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# Chemical Analysis of Internal Environmental Response of Carp *Puntius stigma* to DDT

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Exposure of *Puntius stigma* to sublethal dose of DDT (0.05 ppm) for 15 days decreased the liver concentrations of protein and DNA from  $65 \pm 0.91$  to  $63 \pm 0.78$  mg/100 mg and  $669 \pm 17$  to  $575 \pm 14$   $\mu$ g/100 mg, respectively whereas RNA increased from  $8214 \pm 318$  to  $8929 \pm 209$   $\mu$ g/100 mg. Biotransformation products of this hydrocarbon interfered in the pathways of protein biosynthesis and induced proteolysis in hepatic tissues. The resulting changes were explained in the light of hypertrophication of liver parenchyma and inter-relationships of chemical constituents.

**KEY WORDS:** Internal environment, nucleic acids, carp, DDT.

## INTRODUCTION

Backlash of the use of chlorinated hydrocarbons, especially DDT, for management of insect pests is undoubtedly quite alarming. Stability of this chemical in the aquatic environment not only creates cumulative pollution but the problem assumes infinite proportions through biological magnifications as the substance moves in biogeochemical cycles and along food chains, reaching the human beings

(top carnivore). Little information exists on biochemical and physiological effects of DDT in animals. Barring reports on dysfunction of nervous<sup>1-3</sup> and reproductive<sup>4-10</sup> systems caused by DDT, most of the toxicity tests are carried out at organism level. The present study focuses attention on the deleterious role of DDT contaminated water (0.05 ppm) on the internal environment of *Puntius stigma*, a carp fish which is foraged heavily by carnivorous teleosts and occupies an important place in food chain in the aquatic environment. The effects have been evaluated at molecular level which are absolutely essential for explaining the implications of DDT in the ecosystem with a greater degree of exactitude.

## MATERIALS AND METHODS

Live specimens of *Puntius stigma* (total length, 7.1–9.1 cm; body weight, 4.0–9.5 g) sampled from local ponds at Aligarh (latitude 27° 34' 30" N, longitude 78° 4' 26" E) were acclimated to laboratory conditions for 1 week. After the period of acclimatization the fish were randomly grouped into two batches of 50 each and reared separately in 30 litre capacity aquaria. The temperature and dissolved oxygen of medium were maintained at  $23 \pm 0.5^\circ\text{C}$  and  $4.9 \pm 0.2$  ppm, respectively. A known quantity of DDT was dissolved in a small but measured volume of acetone and diluted to desired level by tap water. After preliminary trials, a sublethal dose of DDT (0.05 ppm) was selected for exposing the specimens of one of the batches. The other group of fish served as control. This stock was run in DDT-free water containing acetone in the same quantity as in test aquaria (0.15 ml/30 litre). The experiment continued for 15 days and no food was supplied during this period. After the last day of experiment all specimens were decapitated. Their length and weight were recorded. Liver was immediately dissected out and its weight was recorded for determining liver-somatic index by the formula:

$$\text{Wet weight of liver (g)} \times 100 / \text{Total weight of intact fish (g)}$$

A known weight of liver samples was pooled together and processed for obtaining dry, fat-free tissue powder<sup>11</sup> which was used for quantitative determination of protein, RNA and DNA. Protein

was assayed by the procedure of Lowry *et al.*<sup>12</sup> RNA was extracted and estimated following the techniques of Schneider.<sup>13</sup> DNA was extracted employing the procedure of Webb and Levy<sup>14</sup> and quantitated by the methodology of Ashwell.<sup>15</sup> Protein was expressed as mg/100 mg and RNA and DNA as  $\mu\text{g}/100\text{ mg}$  dry, fat-free tissue.

## RESULTS AND DISCUSSION

Data presented in Figure 1 indicate that concentrations of protein and DNA declined whereas that of RNA increased in the liver of *Puntius stigma* exposed to the sublethal dose of DDT. The changes were, however, not statistically significant ( $P > 0.05$ ). Evidently, the DDT induces proteolysis, although mechanism of this phenomenon can not be accurately explained. It is recognized that DDT is a powerful stressor<sup>16-18</sup> and through blood circulation it tends to enter the liver,<sup>19</sup> where it preferentially accumulates. DDT or its transformation products may also interfere with pathways of protein biosynthesis by creating what has been termed as "biochemical lesion", and enhancing the proteolysis of preformed liver protein. The stress-mediated release of larger quantity of adrenocorticotrophic hormone (ACTH) by the hypophysis and increased output by adrenal gland of cortisone and corticosterone sequentially<sup>20,21</sup> can also be a potent factor. The same authors have documented that ACTH influences the enzymes involved in degradation of protein and its conversion to glucose. This can be viewed as an adaptive adjustment in the internal environment to ensure supply of the most

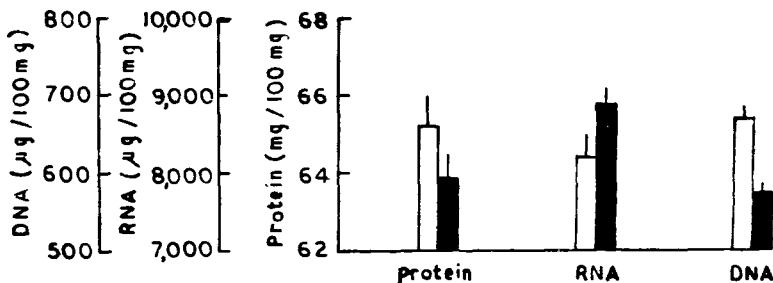


FIGURE 1 Concentrations of protein, RNA and DNA in liver of control (white bars) and DDT-exposed (black bars) specimens of *Puntius stigma*. Vertical lines indicate standard error of mean.

readily utilizable source of energy which alone can sustain the strenuous muscular activity of fish under stress.

The reciprocal change in RNA and DNA concentrations as seen in this study is attributable to hypertrophication of liver parenchyma possibly caused by DDT. Annau<sup>22</sup> has observed enlargement of hepatic cells of mice fed another insecticide, aldrin, but the author is not aware of such a report *vis-à-vis* DDT. Higher liver-somatic index ( $1.176 \pm 0.314$  SE) of DDT-exposed fish specimens compared to control group ( $1.041 \pm 0.278$  SE) also points towards hypertrophy of liver. The catabolised protein and fat are believably replaced largely by water. Such a substitution relationship between these constituents has been termed by Love<sup>23</sup> as "protein/fat-water effect" in fish undergoing profound biochemical changes as part of its normal seasonal cycle. Findings of Chargaff and Davidson<sup>24</sup> leave no doubt that cellular hypertrophy is associated with increase in RNA content. This lends support to the present observations. Further, as the hypertrophied cells of liver accumulate water, RNA and some other substances in the cytoplasm, they put on more weight and the cellular DNA is "diluted". As a matter of fact, a smaller number of these cells of larger size and weight can make a unit weight of tissue compared to larger number of cells of lesser size and weight obtained from normal liver. DNA, which is related to number of cells/unit weight of tissue sample, was thus reduced. "Cause and effect" relations are worth mentioning at this stage of discussion. One must view that this change in DNA is in concentration which is a function of changing number of cells/unit weight of tissue sample processed for biochemical assays, and does not reflect alteration in its amount/cell. In earlier publications Mustafa<sup>25-27</sup> has suggested caution in interpretation of cause and effect of any such change. It is also well authenticated that DNA is metabolically stable and remains remarkably indifferent to metabolic "traffic" in cytoplasm. Indeed, such a stability is essential if DNA is to serve as a genetic material and to govern hereditary destiny.

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