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EFFECTS OF HERBICIDES ON THE CYTOCHROME P-450 CONTENT OF LIVER MICROSOMES IN CARP (CYPRINUS CARPIO L.)

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Abstract

Ultracide (methidathion), paraquat and $CuSO_4$ were administered in 0.5–10 ppm concentrations to carp and the cytochrome P–450 contents of the liver microsomes were determined. After treatment with $CuSO_4$ the cytochrome P–450 content increased. For paraquat and ultracide, decreases were observed after several days of administration. Alterations in cytochrome P–450 levels might be due either to direct action of the herbicides or to changes caused by them in the membrane structure of the endoplasmic reticulum.

Key-words: herbicides, cytochrome P–450, carp.

Introduction

The extensive use of herbicides has increased the incidence of environmental pollution. Herbicides are among the most dangerous agents of water contamination, since they are used on or near the soil and in many instances in water for aquatic weed control. One property of many biologically active compounds such as drugs or pesticides is their ability to induce the microsomal enzyme systems of animals (CONNEY, 1967; MANNERING, 1968). Recent evidence has shown that the enzyme induction following herbicide treatment is the most sensitive parameter among the biological and toxic responses in mammals (CARLSON and SCHOENIG, 1980). Cytochrome P-450, the carbon monoxide binding pigment of microsomes, serves as the terminal oxidase in the metabolism of a wide variety of substrates, including drugs, insecticides, chemical carcinogens, fatty acids and steroids (MANNERING et al., 1969). Two techniques frequently used to determine the potential for agents to induce microsomal enzyme systems are quantitative analysis of the haemoprotein cytochrome P-450 and measurement of the NADPH cytochrome c reductase activity. In its reduced form, cytochrome P-450 complexes with carbon monoxide and the complex exhibits an absorption maximum at 450 nm (ALVARES et al., 1967). Various chemicals have different effects on the quantity or activity of this cytochrome and these measurements can usually be employed to characterize the agents.

In an early report, BRODIE and MAICKEL (1962) suggested that microsomal drugmetabolizing enzymes might be absent in fish. Subsequent studies, however, have demonstrated the presence of various levels of oxidative drugmetabolizing activity in some vertebrate species of fresh water and marine origin, and also in insects. CREAVEN et al. (1967) concluded that microsomal drug-metabolizing enzymes do occur in trout and amphibia but at lower levels than in mammals. The presence

of cytochrome P-450 in the microsomal fraction of trout liver was reported by CHAN et al. (1967).

The purpose of the present study was to characterize the effects of three kinds of herbicides, paraquat, $CuSO_4$ and ultracide, on the cytochrome P-450 content of carp liver.

Materials and methods

Chemicals: Paraquat (1,1' -dimethyl-4,4 -bipyridylium dichloride) was purchased as Gramoxone (ICI Plant Protection Division, England), which contains 24% of the herbicide in aqueous solution. Methidathion (0,0-dimethyl-S-2-methoxy-1,3, 4-thiadiazol-5(4H)-yl-4-methyl-dithiophosphate) was obtained as ultracide (Ciba-Geigy A. G.). All other chemicals were of analytical grade and were obtained from Reanal, Hungary.

Common carp (*Cyprinus carpio* L.) weighing 600–800 g were obtained from the Fisheries Research Institute in Szarvas, Hungary, and were acclimatised for at least 5–7 days before treatment. Herbicides were administered to the water of the fish in 0.5–10 ppm concentrations. (The concentrations of herbicides were calculated on the basis of effective substances.) Carps were killed and the livers were removed. The livers were minced in ice-cold 50 mM Tris HCl buffer, pH 7.4 containing 0.25 M sucrose and homogenized to a final homogenate concentration of 25% (w/v) using a glass Potter-Elvehjem homogenizer with a teflon pestle. Microsomes were prepared according to KAMATH and NARAYAN (1972). Protein content was measured by the method of LOWRY et al. (1951). Cytochrome P–450 was estimated from the absorption difference at 450 and 490 nm, by the application of an extinction coefficient of 91 mM^{-1} (OMURA and SATO, 1964). The difference spectra were recorded using a Pye Unicam SP spectrophotometer. The given values are the averages ±(S. D.) for 3–5 fish specimens.

Results

EFFECT OF PARAQUAT

In our experiments paraquat was applied in concentrations of from 0.5 to 7 ppm. After 24 hours of treatment the cytochrome P-450 content of the liver microsomes was increased (Fig. 1). At a 0.5 ppm herbicide concentration the increase was 10%.

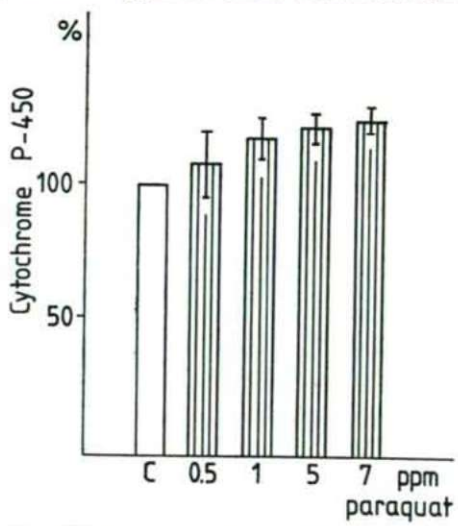

Fig. 1. Effect of paraquat in different concentrations on cytochrome P–450 content of liver. The values are averages of those measured from 3–5 individuals as expressed in the percentage of the controls.

With the higher concentration of paraquat the cytochrome P-450 content of the microsomes was about 25% higher than in the control fish. This increase in haemoprotein content, however, was not permanent and during longer treatment a secondary decrease was found. The time curve of the concentration of cytochrome P-450 in the

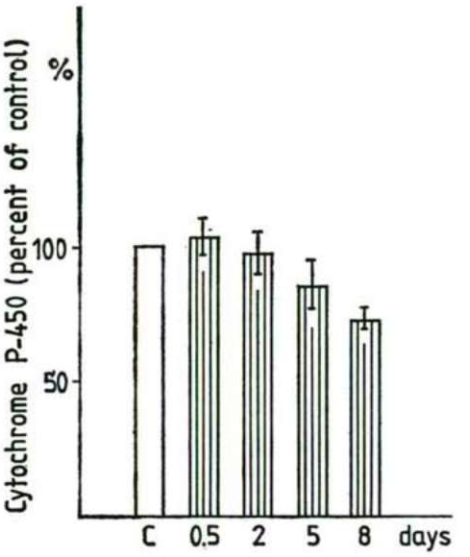

Fig. 2. Effect of 0.5 ppm paraquat on the level of cytochrome P-450 of carp liver microsomes. The values are averages of those measured from 3–5 individuals and expressed in the percentage of the controls.

liver of fish treated with 0.5 ppm paraquat is shown in Fig. 2. It can be seen that on the second day the level of this protein is roughly equal to the original (i. e. untreated) value. During longer treatment with paraquat a significant decrease in the cytochrome P-450 content was observed.

On the eighth day only 72% of the original value was present in the treated fish.

Effect of copper sulphate

During the treatment $CuSO_4$ was used in 3, 5 and 7 ppm concentrations. After 24 hours of administration the cytochrome P-450 level of the liver microsomes was increased (Fig. 3). At 5 ppm concentration an increase of about 35% was measured in the treated carp as compared to the controls. A further increase in the concentration of $CuSO_4$ apparently had no further enhancing effect.

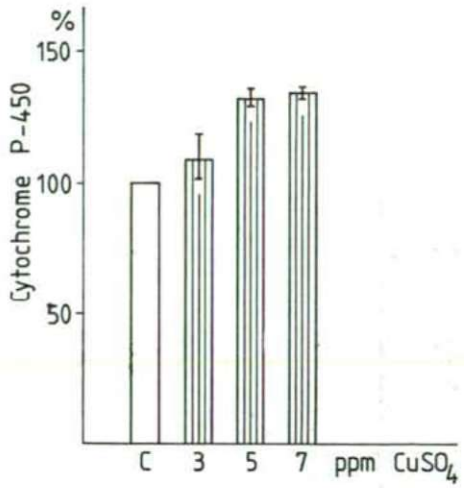

Fig. 3. Changes in the cytochrome P–450 content of liver after $CuSO_4$ treatment in vivo.

In another experiment the effect of 0.5 ppm of $CuSO_4$ was studied during a longer period. The level of cytochrome P-450 on the first day, of treatment was practically the same as in the untreated controls. However, on the second day the value was about 20% higher and this value was also detected on the third day (Fig. 4).

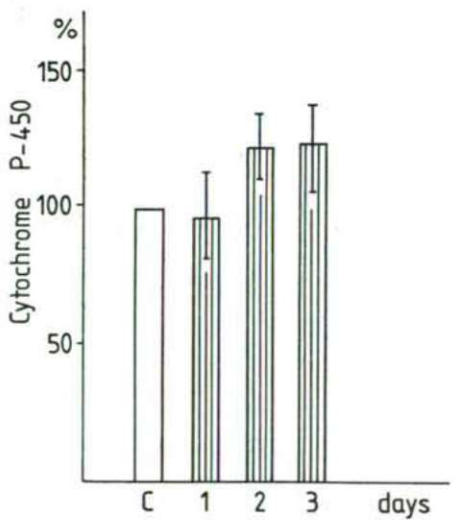

Fig. 4. The action of 0.5 ppm $CuSO_4$ on the level of cytochrome P-450 of carp liver after 24 hours of treatment.

EFFECT OF ULTRACIDE

Changes induced in the cytochrome P-450 concentration of carp liver by ultracide are shown in Fig. 5. After one day, the applied doses of this compound caused increases in the cytochrome P-450 level compared to the control values. After three and four days of ultracide treatment significant decreases were found in the cytochrome

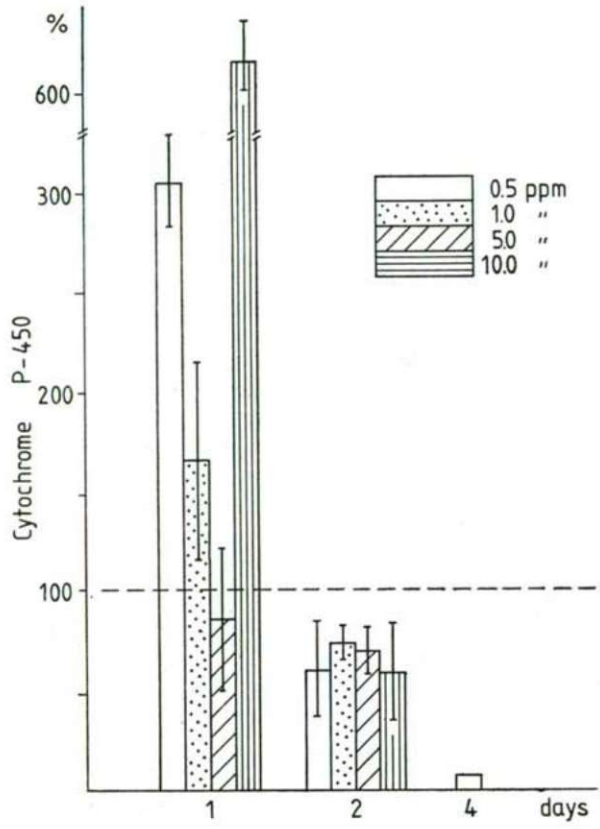

Fig. 5. Effect of ultracide in different concentrations on cytochrome P–450 of liver. The values are averages of those measured from 3–5 individuals and expressed in the percentage of the controls.

P-450 concentration. On the third day the cytochrome P-450 level was decreased to 75—80% as compared to the controls for all applied concentrations. After four days of treatment the presence of cytochrome P-450 could be shown only in fish treated with 0.5 ppm of herbicide, and this value was about 7% of the original. A higher concentration of the herbicide resulted in the total loss of this haemoprotein.

Discussion

Recently many reports have been published on the mechanism of paraquat action (MONTGOMERY, 1976; STEFFEN et al., 1980). Paraquat may alter the lipid metabolism and DNA and protein synthesis in the lung of rats. In an earlier paper we reported that paraquat could affect the glycogen metabolism of the liver in fish (SIMON et al., 1982). NEMCSOK et al. (1981) found morphological alterations in the liver of fish after paraquat treatment. Our results suggest that changes in the cytochrome P-450 level of liver treated with paraquat might be due to damage caused by this herbicide in the endoplasmic reticulum membrane.

As a result of studies in recent years it has become apparent that fish and other aquatic organisms are able to accumulate and retain trace elements from their environment. For both fish and mammals the liver is known to be an organ of considerable importance in the storage and uptake of metals (O'DELL and CAMPBELL, 1970) and it is also known to be the site of a number of detoxification functions. Metals can alter the enzyme activities of the liver. Lead, for example, is an inhibitor of the synthesis of haeme in the liver, inhibiting 5-aminolaevulinate dehydratase (SCOPPA et al., 1973).

Our results suggest that herbicides probably affect the synthesis of haeme in the liver or decrease the haeme oxidase activity, diminishing the turnover of haeme in the liver.

Some agents are known to decrease the affinity of the apoprotein of cytochrome P—450 for haeme, so that some of the haeme present bound in cytochrome P—450 at the time of poisoning will subsequently be released and made available for degradation (JÄRVISALO et al., 1978; BISSEL and HAMMAKER, 1976). It is possible that methidathion acts in the same way as the agents mentioned above.

Our results show that the applied herbicides can alter the cytochrome P-450 content of carp liver. Cytochrome P-450 is known to play an important role in the hydroxylation of various xenobiotics in the organism. Thus, every change in its concentration may result in a change of the liver's ability to metabolize xenobiotics in the body. However, it can not yet be stated for sure what causes the changes in cytochrome P-450 concentration. It is expected that future investigations will provide an answer to this question.

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