Biochemical Pharmacology, Vol. 43, No. 2, pp. 251–257, 1992
Printed in Great Britain.

0006-2952/92 $5.00+0.00

A COMPARATIVE STUDY ON THE CONTRIBUTION OF CYTOCHROME P450 ISOZYMES TO METABOLISM OF BENZENE, TOLUENE AND TRICHLOROETHYLENE IN RAT LIVER

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(*Received* 27 *June* 1991; *accepted* 12 *September* 1991)

Abstract—The contribution of P450IIE1, P450IIC11/6, P450IIB1/2 and P450IA1/2 to the formation of chloral hydrate (CH) from trichloroethylene (TRI) was investigated in microsomes from control, ethanol-, phenobarbital (PB)- and 3-methylcholanthrene (MC)-treated rats using monoclonal antibodies (MAbs) to the respective P450 isozymes, and compared with their roles in benzene and toluene metabolism. Anti-P450IIE1 inhibited the formation of CH from TRI more strongly in microsomes from ethanol-treated rats than in microsomes from control rats at low concentration of TRI when net inhibition was compared. Anti-P450IIC11/6 inhibited CH formation in microsomes from control and PB-treated rats at high, not low, concentration of TRI, but the net inhibition in control microsomes was less than that due to anti-P450IIE1. Anti-P450IIB1/2 and anti-P450IA1/2 also inhibited CH formation from TRI in microsomes from PB- and MC-treated rats, respectively, stronger at high substrate concentration than at low concentration. These results indicate that P450IIE1, P450IIC11/6, P450IIB1/2 and P450IA1/2 are involved in the metabolic step from TRI to CH, and the first isozyme may be a low-K_m TRI oxidase and the others high-K_m one. Comparing the contributions of four isozymes to benzene, toluene and TRI metabolism, all four acted in the metabolism of these compounds, but P450IIE1 did not catalyse *o*-cresol formation nor P450IA1/2 benzyl alcohol formation from toluene, suggesting regioselectivity of toluene metabolism in the action of these two isozymes. The contribution of P450IIE1 in benzene and TRI oxidation was greater than that of P450IIC11/6, but the reverse was seen with respect to benzyl alcohol formation from toluene, indicating that P450IIC11/6 is relatively inactive towards benzene and TRI oxidation, but is primarily involved in toluene metabolism. P450IIB1/2 and P450IIC11/6 attacked all the metabolic positions studied, but only in the side-chain metabolism of toluene was their contribution significant, suggesting that these two isozymes are quite similar in function.

Trichloroethylene (TRI¶) is a common organic solvent used worldwide in industry as a degreasing agent [1], and is also a widespread environmental contaminant [2]. TRI metabolism has a primary role in the hepatotoxic effect of this substance [3, 4] and in biological monitoring to evaluate levels of exposure [5]. Cytochrome P450,** consisting of multiple isozymes, is a key enzyme in the first step of TRI metabolism chloral hydrate (CH) formation, which is a rate-limiting step in TRI metabolism and has toxicological importance in the hepatotoxicity and carcinogenicity of TRI [3, 4, 6].

We previously demonstrated, on the basis of kinetic analysis, that there are least three different P450 isozymes involved in TRI metabolism in rat liver microsomes [7]. The livers from intact rats contain a TRI oxidase with a low-K_m value, which is induced by ethanol treatment. Phenobarbital (PB) treatment induces a TRI oxidase with a high-K_m and 3-methylcholanthrene (MC) with a medium-K_m isozyme. Identification of which P450 isozymes correspond to the different K_m values, is of interest.

We also demonstrated the unique capability of a monoclonal antibody (MAb) to define the cytochrome P450 isozyme responsible for the metabolism of benzene and toluene [8–10]. TRI has a quite different structure to benzene and toluene, except in having an unsaturated carbon skeleton. Our interest focused on whether cytochrome P450 isozymes exhibit substrate- or regioselectivity to these structurally different hydrocarbons, as has already been observed with other chemicals [11, 12]. Our study of inhibition by MAbs for reaction phenotyping is intended to provide information on this structure–activity relationship between P450 isozymes and benzene, toluene and TRI. For this purpose, we have investigated the contributions of ethanol-, PB- and MC-inducible, and male-specific cytochrome P450s to TRI oxidation using anti-P450IA1/2, anti-P450IIB1/2, anti-P450IIC11/6 and

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¶ Abbreviations: TRI, trichloroethylene; MAb, monoclonal antibody; PB, phenobarbital; MC, 3-methylcholanthrene; CH, chloral hydrate.

** The nomenclature used in this report was described by Nebert *et al.*, *DNA* **8**: 1–13, 1989.

anti-P450IIE1 MAbs and furthermore, the contributions of these isozymes to TRI metabolism have been compared with their contributions to benzene and toluene metabolism.

MATERIALS AND METHODS

Animals. Our experiments were performed in accordance with the Guidelines for Animal Experiment of the Shinshu University School of Medicine. Male Wistar rats (6 weeks of age) were obtained from Nippon SLC Inc. (Shizuoka, Japan). They were housed in an air-conditioned (20 ± 2°) room with artificial lighting from 6 a.m. to 6 p.m. and maintained on pellet feed (Clea CE-2, Tokyo, Japan) and water *ad lib.* until they reached the age of 8 weeks. Rats were divided into four groups: control, PB, MC and ethanol groups. All groups except the one given ethanol were fed a well-balanced liquid diet (basal diet) prepared according to the recipe of Lieber *et al.* [13], with a slight modification: the basal diet contained 15.0 g of sucrose, 3.66 g of casein (sodium salt), 51 mg of L-cysteine, 31 mg of DL-methionine, 2.5 g of oil (olive : corn = 4 : 1), 265 mg of ethyl linoleate, 509 mg of vitamin mixture, 1.0 g of mineral mixture, 25 mg DL-α-tocopherol and 265 mg carrageenan in a volume of 100 mL (1 kcal/mL). The ethanol group was given an ethanol-containing liquid diet (ethanol diet) with the same composition as the basal diet, except that 2.5 g ethanol was added to 100 mL basal diet in which the sucrose content had been reduced to 5 g/100 mL, and oil content increased to 5 g/100 mL, so that the ethanol and basal diets were isocaloric. The rats in the PB- and MC-fed groups were administered PB 80 mg/kg/day and MC 20 mg/kg/day i.p. for 4 days before killing, respectively. Rats were given these liquid diets (80 mL/rat) daily at 4 p.m. for 3 weeks as their only source of food and water.

Preparation of microsomes. All rats were killed by decapitation 18 hr after the last inducer injection and giving ethanol diet (10 a.m.). The liver was removed promptly and perfused with cold 1.15% KCl solution. Liver homogenate 25% (w/v) in the salt solution was centrifuged at 10,000 *g* for 10 min, the supernatant was further centrifuged at 105,000 *g* for 60 min and the microsomal pellets obtained were suspended in 50 mM potassium phosphate buffer (pH 7.4) containing 10% glycerin and then stored frozen at −85° until use.

Metabolism assay. TRI metabolism was assessed by measuring the rate of chloral hydrate (CH) formation according to the method of Nakajima *et al.* [7] with a modification: the microsomal protein used was reduced to 600 μg and incubation time at 37° was changed from 10 to 30 min in all microsomes except PB-induced microsomes. The reaction mixture contained, in addition to the protein, 1.0 mM NADP, 20 mM glucose 6-phosphate, 50 mM $MgCl_2$, 50 mM potassium phosphate buffer (pH 7.4), 2 IU glucose 6-phosphate dehydrogenase, and 0.20 (double of low-K_m value [7] for TRI metabolism) or 5.90 mM (saturated concentration of TRI) TRI. After the incubation, CH concentration was measured as previously described [7]. In these conditions, the amount of CH formed in the incubation system increased linearly with incubation time.

Benzene metabolism was assessed by measuring the rate of phenol and hydroquinone formation at 0.2 and 6.26 mM of benzene according to the method of Nakajima *et al.* [9], and toluene metabolism by benzyl alcohol and *o*- and *p*-cresol formation at 2.0 and 5.0 mM of toluene according to the method of Nakajima *et al.* [10].

Monoclonal antibody inhibition. MAbs were produced at the US National Cancer Institute, Laboratory of Molecular Carcinogenesis using a modification of the method of Koehler and Milstein [14]. In the present study, four MAbs that had been shown to be specific towards different cytochromes P450s were used as follows: anti-P450IA1 (clone 1-7-1) [15], which cross-reacted with P450IA2, anti-P450IIB1 (clone 2-66-3) [16], which cross-reacted with P450IIB2, anti-P450IIC11 (clone 1-68-11) [17], which cross-reacted with P450IIC6, and P450IIE1 (clone 1-91-3) [18]. As a control MAb, Hy-Hel to chicken lysozyme was used to determine any non-specific reaction. Each MAb was added to microsomes and buffer at room temperature 30 min before the metabolism assay at 37° was started by adding NADPH-generating system and substrate (TRI, benzene or toluene). In preliminary experiments, maximal inhibition by each MAb was obtained below a MAb protein/microsomal protein ratio of 1.0 for the assays.

P450IIE1 is known to contribute as a low-K_m isozyme to benzene and toluene metabolism, and at high substrate concentration, the inhibitable activity by anti-P450IIE1 decreases [9, 10]. Moreover, in a study of microsomal TRI metabolism, the ethanol-inducible TRI-metabolizing enzyme showed a low K_m, and at high substrate concentration the metabolic activity decreased compared with that at low concentration [7]. Therefore, the inhibition by anti-P450IIE1 was conducted only at low concentrations (0.23 mM for benzene, and 0.20 mM for toluene and TRI metabolism). The inhibition by anti-P450IIC11/6, P450IIB1/2 and P450IA1/2 was conducted with both low and high substrate concentrations (6.26 mM for benzene, 5.0 mM for toluene and 5.90 mM for TRI).

Microsomal protein and cytochrome P450. Protein content and cytochrome P450 content were measured according to the methods of Lowry *et al.* [19] and Omura and Sato [20], respectively.

Statistics. Analysis of variance was performed. When there was significant difference among groups, means were tested by Student's *t*-test or paired *t*-test. The 0.05 level of probability was the criterion of significance.

RESULTS

Inhibition of TRI metabolism

Ethanol or PB treatment increased the formation of CH from TRI at a substrate concentration of 0.20 mM (Table 1), in accordance with previous reports [3, 4, 7]. MC treatment also enhanced it, but to a smaller extent than ethanol or PB treatment.

With anti-P450IIE1, the formation of CH from TRI was greatly inhibited in microsomes from

Table 1. Inhibition of chloral hydrate formation from trichloroethylene by several monoclonal antibodies in rat liver

MAb	Treatment			
	Control	Ethanol	3-Methylcholanthrene	Phenobarbital
TRI concentration (0.20 mM)				
Without MAb	0.56 ± 0.15	2.42 ± 0.60*	0.68 ± 0.06	1.78 ± 0.25*
Hy-Hel	0.52 ± 0.15 (100)	2.52 ± 0.62* (100)	0.93 ± 0.08*† (100)	1.81 ± 0.30*† (100)
Anti-P450IIE1	0.22 ± 0.06‡ (42 ± 6)	0.86 ± 0.21‡ (34 ± 3)	0.80 ± 0.16 (86 ± 11)	1.69 ± 0.29‡ (93 ± 5)
Anti-P450IIC11/6	0.49 ± 0.11 (96 ± 7)	2.39 ± 0.33 (96 ± 5)	0.91 ± 0.11 (97 ± 5)	1.84 ± 0.27 (102 ± 7)
Anti-P450IA1/2	ND	ND	0.70 ± 0.05‡ (76 ± 3)	ND
Anti-P450IIB1/2	ND	ND	ND	0.61 ± 0.07‡ (34 ± 6)
TRI concentration (5.90 mM)				
Hy-Hel	0.46 ± 0.05 (100)	1.59 ± 0.11*§ (100)	2.10 ± 0.30*†§ (100)	4.18 ± 0.40*†§ (100)
Anti-P450IIC11/6	0.27 ± 0.10‡ (58 ± 16)	1.46 ± 0.24 (91 ± 8)	2.05 ± 0.32 (97 ± 7)	2.95 ± 0.67‡ (71 ± 13)
Anti-P450IA1/2	ND	ND	1.20 ± 0.08‡ (58 ± 5)	ND
Anti-P450IIB1/2	ND	ND	ND	1.04 ± 0.49‡ (25 ± 13)

Hepatic microsomal protein (mg/g liver) and cytochrome P450 (nmol/mg protein/min) contents: control, 25.4 ± 2.9, 0.62 ± 0.06; ethanol, 30.2 ± 2.3, 1.00 ± 0.09; 3-methylcholanthrene, 22.8 ± 3.6, 1.11 ± 0.16; phenobarbital, 31.4 ± 3.9, 1.32 ± 0.15. Each figure represents the remaining activity (mean ± SD, nmol/mg protein/min) of four rat livers. Figures in parentheses show the percentages of remaining activity (mean ± SD) expressed as (activity with MAbs/activity with Hy-Hel) × 100.

* Significantly different ($P < 0.05$) from control.

† Significantly different ($P < 0.05$) from ethanol.

‡ Significantly different ($P < 0.05$) from respective Hy-Hel.

§ Significantly different ($P < 0.05$) from respective results at 0.20 mM trichloroethylene.

ND, not determined.

control and ethanol-treated rats. The net inhibition (difference between activity with Hy-Hel and with the MAb) in microsomes from ethanol-treated rats was 1.66 ± 0.43 nmol/mg protein/min, significantly greater than that in microsomes from control rats (0.31 ± 0.11 nmol/mg protein/min), though no difference in the percentage inhibition (difference between activity with Hy-Hel and with the MAb divided by the activity with Hy-Hel × 100), was seen between them. This indicates that cytochrome P450IIE1 is an ethanol-inducible isozyme in good agreement with previous reports [8–10]. However, in microsomes from PB-treated rats slight inhibition, and in those from MC-treated rats no inhibition of CH formation from TRI were found, suggesting that the contribution of P450IIE1 may be neutralized by such treatments, consistent with our previous reports [8–10] and the report of Thomas *et al.* [21]. Anti-P450IIC11/6 did not inhibit CH formation from TRI in any microsomes at low TRI concentration. Anti-P450IA1/2 and anti-P450IIB1/2 inhibited CH formation from TRI in microsomes from MC- and PB-treated rats, respectively, at the low concentration.

At a high concentration of TRI (5.90 mM), all the chemical treatments increased the rate of CH formation from TRI. However, the activity in microsomes from ethanol-treated rats was slightly lower at high TRI concentration than at the low concentration (0.20 mM), suggesting that substrate inhibition may occur at the high concentration. In contrast, the activities in microsomes from MC- and PB-treated rats at high TRI concentration were twice as high as those at low concentration. With anti-P450IIC11/6, CH formation from TRI was inhibited only in microsomes from control and PB-treated rats. The net inhibition in microsomes from control rats (0.19 ± 0.07 nmol/mg protein/min) was even less than that by anti-P450IIE1 at low TRI concentration, suggesting that P450IIC11/6 contributes less than P450IIE1 to CH formation from TRI. In microsomes from PB-treated rats, the net inhibition (1.24 ± 0.56 nmol/mg protein/min) was much greater than that in microsomes from control rats, although no significant difference in percentage inhibition was found between them. Anti-P450IA1/2 and P450IIB1/2 inhibited CH formation from TRI in microsomes from MC- and PB-treated rats, respectively. The net inhibition by these two MAbs was larger at high TRI concentration than that at low concentration. On the basis of these results, it may be concluded that cytochrome P450IIE1, IIC11/6, IA1/2 and IIB1/2 are all involved in the formation of CH from TRI.

Comparative study on benzene, toluene and TRI metabolism. In order to compare the contributions of four cytochrome P450 isozymes to the metabolism of benzene, toluene and TRI, the inhibition of the metabolism of these three chemicals by anti-P450IIE1, anti-P450IIC11/6, anti-P450IA1/2 and anti-P450IIB1/2 MAbs were examined. Figure 1 shows the percentage inhibition (difference between Hy-Hel (100%) and percentage of remaining activity) of benzene aromatic hydroxylase, toluene side-chain oxidase, toluene ring hydroxylase and TRI oxidase by four MAbs. The contributions of cytochromes P450IIE1 and P450IIC11/6 to the metabolism of these three compounds were assessed using microsomes from control rats. P450IIE1 was found to be involved in all of the metabolic pathways studied except in *o*-cresol formation from toluene. Its contribution was slightly greater to benzene aromatic hydroxylation and TRI oxidation than to toluene side-chain oxidation in terms of percentage inhibition. The contribution of this isozyme to *p*-cresol from toluene seems to be negligible since the result in Fig. 1 was obtained in control microsomes, and it would become significant when microsomes from ethanol-treated rats were used, but was still small relative to that of the isozyme to benzyl alcohol formation from toluene [10].

Cytochrome P450IIC11/6 was involved in all metabolic pathways, but only in the side-chain metabolism of toluene was its contribution important.

The contribution of cytochrome P450IIB1/2 was assessed using microsomes from PB-treated rats. This isozyme too was found to be involved in all the metabolic routes. Although the contribution was almost the same to five metabolic pathways, the inhibitable activity of the side-chain oxidation of toluene was especially large, followed by that to TRI oxidation, *p*-cresol formation from toluene, and benzene aromatic hydroxylation, and *o*-cresol formation from toluene, in decreasing order.

The contribution of cytochrome P450IA1/2 was assessed in microsomes from MC-treated rats. Unlike the other three isozymes, P450IA1/2 was not involved in toluene side-chain metabolism, but contributed greatly to formation of *o*-cresol from toluene and CH from TRI, moderately to benzene aromatic hydroxylation, and slightly to *p*-cresol formation from toluene.

DISCUSSION

MAb-mediated inhibition of cytochrome P450 isozymes involved in TRI metabolism was investigated. In this experiment, P450IIE1 acted in CH formation from TRI more effectively than that of P450IIC11/6 in control microsomes. In agreement with previous reports [8–10, 22], P450IIE1 was clearly induced by chronic ethanol consumption, whereas P450IIC11/6 was refractory to this treatment. P450IIB1/2 and P450IA1/2 were also involved in the formation of CH from TRI in microsomes from PB- and MC-treated rats, respectively. Similarly to the metabolism of benzene and toluene [8–10], P450IIC11/6, P450IA1/2 and P450IIB1/2 were high-K_m isozymes for TRI metabolism, whereas P450IIE1 was a low-K_m one.

Miller and Guengerich [3] have examined the ability of eight different purified isozymes of P450 to metabolize TRI to CH and reported that P450IIB1 metabolized TRI to the greatest extent, followed by P450IA2 or P450IA1; the contributions of P450IIC11 and P450IIC6 were small but significantly; P450IIA1, IIIA1 and IIB2 hardly acted in the metabolism. Therefore, the response of P450IIB1/2 resulting from our MAb study is mainly due to that of P450IIB1. Recently, Guengerich *et al.* [23] showed that human cytochrome P450IIE1 is a major catalyst of TRI oxidation, suggesting that P450IIE1 is

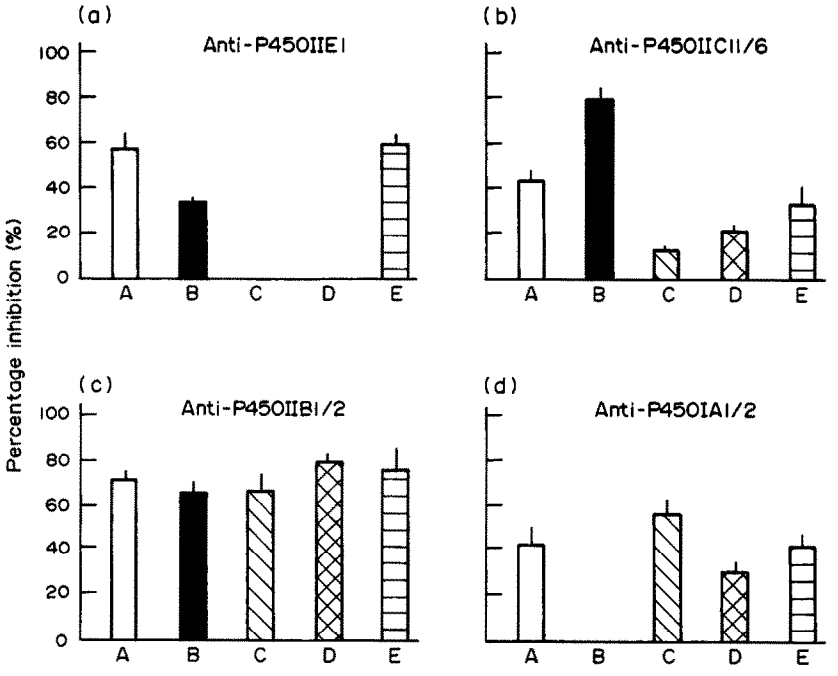

Fig. 1. The contribution of cytochrome P450 isozymes to benzene, toluene and trichloroethylene metabolism using monoclonal antibody-directed inhibition for reaction phenotyping. A, Benzene aromatic hydroxylation; B, benzyl alcohol formation from toluene; C, *o*-cresol formation from toluene; D, *p*-cresol formation from toluene; E, chloral hydrate formation from trichloroethylene. (a) The full activities (nmol/mg protein/min) with Hy-Hel in microsomes from control rats at low substrate concentrations (0.20–0.23 mM) were as follows: benzene aromatic hydroxylase (BAH), 0.39 ± 0.05; toluene side-chain oxidase (TSO), 0.81 ± 0.25; TRI oxidase (TRIO), 0.52 ± 0.15. (b) The full activities with Hy-Hel in microsomes from control rats at high substrate concentrations (5.0–6.26 mM) were as follows: BAH, 0.40 ± 0.08; TSO, 6.50 ± 1.23; toluene ring hydroxylase (*o*-position, TRH-*o*), 0.11 ± 0.02; toluene ring hydroxylase (*p*-position, TRH-*p*), 0.10 ± 0.02, TRIO, 0.46 ± 0.05. (c) The full activities with Hy-Hel in microsomes from phenobarbital-treated rats at high substrate concentrations (5.0–6.26 mM) were as follows: BAH, 2.09 ± 0.21; TSO, 21.82 ± 3.15; TRH-*o*, 1.53 ± 0.25; TRH-*p*, 1.92 ± 0.50; TRIO, 4.18 ± 0.40. (d) The full activities with Hy-Hel in microsomes from 3-methylcholanthrene-treated rats at high substrate concentrations (5.0–6.26 mM) were as follows: BAH, 0.81 ± 0.11; TSO, 4.83 ± 0.51; TRH-*o*, 2.02 ± 0.16; TRH-*p*, 0.40 ± 0.09; TRIO, 2.10 ± 0.30. The results are means ± SD of four rats.

involved in TRI metabolism not only in experimental animals but also in humans.

Anti-P450IIC11/6 inhibited CH formation from TRI to a greater extent in PB-induced microsomes than in control microsomes, and hence it seems that cytochrome P450IIC11 and/or IIC6 can be induced by PB treatment. However, it has been shown that PB treatment decreases cytochrome P450IIC11 activity, and induces IIC6 [10, 24, 25], and these two isozymes could not be separated by this MAb [26]. The inhibition of CH formation from TRI by anti-P450IIC11/6 may therefore imply that the involvement of P450IIC11 cannot be excluded by this experiment, although the involvement of P450IIC6 is indeed implicated.

MC [7] or β-naphthoflavone [3] treatment did not enhance the rate of CH formation in microsomal studies of TRI metabolism. However, MC treatment enhanced CH formation at either low or high concentration of TRI after addition of control MAb (Hy-Hel) to the reaction system (Table 1). This may be due not to the control MAb, but to 0.5% bovine serum albumin, because each MAb was diluted with this albumin before use, and in fact, 0.5% bovine serum albumin itself enhanced CH formation from TRI in microsomes from MC-treated rats (data not shown). The albumin solution may facilitate P450IA1/2 binding with TRI. However, 40% of the activity of microsomes from MC-treated animals was inhibited by anti-P450IA1/2 MAb, suggesting that there is indeed a substantial involvement of the inducible isozymes in the metabolism.

Even though cytochrome P450 isozymes have broad and overlapping substrate specificities, they often exhibit remarkable regioselectivity and stereo selectivity [27]. In this comparative study of the contributions of cytochrome P450 isozymes to benzene, toluene and TRI metabolism, all the isozymes investigated were found to be involved in the metabolism, and product specificity was observed for some isozymes, P450IIE1 performed all steps of metabolism of the three compounds studied except *o*-cresol formation from toluene, suggesting that binding of this isozyme to toluene hardly affects the

o-position, but can activate the other positions without regard to structure. P450IA1/2 contributed to all the metabolic steps except benzyl alcohol formation from toluene. This result suggests that P450IA1/2 easily binds to a wide variety of unsaturated structures in volatile compounds, but hardly binds to the saturated portions. Such a conclusion could be related to the observations that purified MC-inducible P450 is responsible for *n*-hexane metabolism, but is far less active than the PB-inducible isozyme [28], and that MC-inducible purified P450 (P450IA2) is a quite ineffective catalyst of *n*-pentane [29] and carbon tetrachloride metabolism [30]. P450IIB1/2 and P450IIC11/6 both performed all the metabolic steps studied, although their contributions to the side-chain metabolism of toluene were especially great compared with other metabolic steps in terms of net inhibition. P450IIC11/6 seems to display high metabolic activity for volatile compounds, comparable to that of P450IIb1/2 as reported by Waxman [31]. Though the relative specificity of P450IIC11/6 and P450IIB1/2 for benzyalcohol formation from toluene appears relatively high, it shows low regioselectivity for volatile compounds investigated, in contrast to the very different regioselectivities of the two isozymes in steroid hormone metabolism [12]. Moreover, the contribution of P450IIE1 to benzene and TRI metabolism was more significant than that of P450IIC11/6, but the opposite was seen in toluene metabolism. From the fact based on the similar inhibitable activity of benzene and TRI metabolism by four MAbs, an interesting observation is that in spite of their very different structures, benzene and TRI showed quite similar affinities for the four P450 isozymes studied, which were unlike those of the toluene side-chain.

In conclusion, cytochrome P450IIE1, P450IA1/2, P450IIC11/6 and P450IIB1/2 are all involved in the metabolism of benzene, toluene and TRI, indicating broad and overlapping substrate specificity for these volatile compounds. Cytochrome P450IIE1 and P450IA1/2, however, exhibit regioselectivity for toluene metabolism.

Acknowlegement—This work was supported in part by a Grant-in-Aid for General Scientific Research (No. 02807059) from Japan Ministry of Education, Science and Culture.

REFERENCES

1. Waters EM, Gerstner HB and Huff JE, Trichloroethylene. 1. An overview. *J Toxicol Environ Health* **2**: 671–707, 1977.
2. United States Environmental Protection Agency (U.S. EPA), *Health Assessment Document for Trichloroethylene*. Final Report E-600/8-82-006B, Washington, DC, 1985.
3. Miller RE and Guengerich FP, Metabolism of trichloroethylene in isolated hepatocytes, microsomes, and reconstituted enzyme systems containing cytochrome P-450. *Cancer Res* **43**: 1145–1152, 1983.
4. Nakajima T, Okino T, Okuyama S, Kaneko T, Yonekura I and Sato A, Ethanol-induced enhancement of trichloroethylene metabolism and hepatotoxicity: difference from the effect of phenobarbital. *Toxicol Appl Pharmacol* **94**: 227–237, 1988.
5. Imamura T and Ikeda M, Lower fiducial limit of urinary metabolite level as an index of excess-exposure to industrial chemicals. *Br J Ind Med* **30**: 289–292, 1973.
6. Bruckner JV, Davis BD, Blancato JN, Metabolism, toxicity, and carcinogenicity of trichloroethylene. *Crit Rev Toxicol* **20**: 31–50, 1989.
7. Nakajima T, Wang RS, Murayama N and Sato A, Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. *Toxicol Appl Pharmacol* **102**: 546–552, 1990.
8. Nakajima T, Elovaara E, Park SS, Gelboin HV, Hietanen E and Vainio H, Immunochemical characterization of cytochrome P-450 isozymes responsible for benzene oxidation in the rat liver. *Carcinogenesis* **10**: 1713–1717, 1989.
9. Nakajima T, Elovaara E, Park SS, Gelboin HV, Hietanen E and Vainio H, Monoclonal antibody-directed characterization of benzene, ethoxyrosorufin and pentoxyresorufin metabolism in rat liver microsomes. *Biochem Pharmacol* **40**: 1255–1261, 1990.
10. Nakajima T, Wang R-S, Elovaara E, Park SS, Gelboin HV, Hietanen E and Vainio H, Monoclonal antibody-directed characterization of cytochrome P450 isozymes responsible for toluene metabolism in rat liver. *Biochem Pharmacol* **41**: 395–404, 1991.
11. Kaminsky LS, Fasco MJ and Guengerich FP, Comparison of different forms of purified cytochrome P-450 from rat liver by immunological inhibition of regio- and stereoselective metabolism of warfarin. *J Biol Chem* **255**: 85–91, 1980.
12. Waxman DJ, Interactions of hepatic cytochromes P-450 with steroid hormones. Regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of rat P-450 enzyme expression. *Biochem Pharmacol* **37**: 71–84, 1988.
13. Lieber CS, Leonore M and DeCarli BA, The feeding of ethanol in liquid diets. *Alcoholism* **10**: 550–553, 1986.
14. Koehler D and Milstein C, Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **265**: 495–497, 1975.
15. Park SS, Fujino T, West D, Guengerich FP and Gelboin HV, Monoclonal antibodies that inhibit enzyme activity of 3-methylcholanthrene-induced cytochrome P-450. *Cancer Res* **42**: 1798–1808, 1982.
16. Park SS, Fujino T, Miller H, Guenerich FP and Gelboin HV, Monoclonal antibodies to phenobarbital-induced rat liver cytochrome P-450. *Biochem Pharmacol* **33**: 2071–2081, 1984.
17. Park SS, Waxman DJ, Lapenson DP, Schenkman JB and Gelboin HV, Monoclonal antibodies to rat liver cytochrome P-450 2c/RLM5 that regiospecifically inhibit steroid metabolism. *Biochem Pharmacol* **38**: 3067–3074, 1989.
18. Ko IY, Park SS, Song BJ, Patten C, Tan Y, Hah YC, Yang CS and Gelboin HV, Monoclonal antibodies to ethanol-induced rat liver cytochrome P-450 that metabolizes aniline and nitrosamines. *Cancer Res* **47**: 3101–3109, 1987.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. 1. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
21. Thomas PE, Bandiera S, Maines SL, Ryan DE and Levin W, Regulation of cytochrome P-450j, a high-affinity *N*-nitrosodimethylamine demethylase, in rat hepatic microsomes. *Biochemistry* **26**: 2280–2289, 1987.
22. Johansson I and Ingelman-Sundberg M, Benzene

metabolism by ethanol-, acetone- and benzene-inducible cytochrome P-450 (IIE1) in rat and rabbit liver microsomes. *Cancer Res* **48**: 5387–5390, 1988.
23. Guengerich FP, Kim DH and Iwasaki M, Role of human cytochrome P-450IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* **4**: 168–179, 1991.
24. Waxman DJ, Dannan GA and Guengerich FP, Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24**: 4409–4417, 1985.
25. Dannan GA, Guengerich FP, Kaminsky LS and Aust SD, Regulation of cytochrome P-450. *J Biol Chem* **258**: 1282–1288, 1983.
26. Waxman DJ, Lapenson DP, Park SS, Attisano C and Gelboin HV, Monoclonal antibodies inhibitory to rat hepatic cytochrome P-450: P-450 form specificities and use as probes for cytochrome P-450-dependent steroid hydroxylations. *Mol Pharmacol* **32**: 615–624, 1987.
27. Lu AYH and West SR, Multiplicity of mammalian microsomal cytochrome P-450. *Pharmacol Rev* **31**: 277–295, 1980.
28. Morohashi K, Sadano H, Okada Y and Omura T, Position specificity in *n*-hexane hydroxylation by two forms of cytochrome P-450 in rat liver microsomes. *J Biochem* **93**: 413–419, 1983.
29. Terelius Y and Ingelman-Sundberg M, Metabolism of *n*-pentane by ethanol-inducible cytochrome P-450 in liver microsomes and reconstituted membranes. *Eur J Biochem* **161**: 303–308, 1986.
30. Johansson I and Ingelman-Sundberg M, Carbon tetrachloride-induced lipid peroxidation dependent on an ethanol-inducible form of rabbit liver microsomal cytochrome P-450. *FEBS Lett* **183**: 265–269, 1985.
31. Waxman DJ, Rat hepatic cytochrome P-450 isoenzyme 2c. *J Biol Chem* **259**: 15481–15490, 1984.