogenic origin alter the water chemistry and induce stress in the organisms living therein.

Blood chemistry (McLeay 1973; Demael et al. 1980; Omoregie et al. 1990) and changes in haematological profiles of fish exposed to various contaminants (Allen 1994; Santhakumar et al. 1999) have remained the most studied physiological indices of stress response mechanisms in fish. As a result of their immunocompetency, total white blood cell and differential white blood cell counts are considered the most susceptible blood parameters as response indicators of stress (Soivio and Oikari 1976; Flos et al. 1987; Nussey et al. 1995). As a result of the sensitivity and rapidity of response to water quality changes (McLeay 1973), as well as their inextricable involvement in disease resistance and body defence, the total leucocyte and differential leucocyte counts have remained indispensable indices of assessing subtle perturbations in water quality.

The objective of the study was to determine the effect of sublethal concentrations of Gammalin 20 (lindane) on total leucocyte and differential leucocyte counts in *Clarias albopunctatus*. This knowledge is important in ongoing contaminant monitoring. The choice of this small silurid catfish was made because it has the necessary qualities recommended by Johnson et al. (1993) for biomonitoring.

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This catfish, found in most tropical freshwaters (i.e. cosmopolitan), has taxonomic soundness and is easy to recognise by a non-specialist. The fish is suitable for laboratory studies, allowing for determination of causality.

MATERIALS AND METHODS

The "small" catfish, *Clarias albopunctatus,* was obtained from the Anambra River at Ogurugu, Nigeria using local traps. In the laboratory, the fish were acclimated for three weeks in well-aerated tap water. During the acclimation period, the fish were fed at 3% of wet body weight, a 35% crude protein, pelleted feed. Thereafter, the fish were randomly divided into five groups of 30 fish per group. Fish in groups 1 – 4 were exposed to 0.25, 0.5, 0.75, 1.0 μg/L Gammalin 20, respectively in 12-L plastic tanks. Fish in the fifth group were exposed to tap water only in similar sized tanks as the control. Each treatment group was further randomized into three replicates of 10 fish per replicate.

During the experimental period, fish were fed with same diet as during the acclimation period. The fish were subjected to sublethal concentrations of Gammalin 20 in a static bioassay system. Both the water and Gammalin 20 were changed every day to maintain the toxicant concentration and at the same time minimize the accumulation of waste products that may induce stress in the fish. One fish was removed every 7 days from each replicate tank per group and blood obtained by either cardiac puncture or severance of the caudal peduncle without recourse to anti-coagulant. After obtaining the blood, a Giemsa Romanowsky stained film of blood was prepared by the method described by Nussy et al. (1995) and the film was examined a using binocular microscope under oil immersion.

The total leucocyte count was obtained using an improved neubauer counting chamber. The leucocyte sub-populations were counted as described by Nussey et al. (1995). Statistical analysis was done using the one-way analysis of variance (ANOVA) with significance level fixed as $P < 0.05$ and the least significant difference (Steel and Torrie 1980), using the statistical package for social sciences (SPSS) software.

RESULTS AND DISCUSSION

The average white blood cell counts for *C. albopunctatus* exposed to Gammalin 20 (lindane) are shown in Tables 1 - 3. Four types of leucocytes were found and these were divided into two main groupings, agranulocytes (lymphocytes, monocytes) and the granulocytes (neutrophils, eosinophils). Compared with the control, total white blood cell counts in the treatment groups increased significantly ($P < 0.05$), with increasing Gammalin 20 concentration (Table 1). Both large and small lymphocytes were found in the peripheral blood of *C.*

Table 1. Mean differential and total white blood cell (TWBC) counts for *Clarias albopunctatus* exposed to Gammalin 20 for 7 days.

	Concentration of Gammalin 20 (μg/L)*				
Type of white blood cell	Control (0)	0.25	0.50	0.75	1.0
Agranulocytes (%)					
Lymphocytes	63.6 ± 1.0[a]	80.0 ± 2.7[b]	66.4 ± 1.9[a]	68.4 ± 1.8[a]	74.0 ± 1.6[c]
Monocytes	12.5 ± 0.6[a]	5.7 ± 0.4[b]	13.1 ± 0.8[a]	8.2 ± 0.4[c]	5.5 ± 0.7[b]
Granulocytes (%)					
Neutrophils	21.9 ± 0.8[a]	11.4 ± 0.5[b]	20.0 ± 0.7[a]	14.8 ± 0.6[c]	18.5 ± 0.3[b]
Eosinophils	1.8 ± 0.2[a]	2.9 ± 0.5[b]	5.6 ± 0.8[c]	8.2 ± 0.2[d]	9.1 ± 0.8[d]
TWBC	4.7 ± 1.9[a]	11.5 ± 1.1[b]	14.85±2.1[b]	20.0 ± 2.6[c]	31.1 ±2.6[d]

*Means ± standard error of mean in a row with the same superscript are not significantly different (P > 0.05).

Table 2. Mean differential and total white blood cell (TWBC) counts for *Clarias albopunctatus* exposed to Gammalin 20 for 14 days.

	Concentration (μg/L)*				
Type of white blood cell	Control (0)	0.25	0.5	0.75	1.0
Agranulocytes (%)					
Lymphocytes	66.7 ± 1.6[a]	76.5 ± 1.8[b]	90.1 ± 1.9[c]	64.5 ± 1.3[a]	95.7 ± 1.7[d]
Monocytes	11.1 ± 0.6[a]	5.9 ± 0.2[b]	4.8 ± 0.6[b]	19.4 ± 0.8[c]	2.1 ± 1.0[d]
Granulocytes (%)					
Neutrophils	22.2 ± 0.4[a]	17.6 ± 0.9[b]	6.7 ± 0.1[c]	6.5 ± 0.1[c]	2.1 ± 0.4[d]
Eosinophils	1.6 ± 0.1[a]	2.3 ± 0.6[a]	3.3 ± 0.2[b]	-	-
TWBC	7.4 ± 1.1[a]	19.6 ± 1.2[b]	24.4 ±1.3[c]	39.7 ± 1.6[d]	44.1 ± 1.5[e]

*Means ± standard error of mean in a row with the same superscript are not significantly different at (P > 0.05).

Table 3. Mean differential and total white blood cell (TWBC) counts for *Clarias albopunctatus* exposed to Gammalin 20 for 21 days.

	Concentration (μg/L)*				
Type of white blood cell	Control (0)	0.25	0.50	0.75	1.0
Agranulocytes (%)					
Lymphocytes	57.1 ± 1.6[a]	68.4 ± 1.6[b]	85.3 ± 1.6[c]	85.4 ± 1.0[c]	86.7 ± 1.6[c]
Monocytes	11.9 ± 0.1[a]	21.1 ± 1.0[b]	8.8 ± 0.9[c]	-	-
Granulocytes (%)					
Neutrophils	23.8 ± 1.1[a]	7.9 ± 0.3[b]	5.9 ± 0.1[c]	2.1 ± 0.6[d]	3.3 ± 0.9[e]
Eosinophils %	1.5 ± 0.1[a]	2.6 ± 0.0[b]	6.8 ± 0. 7[c]	10.0 ± 0.8[d]	12.0 ± 0.4[e]
TWBC	12.2 ± 1.0[a]	17.2 ± 1.2[b]	29.6 ± 1.8[c]	40.9 ± 1.6[d]	39.4 ± 1.6[d]

*Means ± standard error of mean in a row with the same superscript are not significantly different ($P > 0.05$).

albopunctatus. Both were counted as lymphocytes in the blood of *C. albopunctatus*. Compared with the control, the monocytes were significantly lower ($P < 0.05$) in the Gammalin 20-exposed fish. The monocytes were absent in the blood of the fish exposed to 0.75 and 1.0 μg/l Gammalin 20 on day 21 (Table 3).

The neutrophils were the most numerous granulocytes in the fish and second to the lymphocytes. By day 7, except for the group exposed to 0.5μg/L, the neutrophils were significantly reduced in the Gammalin 20-exposed treatment groups ($P < 0.05$) compared with the control. The neutrophils decreased with increasing Gammalin 20 concentration and duration up to day 14. Thereafter, the neutrophils increased significantly ($P < 0.05$) in the treatment groups but were still lower than the control ($P < 0.05$).

Leucocytosis observed in the study was consistent with earlier results from some other workers. Leucocytosis was reported in fish exposed to toxicants (Van Vuren 1986), endosulphan (Matthiessen 1981), monocrotophos (Santhakumar et al. 1999) and danitho (Figar et al. 1995). In contrast, McLeay (1975) reported a significant reduction in the leucocyte number in coho salmon exposed to kraft pulp mill effluent.

The lymphocytosis observed in this study was similar to the report of Kreutzmann

(1977) that chloramphenicol and oxytetracycline increased in lymphocytes in peripheral blood of the European eel (*Anguilla anguilla).* Also, Nussey et al. (1995) reported that fish exposed to heavy metals had a significant increase in lymphocytes. Similarly, Johanssen-Sjobeek and Larsson (1978) reported lymphocytosis in flounder, *Pleuronectes platesa,* exposed to cadmium. The results from this study further showed that lymphocytes were the most dominant leucocyte sub-population in the peripheral blood of *C. albopunctatus.* The enhanced production of lymphocytes in the treatment groups may be due to the presence of large amounts of cationic proteins in the blood following Gammalin 20 intoxication (Stossel 1983). Since lymphocytes are immunocompetent cells (Ellis 1977), lymphocytosis in the blood of Gammalin 20-exposed fish could result from stimulation of the immune system by the toxicant.

The eosinophils were as abundant as the neutrophils. In the groups where they were identified, the eosinophils increased with Gammalin 20 concentration.

The results of this study further revealed that the fish treated with Gammalin 20 generally suffered monocytopenia. In contrast, Kreutzmann (1977) reported that chloramphenicol and oxytetracycline caused monocytophilia in the catadromous eel, *A. anguilla.* The observation in this study were considered adaptive response mechanisms to protect the fish against the effect of Gammalin 20 intoxication and the associated infections.

REFERENCES

Allen P (1994) Changes in the haematological profile of cichlid *Oreochromis aureaus* (Steindachner) during acute inorganic mercury intoxication. Comp Biochem Physiol 108C:117-121

Demael A, Garin D, Peres G (1980) Response of the tench (*Tinca tinca*) to potassium nitrate enriched water. J Fish Biol 16:15-22

Ellis AE (1977) The leucocytes of fish: a review. J Fish Biol 11:453-491

Figar A, Ali SS, Shokoori AK (1995) Sublethal effect of danithol (fenproparthrin) a synthetic pyrothrod, on Chinese grass carp (*Ctenopharyngodon idella*). Folia Biol 43:151-159

Flos R., Turt L, Balasch J (1987) The effect of zinc sulphate on haematological parameters in the dogfish (*Syliorhinus anacula*) and influence of MS222. Mar Environ Res 21:289-298

Gimeno L, Ferrando MD, Sanchez S, Andreu E (1994) Endosulphan effect on liver blood of the eel (*Anguilla anguilla*). Comp Biochem Physiol 108C:343-348

Johansson-Sjobeck M, Larson A (1978) Effect of cadmium on the haematology and activity of delta-aminolevulinic acid dehydratase (ALA-D) in the blood and haematopoietic tissues of flounder (*Pleuronectes flesus* L). Environ Res 17:191-431

Johnson RK, Wiederholm T, Rosenberg DM (1993) Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. In: Rosemberg DM, Kesh VH (eds) Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York, pp 40-123

Kreutzmann HL (1977) The effects of chloramphenical and oxytetracycline on haematopeiesis in the European eel (*Anguilla anguilla*) Aquaculture 10: 323 - 334

Matthiessen P (1981) Haematological changes in fish following spraying with endosulphan insecticide. J Fish Biol 18:461-469

McLeay DJ (1973) Effect of a 12-h and 28-day exposure to Kraft pulp mill effluent on the blood and tissue of juvenile coho salmon (*Onchorhynchus kisutch*). J Fish Res Bd Can 30:395-400

Nussey G, Van Vuren JHH, duPrez HH (1995) Effect of copper on the differential white blood cell counts of the mossambique tilapia (*Oreochromis mossambicus*) Cichlidae. Comp Biochem Physiol 111C:369-380

Oluah NS, Amalu CC (1998) The effect of the heavy metal on the plasma alanine aminotransferase activity in the freshwater catfish (*Clarias albopunctatus*). J Aquat Sci 13:5-8

Oluah NS (2001) Effect of sublethal cadmium on the haematology of the freshwater catfish (*Clarias gariepinus* Pisces: Clariidae). J Sci Ag Food Tech Environ 1:9-14

Omoregie E, Ufodike EBC, Keke IR (1990) Tissue chemistry of *Oreochromis niloticus* exposed to sublethal concentrations of Gammalin 20 and Actellic 5EC. J Aquat Sci 5:33-36

Santhakumar M, Balaji M, Ramudu K (1999) Effect of sublethal concentration of monocrotophos on erythropoietic activity and certain haematological parameters of fish *Anabas testudineus* (Bloch). Bull Environ Contam Toxicol 63: 379-384

Soivio A, Oikari A (1976) Haematological effect of stress on a teleost *Esox lucius* L. J Fish Biol 8:397-411

Steel GD, Torrie JH (1980) Principles and Procedures of Statistics. McGraw Hill, New York, 451 pp

Stossel TP (1983) Functions of granulocytes. In: Williams WJ, Bentler E, Erslev AJ, Liehtman MA (eds) Haematology: 3rd edition McGraw Hill, New York, pp 685 - 698

Takeuchi T, Morikawa N, Matsumoto H, Shiraishi Y (1962) A pathological study of the Minamata Bay disease in Japan. Acta Neurol Pathol 2:40-57

Van Vuren JHH (1986) The effects of toxicants on the haematology of *Labeo umbratus* (Teleosti Cyprinidae). Comp Biochem Physiol 83C:155

Puchkov, NV (1964) The white blood cells. In: Monson S (ed) Techniques for the Investigation of Fish Physiology, Jerusalem, pp 11-15