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Differential Induction of Hepatic Drug-Metabolizing Enzymes by Fenvaleric Acid in Male Rats

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Racemic fenvaleric acid [2-(4-chlorophenyl)-3-methyl-butanoic acid], the principal metabolite of fenvalerate, was administrated orally at 0.75, 1.5, and 3.0 mmol/kg body weight/day to Fisher-344 male rats for 7 days. Both pure enantiomers of fenvaleric acid were administered at 1.5 mmol/kg body weight/day; the clofibric acid at the same concentration was used as a positive control. Hepatic enzyme activities were measured. Results obtained clearly show that fenvaleric acid induced numerous hepatic drug metabolism enzymes in F344 rats. The (*R*) enantiomer of this compound induces a proliferation of peroxisomes, whereas the (*S*) enantiomer induces CYP2B and mEH activities. Therefore, high exposure to pyrethroid insecticides could interact with the normal metabolism of drugs or xenobiotics.

Key Words: **fenvaleric acid; hepatic enzymes; drug metabolism; pyrethroids.**

Fenvalerate **I** [cyano(3-phenoxyphenyl)methyl 2-(4-chlorophenyl)-3-methylbutanoate] is a synthetic pyrethroid that is highly efficient against a variety of insect pests damaging numerous commercial crops, including apples, peaches, cotton, and almonds (Meister, 1996). Over the last 20 years, large amounts of fenvalerate (approximately 1000 metric tons per year; UN-WHO, 1990) were used as an agricultural insecticide. It is now replaced largely by esfenvalerate, the active enantiomer (represented in Fig. 1). In 1994, over 15 tons of the active ingredient of esfenvalerate (Asana®) were applied to various crops in the United States (Gianessi and Anderson, 1995). Fenvalerate **I** is rapidly degraded ($t_{1/2} \cong 15$ days) in most environments (Hill, 1981), yielding fenvaleric acid **II** [2-(4-chlorophenyl)-3-methyl-butanoic acid] (Agnihotri *et al*., 1986; Okhawa *et al*., 1978) as a major metabolite. The presence of fenvalerate residues have been detected in crops (Greenberg, 1981; Patel *et al*., 1990) and food products such as milk, eggs, and beef (Boyer *et al*., 1992). However, human exposure through food and beverage is considered low (UN-WHO, 1990).

Fenvalerate displays moderate toxicity in mammals (Ecobichon, 1996); oral LD_{50} values from 100 to over 3000 mg/kg were found for several rodent species (UN-WHO, 1990). Fenvalerate is rapidly hydrolyzed in rats and mice to yield fenvaleric acid as a major metabolite (Kaneko *et al*., 1981; Okawa *et al*., 1979). Moreover, the racemic mixture of fenvalerate and its active isomer esfenvalerate displayed similar metabolism (Kaneko *et al*., 1981). After a single exposure, fenvalerate and its metabolites are rapidly eliminated (Okawa *et al*., 1979). However, over a short-term exposure (30 days), fenvaleric acid **II** is bioaccumulated in rat liver and kidney (Misra and Sharma, 1997). No chronic toxicity and no carcinogenic effects were observed in long-term exposure studies in mice (Parker *et al*., 1983; Cabral and Galendo, 1990) and rats (Parker *et al*., 1984). However, high fenvalerate concentrations induce microgranulomas in the liver, spleen, and lymph node in both rats and mice (Cabral and Galendo, 1990; Okuno *et al*., 1986). Moreover, fenvalerate induces microgranulomas in human lymphocytes (Surralles *et al*., 1995). Fenvalerate was shown to enhance altered hepatic foci and inhibit intercellular communication *in vitro* (Hemming *et al*., 1993). Recently, fenvalerate was shown to enhance the activity of hepatic lysosomal enzymes, and may act as a tumor promoter (Balbaa *et al*., 1998).

These results show that fenvalerate and its major metabolite fenvaleric acid have several biologic and biochemical effects on liver and kidneys. These two tissues carry out the metabolism of numerous drugs and chemicals. The induction of some drug-metabolizing enzymes by one chemical could interact with the metabolism of other endogenous or exogenous chemicals. For example, clofibric acid **III** induces hepatic peroxisomal enzyme activities (Moody *et al*., 1991) and some drug-metabolizing enzymes, including P450s (Orton and Parker, 1982), esterases, and epoxide hydrolases (Grant *et al*., 1994). To our knowledge, the ability of fenvalerate or fenvaleric acid to induce hepatic drug-metabolism enzymes has not been studied.

In order to investigate the ability of fenvaleric acid to induce hepatic drug-metabolism enzymes, male F344 rats were fed varying doses of fenvaleric acid (the racemic mixture and both pure enantiomers) for 7 days. Clofibric acid was used as a positive control. The activities of several drug-metabolizing enzymes were then measured on hepatic extracts.

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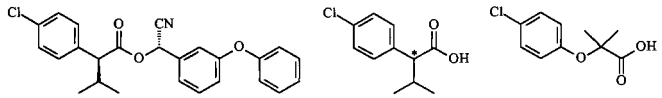

I **II** **III**

FIG. 1. Structure of esfenvalerate **I**, racemic fenvaleric acid **II**, and clofibric acid **III**.

MATERIAL AND METHODS

Chemicals. The 2-(4-chlorophenyl)-3-methyl-butanoic acid **I** was graciously supplied by the Dupont de Nemours and Co. (Wilmington, DE). The (*R*) and (*S*) enantiomers were resolved via the chiral α-methylbenzylamine salt according to published procedures (Shan *et al*., 1999; Miyakado *et al*., 1975). All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Radioactive substrates came from Amersham Life Science (Arlington Heights, IL). The purity of the products synthesized was checked by silica gel thin layer chromatography with UV detection at 254 nm. Their structures were verified by NMR on a QE 300 MHz spectrometer (Bruker NMR, Billericz, MA). The liquid scintillation cocktail (CytoScint) was purchased from Fisher Scientific (Fairlawn, NJ). The bicinchoninic acid (BCA) reagent for determining protein concentration was obtained from Pierce, Inc. (Rockford, IL).

Animals. Thirty-five male F344 rats (160–180 g initial body weight, Charles River) were dosed by gavage, once daily for 7 consecutive days at the levels indicated in Table 1 (5 rats per dose). Three different doses of fenvaleric were evaluated and compared with a single dose of clofibric acid and the two optically rich isomers of fenvaleric acid. The control animals were administered the vehicle (2 % arabic gum in tap water) at 1 ml/kg by gavage. Twenty-four hours after the last dose, the animals were killed by cervical dislocation and livers were removed, blotted dry, weighed, and perfused with ice-cold 1.5 mM KCl. Livers were separately homogenized in a 20-mM sodium phosphate buffer pH 7.4. A 10-min 400 $\times$ g centrifugation allowed the removal of cellular debris from the homogenate. The peroxisomal fraction was prepared by a 15-min 10,000 $\times$ g centrifugation. The resulting pellet was washed twice and resuspended in a Tris/HCl buffer (pH 8.3; 20 mM) containing 0.25 M sucrose and 5 mM EDTA. A 60-min 100,000 $\times$ g centrifugation of the 10,000 $\times$ g supernatant separated the cytosolic and the microsomal fractions. The latter was then resuspended in a sodium phosphate buffer (pH 7.4; 10 mM) containing 5 mM EDTA and 20 % glycerol. Fractions were frozen at −80°C. All handling was performed at 0 to 4°C.

Enzyme assay. Cyanide-insensitive β-oxidation of palmitoyl coenzyme A was determined according to the method of Lazarow (1981) by measuring, in presence of cyanide, the accumulation of NADH resulting from the peroxisomal oxidation of palmitoyl CoA, and monitored at 340 nm. Microsomal omega hydroxylation of lauric acid was determined using a radiometric method with [^{14}C]-lauric acid as substrate (Orton and Parker, 1982). Microsomal 7-ethoxy- (EROD) and 7-pentoxyresorufin-O-dealkylation was measured using the method described by Dutton and Parkinson (1989); the resorufin formed was detected fluorometrically ($\lambda_{ex.}$: 535 nm; $\lambda_{em.}$: 585 nm) and compared to a calibration curve (0–1 nmol resorufin). Microsomal testosterone hydroxylase activity was determined using ^{3}H-labeled testosterone as substrate (Draper *et al*., 1998). Cytosolic and peroxisomal epoxide hydrolase (EH) activities were determined using [^{3}H]-*trans*-1,3-diphenyl-propene oxide (tDPPO) as substrate (Borhan *et al*., 1995). [^{3}H]-*cis* Stilbene oxide (cSO) was used to quantify microsomal EH activity (Wixtrom and Hammock, 1985). Cytosolic glutathione transferase activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate (Habig *et al*., 1974). 4-Nitrophenylacetate was used to quantify microsomal carboxylesterase activity (Krisch, 1966). Protein concentration was quantified using the Pierce BCA assay with bovine serum albumin (BSA) as the calibrating standard.

Western blot. The Western blot was performed using anti-rat cytochrome P-450 4A1 antibodies purchased from Amersham. A semi-dry blotting technique was used to transfer samples from the SDS–PAGE to a 0.2-μm Biorad nitrocellulose membrane. The latter was blocked with a 10 g/l BSA solution. The blot was developed using metal-enhanced 3,3-diaminobenzidiene (Boehringer-Mannheim).

RESULTS

Animal Behavior

Over the 7 days of treatment, there was no effect observed on the overall health of the animals following fenvaleric acid administration. It did not cause any effect on food or water consumption. Moreover, no significant difference in the body weight was observed for any of the doses administered (Table 1), indicating no toxic effect attributable to fenvaleric acid **II**. The racemic fenvaleric acid **II** produced a significant dose-dependent hepatomegaly (Table 1). This effect is attributed to the (S)-**II** enantiomer content, as this enantiomer induced a

TABLE 1
Effect of a 1-Week Exposure of Clofibric Acid and Fenvaleric Acid on Body and Liver Weights and Peroxisomal, Microsomal, and Cytosolic Proteins in F344 Rats

	Body weight (% initial)	Relative liver weight (g/100 g body wt)	Peroxisomal protein (mg/g liver)	Microsomal protein (mg/g liver)	Cytosolic protein (mg/g liver)
Control	119 ± 4	4.5 ± 0.2	10 ± 1	11 ± 1	57 ± 4
Clofibric acid					
1.5 mmol/kg	121 ± 8	6.8 ± 0.6*	9 ± 1	14 ± 1*	64 ± 5
Fenvaleric acid					
(±) 0.75 mmol/kg	122 ± 2	4.9 ± 0.2*	10 ± 1	11 ± 1	56 ± 4
(±) 1.5 mmol/kg	120 ± 3	5.0 ± 0.3*	10 ± 1	13 ± 2	54 ± 4
(±) 3.0 mmol/kg	121 ± 5	5.5 ± 0.1*	11 ± 1	11 ± 1	59 ± 3
(*R*) 1.5 mmol/kg	121 ± 2	4.8 ± 0.3	10 ± 2	10 ± 1	60 ± 4
(*S*) 1.5 mmol/kg	122 ± 3	5.2 ± 0.3*	9 ± 1	11 ± 1	60 ± 6

Note. Each value is sample mean ± standard deviation ($n = 5$).
* Significantly different from controls, $p < 0.05$.

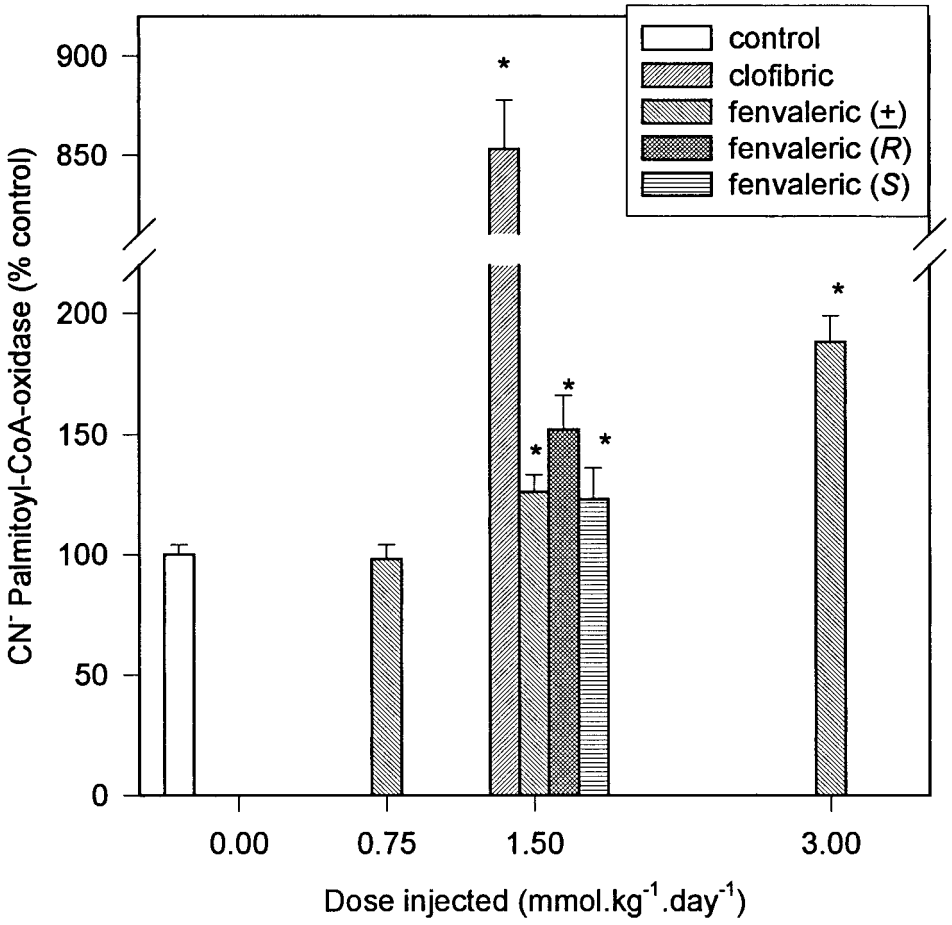

FIG. 2. Effect of 1-week exposure of clofibric acid and fenvaleric acid on hepatic CN^- palmitoyl-CoA oxidase activity in F344 rats.

significant increase in liver weight whereas the (*R*)-**II** enantiomer at the same doses (1.5 mmol/kg) did not.

Hepatic cytosolic, peroxisomal, and microsomal fractions were prepared as indicated in Materials and Methods. No significant difference in the protein content of these fractions was observed for any of the doses admnistered (Table 1).

Effects on Peroxisomal β-Oxidation

The effects on peroxisomal β-oxidation were assayed by measuring palmitoyl-CoA oxidase activity in the presence of cyanide. Results obtained are displayed in Figure 2. With the exception of the lowest dose tested, racemic fenvaleric acid administration induced a significant increase ($\alpha = 0.05$) in β-oxidation activity. At the highest concentration tested, a two-fold increase in activity was observed. At 1.5 mmol/kg, the (*R*) enantiomer induced significantly more CN^- palmitoyl-CoA oxidase activity than the (*S*) enantiomer.

Effect on Cytochrome P450 Activities

Four cytochrome P450 activities were tested on the hepatic microsomal fractions. A summary of the results is shown in Table 2. Following racemic fenvaleric acid administration, ω-laurate hydroxylase (CYP4A1), EROD (CYP 1A), and PROD (CYP2B) activities were significantly increased in a dose-dependent manner. Interestingly, the CYP2B activity was increased more than CYP4A1 and CYP1A. The testosterone hydroxylase activity (CYP3A and 2B11) was not significantly influenced by any dose of the pesticide metabolite administered. The ω-laurate hydroxylase activity was induced significantly more by the (*R*) enantiomer, whereas the PROD activity was induced more by the (*S*) enantiomer. No significant difference in the EROD activity was observed between the two enantiomers.

Effect on Other Enzyme Activities

Five drug-metabolizing enzymes were assayed in the hepatic fractions. The results are presented in Table 3. Following racemic fenvaleric acid **II** administration, peroxisomal, cytosolic, and microsomal epoxide hydrolase activities were significantly increased in a dose-dependent manner. Interestingly, the microsomal activity was increased more than the two soluble activities. The carboxylesterase activity was also significantly increased in a dose- dependent manner, whereas the glutathione transferase activity was significantly decreased

TABLE 2
Effect of 1-Week Exposure of Clofibric Acid and Fenvaleric Acid on Four Hepatic P-450 Activities in F344 Rats

	ω-Laurate hydroxylase[a] P-450 4A1	7-Ethoxy-resorufin-O-dealkylase[b] P-450 1A	7-Pentoxy-resorufin-O-dealkylase[b] P-450 2B	Testosterone hydroxylase[b] P-450 3A + P-450 2B11
Control	3.0 ± 0.2	114 ± 21	10 ± 3	248 ± 22
Clofibric acid				
1.5 mmol/kg	21 ± 2 (7.0)*	14 ± 17 (1.0)	59 ± 9 (5.9)*	222 ± 19 (0.9)
Fenvaleric acid				
(±) 0.75 mmol/kg	3.9 ± 0.3 (1.3)*	377 ± 33 (3.3)*	81 ± 6 (8.1)*	233 ± 17 (0.9)
(±) 1.5 mmol/kg	5.5 ± 0.4 (1.8)*	447 ± 34 (3.9)*	181 ± 12 (18.1)*	240 ± 33 (1.0)
(±) 3.0 mmol/kg	12.0 ± 0.4 (4.0)*	677 ± 20 (5.9)*	329 ± 24 (32.9)*	282 ± 22 (1.1)
(*R*) 1.5 mmol/kg	8.2 ± 0.7 (2.7)*	553 ± 60 (4.9)*	110 ± 13 (11.0)*	256 ± 13 (1.0)
(*S*) 1.5 mmol/kg	5.2 ± 0.2 (1.7)*	615 ± 42 (5.4)*	228 ± 22 (22.8)*	298 ± 20 (1.2)*

Note. Each value = sample mean ± standard deviation.
[a] Given in nmol/min^{-1}/mg^{-1}.
[b] Given in pmol.min^{-1}.mg^{-1}.
* Significant difference at $p < 0.05$.

without any clear dose dependence. The two soluble EH activities were increased more by the (*R*) enantiomer; the microsomal EH activity was increased more by the (*S*) enantiomer. The carboxylesterase activity was induced more by the (*R*) enantiomer.

Western Blot

To verify that the increase in laurate hydroxylase activity (P-450 4A1) was due to an induction of enzyme synthesis, a Western blot analysis was done on the microsomal fractions using a commercial CYP4A1 antibody. Results are displayed in Figure 3. As expected, an immunolabeled band at 45 kDa was obtained for each fraction tested. As determined by densitometry using NIEH Image software (NIEH, Washington, DC), the intensity of the immunolabeled protein increased in correlation with the fenvaleric acid dose, indicating an induction of the synthesis of the P-450 4A1. Moreover, this increase in intensity of the band is well correlated ($r^2 = 0.96$) with the increase of ω-laurate hydroxylase activity observed. Due to the lack of antibodies easily available for the other enzymes induced, Western blots were not performed on other drug-metabolizing enzymes.

DISCUSSION

Results obtained show that fenvaleric acid **II** induced numerous hepatic drug- metabolizing enzymes in F344 rats. The fact that this compound induced hepatomegaly and increased in a dose-dependent manner the peroxisomal β-oxidation of lipids would support the classification of fenvaleric acid as a typical peroxisome proliferator (Reddy & Lalwani, 1983). This is confirmed by the ability of this chemical to induce CYP4A1

TABLE 3
Effect of 1-Week Exposure of Clofibric Acid and Fenvaleric Acid on Five Hepatic Drug Metabolism Enzyme Activities in F344 Rats

	Peroxisomal epoxide hydrolase[a]	Cytosolic epoxide hydrolase[a]	Microsomal epoxide hydrolase[a]	Microsomal carboxylesterase[a]	Cytosolic glutathione tranferase[a]
Control	4.1 ± 0.3	4.0 ± 0.5	7.8 ± 0.6	3.9 ± 0.5	4.3 ± 0.1
Clofibric acid					
1.5 mmol/kg	9.3 ± 0.6 (2.3)*	23.1 ± 1.5 (5.7)*	8.8 ± 0.8 (1.1)	5.2 ± 0.9 (1.3)*	1.8 ± 0.1 (0.4)*
Fenvaleric acid					
(±) 0.75 mmol/kg	4.2 ± 0.1 (1.0)	5.1 ± 0.4 (1.3)*	10.5 ± 0.6 (1.3)*	3.7 ± 0.2 (0.9)	4.2 ± 0.1 (1.0)
(±) 1.5 mmol/kg	4.6 ± 0.2 (1.2)*	5.7 ± 0.2 (1.4)*	16.0 ± 1.1 (2.1)*	5.2 ± 1.4 (1.3)*	3.9 ± 0.2 (0.9)*
(±) 3.0 mmol/kg	5.2 ± 0.3 (1.3)*	7.8 ± 0.7 (2.0)*	24.1 ± 0.8 (3.1)*	6.4 ± 1.3 (1.6)*	3.8 ± 0.3 (0.9)*
(*R*) 1.5 mmol/kg	5.0 ± 0.3 (1.2)*	6.8 ± 0.5 (1.7)*	14.3 ± 1.1 (1.8)*	7.4 ± 1.0 (2.9)*	3.5 ± 0.1 (0.8)*
(*S*) 1.5 mmol/kg	4.4 ± 0.2 (1.1)	4.9 ± 0.2 (1.2)*	18.7 ± 1.8 (2.4)*	5.8 ± 1.4 (1.5)*	4.0 ± 0.2 (0.9)*

Note. Each value = sample mean ± standard deviation.
[a] Given in nmol/min^{-1}/mg^{-1}.
* Significant difference at $p < 0.05$.

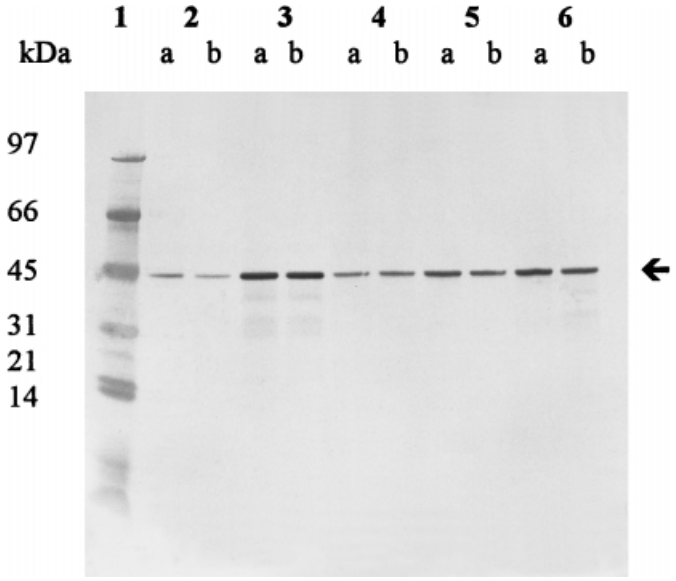

FIG. 3. Western blot analysis for microsomal P450 IV A1. A 3-μg aliquot of microsomal protein was loaded on each track. **1**, molecular-weight markers; **2**, control; **3**, clofibric acid 1.5 mmol/kg; **4**; (±)-**II** 0.75mmol/kg; **5**, (±)-**II** 1.5 mmol/kg; **6**, (±)-**II** 3.0 mmol/kg. For each dose, extract from two different rats was loaded (a and b). As indicated in the text, densitometric analysis indicated a significant increase in CYP4A1.

biosynthesis. Moreover, the induction of soluble epoxide hydrolase and carboxylesterase is also characteristic of peroxisome proliferators (Grant *et al*., 1994). However, fenvaleric acid exposure resulted in a lower level of enzyme induction (2- to 4-fold) than is demonstrated by some other peroxisome proliferators such as clofibric acid **III** (Moody *et al*., 1991). The slight increase in the incidence of liver cell tumors by fenvalerate over a 2-year carcinogenicity study (Cabral and Galendo, 1990), agrees with the lower peroxisome proliferation activity observed in the current study.

Fenvaleric acid also induced CYP2B and microsomal epoxide hydrolase. These are generally only slightly induced by peroxisome proliferators (Grant *et al*., 1994) and more significantly by some compounds such as phenobarbital (Hassett *et al*., 1998). The mEH is involved in the metabolism of xenobiotic epoxides and possibly also in the metabolism of endogenous steroid epoxides (Vogel-Bindel *et al.*, 1982). Furthermore, mEH is implicated in the development of emphysema (Smith and Harrison, 1997), spontaneous abortion (Wang *et al*., 1998), and cancer (Benhamou *et al*., 1998). Induction by fenvaleric acid could disturb the physiologic role of this enzyme.

Interestingly, the (*R*) enantiomer induced the peroxisome proliferation enzyme markers more than the (*S*) enantiomer. A similar effect of the absolute spatial conformation was observed with fibrate analogues (Holloway and Thorp, 1993). It is consistent with the higher potency of the (*R*) enantiomer of fenvaleric acid to induce the production of microgranuloma in rat liver (Okuno *et al*., 1986). The (*S*) enantiomer of fenvaleric acid was a more potent inducer of CYP1A, 2B, and mEH, indicating that each enantiomer induced two different sets of enzymes. It has been proposed that peroxisome proliferators enter the cell by diffusion, then bind directly to the peroxisome proliferator activator receptor (PPAR) located upstream of target genes (Issemann and Green, 1990). The activated receptor would enhance or repress gene transcription, especially those required for the peroxisomal β-oxidation, as well as genes of the P450 CYP4A family (Kimura *et al*., 1989). Induction of the expression of CYP 1A, 2B, and 3A, and mEH genes occurs by different mechanisms largely unknown (Hassett *et al*., 1998). The results obtained indicate that both sets of genes are probably induced by opposite enantiomers of fenvaleric acid.

In conclusion, fenvaleric acid is able to induce numerous drug-metabolizing enzyme activities in rat liver. Exposure to pyrethroid insecticides is considered low (UN-WHO, 1990), so the direct effect of this compound on human health is probably minimal. However, high fenvalerate exposure, and probably exposure to other pyrethroid insecticides also, could perturb normal metabolism of other xenobiotics or endogenous compounds, especially those transformed by CYP2B. Fenvalerate insecticide consists of a mixture of four isomers (two pairs of diastereoisomers), only one of which is insecticidally active. This insecticide has now been largely replaced worldwide by the chiraly synthesized active isomer, esfenvalerate, made from the (*S*)-fenvaleric acid, which is the more active enantiomer in the present study.

ACKNOWLEDGMENTS

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