Bull. Environ. Contam. Toxicol. (1990) 45:88–93

Bulletin of Environmental Contamination and Toxicology

Changes in Brain Monoamine Levels and Monoamine Oxidase Activity in the Catfish, *Clarias batrachus*, During Chronic Treatments with Mercurials

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Mercury (Hg) is a highly toxic, nonessential heavy metal which is bioconcentrated through the food chain (WHO 1976). The environmental level of Hg increases every year as a result of natural weathering processes and large scale uses in industries, agriculture, and pharmaceuticals, and it reaches ultimately the aquatic environment. Fishes which constitute an important link in the food chain concentrate Hg largely as methyl Hg (CH_3Hg). The deleterious effects of Hg poisoning in fishes include inhibition of enzymes and protein synthesis, structural alterations of epidermal mucus, reduction in sperm viability, inhibition of embryogenesis and survival of second generation fry, reduction in olfactory response, vision and respiration; decrease in fin generation time; and decreased ability to osmoregulate (Moore and Ramamoorthy 1984).

In mammals, the central nervous system is the primary target for CH_3Hg poisoning which is clinically known as "Minamata disease" (Chang 1977). Hg is a widely recognised neurotoxin and has been reported to impair brain monoamine neurotransmitter metabolism (Hrdina et al. 1976; Taylor and DiStefano 1976; Okuda et al. 1978; Tsuzuki 1981, 1982; Bartolome et al. 1984). Reports on effects of Hg on brain monoamine activity in fishes are scarce (Ram and Sathyanesan 1985). In the present study, therefore, changes in the brain monoamine levels and the degradation enzyme, monoamine oxidase (MAO), are described in the catfish, *Clarias batrachus*, exposed to sublethal concentrations of mercuric chloride ($HgCl_2$-inorganic Hg), methylmercuric chloride (CH_3HgCl-organic Hg), and a commercial mercurial fungicide formulation, emisan 6 (methoxyethyl Hg-organic Hg) for 45, 90 and 180 d during gonadal recrudescence. These intervals correspond to late preparatory, prespawning and spawning phases, respectively, of the annual reproductive cycle of the catfish.

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MATERIALS AND METHODS

Over 180 adult *C. batrachus* weighing 60 ± 5 g were purchased from the local fish market. They were acclimated to laboratory conditions for 14 d before starting the investigations. They were divided into 4 groups of 45 fish each and kept in glass aquaria containing well-water (pH 7.3, hardness 23.2 mg L^{-1} as $CaCO_3$ and dissolved oxygen 8 mg L^{-1}). The fish were fed minced goat liver daily and were maintained under natural photoperiod which varied from 11L:13D, 16°C to 13L:11D, 22°C. The groups 1, 2 and 3 were exposed to sublethal concentrations of 0.05 mg L^{-1} $HgCl_2$ (98% W/W, The British Drug Houses Ltd., India), 0.04 mg L^{-1} CH_3HgCl (98% W/W, Wilson Laboratories, Bombay) and 0.5 mg L^{-1} emisan 6 (6% methoxyethyl mercury chloride + 94% inert ingredients W/W, Excel Industries Ltd., Bombay), respectively. The sublethal concentrations corresponded to one-tenth of LC_{50} values for $HgCl_2$ (0.507 mg L^{-1}), CH_3HgCl (0.430 mg L^{-1}) and emisan 6 (4.322 mg L^{-1}) during 96-hr exposure. The aquaria water was replenished daily with the required amounts of the pollutants. The group 4 fish were untreated and served as the control. The experiments were started in February (early preparatory phase).

Fifteen fish from each group were sacrificed after 45, 90 and 180 days. The fish were killed by decapitation between 8:00 and 9:00 am to avoid circadian differences of the correlates. The brains were dissected out on ice for the estimation of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and MAO activity. NE and DA, and 5-HT were estimated spectrophotofluorometrically in an AMINCO Spectrophotofluorometer-500 according to the method of Shellenberger and Gordon (1971) and Snyder et al. (1965), respectively. The relative fluorescence was measured against their blanks at 325/380 nm for DA, 380/495 nm for NE and 385/490 for 5-HT. The readings were calculated from standard curves prepared with different concentrations of DA, NE and 5-HT. The standard curves were linear over the ranges observed with the tissue samples. Known amounts of DA, NE and 5-HT were added to tissue samples for recovery. The percentage of recovery was found to be 83.55%, 76.39% and 69.33%, respectively for DA, NE and 5-HT. The final values were adjusted accordingly. MAO activity was estimated by the radioisotopic technique of Parvez and Parvez (1973) using ^{14}C-tryptamine bisuccinate (New England Nuclear, Massachusetts, U.S.A.; Sp. Act.: 56.1 mCi/mM) as the substrate. Radioactivity was measured in terms of 5-hydroxyindoleacetic acid formed during 20 min of incubation of brain homogenates at 37°C in a

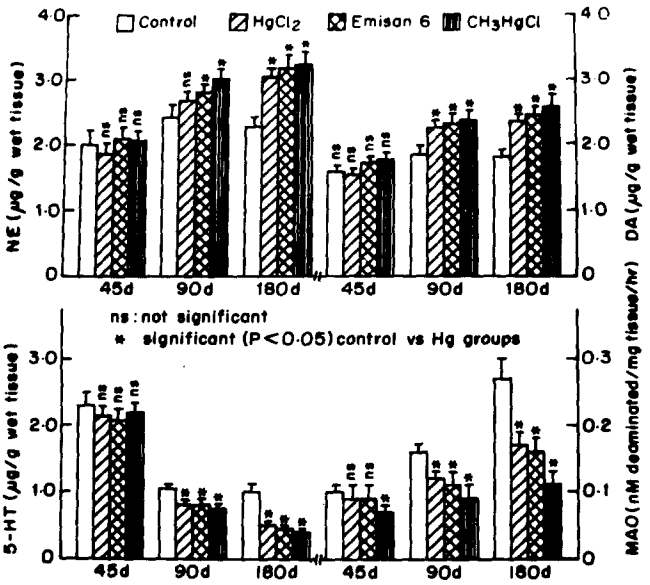

Figure 1 : Changes in brain monoamine levels and MAO activity in *Clarias batrachus* following chronic treatment with mercurials

Beckman-1801 liquid scintillation counter (Beckman Instruments Inc., Irvine, California, U.S.A.). The data obtained were tested for statistical significance ($p<0.05$) using a single-factor ANOVA and Newman-Keuls' tests.

RESULTS AND DISCUSSION

In the Hg-treated catfish, brain concentrations of DA and NE did not change significantly after 45 days, but had registered a significant rise after 90 and 180 days (Fig. 1). This is in agreement with the observations in mammals that catecholamine (CA) levels are increased after Hg intoxication. An increase in brain DA level, striatal and brain-stem NE level (Tsuzuki 1982), and brain-stem NE level (Hrdina et al. 1976) was observed in CH_3Hg-intoxicated rats. Taylor and DiStefano (1976) have observed an initial decrease in DA and NE levels, followed by a significant increase in the amine levels in the developing brains of CH_3Hg-exposed rats. Bartolome et al. (1984) have reported differential effects of $HgCl_2$ with a significant effect on NE level, turnover, and synaptosomal uptake and an insignificant effect on the dopaminergic system of developing rat brain.

The observed increase in the CA levels following Hg exposure may be due to either an increased synthesis or decreased degradation of the amines. In the Hg-treated catfish, brain MAO activity decreased significantly after 45 days in the CH_3HgCl group and in all the groups after 90 and 180 days. The decreased enzyme activity indicates reduced oxidative deamination of monoamines and therefore may account for the increase in CA levels. Decreased activity of brain MAO has been also reported in CH_3Hg-treated rats (Okuda et al. 1978; Tsuzuki 1981) and in emisan 6- and $HgCl_2$-treated *Channa punctatus* (Ram and Sathyanesan 1985). Tsuzuki (1982) had observed decreased level of 3,4-dihydroxyphenyl acetic acid, a deamination product of MAO, in CH_3Hg-treated rat brains.

Unlike CA, the concentration of 5-HT in the brain of Hg-treated catfish decreased significantly after 90 and 180 days (Fig. 1), as has also been reported in CH_3Hg-intoxicated rat brains (Hrdina et al. 1976; Tsuzuki 1982). The level of 5-hydroxyindoleacetic acid was also significantly low after CH_3Hg poisoning (Tsuzuki 1982). Thus it appears that the decreased level of 5-HT in the Hg-treated catfish may not be due to increased oxidative deamination by MAO, but due to its reduced synthesis. CH_3HgCl has been reported to inhibit tryptophan (the precursor of 5-HT) level and tryptophan hydroxylase (the rate-limiting enzyme) activity in developing rat brains (Taylor and DiStefano 1976).

Our observations further show that the magnitude of the effects - increase or decrease in brain monoamine levels and decrease in MAO - is dependent on the exposure period; the maximum effects being noticed in the 180-day treatment groups. Furthermore, the magnitude of the effects also varied with the chemical nature of the mercurials. Organic mercurials (CH_3HgCl and emisan 6) were found to be more toxic to impair brain monoamine activity than the inorganic Hg.

The changes in brain monoamine activity following Hg exposure may alter several monoamine-dependent functions of the catfish. In teleosts, it has been demonstrated that the brain monoamines are directly involved in the neuroendocrine regulation of several pituitary hormones, such as prolactin, gonadotropin and melanophore stimulating hormone (see reviews: Ball 1981; Peter et al. 1986). Therefore, alterations in brain monoamine metabolism caused by Hg may affect the functional interrelationship between the pituitary and its endocrine target organs. Cytological changes in the secretory activity of the corticotrophs, gonadotrophs and thyrotrophs (unpublished observation) accompanied by

inhibitory changes in adrenal (unpublished observation), ovarian (Kirubagaran and Joy 1988) and thyroidal (Kirubagaran and Joy 1989) activity in the Hg-treated catfish suggest such a possibility.

Acknowledgments. The authors are grateful to the Department of Environment, Government of India and the C.S.I.R., New Delhi for financial support.

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Received June 13, 1989; accepted December 4, 1989.