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Respiratory Potentials of the Fish (*Tilapia mossambica*) Under Malathion, Carbaryl and Lindane Intoxication

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Although the existence of pesticides in marine and freshwater ecosystems is established (Andryushchenko 1971), literature concerning the physiological effects of these pesticides on aquatic organisms is less. The target action of some of these compounds is on the enzyme, AChE. But in general, one of the symptoms of pesticide toxicity is respiratory distress (O'Brien 1967). The respiratory potentials of an animal are the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general. This also helps for making valid inferences on its environmental requirements. Hence, the present study is designed to evaluate the sublethal effect of malathion (organophosphate), carbaryl (carbamate) and lindane (BHC: organochloride) on a comparative basis, selecting the rate of oxygen consumption and certain respiratory enzymes like succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and cytochrome-c-oxidase as the experimental parameters in the freshwater edible fish, Tilapia mossambica (peters). In the present study, sublethal level is selected in support of the recognition that all pesticides are potentially lethal even at low concentrations to fish (Holden 1973) and it also closely approximates naturality.

MATERIAL AND METHODS

The details of collection, maintenance and acclimation of the fish is the same as described earlier (Rao and Rao 1979). The commercial grade malathion (S-(1,2-dicarbethoxyethyl) o,o-dimethyldithiophosphate), carbaryl (1-naphthyl N-methyl carbamate) and lindane (1α,2α,3β,4α,5α,6β -hexachlorocyclohexane) were employed for the present study due to their wide use in this region (Andhra Pradesh, India). The preparation of standard stock solutions of these pesticides and the determination of LC_{50} values (Finney 1971) are mentioned elsewhere (Basha et al

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1983). The LC_{50} values of malathion, carbaryl and lindane were found to be 0.367 ppm, 5.495 ppm and 3.199 ppm respectively to T. mossambica for 48 hours at 28°C. Taking one third of the LC_{50} values as sublethal concentration, the fish were exposed separately to the three pesticides belonging to three different groups for 48 hours. Suitable controls were maintained. The oxygen consumption by fish was determined at 12 hour intervals upto 48 hours and the respiratory enzymes were studied only after 48 hours of exposure.

The oxygen consumption by the fish was estimated by Winkler's iodometric method as described in Welsh and Schmidt (1953). The difference in the oxygen content of the initial and final samples is taken as the amount of oxygen consumed, unit metabolism was calculated and expressed as ml of oxygen consumed/g weight/h.

After 48 hours of exposure, liver and brain tissues were isolated and immediately homogenised in 10% trichloroacetic acid (TCA) for glycogen, in 0.25M cold sucrose solution for SDH and MDH and in 0.1M cold phosphate buffer (pH 7.6) for cytochrome-c-oxidase. The supernatants were used for the assay. Glycogen content was estimated as per the method of Carrol et al (1956). Succinate dehydrogenase (Succinate:Acceptor oxidoreductase E.C 1.3.99.1) and malate dehydrogenase (L malate:NAD oxidoreductase E.C.1.1.1.37) activities were estimated as per the method outlined by Nachlas et al (1960) and cytochrome-c-oxidase (Ferrocytochrome'c'oxygen oxidoreductase E.C. 1.9.3.1) activity by the method of Oda et al (1958). The proteins were estimated using Folin-phenol reagent (Lowry et al. 1951). The statistical correlations between control and experimental values were made using student t-test (Bahn 1972) and a p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The results were tabulated in Table 1 and 2. During the 48 hours of exposure of all the three pesticides, changes in the rate of oxygen consumption by the fish showed a similar trend. There is an initial increase in the rate of oxygen consumption upto 24 h of exposure and decreased afterwards upto 48 hours (Table 1).

The initial elevation in the rate of oxygen consumption might be due to acceleration of oxidative metabolism during the first 12 h exposure, as a result of sudden response to the toxic stimulus of the pesticides. But with the onset of symptoms of poisoning, the rate of oxygen consumption decreased in the later periods of exposure. This is in agreement with earlier observations (Rao et al 1981).

Corroborating the results of oxygen consumption, the activity levels of SDH and MDH decreased significantly in liver and brain tissues (Table 2) suggesting the prevalance of hypoxic condition in the tissues and a reduction in the rate of oxidative metabolism at the mitochondrial level, since pesticides are known to block the respiratory centre of the brain, leading to a condition

similar to asphyxia (Rao 1980). Further, the glycogen content also decreased significantly in both the tissues (Table 2) confirming the prevalance of hypoxic condition at the tissue level, since anoxia or hypoxia increases carbohydrate consumption (De Zwaan and Zandee 1972) and thereby creating a sort of stress on the fish even at sublethal level resulting in extra expenditure of energy.

Table 1. Changes in oxygen consumption (ml of oxygen/g/h) of control, malathion exposed (ME), carbaryl exposed (CE) and lindane exposed (LE) fish at different time intervals.

Hours of exposure	Control	ME	CE	LE
12 h	0.3501 ±0.0084	0.4120 ±0.0075 (+18)[a]	0.4017 ±0.0094 (+15)[a]	0.4201 ±0.0082 (+20)[a]
24 h	0.3601 ±0.0199	0.3792 ±0.0298 (+5)	0.3802 ±0.0270 (+6)	0.3851 ±0.0279 (+7)
36 h	0.3642 ±0.0102	0.3497 ±0.0108 (-4)[c]	0.3501 ±0.0126 (-4)[c]	0.3421 ±0.0169 (-6)[c]
48 h	0.3625 ±0.0168	0.3321 ±0.0167 (-8)[b]	0.3361 ±0.0192 (-7)[c]	0.3292 ±0.0210 (-9)[b]

Values in parentheses are percent change over control (N = 6; Mean ± S.D). P values: a = P<0.001; b = P<0.02; c = P<0.05;

The attenuation in SDH and MDH activities is more pronounced with lindane followed by carbaryl and malathion, inferring organochlorides to be relatively potent inhibitors of respiratory enzymes than the organophosphates and carbamates. However, considerable inhibition of SDH and MDH activities by malathion and carbaryl could be due to the secondary effects of these pesticides as a consequence of their action on the target enzyme, AChE. Following SDH and MDH activities, cytochrome-c-oxidase activity also decreased in liver and brain tissues (Table 2) leading to the impairment of electron transport system, thereby reducing the synthesis of ATP molecules which are of paramount importance for all vital functions.

In general, it can be inferred from the above results that there is a shift in the emphasis towards anaerobiosis at the tissue level during the sublethal intoxication of lindane, carbaryl and malathion. These three pesticides seem to show a unique

Table 2. Levels of glycogen, SDH, MDH and cytochrome-c-oxidase activities in liver and brain tissues of control, malathion exposed (ME), carbaryl exposed (CE) and lindane exposed (LE) fish.

Liver				Brain			
Cont.	ME	CE	LE	Cont.	ME	CE	LE
Glycogen (mg/g wet wt)							
32.49 ± 3.21	23.40 ± 4.12 (-28)[a]	27.75 ± 3.46 (-15)[c]	22.31 ± 3.98 (-31)[a]	0.228 ±0.032	0.196 ±0.039 (-14)	0.203 ±0.031 (-11)	0.189 ±0.027 (-17)[c]
SDH (µM formazan/mg protein/h)							
0.103 ±0.004	0.090 ±0.007 (-13)[b]	0.085 ±0.008 (-18)[a]	0.079 ±0.008 (-23)[a]	0.254 ±0.016	0.241 ±0.006 (-5)	0.240 ±0.010 (-5)	0.230 ±0.012 (-9)
MDH (µM formazan/mg protein/h)							
0.045 ±0.003	0.038 ±0.005 (-14)[c]	0.035 ±0.003 (-21)[a]	0.037 ±0.003 (-16)[b]	0.148 ±0.007	0.126 ±0.008 (-15)[a]	0.127 ±0.010 (-14)[b]	0.125 ±0.013 (-16)[a]
Cytochrome-c-oxidase (µM diformazan/mg protein/h)							
75.60 ± 3.74	69.79 ± 3.94 (-8)	71.33 ± 4.74 (-6)	68.25 ± 4.69 (-10)[c]	101.9 ± 6.8	89.52 ± 6.24 (-12)[b]	93.09 ± 5.81 (-9)	90.25 ± 6.65 (-11)[c]

Values in parentheses are percent change over control (N = 6; Mean ± S.D); P values: a = P<0.001; b = P<0.02; c = P<0.05;

effect with slight variation on the respiratory potentials of fish, eventhough there are structural dissimilarities among these compounds.

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REFERENCES

Andryuschenko VV (1971) Proceedings of a scientific conference commemorating the 50th anniversary of Norosriysk biological station, Mater Nauch Konf, Posvyasch

Bahn KA (1972) Basic Medical Statistics, Grune and Stralton Inc. New York and London

Basha SM, Rao KSP, Rao KRSS, Rao KVR (1983) Differential toxicity of malathion, carbaryl and BHC to the freshwater fish, Tilapia mossambica. Bull Envir Contam Toxicol (In press)
Carrol NV, Longley RW, Roe JH (1956) The determination of glycogen in liver and muscle by use of anthrone reagents. J Biol Chem 220:583-593
De Zwaan A, Zandee DI (1972) The utilisation of glycogen and accumulation of some intermediates during anaerobiosis in Mytilus edulis L. Comp Biochem Physiol 43:47-54
Finney DJ (1971) Probit analysis, 3rd ed. Cambridge University Press, Cambridge
Holden AV (1973) Effect of pesticides on fish. In: Edwards CA (ed) Environmental pollution by pesticides. Plenum Press, London, New York
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193: 265-275
Nachlas MM, Margulius SP, Saligman AM (1960) A colorimetric method for the estimation of succinic dehydrogenase activity. J Biol Chem 235:499-503
O'Brien RD (1967) Insecticide action and metabolism. Academic Press, London, New York
Oda T, Seki S, Okazaki H (1958) Colorimetric methods for the estimation of cytochrome-c-oxidase and of cytochrome c-cytochrome oxidase systems. Acta Med Okayama 12:293-301
Rao KRSS, Rao KSP, Sahib IKA, Rao KVR (1981) Comparative evaluation of the toxicity of elsan in continuous flow and static systems with reference to oxygen consumption and excretion in O. punctatus. Indian J Fish 28:254-258
Rao KSP (1980) Studies on some aspects of metabolic changes with emphasis on carbohydrate utilisation in cell-free systems of the freshwater teleost, T. mossambica subjected to methyl parathion exposure. Ph D thesis, S.V. University, Tirupati, India
Rao KSP, Rao KVR (1979) Effect of sublethal concentration of methyl parathion on selected oxidative enzymes and organic constituents in the tissues of the freshwater fish, T. mossambica. Curr Sci 48:526-528
Welsh JH, Schmidt RI (1953) Laboratory exercises in invertebrate physiology, Burgess Publishing Co. Minneapolis

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