

Thiodan Induced Hematological Alterations in a Fresh Water Fish *Oreochromis mossambicus*

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Abstract

The effects of the insecticide thiodan on certain hematological parameters (RBC count, WBC count, hemoglobin content and hematocrit value) of the fish *Oreochromis mossambicus* were studied at sub-lethal concentrations of 1/20, 1/15 and 1/10 of LC_{50} value and at 10, 20 and 30 days of exposure. The RBC count, hemoglobin content and the hematocrit value in the thiodan exposed fish were diminished in response to increase in time of exposure and dose. The WBC count showed a duration and dose dependent increase at all the sub-lethal concentrations. The highest sub-lethal concentration on the 30 of day caused the most pronounced effect in all the parameters. Thus, the dose and duration dependent impact of thiodan on the hematology of the fish is evident.

Key words : Thiodan, *Oreochromis mossambicus*, RBC and WBC count, Hemoglobin content, Hematocrit value.

Thiodan (35% EC), a commercial grade endosulfan widely used in India, is a broad spectrum non-systemic contact and stomach insecticide effective against numerous insect pests. Due to indiscriminate usage of chemical pesticides in agriculture, there has been immense damage to non-target organisms especially fish. Hematological parameters have been used for detecting diseases and environmental stress (1). Such study provides valuable information in the health management of fish. Hence in the present investigation an attempt was made to study the hematological changes of the fish *Oreochromis mossambicus* exposed to thiodan.

Methods

The commercial grade endosulfan (35% EC) thiodan, supplied by Bayer Crop Science India Ltd., Mumbai was used. The required concentration of the pesticide was prepared in chlorine free tap water using acetone as solvent. Fresh water acclimated fish having a size of 8—10 cm and 10—12 g wet weight were used. The maintenance and acclimation of fish was done using standard procedures as described earlier (2). The 96 h LC_{50} values were calculated using probit analysis with SPSS/Windows (SPSS.10.0.LNK).

The fish were grouped into four, each consisting

of ten individuals. First group served as control and the remaining as experimental. The fish were exposed to different sub lethal concentrations (1/20, 1/15 and 1/10 of LC_{50}) of the toxicant. The fish were fed with standard food pellets throughout the experiment, but feeding was stopped 24 h prior to each sampling day. Controls received similar treatment except the addition of thiodan. At the end of 10, 20 and 30 days of exposure blood samples were collected for determination of various parameters. RBC count was done with a Neubauer crystalline counting chamber as described by Sohn and Henry (3). WBC count was done following the procedure described by Hunter and Bomford (4). The hemoglobin content was estimated by acid hematin method (5). The hematocrit value was calculated by the micro hematocrit method (6).

Results and Discussion

RBC Count

Table 1 shows that a duration dependent reduction in RBC count was recorded in the exposed fish at all the three sublethal concentrations on the 10, 20 and 30 day respectively. Of the three sublethal concentrations, the effect was most prominent at the highest sublethal concentration ($1/_{10} LC_{50}$). It can be seen that fish exposed to the highest sublethal concentra-

Table 1. RBC count ($\times 10^6/\text{mm}^3$) in the blood of control *O. mossambicus* and that exposed to sublethal concentrations of thiodan. Values are the mean of six individual observations \pm SE. Minus values denoting percent decrease from control level are given in parentheses.

Concentrations	Days		
	10	20	30
Control	2.89 \pm 0.06	2.94 \pm 0.03	2.92 \pm 0.06
$1/_{20}$ LC ₅₀	2.81 \pm 0.03 (-2.77)	2.75 \pm 0.08 (-6.46)	2.57 \pm 0.04 (-11.98)
$1/_{15}$ LC ₅₀	2.67 \pm 0.03 (-7.61)	2.44 \pm 0.03 (-17.01)	2.13 \pm 0.05 (-27.05)
$1/_{10}$ LC ₅₀	2.17 \pm 0.03 (-24.91)	1.82 \pm 0.06 (-38.09)	1.32 \pm 0.03 (-54.79)

tion on the day 10 recorded 24.91% reduction in RBC level than the control value. It was further reduced to 38.09% on the day 20 and to 54.79% on the day 30 when compared to control.

The major function of red blood cells (RBC) is to transport hemoglobin, which in turn carries oxygen from the gills to the tissues. In the present study the RBC count showed a decreasing trend with increasing concentration and exposure time of thiodan which could be attributed to decreased erythropoietic activity. In most vertebrates, including fish, erythropoietic activity is regulated by erythropoietin produced in the kidney (7). *O. mossambicus* showed a decrease in RBC count suggesting decrease in erythropoietin activity due to the interference of thiodan in the normal functioning of kidney. Verma et al. (8) observed similar fall in RBC level in the fish *Mystus vittatus* subjected to pesticides.

Later, Goel et al. (9) in their 15-day study using alachlor on the fish *Clarias batrachus* noted significant reduction in RBC level. Similarly a dose dependent decrease in RBC level of *Channa punctatus* on exposure to the herbicide stamp 30 E.C (organonitrochlorine group) was recorded by Sharan et al. (10). Recently, Venkataramana et al. (11) in their short term study observed a diminution in RBC count in malathion exposed *Cyprinus carpio*. Thus in most of the earlier studies a reduction in RBC level that corroborated with the result of the present study were noted.

WBC Count

Table 2 shows that on the days 10, 20 and 30 of

Table 2. WBC count ($\times 10^4/\text{mm}^3$) in the blood of control *O. mossambicus* and that exposed to sublethal concentrations of thiodan. Values are the mean of six individual observations \pm SE. Values denoting percent increase from control level are given in parentheses.

Concentration	Days		
	10	20	30
Control	6.11 \pm 0.05	6.13 \pm 0.07	6.14 \pm 0.05
$1/_{20}$ LC ₅₀	6.22 \pm 0.06 (1.80)	6.28 \pm 0.06 (2.45)	6.39 \pm 0.06 (4.07)
$1/_{15}$ LC ₅₀	6.57 \pm 0.08 (7.53)	6.89 \pm 0.03 (12.40)	7.28 \pm 0.05 (18.57)
$1/_{10}$ LC ₅₀	7.19 \pm 0.05 (17.67)	8.29 \pm 0.08 (35.23)	9.13 \pm 0.08 (48.69)

exposure, slight increase in WBC count was measured at the lowest sublethal concentration. A time dependent significant increase in WBC level was recorded at the intermediate sublethal concentration. The WBC count of fish exposed to the highest sublethal concentration on the day 10 registered 17.67% increase over the control value. Subsequently it was increased to 35.23 and 48.69% on the days 20 and 30 respectively. Thus the WBC level showed a duration and dose dependent increase at the sub-lethal concentrations. The most pronounced increase was recorded at the $1/_{10}$ of LC₅₀ concentration on day 30.

The white blood cells (WBC) are the mobile units of the body's protective system. They are specifically transported to areas of serious inflammation, providing a rapid and potent defence against any infectious agent/toxic substance that might be present. In the present experiment increase in the number of WBC might have been to meet the pathological conditions arising from the toxicant stress.

Several workers have reported escalated WBC level in fish subjected to various toxicants. Goel et al. (9) in their study using alachlor on the fish *Clarias batrachus* observed similar elevation in WBC level. Kumari et al. (12) registered significant increase in WBC count in *Clarias batrachus* exposed to organochlorine pesticides namely DDT and lindane. Joshi et al. (13) recorded elevated WBC count in the fish *Clarias batrachus* intoxicated with lindane. All these reports pointed towards the increased WBC count in fish upon exposure to various pesticides which are in agreement with the result of the present experiment.

Table 3. Hemoglobin content (g/100 ml) in the blood of control *O. mossambicus* and that exposed to sublethal concentrations of thiodan. Values are the mean of six individual observations \pm SE. Minus values denoting percent decrease from control level are given in parentheses.

Concentrations	Days		
	10	20	30
Control	9.48 \pm 0.11	9.54 \pm 0.15	9.48 \pm 0.08
$1/_{20}$ LC ₅₀	9.38 \pm 0.09 (-1.05)	9.22 \pm 0.08 (-3.35)	8.92 \pm 0.10 (-5.91)
$1/_{15}$ LC ₅₀	8.78 \pm 0.16 (-7.38)	8.25 \pm 0.12 (-13.52)	7.53 \pm 0.15 (-20.57)
$1/_{10}$ LC ₅₀	7.24 \pm 0.17 (-23.63)	6.06 \pm 0.15 (-36.48)	4.98 \pm 0.11 (-47.46)

Hemoglobin (Hb) Content

Table 3 shows the changes observed in the Hb content of the fish exposed to various sublethal concentrations of thiodan. A duration dependent decrement in Hb level was noted at the lowest sublethal concentration. The same trend was observed for the intermediate and highest sublethal concentrations. The most pronounced diminution in Hb content was noted at the highest sublethal concentration on the day 30.

Hemoglobin is the oxygen carrying pigment of the erythrocytes. Any quantitative reduction in the pigment is likely to affect the oxygen related metabolism of the organism. In the present experiment, Hb decrease was observed in proportion to concentration of thiodan and the duration of exposure. The low levels of Hb indicated anemic condition in fish which might be due to stress caused hemolysis as opined by Panigrahi and Mishra (14). Koundinya and Ramamurthy (15) suggested the possibility of inhibition of aerobic glycolysis curtailing *de novo* synthesis of Hb during toxic stress. Also, the lower Hb levels in the thiodan intoxicated fish might be due to the disruption of the iron synthesizing machinery as supported by Beena and Viswarajan (16). Recently, Tyagi and Srivastava (17) registered reduction in Hb content in the fish *Channa punctatus* subjected to chronic zinc exposure which is in consonance with the present study.

Hematocrit (Ht) Value

Result of the change in Ht values of the fish sub-

Table 4. Hematocrit value (%) in the blood of control *O. mossambicus* and that exposed to sublethal concentrations of thiodan. Values are the mean of six individual observations \pm SE. Minus values denoting percent decrease from control level are given in parentheses.

Concentrations	Days		
	10	20	30
Control	34.19 \pm 1.61	34.65 \pm 0.99	35.54 \pm 1.31
$1/_{20}$ LC ₅₀	33.39 \pm 1.67 (-2.34)	32.61 \pm 0.61 (-5.89)	29.38 \pm 0.85 (-17.33)
$1/_{15}$ LC ₅₀	28.08 \pm 1.57 (-17.87)	25.06 \pm 1.15 (-27.68)	24.02 \pm 0.58 (-32.41)
$1/_{10}$ LC ₅₀	25.54 \pm 1.26 (-28.22)	21.33 \pm 0.84 (-38.44)	16.35 \pm 1.12 (-53.99)

jected to thiodan are given in Table 4. A duration dependent slight reduction in Ht value was measured at the lowest sub-lethal concentration. A more significant duration dependent diminution in Ht values was registered at the intermediate sublethal concentration. The fish exposed to the highest sub lethal concentration also exhibited the same trend but, the decrease in percentage level over control was more pronounced here. The highest sub lethal concentration on day 30 effected the most pronounced decrease in Ht value.

The hematocrit (Ht) value was found decreased with increase in exposure period and concentration of thiodan in *O. mossambicus*. Several workers have reported similar response in fish exposed to various toxicants. Koundinya and Ramamurthy (15) noted significant decrease of Ht values in *Sarotherodon mossambicus* subjected to sumithion and sevin. The decreased Ht level in fish exposed to sublethal concentration of monocrotophos, according to Srinivasan and Radhakrishnamurthy (18) was due to decreased RBC count which in turn might be due to the effect of monocrotophos on blood forming organs.

The Ht values are valuable in determining the effect of stressors on the health of fish and are also used to determine the oxygen carrying capacity of blood. The low Ht value would indicate anaemia or oligohaemia or an alteration in the fish metabolism as suggested by Srivastava and Mishra (19). However, Naidu et al. (20) from a 24-h study reported an increase in the Ht value in *C. carpio* exposed to sublethal concentration of endosulfan. They stated that the fish exposed to the toxicant tried to cope up with the adverse condition by enhancing their respiratory capability through elevated RBC synthesis. They fur-

ther inferred that prolonged exposure of fish to pesticide might pose a great threat to their survival. The recent long term study report of Joshi et al. (13) using lindane in *C. batrachus* has further substantiated these statements inferring thereby that the health of the fish was under continued threat when exposed to the insecticide.

It could be thus concluded that a duration dependent significant effect (decrease/increase) was observed for all investigated parameters at the highest sublethal concentration. The adaptive strategies of the fish to tide over the toxic stress were evident at lower sublethal concentrations, but with increase in concentration, it was found to be detrimental to the fish.

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