

## The effects of fenvalerate on hepatic and cerebral xenobiotic metabolizing enzymes in selenium and/or iodine deficient rats

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### ABSTRACT

**Objective(s):** Particularly in developing countries, selenium and/or iodine deficiencies are encountered and use of pesticides in agriculture are not well-controlled. Fenvalerate is a pyrethroid insecticide used in agriculture and has applications against a wide range of pests. This study was designed to evaluate the effects of fenvalerate on hepatic and cerebral xenobiotic metabolizing enzyme activities in the presence of iodine and/or selenium deficiency on a rat model.

**Materials and Methods:** Iodine and/or selenium deficiency was induced by feeding three-week-old Wistar rats with a diet containing <0.005 mg selenium kg<sup>-1</sup>, and/or administering 1% sodium perchlorate in drinking water for 7 weeks. Test groups received fenvalerate (100 mg kg<sup>-1</sup> BW IP) for the last 7 days. Hepatic and cerebral microsomal aniline hydroxylase (CYP2E1) and cytosolic glutathione S-transferase (GST) activities were determined. Besides, hepatic NADPH-cytochrome P450 reductase (P450R), ethoxyresorufin O-deethylase (EROD, CYP1A1/1A2) and penthoxyresorufin O-depenthylase (PROD, CYP2B1/2B2), activities were also measured.

**Results:** Fenvalerate had a general inductive effect on the hepatic and cerebral xenobiotic metabolizing enzyme activities. Moreover, enzyme activities were also altered by iodine and/or selenium deficiency, but the effects seemed to be enzyme- and tissue-specific.

**Conclusion:** The inductive effect of fenvalerate, particularly in high dose exposures, may change the metabolism of several xenobiotics, including drugs, as well as endogenous substrates. The effects may vary depending on the selenium and/or iodine status of individual.

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### Introduction

Pyrethroids are synthetically derived chemical forms of naturally occurring pyrethrins and widely used insecticides in agriculture and public health due to their high environmental stability and high insecticidal activity. They account for over 30% of the insecticide market as pest control or crop protection agent, veterinary insecticides and topical medicines. They were shown to exhibit low mammalian toxicity (1-5).

All the pyrethroids act on the voltage-gated sodium channels of insects by prolonging the inactivation current and cause membrane depolarization, repetitive discharges and synaptic disturbances that result in hyperexcitatory symptoms in all excitable tissues (3, 6, 7). According to their different behavioral, neurophysiological and biochemical profiles, pyrethroids are divided into two subclasses: Type I and Type II. Type II pyrethroids carry a cyano group and produce a more complex syndrome including clonic seizures (2, 4, 6). Fenvalerate (C<sub>25</sub>H<sub>22</sub>ClNO<sub>3</sub>; IUPAC name: (R,S)- $\alpha$ -cyano-3-phenoxybenzyl (R,S)-2-(4-chlorophenyl)-3-methylbutyrate; CAS no:

51630-58-1) belongs to Type II pyrethroid subclass. Exposure of the general population to fenvalerate is mainly via oral route. However, dermal and inhalation routes are the most important sources of occupational exposure. It is rapidly metabolized to fenvaleric acid in the mammals (8-10) and both fenvalerate and its primary metabolite is excreted in a short time (11). Fenvaleric acid can be accumulated in rodent liver and kidney after repeated exposure for 30 days (12). However, chronic exposure of mice and rats to fenvalerate did not produce any evidence related to chronic toxicity or carcinogenicity (13, 14). Fenvalerate has moderate toxicity in mammals (15, 16). It is a potent neurotoxicant in vertebrates and invertebrates. Hepatotoxic effects and involvement of reactive oxygen species (ROS) in the toxicity were also reported (17). In recent years, several studies suggest that exposure to certain pyrethroids including fenvalerate may contribute to reproductive dysfunction, developmental impairment, and cancer through hormonal pathways (18, 19). Limited studies indicate that fenvalerate may cause alterations in rat thyroid hormone status (20, 21).

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On the other hand, pyrethroids have been shown to interact with the hepatic and cerebral xenobiotic metabolizing enzymes (22-24). However, the results of different studies are controversial. Some reports pointed out that these pesticides did not markedly influence the microsomal liver enzymes (24, 25) whereas others showed significant increases or decreases in the activities of these enzymes in different species (26, 27). The effects of pyrethroids on xenobiotic metabolizing enzyme activities in selenium and/or iodine deficiency have not been studied.

The essential trace elements, iodine and selenium, are involved in the maintenance and modulation of various normal metabolic functions. Iodine is the critical element for thyroid hormone production and therefore it is a primary requirement for the thyroid function. Iodine deficiency is known to induce a variety of thyroid disorders, including endemic goiter, which is considered as the greatest cause of preventable brain damage and mental retardation (28). Selenium has also fundamental importance in thyroid hormone synthesis and metabolism, because the iodothyronine 5'-deiodinases (DIOs) are selenoproteins. Selenium deficiency impairs T<sub>4</sub> conversion to T<sub>3</sub> whereas selenium supplementation decreases plasma T<sub>4</sub> levels, increases DIO activity and improves the conversion of T<sub>4</sub> to T<sub>3</sub> (29, 30). Moreover, selenium is the integral component of several major metabolic pathways of cellular antioxidant defense and redox control (31). However, both of these essential trace elements are inadequately available for man and livestock in many parts of the world. Existing data indicate that iodine deficiency prevails in most parts of Turkey, and none of the regions of the country is free from endemic goiter. On the other hand, Turkish daily intake of selenium, in general, is at the borderline of deficiency (30-33).

Thyroid hormones have regulatory effects on the expression of electron donors of cytochrome P450 enzymes, particularly on NADPH-cytochrome reductase (P450R) (35-38). The studies assessing the effect of selenium on the xenobiotic metabolizing enzyme activities are limited, not comprehensive and the data are mostly contradictory (38-40).

Considering the potential adverse effects of these essential element deficiencies, and taking into account the heavily but improperly controlled usage of insecticides, this study was designed to investigate the effects iodine or selenium deficiency on fenvalerate toxicity in rats.

## Materials and Methods

### Reagents and chemicals

Technical grade fenvalerate (92%, w/w in emulsifiable concentrate) was from Koruma Kimya (İzmit, Turkey). Selenium-deficient diet (<0.005 mg

selenium kg<sup>-1</sup>) was supplied by Scientific Animal Food and Engineering (SAFE) Laboratories (Augy, France). All other reagents and chemicals used were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St Louis, Missouri, USA).

### Experimental groups

Three-week-old male albino Wistar rats were obtained from Hacettepe University Experimental Animal Laboratory and were randomly divided into five groups of six rats in each group. All animals were housed in plastic cages with stainless-steel wire tops. The cages were maintained in a room with a controlled temperature (23±2 °C), relative humidity (50±10%) and light/dark cycle (12 hr/12 hr). All animals were kept under observation until decapitation.

Feeding period was 7 weeks and animals were allowed free access to diet and water. Body weight (BW) of animals was monitored weekly. Experimental groups: 1) **Control group (C)** was fed with regular commercial diet and tap water and received corn oil, intraperitoneally (IP) for the last 7 days; 2) **Control-fenvalerate group (CF)** was fed with regular commercial diet and tap water; during the last week of feeding period received 100 mg/kg BW/day; IP; ~1/3 LD<sub>50</sub> fenvalerate; 3) **Iodine deficient and fenvalerate group (IDF)** was fed with regular commercial diet, received 1 % sodium perchlorate containing drinking water, and during the last week of feeding period received 100 mg/kg BW/day IP fenvalerate; 4) **Selenium deficient and fenvalerate group (SeDF)** was fed with selenium deficient diet containing <0.005 mg of selenium/kg, received normal tap water and during the last week of feeding period received 100 mg/kg BW/day IP fenvalerate; 5) **Iodine plus selenium deficient and fenvalerate group (ISeDF)** was fed with selenium deficient diet, received 1% sodium perchlorate containing drinking water, and during the last week of feeding period received 100 mg/kg BW/day IP fenvalerate. The animals were treated humanely and with regard for alleviation of suffering, and all studies have been approved by Hacettepe University Ethical Committee.

Twenty-four hours after the last dose of fenvalerate, animals were weighed and sacrificed by decapitation under thiopental anesthesia. Liver and brain tissues were removed, immediately frozen, and kept at -80 °C until analysis.

### Preparation of tissue homogenates and microsomal/cytosolic fractions

The homogenization of liver and brain tissues was carried out in a Teflon-glass homogenizer (Thomas Scientific, Swedesboro, NJ) in a volume of ice-cold potassium chloride (0.154 mol/l)-Tris (50 mmol/l) buffer (pH 7.4) to obtain a 3 g/ml tissue

homogenate. Following centrifugation at 2500 x g at 4 °C for 10 mins, supernatant was further centrifuged at 10.000 x g at 4 °C for 10 min. The latter supernatant was centrifuged at 105.000 x g at 4 °C for 60 min and cytosolic supernatant was collected and used for the measurement of GST activity. The microsomal pellet was re-suspended in a buffer containing Tris-EDTA-sucrose (20 mmol/l Tris, 5 mmol/l EDTA and 0.25 mol/l sucrose; pH 7.4; 1 g tissue/ ml).

For selenium analysis, hepatic tissue homogenates were prepared in a volume of ice-cold buffer containing Tris (10 mmol/l), diethylenetriaminepentaacetic acid (1 mmol/l, and phenylmethanesulphonyl fluoride (1 mmol/l; adjusted to pH 7.4) using a Teflon pestle homogenizer to obtain 10% (w/v) whole homogenate. All samples were aliquoted and stored at -80 °C until analysis.

### Selenium levels

The hepatic selenium levels were determined by a spectrofluorometric method (41). According to this method piarselenol complex formed by Se(IV) with 2,3-diamino naphthalene (2,3 DAN) was detected at excitation and emission wavelengths of 377 and 523 nm and the results were given as µg/g liver tissue.

### NADPH-cytochrome reductase activity

Microsomal P450R activity was measured at 550 nm and 37°C by monitoring the reduction of cytochrome c in the presence of NADPH (42). The results were given as nmol/mg protein/min.

### Xenobiotic metabolizing enzyme activities

Microsomal 7-ethoxyresorufin O-deethylase (EROD) as a measure of CYP1A1, and 7-pentoxoresorufin-O-depentylase (PROD) as a measure of CYP2B1/2 activities were determined spectrofluorometrically from the amount of resorufin produced using ethoxyresorufin and pentoxoresorufin as substrates, respectively, at excitation and emission wavelengths of 530 and 585 nm (43,44). EROD and PROD activities were given as pmol resorufin mg protein/min.

Microsomal aniline hydroxylase (CYP2E1) activity was determined by measuring p-aminophenol production (45). The results were given as pmol mg protein/min.

Cytosolic glutathione-S-transferase (GST, EC 2.5.1.18) activity was determined using 1-chloro-2,4 dinitrobenzene as a substrate (46). The results were given as µmol/mg protein/min.

### Protein determination

Protein concentration was determined colorimetrically using the Folin-Phenol reagent according to Lowry et al (47).

### Statistical analyses

The experimental data were analyzed with Kruskal-Wallis test followed by the Mann-Whitney U

using a Statistical Package for Social Sciences Program (SPSS, SPSS Inc., Chicago, IL, USA) version 16.0. The *P*-values <0.05 were considered significant and all the results are expressed as mean ± standard error of mean (SEM).

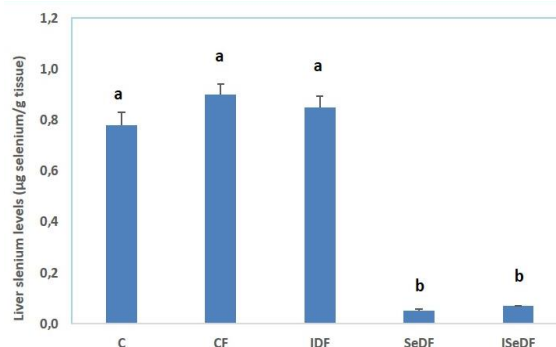
## Results

### Body and tissue weights

There was no mortality and no clinical signs of fenvalerate poisoning except minor symptoms of neurotoxicity, such as abnormal gait, slight irritability, and agitation in fenvalerate-exposed groups. Significant differences in the food intake were not observed between the groups. Changes in the body and relative tissue weights (tissue weight/100 g BW) of the control and fenvalerate-exposed groups are shown in Table 1. A statistically significant decrease (~25 %) was observed in the mean body weights of rats by fenvalerate exposure. Moreover, iodine and/or selenium deficient rats exposed to fenvalerate showed more pronounced decreases in body weights compared to C group (34% in IDF, 45% in SeDF; 42% in ISeDF rats, *P*<0.05) and CF (14% in IDF, 28% in SeDF; 24% in ISeDF rats, *P*<0.05) groups. The relative brain weights were found to be significantly higher in all study groups whereas relative liver weight were found to be higher only in SeDF group as compared to C (*P*<0.05).

### Verification of iodine and selenium deficiency

Selenium deficiency was evident by lower hepatic selenium levels in SeDF (93%) and ISeDF (92 %) groups compared to control. The average selenium level of healthy control was 0.74 ± 0.05 µg/g liver tissue, whereas the levels of SeDF and ISeDF groups were found to be 0.05±0.02 µg/ g tissue and 0.06±0.01 µg/g tissue, respectively. No significant alterations were observed in the selenium levels of CF and IDF rats compared to control (Figure 1).



**Figure 1.** Liver selenium levels

Experimental groups for 7 weeks were on: control group (C); control-fenvalerate group (CF); iodine deficient and fenvalerate group (IDF); selenium deficient and fenvalerate group (SeDF); iodine plus selenium deficient and fenvalerate group (ISeDF). All the values are given as mean±standard error of mean (SEM). <sup>a,b</sup> Bars that do not share the same letters (superscripts) are significantly different from each other (*P*<0.05; n=6)

**Table 1.** The effects of fenvalerate on body and relative tissue weights in selenium and/or iodine deficient rats

	Body weight (g)	Relative liver weight (g 100 g <sup>-1</sup> BW)	Relative brain weight (g 100 g <sup>-1</sup> BW)
C	221.4 ± 8.2 <sup>a</sup>	4.2 ± 0.1 <sup>a</sup>	0.85 ± 0.0 <sup>a</sup>
CF	169.2 ± 6.8 <sup>b</sup>	4.2 ± 0.1 <sup>a</sup>	1.01 ± 0.0 <sup>b</sup>
IDF	145.3 ± 7.3 <sup>c</sup>	3.5 ± 0.2 <sup>b</sup>	1.18 ± 0.0 <sup>c</sup>
SeDF	121.4 ± 6.1 <sup>d</sup>	4.9 ± 0.1 <sup>c</sup>	1.39 ± 0.1 <sup>d</sup>
ISeDF	128.5 ± 5.3 <sup>cd</sup>	4.5 ± 0.2 <sup>a</sup>	1.33 ± 0.1 <sup>d</sup>

Experimental groups for 7 weeks were on: control group (C); control-fenvalerate group (CF); iodine deficient and fenvalerate group (IDF); selenium deficient and fenvalerate group (SeDF); iodine plus selenium deficient and fenvalerate group (ISeDF). All the values are given as mean ± standard error of mean (SEM). Means within each row that do not share same letters (superscripts) are significantly different from each other ( $P < 0.05$ ;  $n = 6$ )

IDF, SeDF and ISeDF groups were significantly different than control even after Bonferoni correction for body weight of the groups

SeDF group was significantly different than control even after Bonferoni correction for relative liver weight of the groups

IDF, SeDF and ISeDF groups were significantly different than control even after Bonferoni correction for relative liver weight of the groups

Iodine deficiency and resulting hypothyroidism was produced by perchloride containing drinking water as evidenced by higher thyroid stimulating hormone (TSH) and lower plasma TT4 levels along with the increased thyroid weights in IDF and ISeDF groups as shown earlier (15). TSH levels elevated 2-fold in IDF and 2.4-fold in ISeDF whereas plasma TT4 levels were found to be lower in IDF (40%) and in ISeDF (60%) compared to C group. The lowest TT4 levels were measured in ISeDF group ( $P < 0.05$ ).

#### Hepatic xenobiotic metabolizing enzyme activities

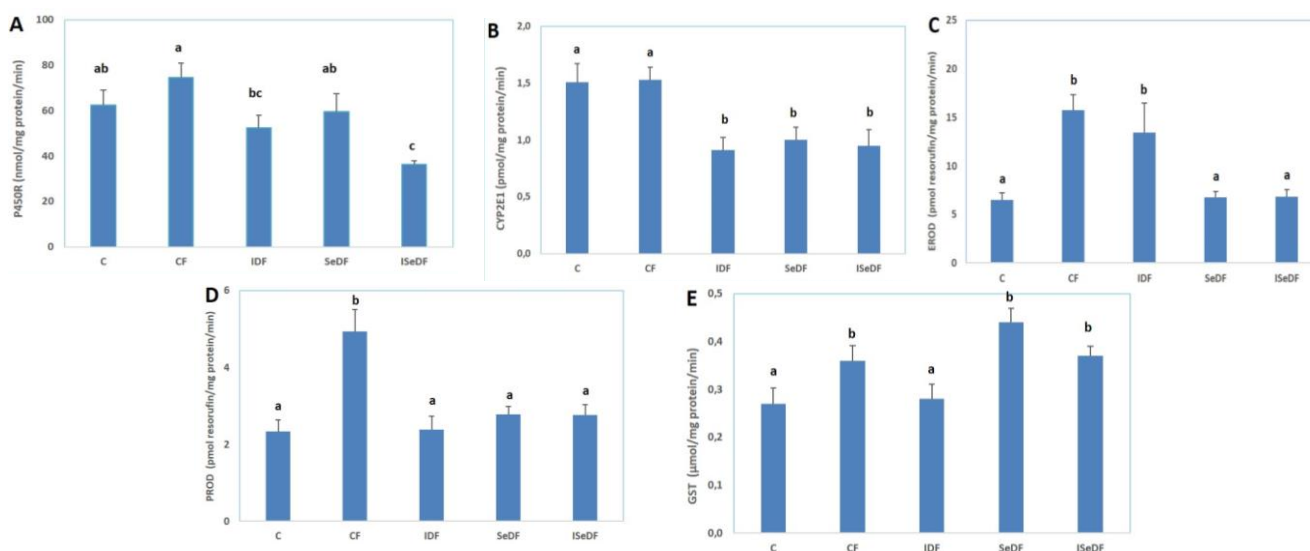
Fenvalerate exposure caused significant ( $P < 0.05$ ) elevations in the hepatic EROD, PROD and GST activities (143%, 112%, and 38%, respectively) compared to control while no significant alterations were noted in hepatic P450R and CYP2E1 activities of CF group (Figure 2).

In iodine deficiency, fenvalerate did not cause any significant alterations in hepatic PROD, P450R and GST activities while CYP2E1 activity decreased

(40%) and EROD activity increased (107%) significantly in IDF rats as compared to control. In IDF group, P450R, CYP2E1, PROD and GST enzyme activities were lower than CF group ( $P < 0.05$ ); however EROD activity of IDF group was not different than CF group (Figure 2).

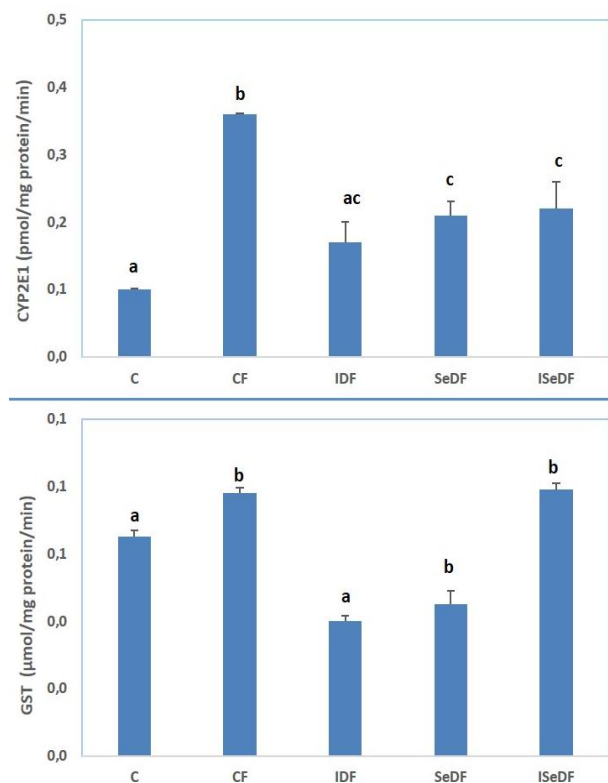
The activity of hepatic CYP2E1 in SeDF group was found to be lower (~35%) than control while hepatic GST activity enhanced (69%) significantly vs. control. In SeDF group, CYP2E1, EROD and PROD activities were lower than CF group ( $P < 0.05$ ) (Figure 2).

In ISeDF group, significant decreases in hepatic CYP2E1 and P450R activities (37%, 41%, respectively) and increases in GST activity (42%) were observed compared to C group ( $P < 0.05$ , all) whereas no significant alterations were observed in the activities of both PROD (18%) and EROD (13%) compared to C group. Besides, P450R, CYP2E1, EROD and PROD activities of ISeDF group were markedly lower compared to CF group ( $P < 0.05$ ) (Figure 2).



**Figure 2.** Activities of hepatic drug metabolizing enzymes in the experimental groups: (A) P450R activity; (B) CYP2E1 activity; (C) EROD activity; (D) PROD activity; (E) GST (cytosolic) activity. Experimental groups for 7 weeks were on: control group (C); control-fenvalerate group (CF); iodine deficient and fenvalerate group (IDF); selenium deficient and fenvalerate group (SeDF); iodine plus selenium deficient and fenvalerate group (ISeDF). All the values are given as mean ± standard error of mean (SEM). <sup>a,b,c</sup> Bars that do not share the same letters (superscripts) are significantly different from each other ( $P < 0.05$ ;  $n = 6$ )





**Figure 3.** Activities of cerebral drug metabolizing enzymes in the experimental groups: (A) CYP2E1 activity; (B) GST (cytosolic) activity. Experimental groups for 7 weeks were on: control group (C); control-fenvalerate group (CF); iodine deficient and fenvalerate group (IDF); selenium deficient and fenvalerate group (SeDF); iodine plus selenium deficient and fenvalerate group (ISeDF). All the values are given as mean±standard error of mean (SEM). <sup>a,b,c</sup>Bars that do not share the same letters (superscripts) are significantly different from each other ( $P<0.05$ ;  $n=6$ )

### Cerebral xenobiotic metabolizing enzyme activities

After 7 days of treatment with the fenvalerate, cerebral CYP2E1 and GST activities were increased compared to control (229%, and 44% respectively,  $P<0.05$ , all) (Figure 3). Since the activities of EROD and PROD were too low in brain tissue, results were not obtained and the data could not be shown. Cerebral CYP2E1 and GST activities did not change in IDF group compared to control; but significantly lower than CF group ( $P<0.05$ ). Cerebral CYP2E1 (119 %) activity was increased in SeDF compared to control; however both CYP2E1 and GST activities of ISeDF were lower than CF group ( $P<0.05$ ). In ISeDF group, CYP2E1 (113%) and GST (46%) activities were elevated significantly compared to control ( $P<0.05$ ) CYP2E1 activity of ISeDF group were found to be lower than CF group ( $P<0.05$ ) (Figure 3).

### Discussion

The main goal of the present study was to investigate the effects of iodine or selenium deficiency on fenvalerate toxicity in rats. The discussion can be divided into two parts:

### Effects of fenvalerate on xenobiotic metabolizing enzymes

The resistance to pyrethroids in insects has been attributed to the induction of CYP450 enzymes; however not much known about the effects of the pyrethroids on the xenobiotic metabolizing systems in mammalian organisms. Existing data suggest that pyrethroids have a weak or moderate induction on the enzymes involved in the drug metabolism (48-52). Moreover, naturally occurring forms of pyrethroids have been demonstrated as CYP2B and CYP3A inducers in rat liver and in human hepatocytes (53). In rats exposed to high dose pyrethroid, a slight induction of the total CYP450 content was determined (33, 48, 49). However the CYP isoforms involved in the enhancement of total CYP450 content have not described well yet.

The studies about the effects of pyrethroid insecticides on P450R activity generally have pointed to an inductive effect (48, 50, 52). The changes in the activity of P450R were associated with duration of pyrethroid exposure and different tissue responses. Oral permethrin administration for 4 days did not markedly induce hepatic P450R activity in male rats, whereas the activity significantly increased after 8 and 12 days treatment (54). Oral 80 mg kg<sup>-1</sup> cypermethrin exposure for 6 and 10 days had a slight inductive effect on hepatic P450R activity while the induction with 100 mg kg<sup>-1</sup> permethrin became significant after 10 days treatment (55). P450R induction was observed in the intestinal tissue of Japanese quail by five different pyrethroids including fenvalerate, but not in the liver (24). In another study 20 mg kg<sup>-1</sup> fenvalerate treatment orally for 20 days significantly increased the cerebral P450R activity (~32%) while decreasing the hepatic activity in rats (50). In our study, the enhancement of hepatic P450R activity (~20%) in CF rats was not found to be statistically significant (Figure 2).

Heder *et al* (2001) studied CYP2B1 mRNA levels, protein expression and PROD activity in the primary rat hepatocyte cell cultures and observed that cypermethrin, permethrin and fenvalerate generated marked dose-dependent elevations in CYP2B1 mRNA levels (23). Moreover, protein levels and PROD activity were found to be higher compared to control although this elevation did not depend on the dose administered. Our data are comparable with this study in terms of hepatic PROD activity induction. In agreement with our results, in another study conducted on F344 rats, the researchers also observed the induction of hepatic EROD and PROD activities by oral fenvaleric acid administration for 7 days in a dose-dependent manner (57). Dayal *et al* (1999) studied the effects of different dose levels and intervals of deltamethrin on the activity of both dealkylase enzymes in liver and brain tissues of rats (24). Significant induction of the enzyme activities

was observed at higher doses or with prolonged exposure. A similar dose-related and time-dependent elevation in the expression of P450 2B1/2B2 and 1A1 isoenzymes was also observed. In our study, marked increases were found in both hepatic EROD and PROD activities (~145% and ~130%, respectively) by fenvalerate treatment compared to the control group. These results might suggest an inductive mechanism on EROD and PROD metabolism by pyrethroid exposure via altering the expression levels of CYP2B1/2B2 and CYP1A1/A2.

Limited number of studies have demonstrated the effects of pyrethroids on CYP2E1 activity and the results of these studies were conflicting (24, 58, 59). Tang *et al* (1987) showed a decrease (53), while Krechniak *et al* (1991) demonstrated an increase in the hepatic activity of CYP2E1 by pyrethroid exposure in rats (55). On the other hand, fenvalerate exposure for 7 days did not induce the activity of CYP2E1 in Japanese quail (24). There are also conflicting data available for the effects of pyrethroids on the GST activity. Some studies suggested an elevation whereas the others showed decreases in the activity of this particular enzyme (23, 34, 40, 56). In our experimental conditions, fenvalerate administration for 7 days significantly increased hepatic and cerebral GST activity (30%, 44%, respectively), along with the activity of cerebral CYP2E1 (229%) but did not cause marked elevation in hepatic CYP2E1 activity compared to control group (Figure 2 and Figure 3). Different results may depend on dose and duration of treatment as well as sex, and species. Thus, exposure dose to pyrethroids including fenvalerate may be the main deterministic factor for their effects on xenobiotic metabolizing enzymes. Results presented herein show that fenvalerate had a general inductive effect on the enzyme activities studied both in liver and brain tissues of rats. As also indicated by Martignoni *et al* (2006), CYP2E1 shows no large differences between species, and extrapolation between species appears to hold quite well. However, the species-specific isoforms of CYP1A may show significant interspecies differences in terms of catalytic activity and it is not sometimes possible to extrapolate the alterations in the activity of EROD in rats to humans (61). Therefore, we can suggest that the induction of CYP2E1 in rat brain might also be valid for human brain tissue. For GST activity, both Reitz *et al* (1989) and Andersen *et al* (1987) suggested that the metabolic rate constants for GST obtained from human and rodent cells were similar, particularly in the metabolism of methylene chloride (62, 63).

#### **Effects of fenvalerate on xenobiotic metabolizing enzymes in the iodine, selenium and combined iodine plus selenium deficiency status**

Although there are some studies investigating the effects of pyrethroids including fenvalerate on xenobiotic metabolizing enzymes, this study is the first to evaluate the effects of fenvalerate on the hepatic and cerebral enzymes in selenium and/or iodine deficiency. Iodine deficiency was induced by applying 1% sodium perchlorate containing drinking water herein and as shown earlier, it was evident by higher TSH and lower plasma TT<sub>4</sub> and TT<sub>3</sub> levels along with increased thyroid weights in rats (64). We also reported that iodine deficiency caused significant decreases in hepatic CYP2E1, EROD and GST activities and insignificant decrease in hepatic P450R activity when compared to control (58). Our recent study supported the findings of the previous studies that showed the substantial role of thyroid hormones in the expression/activity of CYP450 enzymes. Possibly due to strong inductive effect of fenvalerate, we observed significant elevations in hepatic EROD activity in IDF rats although iodine deficiency significantly decreased EROD activity as shown previously (60). Moreover, fenvalerate exposure compensated the reduction of hepatic GST activity in iodine deficiency. Although fenvalerate alone did not cause any significant alteration in hepatic CYP2E1 activity, in hypothyroid rats it triggered significant decreases in the activity of this enzyme compared to control. In cerebral tissue, GST and CYP2E1 activities did not change in iodine-deficient animals by fenvalerate exposure (Figure 3). On the other hand, it was reported that thyroid hormones were required for full expression of P450R in liver tissue and T<sub>3</sub> regulated the expression of liver P450R both transcriptionally and post-transcriptionally. In hypothyroid rats, the decrease in hepatic levels of P450R can primarily be based on this mechanism (48, 78, 79). Research has also demonstrated that in healthy animals, after T<sub>3</sub> injection, an increase was observed in the P450R mRNA levels, protein levels and activity (58). In our previous study, we observed an insignificant decrease in hepatic P450R activity in iodine deficient (ID) rats (65) whereas in the present study we observed a slight insignificant increase of (~20 %) in hepatic P450R activity of IDF rats compared to ID, possibly due to the inductive effect of fenvalerate. Although fenvalerate alone caused a slight but insignificant elevation (~20%) in hepatic P450R activity, no alteration was observed in hepatic P450R activity of IDF rats compared to C group.

Selenium deficiency was evident by lower hepatic selenium levels in SeDF (93 %) and ISeDF (92 %) groups compared to control (Figure 1). We also previously determined hepatic glutathione peroxidase 1 (GPx1) activity as a biological criterion of selenium deficiency and found significant decreases in this enzyme activity (19). In spite of conflicting findings, available data indicate marked

alterations in xenobiotic metabolizing enzymes in selenium deficiency (39, 42, 65, 66). In our previous study, we also observed that selenium deficiency caused significant decreases in rat hepatic EROD and GST activities along with marked elevation in CYP2E1 (60). Fenvalerate administration to selenium deficient rats did not change the activities of hepatic P450R, EROD and PROD vs. control. Fenvalerate exposure and selenium deficiency had opposing effects on hepatic GST activity. Fenvalerate alone caused significant enhancement in the activity of GST in rat liver, whereas in our earlier study we observed significant decrease in hepatic GST activity in selenium deficient rats. In SeDF group, GST activity was not different from the control possibly due to more pronounced effect of fenvalerate. Fenvalerate did not exhibit a marked effect on the activity of CYP2E1 in liver whereas it reversed the inductive effect of selenium deficiency as reported earlier (59). On the other hand, in selenium-deficient rats the inductive effect of fenvalerate on cerebral CYP2E1 activity was observed. Oxidative stress induced by selenium deficiency was suggested to be one of the possible mechanisms triggering the alterations in the metabolizing enzyme systems as we also demonstrated earlier (15). A general impairment of membrane function, as a consequence of GPx1 depletion, was observed in selenium deficiency. After hepatic GPx1 activity completely disappeared, this phenomenon may lead to alterations in the activity of CYP450 enzymes (67). As shown previously, in SeDF rats a further suppression of hepatic GPx activity was observed when compared to selenium deficient rats (15). Moreover, some studies suggest that nutritional deficiency may have different effects on the xenobiotic metabolism depending on its extent (65).

In combined selenium and iodine deficiency, significant decreases in hepatic P450R and CYP2E1 activities and increases in hepatic GST activity were observed by fenvalerate treatment compared to control (Figure 2). The results of our earlier study showed that combined deficiency state caused marked decreases in hepatic P450R, EROD, PROD and GST activities (68). Due to the opposite effects of fenvalerate and combined selenium and iodine deficiency on the enzyme activities in liver, no significant alterations were observed in hepatic EROD and PROD activities compared to control. However, the inductive effect of fenvalerate was more prominent on hepatic GST activity. In spite of suppression of enzyme activity by combined deficiency state in hepatic tissue, marked enhancement of hepatic GST activity was observed in ISeDF rats compared to control. Similar inductive effect of fenvalerate on GST activity was also determined in cerebral tissues of ISeDF group. On the other hand, the effects of iodine and selenium deficiencies were in opposite directions on CYP2E1

in liver tissue. Iodine deficiency caused significant decreases in hepatic CYP2E1 activity in contrast with selenium deficiency compared to control (68). Fenvalerate did not cause any significant change in the activity of hepatic CYP2E1. Therefore, in ISeDF group, the decrease in hepatic CYP2E1 activity might mainly be based on iodine deficiency (Figure 2). On the other hand, we observed significant elevations in cerebral CYP2E1 activity of ISeDF rats. These results suggest that the effect of fenvalerate on CYP2E1 activity may be tissue-specific in healthy or deficient rats.

## Conclusion

In conclusion, our results indicate that hepatic and cerebral xenobiotic metabolizing enzyme activities are significantly affected by exposure to fenvalerate in iodine and/or selenium deficiency. Also the inductive effects of fenvalerate, particularly high dose exposures, might change the metabolism of concomitantly exposed xenobiotics including drugs, as well as endogenous substrates depending on the iodine and/or selenium status of individual.

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## Conflict of interest

The authors report no conflicts of interest.

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