Chapter 6

BIOCONCENTRATION OF MONOCROTOPHOS RESIDUE

Contents

6.1 Introduction
6.2 Materials and methods
   6.2.1 Extraction
6.3 Results
6.4 Discussion
6.1 Introduction

The unsystematic use of insecticides can be taken as one of the factors that alter the quality of environment, causing imbalances in the ecosystem, especially to the denizens of the aquatic environment. Organophosphorus insecticides (Ops) cause a non-reversible phosphorylation of esterases in the central nervous system of insects and mammals and act as cholinesterase inhibitors. The level at which a chemical is concentrated in fish depends on the absolute rates of uptake and elimination of the compound (Spacie and Hamelink, 1985). The usage of organophosphorus pesticides is preferred to the usage of other pesticides such as organochlorine compounds, because organophosphorus pesticides degrade much faster in the environment. Hence, there is an increasing demand for developing methods for the determination of such contaminants in food analysis and environmental analysis. It is often assumed that bioconcentration of organophosphate pesticides by aquatic organisms means no risk for the ecosystem and either a low partition coefficient or a high biotransformation rate prevents these chemicals from accumulating through food chains in higher organisms (Bruijn and Hermens, 1991). However the data on their bioconcentration and excretion by fish are useful for the evaluation of their risk to humans and, further, for the assessment of the contamination of fish by pesticides.

6.2 Materials and methods

Collection, transportation, acclimation and bioassay experiments of on *Heteropneustes fossilis* were carried out as described in the chapter I. *H. fossilis* of 35.5 ± 3 g were subjected to three sub lethal concentrations (6.6 ppm, 4 ppm, and 2 ppm) of monocrotophos. Control group of fish with out toxicant were also maintained. Sampling was done on 21st day to examine the concentration of monocrotophos in the edible part of the fish i.e.
Bioconcentration of monocrotophos residue

Chapter 6

Muscle. Whole body without viscera and internal organs were taken for the analysis.

6.2.1 Extraction

The determination of monocrotophos in the muscle samples was carried out based on the method of Richardson and Seiber (1993), adapted to the current samples as follows: Muscle tissue of the fish was lyophilized and homogenized with 50% HCl (0.01 ml) and 0.5% ethanol-ethyl acetate (20 ml). This homogenate was centrifuged at 1200 rpm (5 min). After centrifugation a drop of 5% decanol in acetone was added to the supernatant. This mixture was dried on a rotary vacuum evaporator. The dried residue was redissolved in 3 ml of a 1:1 mixture cyclohexane: ethyl acetate and ultrasonicated (1 min). Then, it was filtered through nylon filter by using a Varian Vac-Elut. This volume was redissolved in 3:1 ethyl acetate: cyclohexane (6 ml) and introduced into a gas permeation chromatograph. The eluate obtained was dried and redissolved in ethyl acetate (1 ml). This final volume was injected into a Perkin Elmer gas chromatograph equipped with an MS detector. Column was Elite-5 ms with a length of 30 m, diameter of 0.25 mm and film thickness 0.25 μm. The detector and injector temperatures were 270 and 250° C. Helium was used as carrier gas with spilt flow at the rate of 0.55 ml/min. All solvents used were pesticide residue analysis grade and were purchased from Sigma-Aldrich Co.

6.3 Results

Constant occurrence of sub lethal concentrations of monocrotophos in the surrounding water for 21 days appears to be physiologically stressful to the stinging catfish. Concentration of monocrotophos in the different concentration groups 2 ppm, 4 ppm and 6.6 ppm were 0.0868 ± 0.0071,
0.1412 ± 0.0057 and 0.1997±0.0033 μg /gm respectively. Chromatograms showing bioconcentration of monocrotophos at 6.6 ppm, 4 ppm and 2 ppm are given as Fig. 6.1, Fig. 6.2 and Fig. 6.3 respectively. Data were statistically analysed and values were expressed as means ± Standard deviation.

Table 6.1 Concentration of MCP in the muscle of *H. fossilis*

<table>
<thead>
<tr>
<th></th>
<th>2ppm</th>
<th>4 ppm</th>
<th>6.6 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Detected</td>
<td>0.0868 ± 0.0071</td>
<td>0.1412 ± 0.0057</td>
<td>0.1997±0.0033</td>
</tr>
</tbody>
</table>

- All values are in μg / gm

6.4 Discussion

Researchers like Shannon, (1977); Muir and Grift, (1981); Barron *et al.*, (1993); Tsuda *et al.*, (1994); and Sancho *et al.*, (1998) reported on the bioaccumulation of pesticides in fish tissues. Bioconcentration of hazardous substances like organophosphorus insecticides causes serious ecological problems when the degree of partitioning of a substance or its transformation products results in translocation to, and storage in critical tissues of organisms. (Ramaneswari and Rao, 2000). It is not simple to model the distribution of a pesticide in fish or measure the time course in the body or a specific organ, but such methods are especially useful for describing residue dynamics over time. Bioaccumulation of chemicals in biota may be a prerequisite for adverse effects on ecosystems and when uptake rates are significantly higher than metabolic clearance rates bioaccumulation can still occur even though the substance is readily biodegradable.
Ramaneswari and Rao (2000) investigated the bioconcentration of monocrotophos in *Labeo rohita* and *Channa punctata* and observed the bioconcentration of monocrotophos in the form of o-des methyl monocrotophos and hydroxy monocrotophos and the bioconcentration of monocrotophos in *Labeo rohita* and *Channa punctata* were found to be 1.68 and 1.33 µg/g respectively. However there is a dearth of available literature on the bioconcentration studies of monocrotophos in the stinging catfish *H. fossilis*.

In the present investigation, edible parts of the fish were analysed for monocrotophos residue. Bioaccumulation-elimination studies of sub lethal exposure to organophosphate insecticides like monocrotophos in muscle could provide information on the environmental impact of these pesticides. The most obvious signs of monocrotophos intoxication were restlessness, erratic swimming, spasms, and loss of balance. Some of the experimental fish exhibited low motor and sensory activities but all of them endured the set exposure period. These symptoms had been reported in the European eel under sub lethal exposure to other organophosphate insecticides (Da Silva *et al.*, 1993; Sancho *et al.*, 1994a). The results of the present study prove that sub lethal concentrations of monocrotophos bioaccumulates in the muscle of *H. fossilis*. Though the values of concentration were low at all the three sublethal concentration group (0.0868 ± 0.0071, 0.1412 ± 0.0057 and 0.1997±0.0033 µg /gm respectively for 2 ppm, 4 ppm and 6.6 ppm) it was observed to have serious deleterious effects on the metabolic machinery of the fish which could be inferred from studies on various aspects as described in the forgone chapters. Histopathological alterations in the tissues of MCP treated *H. fossilis* (chater-5) supports this observation.
Bioconcentration of monocrotophos residue

Fig 6.1 Chromatogram - 6.6 ppm

Fig 6.2 Chromatogram - 4 ppm

Fig 6.3 Chromatogram - 2 ppm

Stress responses of stingling catfish Heteropneustes fossilis (Bloch) to organophosphorus insecticide Monocrotophos