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Toluene toxicity: Effects of sublethal levels on enzyme activities in seawater adapted tilapia (*Sarotherodon mossambicus* Peters)

AJIT D. DANGE and VASANT B. MASUREKAR
Department of Zoology, Institute of Science, Madam Cama Road, Bombay 400 032

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Abstract. The effects of exposing seawater adapted cichlid fish, tilapia (*Sarotherodon mossambicus* Peters) to sublethal concentrations of toluene on the activities of lactate dehydrogenase, succinate dehydrogenase and acetylcholinesterase were studied. The activity of lactate dehydrogenase increased while those of succinate dehydrogenase and acetylcholinesterase were inhibited in most tissues of the exposed fish. The alterations in the dehydrogenases suggested that some changes in carbohydrate metabolism may have occurred. Acetylcholinesterase inhibition in brain and other tissues indicated impairment of nervous function in toluene-intoxicated tilapia.

Keywords. Tilapia; toluene toxicity; lactate dehydrogenase; succinate dehydrogenase, acetylcholinesterase.

Introduction

A large number of studies on the biological effects of oil pollution in the aquatic environment deal with the effects of whole crude or refined oils or their water-soluble fractions. However, low boiling, aromatic hydrocarbons which are probably the most toxic constituents of oil have until now not been examined in sufficient detail. Toluene, benzene and naphthalene constitute a major component of various oils (Anderson *et al.*, 1974). They may be readily lost by weathering but are toxic in waters that are relatively stagnant and are chronically polluted. Korn *et al.* (1977) have stated that toluene is more toxic than many other hydrocarbons such as benzene, though the latter are more water-soluble. Toxicity of toluene to fish has been demonstrated (Pickering and Henderson, 1966; Stoss and Haines, 1979).

The present study forms part of a detailed programme for investigating the metabolic impact of some important petroleum hydrocarbons on aquatic animals. In this paper, the effects of sublethal concentrations of toluene on the activities of lactate dehydrogenase (EC 1.1.1.27), succinate dehydrogenase (EC 1.3.99.1) and acetylcholinesterase (EC 3.1.1.7) in different tissues of the cichlid fish tilapia (*Sarotherodon mossambicus* Peters) adapted to seawater are reported. Tilapia, a euryhaline fish, easily adapts to saline waters and rapidly recovers from the initial osmotic shock (Meenakshi *et al.*, 1979). However, the fish adapted to 100%

seawater showed high mortality rates (65-85%) during the first four weeks of exposure to toluene. Hence, the effects of toluene on fish adapted to 50% seawater only are reported here.

Materials and methods

Tilapia were collected from Shivaji lake, Thane, Maharashtra and maintained in the laboratory in large glass aquaria containing weathered, well-aerated tap water for two weeks. They were then transferred to aquaria containing 10%, 20%, 30%, 40% and 50% seawater. An acclimation period of one week was allowed at each transfer, but in 50% seawater, the fish were maintained for four weeks. The sea-water acclimated fish did not show any visible signs of stress and were seen to swim about actively. During the entire acclimation period, the fish were fed daily with live tubificid worms (*Tubifex* sp.). The fish were also supplied with groundnut cake once a week.

Since enzyme activities in tilapia are related to size (Mansuri and Pandya, 1977), fish of a relatively uniform size (12-15 cm long; 22.7-24.1 g) were selected from the stock for the exposure. They were further acclimated in the experimental aquaria for two days and then exposed to toluene (25 or 50 mg/L) for ten weeks. The level of toluene was kept constant by changing the water everyday and adding the requisite amounts of a toluene stock solution (prepared in Analar acetone to get more accurate concentrations). The fish were fed daily, prior to change of water to prevent ingestion of toluene through food. A control group exposed to acetone alone was also maintained.

Water samples from each aquarium were analyzed once a week. The conditions prescribed by the American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971) for the quality of water during static exposure of aquatic animals to pollutants were maintained through-out the experiment. The values of some of the physicochemical characteristics of the water from the control aquaria were: dissolved oxygen–7.3 mg/L; dissolved chlorine –0.018 mg/L. salinity –16,8 parts per thousand; pH 7.7; conductivity–18.5 mmhos/cm; temperature –27.5°C. There was no noticeable change in the quality of the water upon addition of toluene.

At the end of ten weeks, the fish were killed by decapitation and the tissues rapidly excised. The tissues were rinsed, blotted and homogenzied in a motor-driven, all glass homogenizer with two volumes of chilled saline (0.7% NaCl). Homogenates were centrifuged (Model 101, MB Corporation, Bombay) at 10,000 g for 15 min. The supernatant fractions were diluted with ten volumes of chilled saline and used as the enzyme source. The activities of lactate dehydrogenase, succinate dehydrogenase and acetylcholinesterase were determined according to the methods described by Srikantan and Krishnamurthy (1955), Nachlas *et al.* (1960) and Pilz (1965), respectively. Protein content was estimated by the method of Lowry *et al.* (1951), using bovine serum albumin as the standard.

The difference in enzyme activities between the control and the exposed fish were analyzed for statistical significance according to the Student's't'-test (Spiegel, 1961).

Results

The stress of exposure to toluene stimulated lactate dehydrogenase activity in all the tissues (table 1). The increase in activity was most significant in liver and brain. The enzyme activity was not markedly changed in the kidney and the gonads. On the other hand, succinate dehydrogenase activity decreased in all the tissues of the exposed fish (table 2), the most marked reduction being in liver and brain.

Table 1. Effect of toluene exposure on lactate dehydrogenase activity in tilapia.

Tissue	Control	Expt. 1 (25 mg/litre)	Expt. 2 (50 mg/litre)
Liver	74 ± 7	121 ± 6[a] (+64%)	252 ±21[a] (+241%)
Brain	67 ± 5	126 ± 6[a] (+88%)	186 ±15[a] (+178%)
Cardiac muscle	59 ± 4	89 ± 5[b] (+51%)	107 ± 8[b] (81%)
Skeletal muscle	32 ± 2	53 ± 2[b] (+66%)	82 ± 4[b] (+156%)
Gill	17 ± 3	22 ± 4 (+29%)	24 ± 4[b] (+41%)
Intestine	89 ± 9	129 ±11[a] (+45%)	159 ±17[a] (+79%)
Kidney	142 ±12	175 ±14[b] (+23%)	189 ±12[a] (+33%)
Testes (immature)	23± 2	28 ± 4 (+22%)	32± 4[b] (+39%)
Ovary (immature)	29 ± 2	34 ± 4 (+17%)	36 ± 5[b] (+24%)

[a] –P<0.001;[b]–P<0.0 1.

Sp. activity – µmol of acetylcholine hydrolyzed/mg protein/h. Values are mean ±SD of 7 and 6 determinations in the control and exposed fish respectively. Values in the paranthesis represent change over control.

Table 2. Succinate dehydrogenase activity in tilapia following toluene exposure.

Tissue	Control	Expt. 1 (25 mg/litre)	Expt. 2 (50 mg/litre)
Liver	187 ±10	101 ±15[a] (−46%)	58 ±14[a] (−69%)
Brain	376 ±29	163 ±13[a] (−57%)	129 ±17[a] (−66%)
Cardiac muscle	68 ± 8	47 ±12[b] (−31%)	36 ± 6[a] (−47%)
Skeletal muscle	62 ± 6	34 ± 5[a] (−45%)	22 ± 8[a] (−65%)
Gill	76 ± 9	62 ± 9 (−13%)	48 ± 7[a] (−36%)
Intestine	205 ±21	172 ±20 (−16%)	135 ±16[a] (−34%)
Kidney	192 ± 8	164 ±19[b] (−15%)	132 ±16[a] (−31%)
Testes (immature)	141 ±13	116 ±12[b] (−18%)	81 ± 9[a] (−43%)
Ovary (immature)	109 ± 7	72 ± 7[a] (−34%)	62 ± 9[a] (−43%)

[a] – P< 0.001; [b] – P<0.01.

(*Sp.* activity—nmol of formazan formed/mg protein/h. Values are mean ± SD of 7 and 6 determinations in the control and exposed fish respectively. Values in the parenthesis: represent change over control).

Acetylcholinesterase activity was significantly inhibited in most tissues of tilapia following exposure to toluene (table3). Tissues like brain, skeletal and cardiac muscles showed the highest inhibition in the enzyme activity.

Table 3. Acetylcholinesterase activity in control and toluene treated tilapia.

Tissue	Control	Expt. 1 (25 mg/litre)	Expt. 2 (50 mg/litre)
Liver	31.82 ±2.17	26.42 ±1.87[b] (−17%)	22.38 ±1.90[a] (−30%)
Brain	81.97 ±5.03	59.98 ±3.19[a] (−27%)	27.54 ±4.32[a] (−66%)
Cardiac muscle	65.21 ±3.19	51.03 ±2.19[a] (−22%)	34.55 ±2.06[a] (−47%)
Skeletal muscle	56.01 ±3.77	44.88 ±4.35[b] (−20%)	31.14 ±3.43[a] (−44%)
Gill	46.72 ±3.63	38.26 ±2.49[b] (−18%)	27.52 ±3.02[a] (−41%)
Intestine	34.25 ±3.08	29.11 ±2.03[b] (−15%)	23.52 ±1.94[a] (−31%)
Kidney	18.43 ±1.04	16.63 ±1.11 (−13%)	14.74 ±0.07[a] (−20%)
Testes (immature)	32.93 ±2.30	27.02 ±1.51[b] (−18%)	22.07 ±1.47[a] (−33%)
Ovary (immature)	24.47 ±2.34	21.29 ±1.21 (−15%)	18.86 ±0.86[a] (−23%)

[a] – $P < 0.001$; [b] – $P < 0.01$.

Sp. activity µmol of acetylcholine hydrolyzed/mg protein/h. Values are mean ±SD of 7 and 6 determinations in the control and exposed fish respectively. Values in the paranthesis represent change over control.

Discussion

Stimulation of lactate dehydrogenase, an enzyme associated with the anaerobic pathway of carbohydrate metabolism and simultaneous inhibition of succinate dehydrogenase, one of the important enzymes involved in the Kreb's cycle, in the tissues of the toluene-intoxicated tilapia indicate disturbances in the cellular oxidative processes. The changes appear to favour a less efficient anaerobic metabolism in tilapia in response to the environmental stess, probably due to the inability of the tissues of such fish to derive sufficient oxygen for the normal metabolic functions. Such tissue hypoxia was indicated by an increase in the lactic acid/pyruvic acid ratio, accompanied by an increase in lactate dehydrogenase and decrease in succinate dehydrogenase activities, in tissues of tilapia exposed to lethal and sublethal levels of naphthalene (Dange, 1979).

In the natural environment, oil pollution may lead to a drastic reduction in the oxygen tension of water, especially in the stagnant, confined types of waters. The possibility of the dissolved hydrocarbon affecting the tissue enzymes by decreasing dissolved oxygen content of the water may be ruled out while interpreting data from the present study, since it was observed that toluene did not reduce the oxygen content significantly. The suggested lack of a proper oxygen supply to the tissues may be due to some damage to the gills similar to that observed in fish exposed to different petroleum hydrocarbons (Gardner, 1975; DiMichele and Taylor, 1978). Gill hyperplasia was also seen in tilapia treated with naphthalene

and toluene (Dange, unpublished data). This is consistent with Hodson's (1976) view that aquatic pollutants cause gill damage leading to tissue hypoxia and possibly death.

Moreover, aromatic hydrocarbons like toluene, being lipid-soluble, may affect the erythrocyte membrane (Gerarde, 1960). Berry (1980) suggested that due to this, the supply of oxygen to various tissues would be inadequate to meet the increased metabolic demands of the stressed fish. Under such conditions, fish like tilapia which are capable of anaerobic metabolism may be able to produce the necessary amount of energy by increasing lactate dehydrogenase activity, probably by utilising stored glycogen as in the case of tilapia exposed to naphthalene (Dange, 1979).

Levitan and Taylor (1979) showed that the effects of petroleum hydrocarbons on fish are salinity-dependent, due to the increased energy demands on an already stressed osmoregulatory system of the euryhaline fish. Probably the metabolic burden of pollution is aggravated by the stress due to the hypertonic medium. However, preliminary studies had shown that no significant effects on the enzyme activity could be ascribed to osmotic stress in the seawater adapted tilapia.

The response of tilapia to toluene included the inhibition of acetylcholinesterase in all the tissues, indicating a disruption in the nervous system in the exposed fish. As this enzyme is involved in the maintenance of the structural and functional integrity of cellular membranes (Kutty *et al.,* 1976), it may be inferred that toluene causes disturbances in the normal cellular functions. Although a large decrease in the acetylcholinesterase activity was not observed by us, as observed by Coppage (1972), the decrease was larger than the 10% decrease suggested as an acceptable criterion for assessing water quality (Nicholson, 1967). Thus even the lower sub-lethal concentration of toluene (25 mg/L) would be objectionable.

The effects on these enzymes probably are only a part of the general metabolic response to stressful conditions and may not be specific for toluene. Hydrocarbons such as benzene and toluene are irritants of mucus membranes and the changes in enzymes like the dehydrogenases may be induced secondarily as a result of lesions in gills and other tissues. The effects may also be caused by accumulation of toluene in different tissues. The generally more severe effects on enzyme activities in liver may be due to extensive accumulation and metabolism of these hydrocarbons in this organ.

Changes in the lactate dehydrogenase and succinate dehydrogenase activity may indicate the facility with which tilapia can shift to anaerobic metabolism under adverse conditions and it would be interesting to observe if similar enzymatic changes occur more often in the anaerobic type of fish following exposure to pollutants.

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