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Toxicology

Effects of several organophosphates on hepatic cytochrome P450 activities in rats

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ABSTRACT. Four commonly used organophosphates (fenitrothion, dichlorvos, chlorpyrifos, and trichlorfon) were orally administered to male Sprague-Dawley rats for five days in order to explore their effects on the activities of liver cytochrome P450 (CYP). In addition, Michaelis-Menten kinetics of the metabolic reactions catalyzed by liver CYPs were analyzed following the addition of these compounds to the assay system to examine their potential inhibitory effects on liver CYPs activities. These reactions included ethoxyresorufin O-deethylation, midazolam 4-hydroxylation, tolbutamide hydroxylation, and bufuralol 1′-hydroxylation for CYP1A, 3A, 2C, and 2D activities, respectively. Total CYP content was also examined after oral administration of each organophosphate. Results revealed that oral giving of fenitrothion inhibited significantly CYP1A and 3A activities while elevated activity of CYP2C. Fenitrothion is a potent inhibitor for CYP1A and 2C with Ki values of 0.42 and 36.1 μM, respectively but had a weak inhibitory effect on CYP2D and 3A with Ki values of 290 and 226 μM, respectively. Chlorpyrifos is a potent inhibitor of CYP1A with Ki 0.24 μM and moderately inhibited CYP2C or 3A with Ki values of 84.8 and 77.7 μM, respectively. On the other hand, dichlorvos and trichlorfon caused extremely low or negligible inhibition of different CYP activities. From these results, it is concluded that both fenitrothion and chlorpyrifos may increase the toxicity of chemicals in environmental living organisms through their potent inhibitory effects on these CYP activities, but dichlorvos and trichlorfon may not.

KEY WORDS: cytochrome P450, induction, inhibition, organophosphate, rat

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It is well recognized that cytochrome P450s (CYPs) have an important role in the biotransformation of many xenobiotics, including drugs and environmental chemicals. Although CYP catalyzing reactions detoxify many xenobiotics, they convert some xenobiotics to reactive metabolites that are carcinogenic.

The activities CYP may be altered by drugs and other chemicals. The alteration may result in potentiated toxicity or carcinogenicity of some xenobiotics. Therefore, the effects of xenobiotics on CYP activities are a matter of great concern in both medical and toxicological fields. Up to now, drug-related alteration in CYP activities (enzyme induction and inhibition) have been extensively studied by many researchers. In addition, significant drug-drug interaction resulted from altered CYP activities has been also demonstrated. However, there are few studies on the effects of other classes of xenobiotics on CYP activities.

The effects of environmental chemicals on CYP activities may be also a matter of concern. Previously, therefore, we checked the impacts of some pyrethroids on hepatic CYPs activities [2]. As a result, it was demonstrated that bifenthrin and etofenprox were inducers of CYP1A, while fenpropathrin and permethrin were a potent inhibitor of CYP1A [2].

Organophosphorus compounds are organic esters of phosphorus-based acid derivatives, most widely used as a pesticide in the world. Exposure to organophosphate, therefore, may be ubiquitous [3, 7, 14]. At the end of the 1970s, the organophosphates (OPs) usage had overtaken the organochlorine insecticides as their residues don't persist in the environment after a few months. In particular, in developing countries, OPs are used more often because they are cheaper than newer alternatives [18].

Some OPs may also affect CYP activities like pyrethroids reported in the previous paper [2]. Also, Lapadula *et al*. found a 2–3-fold increase of CYP content in hepatic microsomes from hen treated with tribufos [22]. This finding suggests that CYP activities

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may be altered by the OPs. Therefore, the effect of OPs on CYP activities should be also clarified. This kind of information is, however, still insufficient. The current study was conducted to check the possible impacts of various organophosphates (fenitrothion, dichlorvos, chlorpyrifos, and trichlorfon) on CYP activities in rats.

MATERIALS AND METHODS

Animals

In total, 60 male Sprague-Dawley rats were collected from Japan Cler inc. (Tokyo, Japan) and allowed to acclimatize to their environment for at least one week prior to the experiment. Their age and bodyweight were 9 weeks and 270–320 g, respectively at the start of the experiment. The rats were housed in stainless-steel cages (five per cage). The housing temperature and humidity were 25°C and 55–70%, respectively with a 12 hr light-dark cycle. They were allowed free access to water and feed. All experiments were conducted according to the guidelines for the care and use of laboratory animals and approved by the ethical committee of animal experiment, Tokyo University of Agriculture and Technology.

Chemicals

Fenitrothion, dichlorvos, chlorpyrifos, and trichlorfon were obtained from Wako Pure Chemical Co. (Osaka, Japan). Both ethoxyresorufin and resorufin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Midazolam, 4-hydroxymidazolam, tolbutamide, hydroxytolbutamide, bufuralol and 1'-hydroxybufuralol were obtained from Daiichi Chemical Co. (Tokyo, Japan). All other chemicals were of HPLC grade.

Experimental design

Effects of organophosphates on hepatic CYP activities after oral administration: Effects of fenitrothion, dichlorvos, chlorpyrifos or trichlorfon on activities of CYP1A, 2C, 2D and 3A subfamilies were examined in rats after oral administration for five days. For each tested compound, 15 rats were used after being divided into three groups (five of each); the first group was served as control and administered vehicle (corn oil or carboxymethyl cellulose), the other two groups were orally administered an OP at one-tenth or one-thirtieth of their LD50. As a result, used doses were as follows; 15 and 50 mg/kg for fenitrothion, 1.5 and 5 mg/kg for dichlorvos, 3 and 10 mg/kg for chlorpyrifos, and 5 and 15 mg/kg for trichlorfon. After 24 hr of the final dosing, rats were sacrificed under deep anesthesia with isoflurane, and the liver was isolated and instantly perfused with ice-cold buffer (0.2 mM sodium EDTA, 1.15% KCl, 0.1 mM phenyl methyl sulphonyl fluoride, 0.1 mM dithiothreitol, and 20% glycerol) until the efflux perfusion buffer became free from blood. Then, the liver was sliced into pieces and stored at −80°C until preparation of microsomes.

Inhibition study: In the present study, the inhibitory effects of the tested OPs on CYP1A, 2C, 2D, and 3A activities were also examined using the hepatic microsomes obtained from the control groups of the above experiment. Each OP was dissolved in dimethyl sulfoxide (DMSO) then added to the enzyme assay system in a volume of 10 μl. The final concentrations of OPs for inhibitor were decided ranged from 0.1–1,000 μM in the assay system. In preliminary study, the concentration of the inhibitor that reduced V_{max} by about half was obtained for each assay system less than 1,000 μM.

Preparation of hepatic microsomes

The microsomal fractions were prepared from the liver specimens using the differential centrifugation method described before [27]. The obtained microsomal suspension was stored at −80°C until being used. The concentrations of protein and contents of CYP were measured as reported before, [5] and [24], respectively.

Assays of CYP enzymes activities

The activities of CYP1A, 3A, 2C, and 2D enzymes were assessed using ethoxyresorufin O-deethylation (EROD), midazolam 4-hydroxylation (MDZH), tolbutamide hydroxylation (TBH), and bufuralol 1'-hydroxylation (BLH), respectively. The reaction proceeded at 37°C in a reaction mixture that contained NADPH generating system (0.5 mM β-$NADP^+$, 50 mM phosphate buffer (pH 7.4), 1.5 U/m*l* glucose 6-phosphate dehydrogenase, 5 mM glucose-6-phosphate, and 5 mM MgCl2), liver microsomes (0.03 mg/m*l* for EROD and 0.4 mg/m*l* for other reactions), and a substrate at various concentrations in a total volume of 0.25 m*l*, except for EROD where total volumes of 1 m*l* was used. Prior to starting the reaction by adding the substrate, a 5-min pre-incubation period at 37°C was done.

Determination of CYP1A activity: The metabolite of ethoxyresorufin, resorufin, was measured using the fluorometric method [6]. The concentration of ethoxyresorufin in the assay system was 2 μM, which is approximately 10-fold higher than the Km value (0.227 μM) reported in rats [13]. In the inhibition study, concentrations of ethoxyresorufin in the assay system ranged from 0.82 to 4.1 μM. The reactions were terminated by addition of 3 m*l* of methanol 15 min after the substrate was added, followed by placement on ice for 5 min. Following centrifugation for 5 min at 2,000 *g*, one m*l* of the supernatant was taken and diluted with four m*l* of methanol in a clean tube and applied to a spectrofluorometer (RF-1500; Shimadzu Co., Kyoto, Japan). The wavelengths of excitation and emission were adjusted at 550 and 586 *n*m, respectively. The detection limit was 0.05 nm at a signal-to-noise ratio of 3. The recovery of resorufin was 103 ± 6% at 20 nM (n=4). The intraday CV values were 3.7% and 5.1% at 20 and 400 nM (n=4), respectively. The inter-day CV values ranged from 3.4% to 6.4% and 1.6% to 5.1% at 20 and 400 nM, respectively (3 days, 4 determinations/day).

Determination of CYP2C activity: The metabolite of tolbutamide, hydroxytolbutamide, was measured using HPLC with UV

detection [23]. The concentration of tolbutamide in the assay system was 10 mM, which is approximately 5-fold higher than the Km value (1.91 mM) reported in rats [11]. In the inhibition study, concentrations of tolbutamide in the assay system ranged from 1 to 10 mM. Following the addition of tolbutamide, the assay system was incubated for 30 min, and the reactions were terminated by adding 0.25 m*l* of 0.15 M phosphoric acid. Chlorpropamide was used as an internal standard. The samples were mixed with 3 m*l* of diethyl ether. After centrifugation at 2,000 *g* for 5 min, the upper organic layer was transferred to a clean pear-shaped flask and evaporated until dryness under reduced pressure. The residue was reconstituted with 500 μl of the mobile phase, then 50 μl of the solution was injected into a C_{18} column (TSK-gel ODS-120, 4.6 × 250 mm; TOSOH Co., Tokyo, Japan). The column effluent was monitored by UV absorbance at 230 nm. The mobile phase consisted of 50 mM phosphate buffer (pH 4.3) and acetonitrile (75:25, v/v). The flow rate was one m*l*/min. The recoveries of hydroxytolbutamide were 91.2 ± 1.2% at 1 μg/m*l* (n=4). The intra-day CV values were 1% and 4% at 0.1 and 1 μg/m*l*, respectively (n=4). The inter-day CV values ranged from 1.9% to 8.2% and 1.3% to 6.3% at 0.1 and 1 μg/m*l*, respectively (4 times/day for three days). The quantification limit was 20 *n*g/m*l* at a signal-to-noise ratio of 3.

Determination of CYP2D activity: The metabolite of bufuralol, 1'-hydroxybufuralol, were analyzed using HPLC with fluorometric detection [20]. The concentration of bufuralol in the assay system was 50 μM, which is approximately 10-fold higher than the Km value (6.4 μM) reported in rats [8]. In the inhibition study, concentrations of bufuralol in the assay system ranged from 4 to 134 μM. Following the addition of bufuralol, the assay system was incubated for 10 min, and the reaction was halted by adding 30 μl of 60% perchloric acid. The denatured protein was precipitated by centrifugation at 10,000 *g* for 2 min, then 50 μl of the supernatant was injected into the C_{18} column. The mobile phase composed of acetonitrile and 1 mM perchloric acid (35:65, v/v). The flow rate was set at one m*l*/min. Excitation and emission wavelengths were 252 and 302 *n*m, respectively. Recovery of the metabolite was 91.4 ± 3.0% at 0.1 μg/m*l* (n=4). The intraday CV values were 1.8% and 2.1% at 0.1 and 1 μg/m*l*, respectively (n=4). The inter-day CV values ranged from 1.9% to 7.9% and 1.9% to 3.1% at 0.1 and 1 μg/m*l*, respectively (3 days, 4 determinations/day), with detection limit of 5 *n*g/m*l* at a signal-to-noise ratio of 3.

Determination of CYP3A activity: The metabolite of midazolam, 4-hydroxymidazolam, was determined using HPLC with UV-detection as described by [21]. The concentration of midazolam in the assay system was 214 μM, which is approximately 10-fold higher than the Km (22 μM) reported in rats [19]. In the inhibition study, concentrations of midazolam in the assay system ranged from 15 to 214 μM. Following midazolam addition, the mixture was incubated for 10 min, and the reaction was ceased by adding 0.25 m*l* of acetonitrile and put on ice for three min After centrifugation at 10,000 *g* for 2 min, the resulting supernatant was applied to the C_{18} column. The mobile phase formed of 100 mM acetate buffer (pH, 4.7), methanol, and acetonitrile (59.4:5.6:35, v/v/v, respectively). A UV detector wavelength of 254 nm and a flow rate of one m*l*/min were used to monitor the column effluent. The limit of detection of 4-hydroxymidazolam was 2.5 *n*g/m*l*, at a signal-to-noise ratio of 3. The recovery of 4-hydroxymidazolam was 102 ± 3.4% at 1 μg/m*l* (n=4). The intra-day CV values were 1% and 3.9% at 0.1 and 1 μg/m*l*, respectively (n=4). The inter-day CV values ranged from 0.2% to 0.5% and 2.6% to 8.5% at 0.1 and 1 μg/m*l*, respectively (3 days, 4 determinations/day).

Michaelis–Menten kinetic analysis: The following equation was applied to the relation between reaction velocities (V) and substrate concentrations (S) to analyze the enzyme kinetics;

$$V = \frac{V_{max} \times S}{K_m + S}$$

In this equation, V_{max} and K_m represent maximal reaction velocity and Michaelis–Menten constant, respectively.

Since all the tested OPs inhibited the enzymes reactions in a non-competitive manner, the kinetic profile of enzyme inhibition was expressed by the following equation;

$$V = \frac{V_{max} / \left(1 + \frac{I}{k_i}\right) \times S}{K_m + S}$$

where K_i and I are the inhibition constant and inhibitor concentration, respectively.

The two curves, which were obtained with or without inhibitors in each reaction, were simultaneously analyzed using the fitting program MULTI [28] to calculate V_{max}, K_m, and K_i.

Statistical analysis

Data of treated groups were compared with those of control group to show the significant induction of hepatic CYPs by orally administered OPs. After confirming equal variances by Bartlett's test, ANOVA was done. If ANOVA showed statistical differences, the differences in averages among groups were analyzed by Sheffe's multiple comparisons. If unequal variances were obtained from Bartlett's test, the nonparametric Kruskal-Wallis test was used for comparison between treatment and control groups. Significance between groups was accepted when $P \leq 0.05$.

RESULTS

Effects of oral organophosphates on hepatic CYP activities

Figure 1 shows the effects of several OPs on the hepatic microsomal activities of CYP1A, 2C, 2D, and 3A after oral administration of each OP for five consecutive days. Microsomal activities of EROD and MDZH were significantly lower in groups

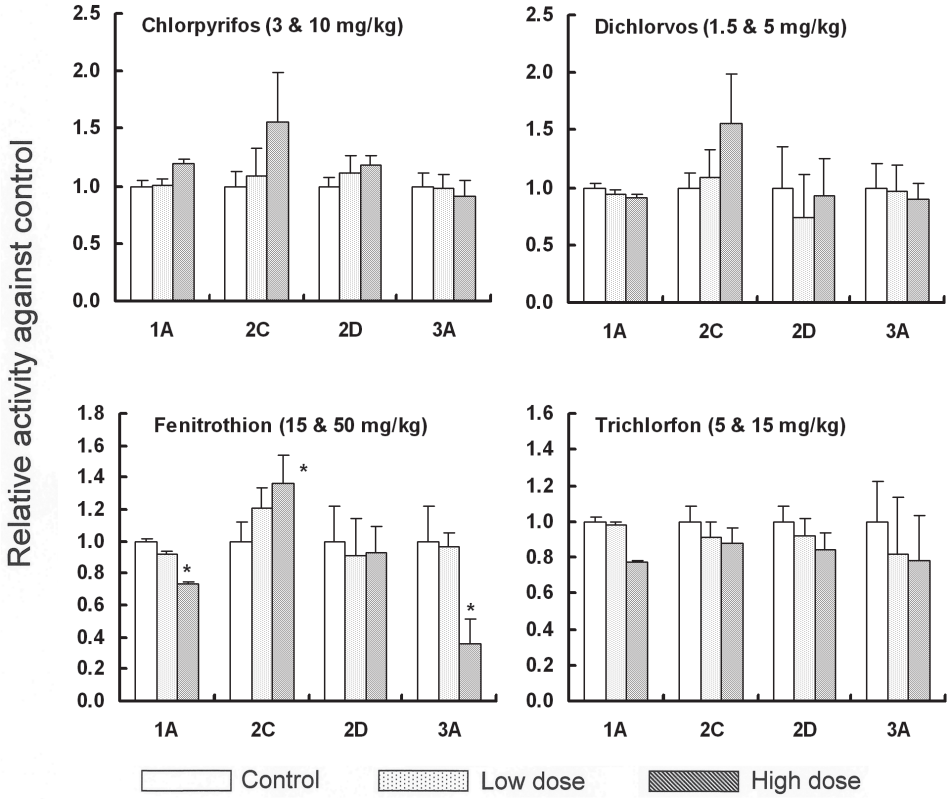

Fig. 1. Effects of organophosphates on metabolic reactions catalyzed by cytochrome P450 1A, 2C, 2D, and 3A after oral administration of each organophosphate for 5 days to rats. Bars represent relative reaction activities against control; error bars indicate SD of the mean (n=5), * $P \leq 0.05$.

Table 1. Total hepatic microsomal cytochrome P450 contents in male SD rats before and after oral administration of organophosphorus in low and high doses. Each value is represented by the mean ± SD (n=5)

Organophosphate	Total cytochrome content (nM/mg protein)		
	Control	Low dose	High dose
Fenitrothion	1.52 ± 0.86	1.38 ± 0.51	1.18 ± 0.23
Dichlorvos	1.56 ± 0.32	1.69 ± 0.40	1.75 ± 0.18
Chlorpyrifos	2.04 ± 0.39	1.69 ± 0.18	1.30 ± 0.48[a)]
Trichlorfon	2.00 ± 0.25	1.63 ± 0.22	1.42 ± 0.10[a)]

a) $P \leq 0.05$.

treated with fenitrothion, while those of TBH was significantly higher than the control group. The rest of the OPs (dichlorvos, chlorpyrifos, and trichlorfon) showed no significant effects on examined CYP activities. As shown in Table 1, the total CYP content was significantly decreased by oral treatment with chlorpyrifos and trichlorfon at a high dose, respectively. On the other hand, the content was not significantly affected by the treatment with fenitrothion or dichlorvos.

Inhibitory effects on CYP activities

The OPs examined in the current study inhibited CYP activities in a non-competitive manner. As shown in Fig. 2, fenitrothion and chlorpyrifos evidently inhibited EROD at less than 1 μM. Their K_i values were calculated to be less than 1 μM (Table 2). Although they also inhibited TBH, BLH and MDZH, the effect was derived by much higher concentration, compared with those in EROD (Figs. 3–5). The K_i values were much higher than those for EROD. In cases of dichlorvos and trichlorfon, their inhibitory effect on EROD was evident at quite high concentrations. However, their inhibitory effects on the other reaction were not evident even at 1,000 μM (Figs. 2–5). Corresponding to these observations, their Ki values were quite high for all reactions examined (Table 2).

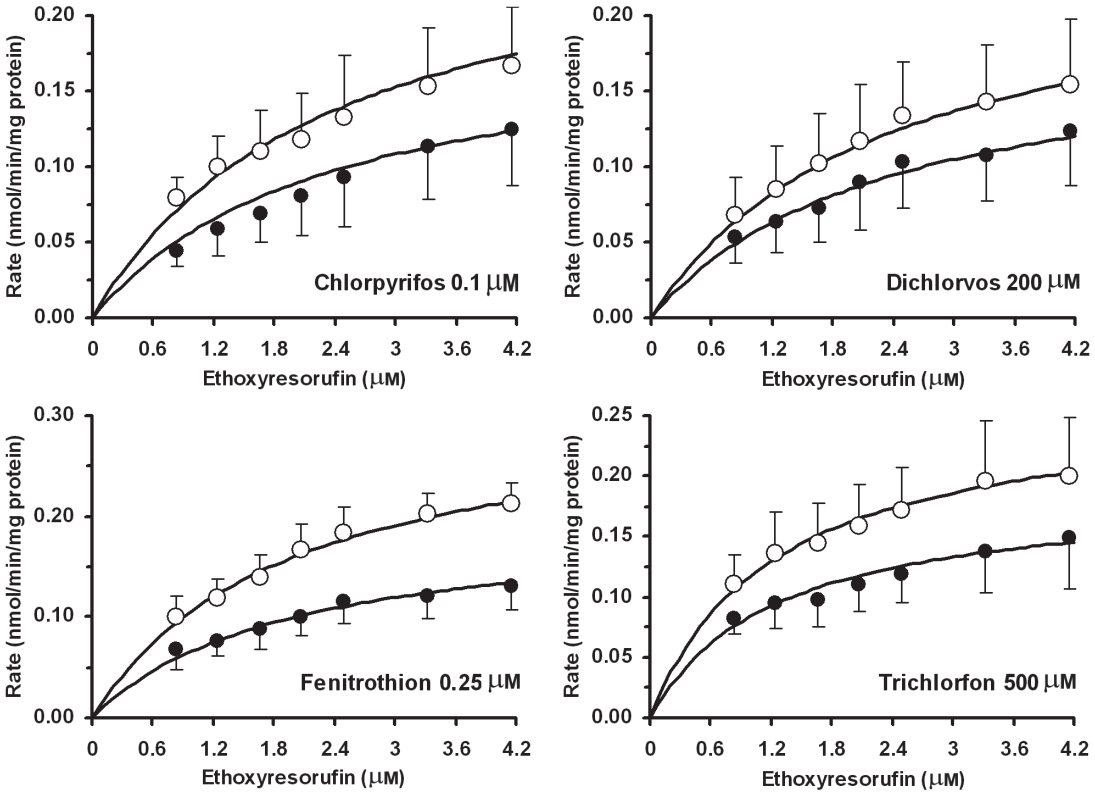

Fig. 2. Michaelis-Menten kinetics of ethoxyresorufin O-deethylation (EROD) in hepatic microsomes from rats. The solid curves represent the theoretical metabolic rates calculated using *Vmax* and *Km* values showed in Table 2. Closed and opened circles represent the observed metabolic rates (mean ± SD) in the presence or absence of organophosphates, respectively.

Table 2. Michaelis–Menten kinetic parameters for several metabolic reactions catalyzed by cytochrome P450 (CYP) subfamilies in hepatic microsomes of male SD rats after addition of organophosphorus to the incubation system of each CYP isoenzyme

Organophosphate	Reaction velocity (nM/min/mg protein)			
	EROD	TBH	BLH	MDZH
Fenitrothion				
Vmax	0.32 ± 0.04	1.01 ± 0.22	0.94 ± 0.14	0.51 ± 0.06
Km (μM)	1.95 ± 0.74	7.97 ± 1.60	6.70 ± 1.77	24.2 ± 5.44
Ki (μM)	0.42 ± 0.11	36.1 ± 12.6	290 ± 45.8	226 ± 43.2
Dichlorvos				
Vmax	0.24 ± 0.06	0.93 ± 0.30	0.81 ± 0.06	0.53 ± 0.07
Km (μM)	2.33 ± 0.61	8.85 ± 2.14	4.91 ± 0.98	35.5 ± 4.10
Ki (μM)	657 ± 70	>5,000	>5,000	>5,000
Chlorpyrifos				
Vmax	0.27 ± 0.09	1.68 ± 0.31	0.70 ± 0.11	0.59 ± 0.11
Km (μM)	2.34 ± 0.53	7.56 ± 2.12	4.43 ± 0.73	29.9 ± 4.54
Ki (μM)	0.24 ± 0.04	84.8 ± 19.4	1,446 ± 362	77.7 ± 17.2
Trichlorfon				
Vmax	0.26 ± 0.08	1.64 ± 0.25	0.69 ± 0.12	0.65 ± 0.18
Km (μM)	1.24 ± 0.05	7.96 ± 2.58	3.10 ± 0.74	29.6 ± 5.66
Ki (μM)	1,252 ± 148	>5,000	>5,000	>5,000

Each value is represented by the mean ± SD (n=5). *Vmax* (nM/min/mg protein), *Km* (μM), and *Ki* (μM) were estimated using a nonlinear least square fitting program. EROD, ethoxyresorufin O-deethylation; BLH, bufuralol 1'-hydroxylation; TBH, tolbutamide hydroxylation; MDZH, midazolam 4-hydroxylation.

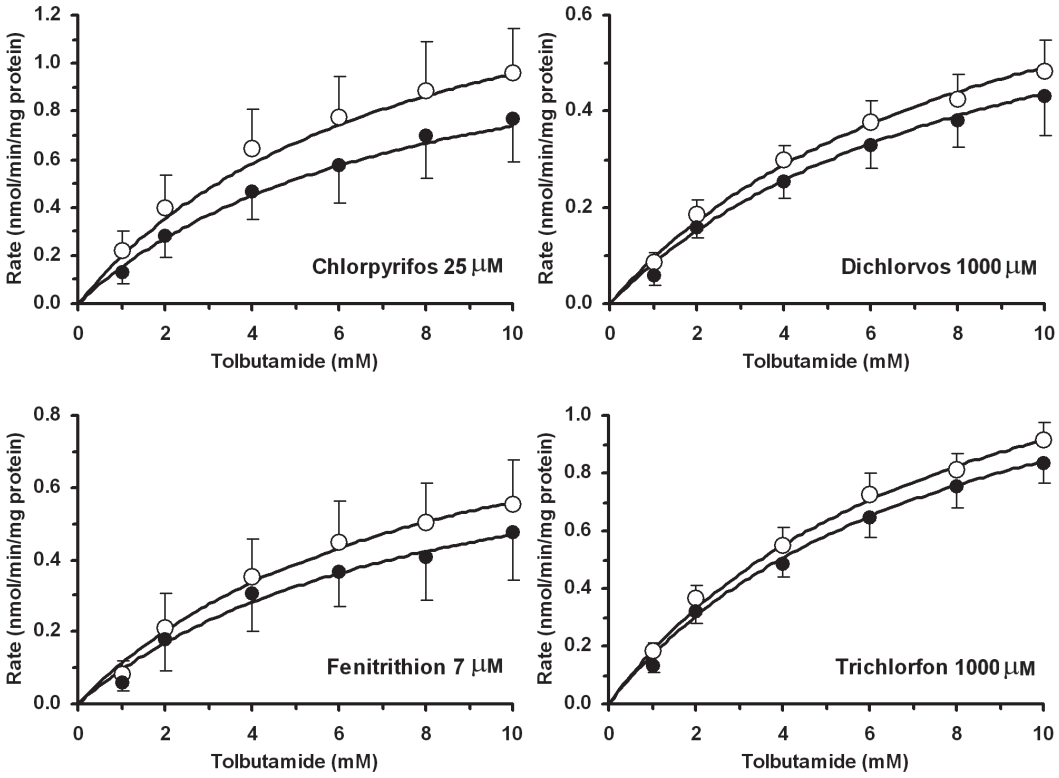

Fig. 3. Michaelis-Menten kinetics of tolbutamide hydroxylation (TBH) in hepatic microsomes from rats. The solid curves represent the theoretical metabolic rates calculated using *Vmax* and *Km* values showed in Table 2. Closed and opened circles represent the observed metabolic rates (mean ± SD) in the presence or absence of organophosphates, respectively.

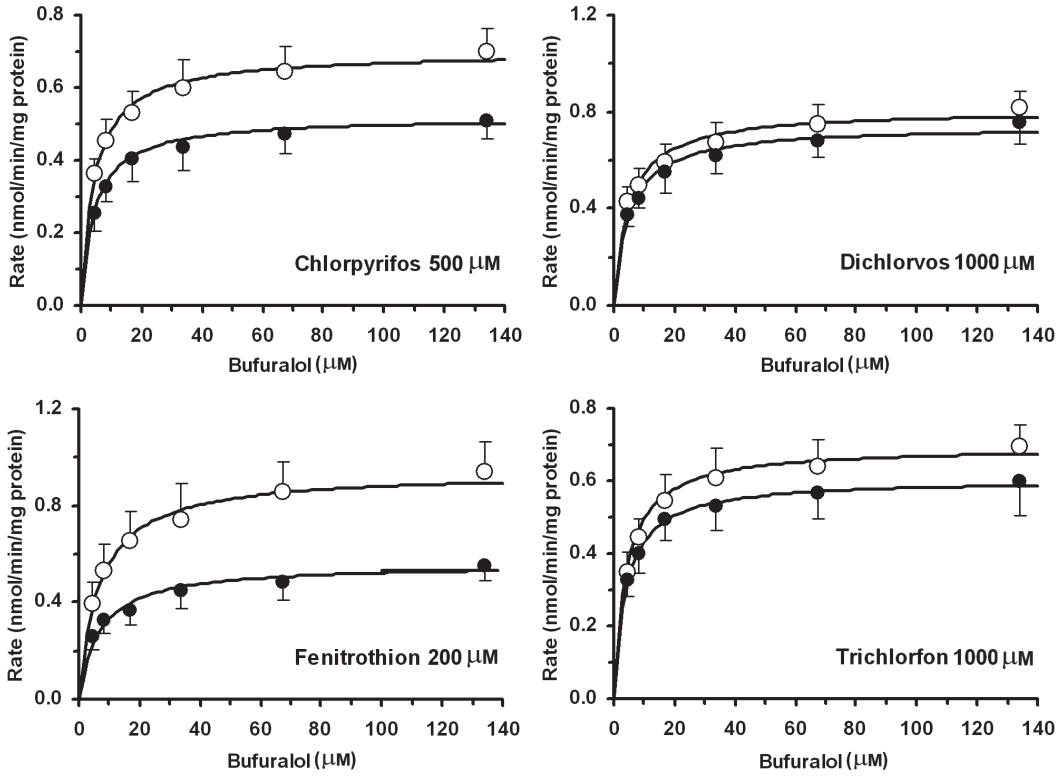

Fig. 4. Michaelis-Menten kinetics of bufuralol 1'-hydroxylation (BLH) in hepatic microsomes from rats. The solid curves represent the theoretical metabolic rates calculated using *Vmax* and *Km* values showed in Table 2. Closed and opened circles represent the observed metabolic rates (mean ± SD) in the presence or absence of organophosphates, respectively.

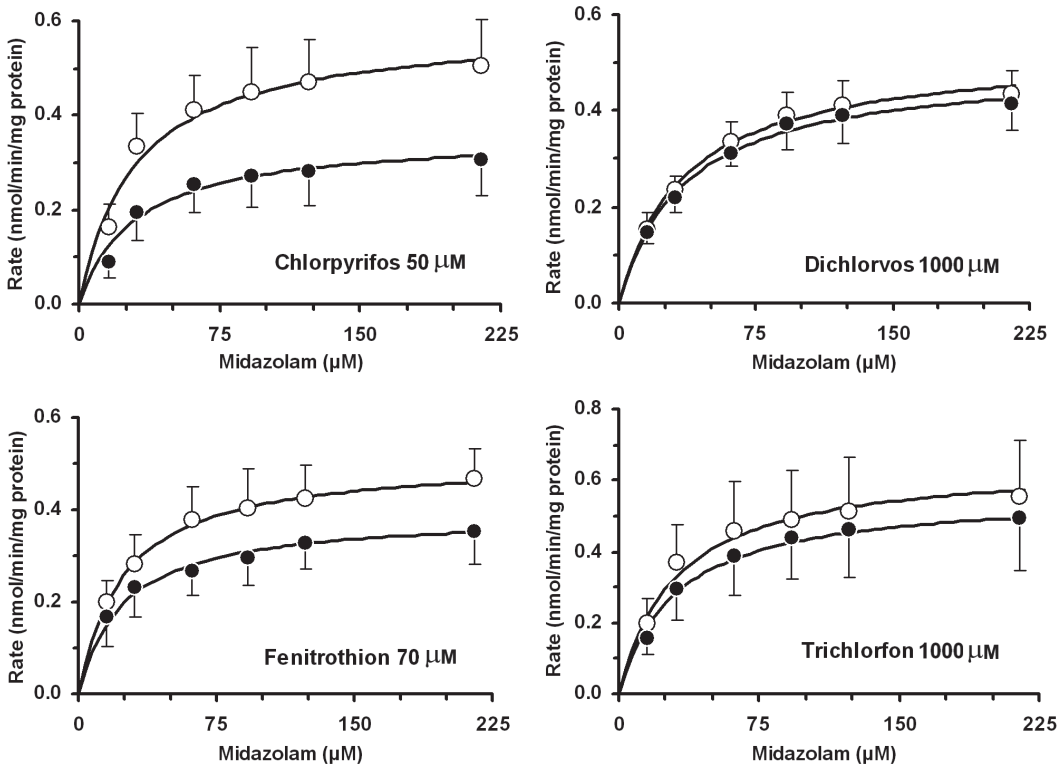

Fig. 5. Michaelis–Menten kinetics of midazolam 4-hydroxylation (MDZH) in hepatic microsomes from rats. The solid curves represent the theoretical metabolic rates calculated using *Vmax* and *Km* values showed in Table 2. Closed and opened circles represent the observed metabolic rates (mean ± SD) in the presence or absence of organophosphates, respectively.

DISCUSSION

The aim of the present study was to evaluate the effects of several OPs including fenitrothion, dichlorvos, chlorpyrifos, and trichlorfon on the activities of liver CYP enzymes, including CYP1A, 3A, 2C, and 2D, in rats. CYP levels were significantly decreased chlorpyrifos and trichlorfon at a high dose. The oral administration of the tested organophosphates showed that microsomal activities of EROD and MDZH were significantly lower in a fenitrothion-treated group compared with the control group, whereas those of TBH was significantly higher when compared with the control group. In contrast, the rest of the tested organophosphates (dichlorvos, chlorpyrifos, and trichlorfon) showed no significant effects on examined CYP activities. Therefore, it was suggested that only fenitrothion induce CYP2C. The mechanism by which CYP2C was induced by fenitrothion is not clear. However, the decreases in some CYP catalyzed reactions by fenitrothion may indicate that this OP may potently inhibit the CYP activities, because the residue of the pesticides in the obtained microsomes may be quite small. The other possible mechanism of the reduction is down-regulation which may be due to a decrease in the expression of mRNA [17]. They have reported that the addition of methyl parathion and methyl paraoxon decreased mRNA expression and activities of CYP1A. Additionally, the decrease in total CYP levels seen with chlorpyrifos and trichlorfon might reflect a decrease in CYP species whose activity was not measured in this study.

As the enzyme inhibition is of great consequence in toxicology, we also examined the possible inhibitory effects of organophosphates on the activities of CYP1A, 2C, 2D, and 3A. As a result, it was indicated that fenitrothion and chlorpyrifos are a potent inhibitor of CYP1A. This was because EROD was strongly inhibited in a noncompetitive manner by fenitrothion and chlorpyrifos with Ki values of less than 1 μM (0.42 and 0.24 μM respectively). On the other hand, the inhibitory effects of these pesticides on other CYP activities were not so high or negligible, based on their Ki values for TBH, BLH, and MDZH. The other organophosphorus pesticides, dichlorvos, and trichlorfon were a weak or negligible inhibitor for CYPs.

Abass *et al.* examined the inhibitory effects of 18 pesticides on CYP activities using human microsomes and indicated that organophosphorus pesticides are the most potent and powerful inhibitors [1]. They found that the IC_{50} of fenitrothion, profenofos, and chlorpyrifos were about 3 μM for CYP1A1/2, IC_{50} of fenitrothion and chlorpyrifos was 2.5 μM for CYP2B6, the IC_{50} of fenitrothion was 4.3 μM for CYP2C8, IC_{50} of malathion and fenitrothion were 2.5 and 4.8 μM, respectively for CYP2C9, IC_{50} value of phenthoate and chlorpyrifos was about 3 μM for CYP2D6 and IC_{50} of phenthoate, chlorpyrifos, and fenitrothion were ranged from 3–4 μM for CYP3A4. Our results together with their data indicate the considerable variation in the potency and extent of inhibition among different OPs. Our findings were matching with previous studies, where chlorpyrifos [10] and fenitrothion [12] inhibited the CYP1A in the rat's liver. Additionally, in another study, chlorpyrifos inhibited CYP 1A, 2B and 3A

after intraperitoneal injection to rats at a dose of 50 mg/kg [26] and in case of CYP2B6, the heme loss was the major mechanism leading to the inactivation of CYP2B6 by chlorpyrifos [9]. Furthermore, trichlorfon was demonstrated to cause a significant rise in the mRNA expression of CYP1A in the liver tissues indicating its cytotoxic effect on hepatocytes due to the destruction of the structure and integrity of cell organelles, such as the endoplasmic reticulum, and membrane transport systems [29]. Exposure to fenitrothion might change estradiol biotransformation by inhibition of certain CYP enzymes [4]. It has also been revealed that both chlorpyrifos-oxon and carbaryl inhibited the esterase(s) necessary for the primary step in permethrin metabolism in the human liver [15]. CPS can be activated by CYP through a desulfuration reaction to form chlorpyrifos-oxon, a potent anticholinesterase. The metabolism catalyzed by CYP of both endogenous substrates, such as testosterone, estradiol and exogenous substrates, such as carbaryl, are strongly inhibited by chlorpyrifos and other phosphorothioates, possibly due to the interaction of highly reactive sulfur (streamed during the oxidative desulfuration process), with the heme iron of CYP [25]. For instance, the hydrolysis of permethrin in the human liver can be inhibited by chlorpyrifos oxon and carbaryl [16]. The acceptable daily intake (ADI) for fenitrothion and chlorpyrifos as determined by the Food Safety Commission of Japan are 0.005 and 0.001 mg/kg/day, respectively. If foods are ingested so that these ADIs are respected, the assumed concentration in the body from these intakes will be much lower than Ki values calculated in this study. It is difficult to imagine that foods containing these insecticides would cause adverse health effects to humans.

Based on our results, the exposure to fenitrothion may alter the metabolism of some xenobiotics including drug and environmental contaminants through its inducing effect on CYP 2C as well as its potent inhibitory effects on activities of CYP1A. That with chlorpyrifos may also alter the metabolism of some xenobiotics through its potent inhibitory effect on CYP1A activities. As a result, toxicities of some xenobiotics may be potentiated by these OPs. On the other hand, dichlorvos and trichlorfon may not show such potentiation, since they had negligible effects on the CYP activities examined in this study.

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