THE ROLE OF MICROSOMAL MIXED FUNCTION OXIDASES IN THE METABOLISM AND MECHANISM OF ACTION OF CERTAIN ORGANOPHOSPHORUS INSECTICIDES

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SUMMARY

The influence of microsomal mixed function oxidases (MFO) on the biotransformation of pesticides was investigated in experiments with perfusion of isolated rat livers and in whole animals. The pesticides examined were 3 organophosphorus insecticides (OP): S-propyl-O-phenyl-O-ethylthiophosphate (heterophos), a mixture of O,O-dimethyl-O-cyclohexylthionphosphate and O,S-dimethyl-O-cyclohexylthiol-phosphate (cyclophos), and dithiophosphonate. The indices of NADPH-dependent hydroxylating systems measured in the microsomal fraction of rat liver homogenates were the activity of aniline hydroxylase, aminopyrine demethylase and the amount of cytochrome P-450.

It was shown that not only did the specific chemical structure of the OP and induction or inhibition of MFO determine OP activation and detoxification (and thus its biological action), but also the route and frequency of exposure could influence these parameters.

KEY WORDS

organophosphorus insecticides, mixed function oxidases, metabolism, rat liver microsomes

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INTRODUCTION

Organophosphorus insecticides (OP) are widely used in agriculture, but may cause poisoning. Elucidation of the mechanism of their action, including the specificity of their biotransformation, allows an understanding of the basis for high or low toxicity, and grants chemists a foundation for the directed synthesis of substances acting as insecticides but with low toxicity for non-target warm-blooded species /1,2/. OP insecticides have a high reactivity and are readily involved in metabolic processes. Plurality of biotransformations is explained by the diversity of OP structures /2,3/. The major role in the metabolism of insecticides, including OP compounds, is played by cytochrome P-450-dependent monooxygenase systems /4-6/.

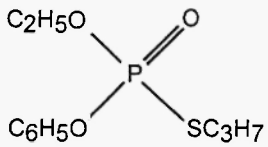
As a result of metabolism by the mixed function oxidase (MFO) enzymes, insecticides may acquire a greater polarity, contributing to their conjugation and elimination from the organism. An important property of MFO enzymes, their capacity for induction under the influence of xenobiotics, enables the biochemical adaptation of organisms to repeated exposure to toxic substances. However, in some cases, as a result of metabolic enhancement, more toxic or mutagenic or carcinogenic compounds may appear /7/. Therefore, the assessment of MFO induction or inhibition should be examined in the light of the particular prevailing conditions such as the structure of the compounds, regime of administration and the route of exposure.

This paper reports on the interaction of MFO enzymes with OP insecticides and on the role of induction and inhibition of these enzymes in the metabolism and toxicity of the compounds investigated.

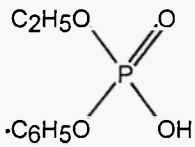
MATERIALS AND METHODS

The influence of MFO on the biotransformation of OP insecticides was investigated by perfusion of isolated male rat liver *in situ* /8/ and in whole animals *in vivo*. The three insecticides used were S-propyl-O-phenyl-O-ethylthiophosphate (heterophos), dithiophosphonate, and a mixture of O,O-dimethyl-O-cyclohexylthionphosphate and O,S-dimethyl-O-cyclohexylthiolphosphate (cyclophos). The structures of these compounds and their metabolites are shown in Figures 1 and 2.

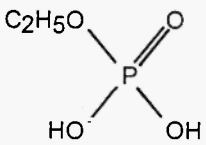
Heterophos and dithiophosphonate metabolism was studied *in situ* under conditions of MFO induction and inhibition. Donor rat blood was diluted with a Ringer-Lock solution (4:1) to make a 40 ml



HETEROPHOS



METABOLITE 1



METABOLITE 2

Fig. 1: The chemical structure of heterophos and its metabolites.

perfusate. Cannulas were inserted into the portal vein, inferior vena cava and the bile duct. A peristalsis pump (artificial heart) ensured a constant velocity of perfusion. The blood was saturated in an oxygenator (artificial lungs). Heterophos was added to the perfusate at 18 μg/ml and dithiophosphonate at 40 μg/ml. The perfusate was sampled after 5, 15, 30 and 45 minutes and the concentration of the parent compounds and their metabolites was determined by gas and thin layer chromatography /9,10/. Bile was also sampled during perfusion. Livers were examined for residues immediately after the perfusion was terminated. The MFO inhibitor was tetramethylthiuram disulphide and the inducer was mitran /11,12/. Mitran was administrated to male rats on an empty stomach on three consecutive days at a dosage of 60 mg/kg. On the fifth day the MFO activity and toxicity of the substances were examined. Tetramethylthiuram disulphide was administrated to male rats, on an empty stomach, three times: in the morning and evening of the first day, and in the morning of the second day of investigation. On the third day, the above mentioned indices were investigated. The MFO activity of male rats was measured *in vivo* after administration of cyclophos:

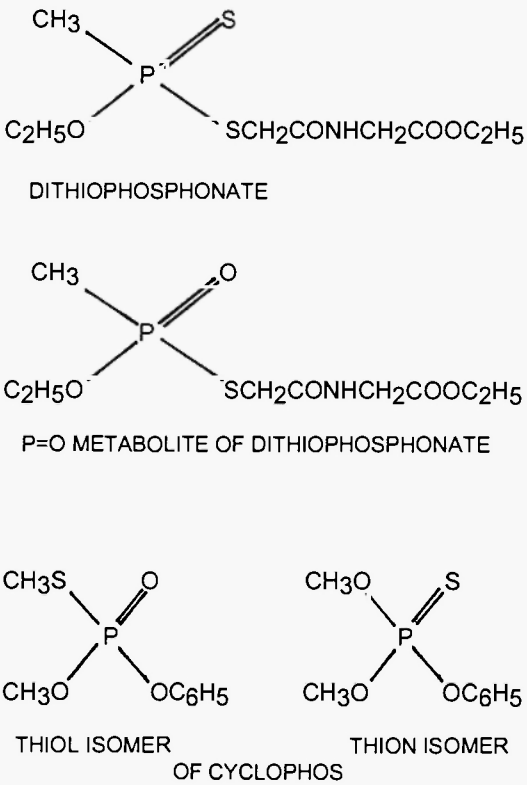


Fig. 2: The chemical structure of dithiophosphonate and its metabolites, and cyclophos (a mixture of thiol and thion isomers).

a) *per os* - at a dosage of 315 mg/kg - once; 126 mg/kg - daily for 3 days; 63 mg/kg (0.1 LD_{50}), 6.3 mg/kg (0.01 LD_{50}) and 0.63 mg/kg (0.001 LD_{50}) repeatedly for 4 months. MFO activity was measured in the liver;
b) by inhalation at a concentration of 19 mg/m^3 for 30 days or intermittently (7 days on, 7 days off). MFO activity was measured in liver and in lungs.

Standard methods were used for determination of cytochrome P-450 /13/, aniline hydroxylase /14/, aminopyrine N-demethylase /15/, and protein /16/. Data were analysed using Student's t-test; $p<0.05$ was taken as the significance level.

RESULTS AND DISCUSSION

Many thioorganophosphorus compounds are subject to oxidative desulphuration, catalysed by MFO. They are transformed into more active anticholinesterase agents, as a rule of higher toxicity. At the same time, MFO enzymes are able to enhance detoxification of organophosphorus compounds. The ultimate order of toxicity is defined by the ratio of the velocities of the activation and detoxification processes.

Dithiophosphonate is metabolised with the formation of the desulphuration product (P=0 metabolite), found in the perfusate. This compound has a high toxicity (LD_{50} 3.3 mg/kg) and pronounced anticholinesterase activity [K_2 = (0.172 ± 12) x 10^{-5} l/M.min]. When the MFO were inhibited, cholinesterase inhibition was less marked (Fig. 3) and the P=O metabolite was found in the liver at only 48% of levels when no inhibitor was given (Fig. 4). Inhibition of MFO caused the toxicity of dithiophosphonate to be lowered by 50%. When MFO induction was produced, there was no change in the toxicity of dithiophosphonate.

Heterophos was poorly detoxified in the liver and was found at relatively high concentrations in the liver after perfusion. Figures 5-7 depict the MFO induction which led to lower levels of heterophos and higher levels of the two metabolites in the perfusate. Consequently, the acute toxicity was reduced (LD_{50} of heterophos is 35 mg/kg, whereas after induction the LD_{50} was 100 mg/kg) and there was less acetylcholinesterase inhibition.

The different responses seen by these two insecticides to MFO inhibition and induction may be explained by structural differences. The metabolism of dithiophosphonate occurs mainly through desulphuration (conversion of P=S into the P=O analogue). The metabolism of substances like heterophos is of special interest because within this class of compounds (thiopropyl derivates of acids of phosphorus) are found several effective and selective insectoacaricides.

It was shown /17/ that the process of detoxification in most cases was the splitting off of the thiopropyl group (P-SC_3H_7) from the OP molecule, depending on the structure of the aryl group (P-OAr); this reaction is more characteristic in warm-blooded animals than in insects. Heterophos is metabolised mainly by breaking the P-OC_6H_5 bond. This manner of metabolism may be responsible for its high toxicity. It cannot be excluded that heterophos activation, as a result of oxidation

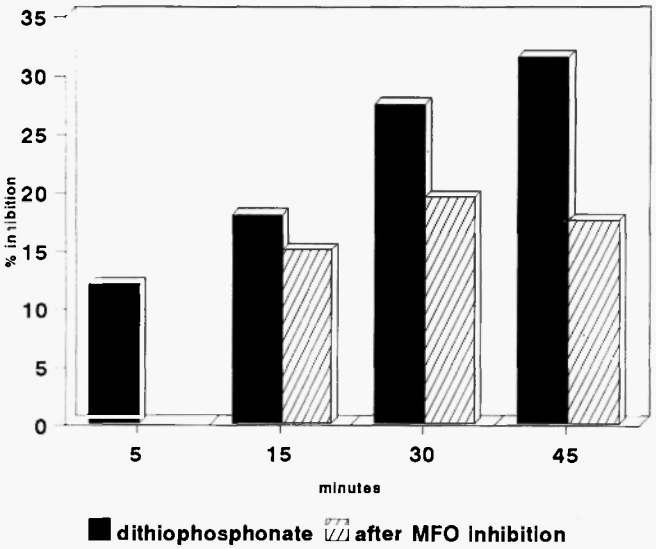


Fig. 3: Cholinesterase inhibition in perfusate before and after MFO inhibition.

Fig. 4: Concentration of dithiophosphonate P=O metabolite before and after MFO inhibition.

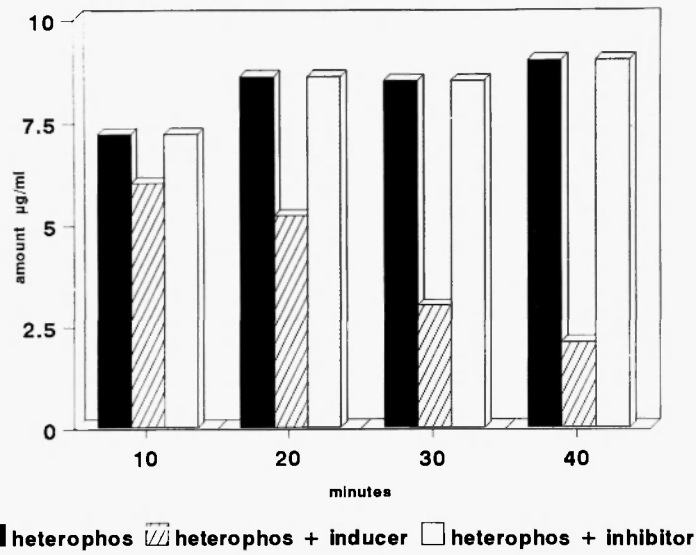


Fig. 5: Amount of heterophos in rat liver perfusate.

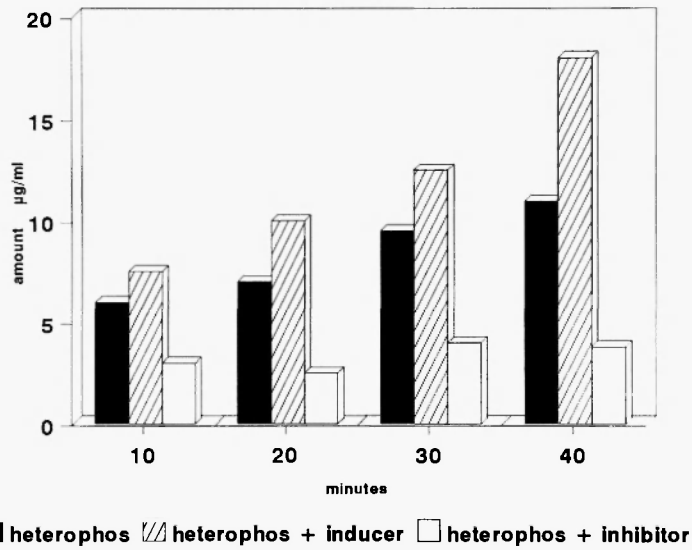


Fig. 6: Amount of heterophos metabolite 1 in rat liver perfusate.

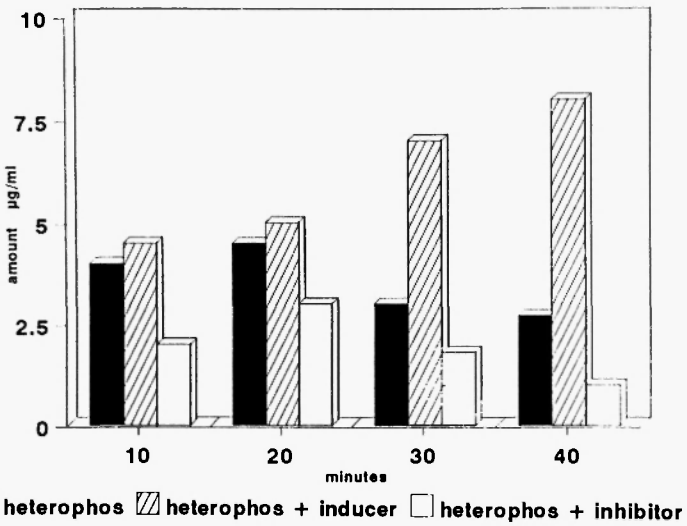


Fig. 7: Amount of heterophos metabolite 2 in rat liver perfusate.

of the sulphur atom of the thiopropyl group, may occur. Some data confirm the possibility of oxidizing activation of S-propyl compounds and point out the stereo-specificity of this process /18,19/. The results from this work permit supposition that MFO induction leads to a primary breaking-off of the P-SC_3H_7 bond of the heterophos molecule and may explain the lowering of toxicity under the influence of the MFO inducer.

There exists convincing proof that diverse cytochrome P-450 isoforms participate in the metabolism of xenobiotics /7,20,21/. The role of cytochrome P-450 and cytochrome P-450 isozymes in breaking the P-S and S-C bonds during the biotransformation of thiopropyl compounds has been reported /17,18/. However, the participation of diverse cytochrome P-450 isoforms in the metabolism of OP compounds and OP insecticides in particular needs to be further investigated.

OP compounds are not only subject to the effect of MFO enzymes but are able to affect the enzyme activity themselves. In many cases there is an inhibitory effect /23,24/ but the subdivision of OP into inducers and inhibitors is so far provisional since the effect depends on the dose, duration and regimes of exposure /25,26/, as was seen in these experiments with cyclophos.

After oral administration of cyclophos once at a dosage of 0.5 LD_{50} (315 mg/kg), MFO activity was inhibited for 15 days. When given thrice at a dosage of 0.2 LD_{50} (126 mg/kg), changes in the phase character were revealed with all three enzymes (Figs. 8-10); after one day, inhibition was evident, after 7 days induction was found and at 15 days normal values were noted. Cyclophos also inhibited MFO activity when administered by inhalation daily for 30 days. However, the intermittent exposure (7 days on, 7 days off) led to MFO induction in both liver and lungs (Figs. 11,12). The chronic exposure (for 4 months) of cyclophos administered *per os* at 63 mg/kg, 6.3 mg/kg and 0.63 mg/kg (0.1 LD_{50}, 0.01 LD_{50}, 0.001 LD_{50}) led to a stable (for 1-2 months) inhibition of aminopyrine demethylase activity (Table 1). The primary inhibitory effect of cyclophos on the MFO enzymes may be connected with the particular chemical structure of cyclophos, which includes a thion isomer as well as the thiol isomer. It is known that compounds containing thion groups inhibit cytochrome P-450 /22/.

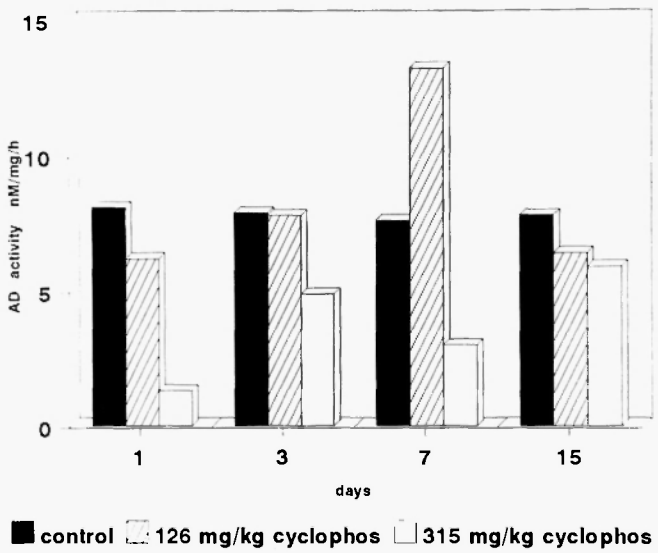


Fig. 8: Aminopyrine demethylase activity in rat liver after cyclophos given *per os*.

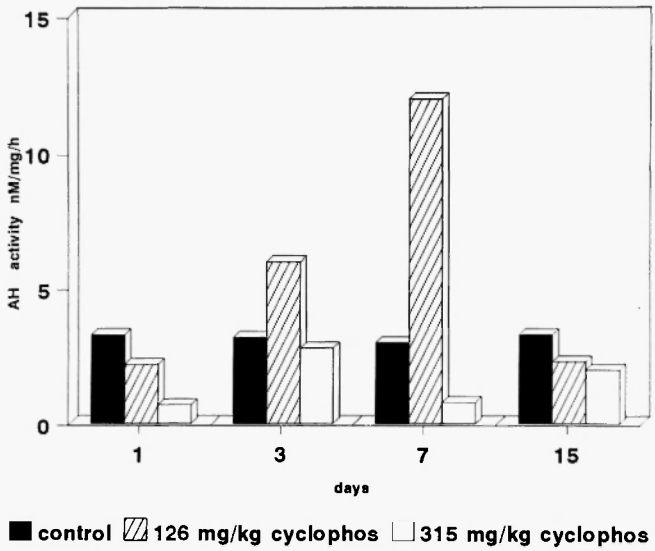


Fig. 9: Aniline hydroxylase activity in rat liver after cyclophos given *per os*.

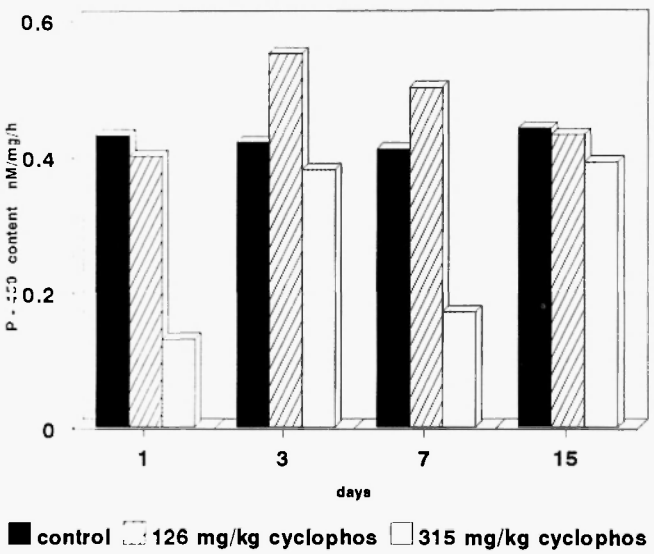


Fig. 10: Cytochrome P-450 content in rat liver after cyclophos given *per os*.

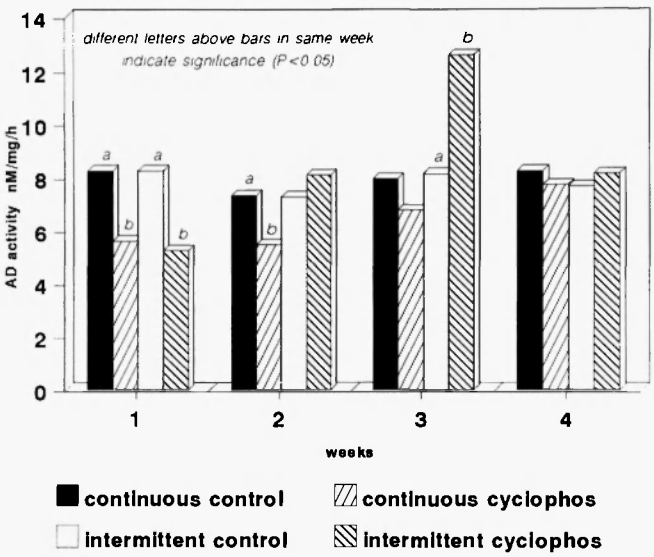


Fig. 11: Aminopyrine demethylase activity in rat liver after cyclophos inhalation. Different letters above bars at the same time indicate significance ($p<0.05$).

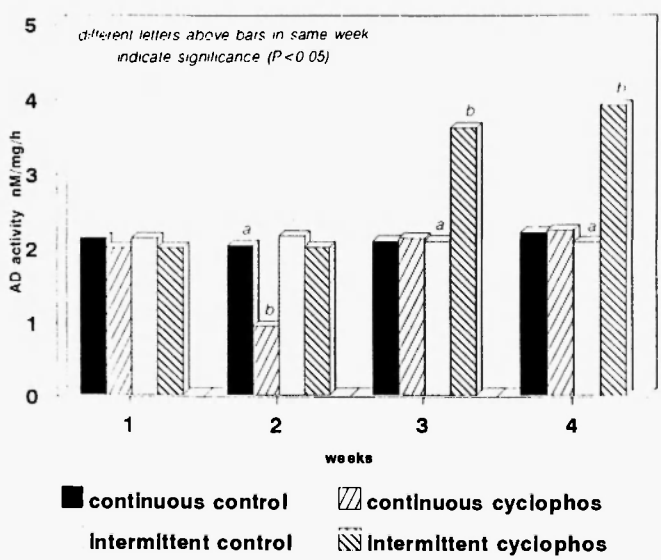


Fig. 12: Aminopyrine demethylase activity in rat lung after cyclophos inhalation. Different letters above bars at the same time indicate significance ($p<0.05$).

TABLE 1

Influence of chronic oral administration of cyclophos on aminopyrine demethylase activity in rat liver microsomes

Length of investigation (months)	Aminopyrine demethylase activity, nM/mg/h					
	63 mg/kg		6.3 mg/kg		0.63 mg/kg	
	Control	Cyclophos	Control	Cyclophos	Control	Cyclophos
1	9.9 ± 0.5^a	6.3 ± 0.5^b	9.9 ± 0.5^a	7.1 ± 0.9^b	10.2 ± 0.2^a	11.9 ± 0.7^a
2	8.5 ± 0.2^a	5.3 ± 0.3^b	10.2 ± 0.2^a	9.0 ± 0.6^a	9.5 ± 0.3^a	5.6 ± 0.5^b
4	11.6 ± 0.4^a	10.7 ± 0.7^a	-	-	8.4 ± 0.6^a	9.0 ± 0.5^a

Different superscripts in the same row for each time and dosage group indicate significance ($p<0.05$)

Further investigations of P-450-dependent monooxygenase system interactions with OP will be useful for revealing:

a) substances metabolising with primary detoxification in warm-blooded organisms, in contrast to insects (selective toxicity);

b) new synergists of insecticides with metabolic products less toxic than those of the initial compounds;

c) MFO enzyme inducers which may be effectively used in combination with cholinolytics and cholinesterase reactivators for accelerating and enhancing detoxification in the therapy of OP poisoning.

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