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MONOCLONAL ANTIBODY-DIRECTED ASSESSMENT OF TOLUENE INDUCTION OF RAT HEPATIC CYTOCHROME P450 ISOZYMES

RUI-SHENG WANG,*† TAMIE NAKAJIMA,* SANG S. PARK,‡ HARRY V. GELBOIN‡ and NINZO MURAYAMA*

*Department of Hygiene, Shinshu University School of Medicine, Matsumoto 390, Japan and, ‡Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892, U.S.A.

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Abstract—Cytochrome P450 isozymes induced in rat liver by a range of concentrations of toluene were studied with monoclonal antibodies (MAbs) to specific P450 isozymes and by enzyme assays. Nitrosodimethylamine demethylase activity was significantly increased in microsomes from rats exposed to more than 1000 ppm of toluene, an increase that was dose-dependent. Anti-CYP2E1 significantly inhibited the metabolism of toluene to benzyl alcohol (BA) by about 50%, in microsomes from 1000 to 4000 ppm toluene-exposed rats, at low substrate concentration (0.2 mM). With anti-CYP2B1/2, the rate of BA formation was decreased by 15–17% in microsomes from rats of 2000 and 4000 ppm toluene exposures at high substrate concentration (5.0 mM). On the other hand, anti-CYP2C11/6 inhibited the rate of formation of BA in all of the microsomes, but the extent of inhibition was progressively decreased from 55% in control to 33% in 4000 ppm exposure. Immunoblot analysis with anti-CYP2E1 and anti-CYP2B1/2 revealed stronger immunoreactive bands in microsomes from rats exposed to more than 1000 and 2000 ppm of toluene, respectively. Stronger bands were also observed in microsomes from rats of 2000–4000 ppm toluene exposures with anti-CYP3A1/2, but no immunoreactivity appeared with anti-CYP1A1/2. These results suggest that toluene induces CYP2E1, CYP2B1/2 and CYP3A1/2, but reduces CYP2C11/6, and has no effect on CYP1A1/2.

Cytochrome P450 in liver microsomes plays a key role in the metabolism of endogenous and exogenous substances including hormones, drugs, carcinogens and many other foreign chemicals. Changes in the activity and function of hepatic cytochrome P450 are, therefore, of great importance in pharmacology and toxicology.

Cytochrome P450s, a superfamily of enzymes, have different substrate specificities and are regulated by different genes [1]. Toluene, a widely used chemical, is found to induce the activity of cytochrome P450-mediated hepatic mono-oxygenases [2, 3], and thus may modify the metabolism and toxicity of other chemicals [4]. However, the specific type of cytochrome P450 which is induced by toluene has not been defined. Furthermore, studies on the induction of mono-oxygenases by toluene are usually conducted at a single concentration, and the dose–response relationship between toluene concentration and induction is not well understood.

Monoclonal antibodies (MAbs§) against different forms of P450 isozymes are useful for quantitative identification of P450 engaged in specific metabolism [5]. In this report, we used MAbs to identify the toluene-inducible isozymes in the livers of rats exposed to different concentrations of toluene, by their inhibitory effect on metabolism. Our results indicate that exposure to toluene induces at least three forms of P450 isozymes, which are respectively related to those induced by ethanol, phenobarbital (PB) and pregnenolone-16α-carbonitrile (PCN), and one type of isozyme is reduced.

MATERIALS AND METHODS

Animals. Male Wistar rats were obtained from Nippon SLC Inc. (Shizuoka, Japan), and housed in a temperature- and light-controlled room (20 ± 2°, light 6:00 a.m.–6:00 p.m.) with free access to food (Nippon Clea, CE-2, Tokyo, Japan) and water for 2 weeks. At the age of 8 weeks, they were fed a nutritionally adequate liquid diet according to the method of DeCarli and Lieber [6] with slight modifications [7]. Some animals were fed an ethanol-containing diet (2 g ethanol/day/rat) which is isocaloric to the control diet (1 kcal/mL). These liquid diets were given once a day, 80 mL/rat at 4:00 p.m. for 3 weeks as the only source of nutrition.

Toluene exposure. Rats were exposed to toluene in a dynamic exposure chamber as described elsewhere [8], at concentrations of 500, 1000, 2000 and 4000 ppm for 6 hr (10:00 a.m.–4:00 p.m.). Toluene concentration in the chamber was monitored every 15 min by a gas chromatograph equipped with a hydrogen flame ionization detector. The standard deviation of the time-weighted toluene concentration during the 6 hr exposure was less than 10%. A control group exposed to 0 ppm (air only) was included. No food was allowed during 24 hr including

† Corresponding author. Tel. (81) 263-35-4600, ext. 5208; FAX (81) 263-33-6458.

§ Abbreviations: MAb, monoclonal antibody; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; NDMAD, nitrosodimethylamine demethylase; PROD, 7-pentoxyresorufin *O*-depentylase; EROD, 7-ethoxyresorufin *O*-deethylase; BA, benzyl alcohol.

the 6 hr exposure, before the animals were killed, but water was offered.

Isolation of liver microsomes. Twenty-five percent homogenate of rat liver in 1.15% KCl was centrifuged at 10,000 *g* for 15 min, and the resulting supernatant was centrifuged at 105,000 *g* for 60 min. Microsomes obtained were washed and then suspended in phosphate buffer (0.05 M, pH 7.4) containing 10% glycerol and stored at −85° at a concentration of about 20 mg protein/mL.

Protein and enzyme assays. Microsomal protein concentration and the total cytochrome P450 content were measured by the method of Lowry *et al.* [9] and the method of Omura and Sato [10], respectively.

Nitrosodimethylamine demethylase (NDMAD) activity was determined with the method of Tu and Yang [11] with some modifications. The demethylase assay mixture contained 60 mM Tris–HCl buffer, pH 7.4, 0.5 mM NADP, 10 mM glucose 6-phosphate, 2 IU of glucose 6-phosphate dehydrogenase, 25 mM $MgCl_2$, 180 mM KCl, 4 mM substrate (NDMA), and 400 μg microsomal protein as the enzyme, in a final volume of 1 mL. The reaction was initiated with the addition of substrate and carried out in vials at 37° in a shaking water bath for 20 min. The reaction was terminated by adding 0.1 mL of each 15% $ZnSO_4$ and saturated $Ba(OH)_2$. The mixture was centrifuged at 3000 rpm for 15 min, and 0.7 mL of the supernatant was mixed to 0.3 mL of a concentrated Nash reagent (15 g of ammonium acetate, 0.2 mL of acetylacetone and 0.54 mL of acetic acid in a final volume of 18 mL of water solution). After the incubation at 50° for 30 min, absorbance at 412 nm was measured to determine the amount of formaldehyde formed. Under these conditions, the amount of HCHO formed was proportional to microsomal protein at least to 600 μg, and to incubation time till 30 min.

The activities of 7-ethoxyresorufin *O*-deethylase (EROD) and 7-pentoxyresorufin *O*-depentylase (PROD) were measured kinetically at 1 μM substrate concentration with a fluorescence spectrophotometer (Hitachi F-3000) by the methods of Prough *et al.* [12] and Burke *et al.* [13], respectively.

Monoclonal antibody inhibition. Some P450 isozymes were found responsible for the metabolism of toluene [14]. The inhibition of toluene metabolism by MAbs against specific epitopes in P450 isozymes was used to investigate the quantitative changes of P450 isozymes in microsomes from toluene-treated rats. Three MAbs were used for the inhibition study: anti-CYP2E1 (clone 1-91-3), anti-CYP2C11 (clone 1-68-11) which cross-reacts with CYP2C6, and anti-CYP2B1 (clone 2-66-3) which cross-reacts with CYP2B2. As a reference protein, a control MAb (Hy-Hel) against chicken lysozyme was used to determine non-specific reaction. The characterization of these MAbs has been described [15–17]. The metabolism of toluene was assessed by measuring the producing rate of benzyl alcohol [BA] which is the mediate product of the side-chain oxidation, and *o*- and *p*-cresol which are the metabolites of ring hydroxylation. The maximal inhibition was found under a ratio of MAb to microsomal protein of 1:1 for anti-CYP2E1 and 1:2 for the other two MAbs. The analytic procedures were reported previously [14].

Table 1. Contents of microsomal protein and cytochrome P450 in rat liver after toluene exposure

Treatment (ppm)	Microsomal protein (mg/g liver)	Cytochrome P450 (nmol/mg protein)
Control	24.32 ± 0.91	0.56 ± 0.05
500	24.90 ± 1.46	0.65 ± 0.05
1000	25.82 ± 2.83	0.72 ± 0.08*
2000	28.88 ± 2.61*	0.71 ± 0.06*
4000	31.04 ± 3.08*	0.68 ± 0.05*

Figures represent the means ± SD for five rats.
* Significantly different from control group ($P < 0.05$).

Western blot analysis. Western blotting was performed as described earlier [18], with slight modifications. Electrophoretic transfer to nitrocellulose sheet was carried out at 100 V for 3 hr with the circulating cooling water. After immersing in 50 mM Tris–200 mM NaCl, pH 7.4, containing 0.05% Tween 20, for 30 min, the nitrocellulose paper was treated with MAbs for 1 hr and then detected with Vectastain ABC kit (Vector, Burlingame, CA, U.S.A.), followed by a color developing reaction with 4-chloro-1-naphthol as the substrate. MAbs used in immunodetection were anti-CYP2E1 (clone 1-98-1) [15], anti-CYP2B1/2, anti-CYP1A1 (clone 1-7-1) which cross-reacts with CYP1A2 [19], and anti-CYP3A1 (clone 2-13-1) which cross-reacts with CYP3A2 [20]. Anti-CYP2C11/6 was not used in immunoblot analysis because it has only little binding affinity for sodium dodecyl sulfate–denatured antigen [21].

Statistics. Data were analysed by one-way analysis of variance, and the means were tested with Student's *t*-test when the variances seem to be equal, otherwise Cochran–Cox test was used. Differences among the mean values were considered significant at $P < 0.05$.

RESULTS

Microsomal protein and cytochrome P450

Table 1 shows the changes in microsomal protein and total cytochrome P450 content in livers of rats after exposure to different concentrations of toluene. Microsomal protein content was increased after 2000 ppm exposure, whereas cytochrome P450 content increased after 1000 ppm toluene exposure.

Nitrosodimethylamine, 7-pentoxyresorufin and 7-ethoxyresorufin metabolism

NDMAD activity was significantly enhanced in microsomes from rats exposed to 1000–4000 ppm toluene, and showed a dose-dependent effect: increment of the enzyme activity increased significantly upon toluene exposure concentration over 500 ppm. This increase was not parallel to the change of gross P450 content. The greatest activity was found in microsomes of 4000 ppm exposure.

PROD activity was only increased at the two high concentrations of toluene exposure, with 4.5- and 8.4-fold at 2000 and 4000 ppm exposure, respectively.

Table 2. Effect of toluene exposure on mono-oxygenase activities in rat livers

Treatment (ppm)	NDMAD	PROD	EROD
	(nmol/mg protein/min)		
Control	1.92 ± 0.18	0.08 ± 0.04	0.26 ± 0.12
500	2.06 ± 0.18	0.10 ± 0.02	0.26 ± 0.04
1000	2.48 ± 0.14*†	0.14 ± 0.04	0.33 ± 0.07
2000	3.30 ± 0.26*†	0.36 ± 0.06*	0.32 ± 0.04
4000	4.27 ± 0.36*†	0.67 ± 0.13*	0.32 ± 0.07

Each figure represents the mean ± SD for five rats.
* Significantly different from control group ($P < 0.05$).
† Significantly different from the group above ($P < 0.05$).

On the other hand, toluene exposure had little influence on EROD activity (Table 2).

Inhibition of toluene metabolism by MAbs

Anti-CYP2E1 was used in the metabolism of toluene at low concentration (0.2 mM), whereas anti-CYP2B1/2 and anti-CYP2C11/6 were employed at high toluene concentration (5.0 mM), considering that CYP2E1 involved in toluene metabolism is a low-K_m isozyme, whereas CYP2B1/2 and CYP2C11/6 are high-K_m isozymes in rat liver microsomes [14, 22].

At low toluene concentration, the metabolic formation of BA was significantly increased in microsomes from 1000 to 4000 ppm toluene-exposed rats; the rate of formation of *p*-cresol was increased even in microsomes from 500 ppm exposure, but that of *o*-cresol was increased only at 4000 ppm exposure. With anti-CYP2E1, the metabolic rates of BA and *p*-cresol were both significantly inhibited to about 50 and 60% of that with Hy-Hel, respectively, in all of the microsomes with the exception of *p*-cresol in control microsomes, but the rate of *o*-cresol formation was not influenced by anti-CYP2E1 (Table 3).

At high substrate concentration (Table 4), anti-CYP2B1/2 inhibited the formation of BA in microsomes from rats exposed to the two high concentrations of toluene. Anti-CYP2C11/6 resulted in a significant decrease in the rate in all of the microsomes compared with Hy-Hel, but the extent of inhibition of this MAb was progressively decreasing upon toluene exposure concentrations, from 50 to 55% in control and 500 ppm exposure to 23% in 4000 ppm exposure, and this was opposite to that of anti-CYP2B1/2.

For *p*-cresol formation, anti-CYP2B1/2 showed inhibitive effect only in microsomes from rats exposed to the two high concentrations of toluene, and inhibited the metabolism by 40% and 53% upon 2000 and 4000 ppm exposure, respectively. On the other hand, anti-CYP2C11/6 exhibited inhibition only in microsomes from control and 500 ppm toluene-exposed rats, displaying a different modifying mode to anti-CYP2B1/2 as on BA metabolism.

For *o*-cresol formation, anti-CYP2B1/2 showed inhibition only in microsomes from 4000 ppm

Table 3. Inhibition of toluene metabolism by MAb anti-CYP2E1 in microsomes from toluene-exposed rats

Treatment (ppm)	Benzylalcohol		*p*-Cresol		*o*-Cresol	
	Hy-Hel	Anti-CYP2E1	Hy-Hel	Anti-CYP2E1	Hy-Hel	Anti-P450IIE1
Control	1.55 ± 0.10 (100)	0.84 ± 0.14 (54.2)*	0.05 ± 0.01 (100)	ND	0.03 ± 0.01 (100)	0.03 ± 0.01 (100.0)
500	1.68 ± 0.13 (100)	0.89 ± 0.09 (53.0)*	0.08 ± 0.01 (100)	0.06 ± 0.01 (75.0)*	0.05 ± 0.02 (100)	0.05 ± 0.01 (100.0)
1000	1.94 ± 0.14 (100)	0.94 ± 0.09 (48.5)*	0.08 ± 0.01 (100)	0.05 ± 0.02 (62.5)*	0.05 ± 0.01 (100)	0.04 ± 0.01 (80.0)
2000	2.35 ± 0.29 (100)	1.02 ± 0.20 (43.4)*	0.10 ± 0.02 (100)	0.05 ± 0.01 (50.0)*	0.04 ± 0.01 (100)	0.03 ± 0.01 (75.0)
4000	2.86 ± 0.18 (100)	1.41 ± 0.23 (49.3)*	0.15 ± 0.02 (100)	0.09 ± 0.02 (60.0)*	0.07 ± 0.01 (100)	0.06 ± 0.01 (85.7)

Each figure represents the remaining activity (nmol/mg protein/min) and is presented as mean ± SD for five rats.
Figures in parentheses show the percentages of remaining activity to the activity with Hy-Hel.
The enzyme assays were performed at toluene concentration of 0.2 mM.
ND, not detectable.
* Significantly different from Hy-Hel ($P < 0.05$).

Table 4. Inhibition of toluene metabolism by two MAbs in microsomes from toluene-exposed rats

Treatment (ppm)	Hy-Hel	MAb Anti-CYP2B1/2	Anti-CYP2C11/6
Benzylalcohol (nmol/mg protein/min)			
Control	6.13 ± 0.35 (100)	5.96 ± 0.32 (97.2)	2.73 ± 0.12 (44.5)*
500	5.79 ± 0.52 (100)	5.50 ± 0.52 (95.0)	2.86 ± 0.25 (49.4)*
1000	6.32 ± 0.50 (100)	6.14 ± 0.62 (97.2)	3.27 ± 0.32 (51.7)*
2000	6.26 ± 0.61 (100)	5.35 ± 0.52 (85.5)*	3.76 ± 0.10 (60.1)*
4000	6.55 ± 0.73 (100)	5.41 ± 0.44 (82.6)*	5.05 ± 0.48 (77.1)*
p-Cresol (nmol/mg protein/min)			
Control	0.14 ± 0.02 (100)	0.14 ± 0.01 (100.0)	0.09 ± 0.02 (64.3)*
500	0.12 ± 0.01 (100)	0.13 ± 0.01 (108.3)	0.09 ± 0.01 (75.0)*
1000	0.15 ± 0.02 (100)	0.12 ± 0.01 (80.0)	0.11 ± 0.03 (73.3)
2000	0.20 ± 0.05 (100)	0.12 ± 0.02 (60.0)*	0.18 ± 0.14 (90.0)
4000	0.30 ± 0.09 (100)	0.14 ± 0.02 (46.7)*	0.32 ± 0.09 (106.7)
o-Cresol (nmol/mg protein/min)			
Control	0.15 ± 0.04 (100)	0.15 ± 0.03 (100.0)	0.07 ± 0.03 (46.7)*
500	0.13 ± 0.01 (100)	0.11 ± 0.01 (84.6)	0.07 ± 0.01 (53.8)*
1000	0.13 ± 0.01 (100)	0.12 ± 0.01 (92.3)	0.08 ± 0.02 (61.5)*
2000	0.18 ± 0.06 (100)	0.12 ± 0.03 (66.7)	0.10 ± 0.01 (55.6)*
4000	0.22 ± 0.05 (100)	0.10 ± 0.01 (45.5)*	0.20 ± 0.05 (90.9)

Figures represent the means ± SD for five rats.
Figures in parentheses are the percentages of remaining activity expressed as: (activity with MAb/activity with Hy-Hel) ×100.
The enzyme assays were carried out at toluene concentration of 5 mM.
* Significantly different from that with Hy-Hel ($P < 0.05$).

toluene-exposed rats, but anti-CYP2C11/6 showed effects in all of the microsomes except those from 4000 ppm toluene-exposed rats.

Although the fractional inhibition of BA metabolizing isozyme activity with anti-CYP2E1 in toluene-exposure groups remained very similar to that in the control (Fig. 1), the increment of net inhibition (i.e. the activity inhibited by MAb), increased significantly with toluene exposure concentration over 1000 ppm: 0.71 nmol/mg/min was inhibited in control microsomes, but the inhibition increased to 141, 189 and 204% in 1000, 2000 and 4000 ppm toluene exposure groups, respectively.

Anti-CYP2B1/2 did not inhibit the activity of BA metabolizing isozyme in control, 500 and 1000 ppm toluene exposure groups, but this MAb inhibited 15–18% activity at two high concentrations of toluene exposures (Fig. 1).

Anti-CYP2C11/6 showed the greatest inhibitory effect (55%) on BA metabolic isozyme activity in microsomes from rats of control at high substrate concentration, suggesting that cytochrome P450IIC11/6 plays an important role in toluene metabolism in non-treated rats at high substrate concentration. The inhibitory extent, however, gradually decreased with toluene exposure concentration, and at 4000 ppm toluene exposure, decreased to less than half of that in the control (Fig. 1).

Immunodetection of cytochrome P450 in microsomes

Immunoblot analysis of the microsomes was carried out with MAbs to confirm the induction of specific isozymes of P450 by toluene exposures (Fig. 2). Analysis with anti-CYP2E1 revealed more distinct immunoreactive bands in microsomes from 1000 ppm or higher toluene exposure. With anti-CYP2B1/2, distinguishable bands were observed in microsomes from 4000 and 2000 ppm toluene exposures. Anti-CYP3A1/2 revealed stronger bands in microsomes of the two high concentration toluene exposures. These results suggest that induction of CYP2E1, CYP2B1/2 and CYP3A1/2 by toluene. No immunoreactivity was observed with anti-CYP1A1/2.

DISCUSSION

NDMA has been reported to be a specific probe for CYP2E1 [23]. That the NDMA metabolic rate was increased with a dose-dependent relationship to toluene exposure concentration in this study indicates the induction of CYP2E1 by the treatment of rats with toluene. From MAb inhibition study, it was evident that CYP2E1, which is responsible for toluene metabolism at low substrate concentration, was induced at 1000 ppm toluene treatment, and this induction was dose-dependent to the concentration of toluene exposure. Immunoblot analysis further confirmed the results of enzyme assay and MAb inhibition. It is interesting that the percentage of CYP2E1 activity toward BA formation at low substrate concentration showed little variation (about 45–55% of total activity) in all groups (Fig. 1), despite the induction of this isozyme by toluene exposures. The total activity, on the other hand, showed significant increase upon toluene exposures (Table 3). This is because CYP2B1/2 and CYP2C11/6 also work in toluene metabolism at low (0.2 mM) substrate concentration [14].

It has been reported that toluene exposure

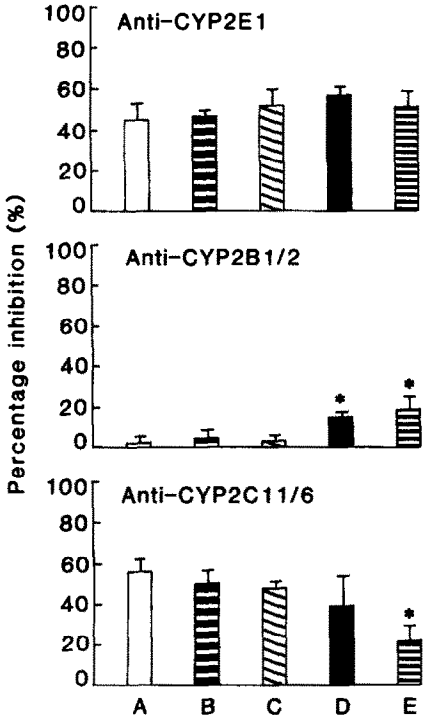

Fig. 1. The inhibition of enzyme activity toward toluene side-chain oxidation by MAbs against specific cytochrome P450 isozymes in rat hepatic microsomes. Substrate concentration used in toluene metabolism was 0.2 mM for anti-CYP2E1, and 5.0 mM for anti-CYP2B1/2 and anti-CYP2C11/6. Each bar represents the mean and SD from five rats. Letters A–E are control, 500, 1000, 2000 and 4000 ppm toluene exposure groups, respectively. *Significantly different from control group ($P < 0.05$).

increases the activities of aminopyrine *N*-demethylase [2], and therefore an inductive effect resembling PB is proposed for toluene [24]. 7-Pentoxyresorufin is exclusively metabolized by CYP2B [13], and the increased PROD activity following toluene exposure of high concentrations is suggestive of the induction of CYP2B. The increase in the fractional inhibition of toluene metabolism and the emergence of band on western blot analysis with anti-CYP2B1/2 further suggest that toluene exposure results in significant induction of CYP2B1/2. This conclusion gives evidence to the supposition of other researchers earlier [24].

It was reported that a significant increase in aryl hydrocarbon hydroxylase and EROD activities is observed in neonatal rats exposed to toluene [25], which are associated with CYP1A, and are known to be induced by 3-methylcholanthrene [26]. No significant increase in EROD activity and no emergence of immunoreactive band with anti-CYP1A1/2 in microsomes from toluene-exposed rats suggest that toluene exposure does not induce CYP1A1/2 in adult rat liver, in agreement with the earlier reports of Pyykko [3] and Pyykko *et al.* [27].

Of particular interest is the change of CYP2C11/6 after toluene exposure. In contrast to the induction of CYP2B1/2, this isozyme was decreased after high concentration toluene exposure (Table 4, Fig. 1). Furthermore, in microsomes from rats treated with ethanol before exposure to toluene, the degree of CYP2B1/2 induction was lower than in microsomes of rats not treated with ethanol, while the extent of CYP2C11/6 reduction was also small (data not shown). These two isozymes are both high-K_m isozymes for the metabolism of toluene [14, 22] and other chemicals [28, 29], indicating that they both play the catalytic role mainly at high concentration of substrate. The decrease of CYP2C11/6 when CYP2B1/2 is induced may be necessary for maintaining the balance between the two isozymes in liver. The induction of CYP2B by other inducers (e.g. PB) also seems to be accompanied by the decrease of CYP2C11 [14, 30], implying that this may be a common phenomenon for the two isozymes. The mechanism for this change, however, is not clear and is in need of further study.

CYP3A can be induced by PCN [31, 32], and often catalyses the 6 β-hydroxylation of steroids. Western blot analysis of microsomes from toluene exposure of high concentrations (2000 and 4000 ppm) showed a stronger immunoreactive band with anti-CYP3A1/2, suggesting that CYP3A1/2, as CYP2E1, are also toluene-inducible isozymes that have not been reported. On the other hand, no inhibition of toluene metabolism was found with anti-CYP3A1/2 (data not shown), implying that this MAb does not conjugate the catalytic site of the enzyme responsible for toluene metabolism as for other xenobiotics [20], or CYP3A1/2 may not catalyse the metabolism of toluene.

Toluene exposure will potentiate the metabolism and toxicity of itself as well as other compounds, through the induction of P450 isozymes. In this study, toluene metabolism was enhanced in microsomes from rats exposed to 1000 ppm or higher toluene at low substrate concentration, because of the induction of CYP2E1. The metabolism, however, showed no change at high substrate concentration. This is because the potentiating effect of CYP2B1/2 induced on toluene metabolism is offset by the reduction of CYP2C11/6, and consequently, the total metabolism of toluene showed no significant change. For some other compounds, the change in types of P450 isozymes may be related to different patterns of metabolism and/or toxicities.

The mechanism of induction of P450 isozymes by toluene is not yet understood. Of interest is the difference in the induction of toluene and PB on CYP2B1/2 and CYP2E1: toluene induced both the isozymes, in accordance with the observations with 2-hexanone and acetonyl acetone [33], but PB induced only CYP2B1/2, and decreased CYP2E1 [14]. The elucidation of the difference is important to understand the mechanism of P450 induction by toluene.

In conclusion, toluene is a mixed type inducer; at least three P450 isozymes are induced by exposure to toluene: CYP2E1 seems to be induced at lower toluene concentration than CYP2B1/2 and

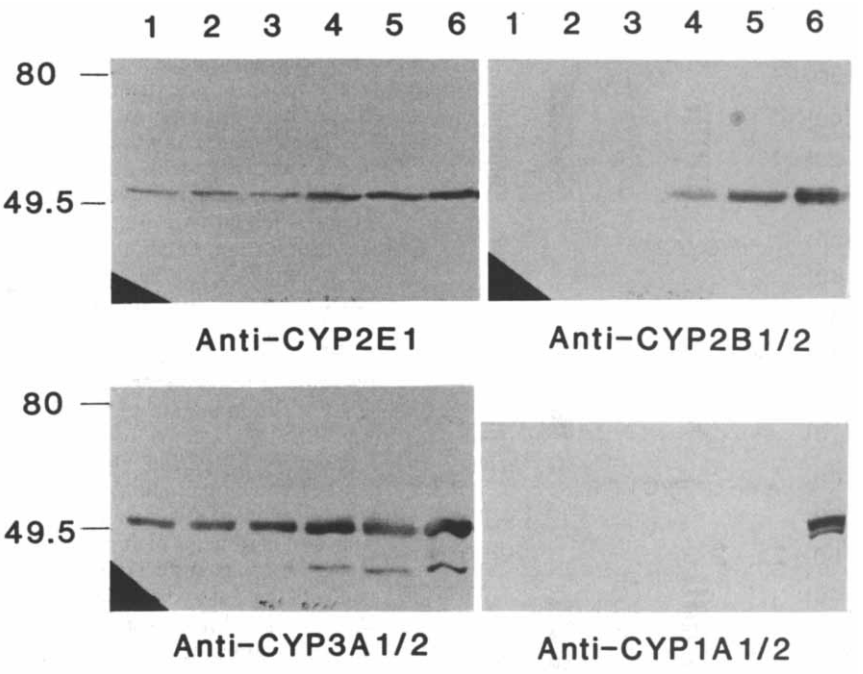

Fig. 2. Western blot analysis of hepatic microsomes from rats exposed to toluene of 0 (lane 1), 500 (lane 2), 1000 (lane 3), 2000 (lane 4) and 4000 ppm (lane 5). Lanes 1–5 in anti-CYP2E1, anti-CYP2B1/2 and CYP1A1/2 each contained 20 μg, and in anti-CYP3A1/2, 10 μg microsomal protein. Lane 6 in anti-CYP2E1 and anti-CYP1A1/2, and lane 6 in anti-CYP2B1/2 and anti-3A1/2 staining each contained 20 and 10 μg microsomal protein from ethanol-, 3-methylcholanthrene-, PB- and PCN-treated rats, respectively.

CYP3A1/2; CYP2C11/6 is reduced by toluene exposure; CYP1A1/2 is not effected.

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