Effects of Tetrachloroethylene and 1,1,1-Trichloroethane on the Expression of P450 Isoforms in Rat Lung and Liver

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Tetrachloroethylene (PCE) and 1,1,1-trichloroethane (1,1,1-TCA) were administered intraperitoneally at a moderately toxic dose of 1 g/kg/d for three consecutive days to male and female Wistar rats weighing about 200 g (7 weeks old). The agents were given alone or simultaneously with phenobarbital (PB, 80 mg/kg/d) and the animals were sacrificed 24 h after the last treatment. Microsomal fractions prepared from both lungs and livers were tested for the monooxygenase activity and protein content of four cytochrome P450 (CYP) isoforms, *i.e.*, CYP1A, 2B, 2E1 and 3A. As far as the monooxygenase activity was concerned, CYP1A, 2E1 and 3A but not 2B were constitutive in liver while only CYP2B was detected in lung. PCE suppressed significantly pulmonary CYP2B and hepatic CYP2E1 but enhanced hepatic CYP1A. In contrast, 1,1,1-TCA suppressed hepatic CYP2E1 under the conditions used. Although PB induced hepatic CYP2B and enhanced CYP1A several-fold, it had no effect on CYP2E1 and 3A, as well as pulmonary CYP2B. The effects of PCE and 1,1,1-TCE on CYP isoforms in microsomal fractions prepared from PB-cotreated animals were also studied comparatively in terms of sex and organ. The response of pulmonary enzymes to both chemicals was the same as that of PB-untreated animals. As far as PB-induced hepatic CYP2B was concerned, PCE was suppressive while 1,1,1-TCA showed a degree of potentiation. Furthermore, in the case of CYP1A a sex difference was noted in the response to these chemicals. As far as the protein levels of CYP isoforms were concerned, they were generally proportional to the enzyme activities.

Key words —— tetrachloroethylene, 1,1,1-trichloroethane, cytochrome P450 isoform, organ specificity, rat, lung

INTRODUCTION

Tetrachloroethylene (PCE) and 1,1,1-trichloroethane (1,1,1-TCA) are members of volatile halogenated hydrocarbons (VHHs) like trichloroethylene (TCE). These chemicals had been widely used in industry as metal-degreasing solvents and in dry-cleaning laundries due to their stability and noninflammable nature. As an ozone-depleting substance, 1,1,1-TCA ceased to be commercially available in Japan from 1996, although it remains as an environmental pollutant. PCE and TCE are known to be carcinogenic and their use has been strictly controlled by law in Japan. However, the environmental pollution caused by these compounds has been serious during the past few decades.

We have described previously the extent of daily human exposure to atmospheric PCE, 1,1,1-TCA and TCE in an urban metropolitan area.[1] An increasing risk of cancer was found among people drinking water from wells polluted with TCE and more toxic PCE.[2] PCE poses a risk of hepatic cancer and non-Hodgkin's lymphoma according to Linge *et al.* and Anttila *et al.*[3,4] PCE was reported to be an additional risk factor for cancer in breast-fed infants by Byczkowski *et al.*[5] A portion of hepatic tumors developed in B6C3F1(C57BL/6 × C3H/HeJ) mice by halogenated compounds such as PCE, TCE and their metabolites exhibited loss of heterozygosity in chromosome 6, suggesting the presence of a tumor suppressor gene.[6]

The toxicity and carcinogenicity of polychlorinated ethylenes such as PCE and TCE are facilitated by cytochrome P450 (CYP)-mediated bioactivation. PCE epoxide, a typical CYP-

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dependent active metabolite of PCE, binds electrophilically to cellular macromolecules such as DNA and proteins.[7,8] An increase in the frequency of PCE-induced micronuclei was observed in human lymphoblastoid cell lines stably expressing cDNA-encoding CYP isoforms.[9] It is well known that phenobarbital (PB)-inducible CYP isoforms, in specific CYP2B, contribute markedly to the toxicity of polyhalogenated ethylenes.[10-12] 1,1,1-TCA enhances the metabolic activation of vinyl chloride by PB-inducible CYP isoforms, on one hand, and adversely affects the CYP2E1-catalyzed bioactivation on the other.[13] In contrast to PCE and TCE, the carcinogenic effect of inhaled 1,1,1-TCA in rats is still doubtful. Although only limited binding of 1,1,1-TCA to cellular macromolecules, such as DNA, RNA and proteins, was observed in organs such as liver, kidney and lung,[14,15] the behavior of 1,1,1-TCA as an uncoupler of NADPH electron transmission systems in mitochondria has been reported.[16] 1,1,1-TCA is slowly oxidized to trichloroethanol and trichloroacetic acid by CYPs. However, it is reductively dehalogenated by rat CYPs under hypoxial conditions to a radical intermediate which, in turn, results in the formation of acetylene and 1,1-dichloroethane.[17,18]

1,1,1-TCA tends to accumulate in the medulla oblongata, causing damage to the central nervous system.[19,20] In general, CYPs act on VHHs in a detoxifying manner via the formation of highly toxic intermediates. As far as biodefences are concerned, therefore, it is of interest to see how *in vivo* CYP expression responds to exposure to VHHs. Although the lung is the primary target organ of inhaled air-born VHHs, the responses of CYPs have been studied mainly in liver, which plays a crucial role in the metabolism of xenobiotics. In a previous study concerning the comparison of CYP-responses to TCE-treatment between lung and liver, we observed the suppression of CYP2B and 2E1 in an organ-specific manner.[21] In this study, the CYP-responses to VHH-treatment will be compared for PCE, 1,1,1-TCA and TCE with respect to organ and sex differences.

We are exposed simultaneously to many environmental chemicals or xenobiotics, interacting metabolically with each other. Phenobarbital (PB), for example, is well known to induce certain CYP isoforms, which are closely involved in the metabolism of VHHs. We have observed the inhibition by TCE of PB-induced CYP isoforms as well as constitutive ones.[21] Therefore, the effects of VHHs on the function of CYP isoforms are also analyzed using PB-cotreated animals.

MATERIALS AND METHODS

Reagents —— Resorufin, 7-ethoxyresorufin, 7-pentoxyresorufin and erythromycin were purchased from Sigma Chemical Company (U.S.A.). PCE (99.0 %), 1,1,1-TCA (95%, stabilizer 3%), *p*-Nitrophenol and 4-nitrocatechol are products of Wako Pure Chemical Ind. (Osaka). Goat anti-rat CYP 2B1 and 2E1 sera and peroxidase-labeled anti-goat IgG serum were obtained from Daiichi Chemical Co. (Tokyo). All the other reagents were purchased from Wako Pure Chemical Ind. (Osaka).

Animals and Treatments —— Wistar rats (Nihon Clea, 7 weeks old) were divided into six groups, each consisting of 4—6 animals; the PCE-treated, 1,1,1-TCA-treated, PB-treated, PCE plus PB-treated, 1,1,1-TCA plus PB-treated and control groups were given i.p. injections of PCE (1 g/kg body weight/d), 1,1,1-TCA (1 g/kg body weight/d), PB (80 mg/kg/d), PCE (1 g/kg body weight/d) plus PB (80 mg/kg body weight/d), 1,1,1-TCA(1 g/kg body weight/d) plus PB (80 mg/kg body weight/d) and corn oil, respectively, for 3 d.

Preparation of Microsomes —— Lungs and livers were removed from rats 24 h after the last treatment, and the wet weights of these organs were recorded. Both lungs and livers were homogenized with 4 volumes of cold 1.15% KCl−50 mM Tris · HCl (pH 7.4) −1 mM EDTA. The homogenates were centrifuged at 900 *g* for 5 min and then 9000 *g* for 15 min at 4℃. Microsomal pellets were obtained from the 9000 *g*-supernatants following centrifugation at 105000 *g* for 60 min at 4℃. The microsomal pellets were homogenized in a cold mixture containing 1.15% KCl, 50 mM Tris · HCl (pH 7.4), 1 mM EDTA and 20% glycerol. The microsomes were kept at −80℃ until use. The measurement of protein was carried out using Miller's modification of the Lowry method.[22]

Enzyme Assays —— Ethoxyresorufin *O*-deethylase (EROD): [23] The reaction mixture consisted of 50 mM phosphate buffer (pH 7.4) containing 5 mM $MgCl_2$, microsomal suspension and 0.3 mM 7-ethoxyresorufin in a final volume of 2.95 ml. Reaction was started by the addition of 50μl 7.5 mM NADPH in a fluorimeter cuvette at 25℃. The fluorescence intensity was recorded in a spectrophotofluorimeter (Hitachi, F-2000;

excitation wavelength, 530 nm; emission wavelength, 585 nm) and the initial rate of resorufin formation was calculated.

Pentoxyresorufin *O*-dealkylase (PROD): [24] The PROD activity was measured under the same conditions as the EROD activity except that 0.3 mM 7-pentoxyresorufin was used as a substrate instead of 7-ethoxyresorufin.

p-Nitrophenol hydroxylase (PNPH): [25] The reaction mixture containing 100 mM phosphate buffer (pH 6.8), microsomal suspension and 10 mM *p*-nitrophenol in a final volume of 1.90 ml was preincubated at 30℃ for 3 min, and then the reaction was started by the addition of 100 μl 20 mM NADPH. After 10 min, in the case of hepatic microsomes, or 20 min, for pulmonary microsomes at the same temperature, the reaction was terminated by the addition of 1 ml 10 *N* perchloric acid. After centrifugation at 1200 *g* for 5 min, 0.2 ml 10 *N* NaOH was added to the supernatant and the absorbance at 546 nm was measured in a spectrophotometer (Hitachi, 100-20).

Erythromycin *N*-demethylase (EMND): [26] The 0.95 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.4), microsomal suspension and 1 mM erythromycin (stock solution, 200 mM in dimethyl sulfoxide) was preincubated at 37℃ for 3 min, and the reaction was started by the addition of 50μl 7.5 mM NADPH. After being incubated at 37℃ for 10 min, the reaction was ended by the addition of 17% perchloric acid. The entire sample was centrifuged at 1200 *g* for 5 min and Nash's reagent was added to the supernatant to give a color with the formed formaldehyde by heating at 70℃ for 20 min. The absorbance was measured at 412 nm.

Western Immunoblot —— Immunoblot analysis of microsomes was performed to detect CYP isoform apoproteins. SDS-PAGE was carried out with 10% acrylamide according to the method of Laemmli.[27] CYP apoproteins were transferred electrophoretically with the help of a blotting system (BioRad, Transblot-SD) from the gel to the nitrocellulose sheet. The sheet was treated with goat anti-rat CYP isoform sera and peroxidase-labeled anti-goat IgG. Color development was achieved using an HRP Conjugate Substrate Kit (BioRad). The protein band-images read by the image scanner were analyzed by NIH-image software.

Statistics —— Statistical significance was determined using Student's *t*-test. The 0.05 level of probability was the criterion of significance.

RESULTS

Effects of PCE and 1,1,1-TCA on Whole Body and Organ Weights

The changes in the whole body and organ wet weights of rats treated with PCE or 1,1,1-TCA, in the absence or the presence of PB, are shown in Table 1, as well as those of the control and PB-treated animals. In contrast to the steady gain in body weight in the control group, a reduced gain or loss in body weight was observed in all other groups, except for the female PB-treated group. The most significant toxicity in terms of body weight loss was observed in the male rats treated with 1,1,1-TCA in either the absence or presence of PB. Treatment with PB

Table 1. Effect of VHHs and/or PB Treatment on Body Weight and Organ Wet Weight

	Administration alone			Co-administration		
	Body weight change[a] (%)	Liver wet weight (g/100 g body wt.)	Lung wet weight (g/100 g body wt.)	Body weight change (%)	Liver wet weight (g/100 g body wt.)	Lung wet weight (g/100 g body wt.)
Control[b]				PB		
Male	9.9±0.4	4.6±0.1(100)	0.41±0.01(100)	3.8±0.3	5.2±0.1(113)	0.38±0.01(92)
Female	4.8±0.9	5.1±0.1(100)	0.47±0.01(100)	5.3±0.6	5.6±0.1(109)	0.46±0.02(98)
PCE				PCE+PB		
Male	1.1±1.0***	4.8±0.1(103)	0.42±0.02(101)	−2.5±1.2§§	5.6±0.2(120)	0.56±0.02(135)§§§
Female	0.0±0.3**	4.9±0.1(95)	0.52±0.03(111)	−3.1±2.8§	5.5±0.2(108)	0.58±0.04(124)§
1,1,1-TCA				1,1,1-TCA+PB		
Male	−7.8±2.8**	4.2±0.2(92)	0.47±0.02(113)*	−8.9±1.6§§	4.7±0.1(102)§	0.44±0.04(107)
Female	−1.4±1.7*	4.3±0.2(84)**	0.41±0.01(104)	−2.9±1.3§§§	5.0±0.1(97)§§	0.48±0.01(103)

Rats were given a daily i.p. injection of trichloroethylene (1 g/kg) and/or phenobarbital (80 mg/kg) for 3 d. Data are shown as the mean± S.E. for 4–6 individual rats with statistically significant differences compared with the control group or PB-treated group at $p<0.05$(*), (§), $p<0.01$(**), (§§), and $p<0.001$(***), (§§§). Figures in parentheses are the percentage of the control.

a) (B−A)/A×100, where A is the body weight at the start of treatment and B at the time of sacrifice. *b*) Corn oil administered.

alone resulted in the reduced gain in body weight in male rats while the females were not affected by PB-treatment. The highest degree of hepatic hypertrophy was observed in male rats treated with a combination of PCE and PB (+20%), whereas rats treated with 1,1,1-TCA showed hepatic hypotrophy irrespective of sex (92 and 84% for male and female rats, respectively). Furthermore, pulmonary hypertrophy was observed in rats treated with a combination of PCE and PB (+35% and +24% for male and female rats, respectively).

Effects of PCE and 1,1,1-TCA on CYP Isoform-Specific Monooxygenase Activities

Male and female rats were treated with PCE or 1,1,1-TCA for 3 d while the animals in the control group were given corn oil vehicle. Using the microsomal fractions prepared from the lungs and livers removed from each animal, we measured the CYP isoform-specific monooxygenase activities, EROD, PROD, PNPH and EMND, corresponding to the functions of CYP1A, 2B, 2E1 and 3A, respectively.[23-26] The results are shown in the upper part of Table 2 with special emphasis on sex differences.

As shown in the previous paper, there was a marked contrast in the expression profiles of constitutive CYP isoforms (enzyme activities in the control group) between liver and lung; PROD was exclusively detected in pulmonary microsomes and the other three enzymes, EROD, PNPH and EMND, were detected in hepatic microsomes without detection of PROD. The constitutive expression of pulmonary PROD and hepatic PNPH and EMND were higher in male

Table 2. Effects of VHHs and/or PB on P450-dependent Monooxygenase Activity in Microsomes from Rat Livers and Lungs

Enzyme activity		Liver		Lung	
		Male	Female	Male	Female
Admionistration alone					
Ethoxyresorufin *O*-deethylase	Control	36.2 ± 4.2	33.9 ± 4.4	n.d.	n.d.
(pmol/min per-mg protein)	PCE	73.8 ±22.4	39.3 ± 9.9	n.d.	n.d.
	1,1,1-TCA	24.4 ± 4.3	30.6 ± 2.8	n.d.	n.d.
Pentoxyresorufin *O*-dealkylase	Control	n.d.	n.d.	49.2±3.5	23.9±3.0
(pmol/min per mg-protein)	PCE	5.9 ± 2.2	n.d.	n.d.***	n.d.**
	1,1,1-TCA	n.d.	n.d.	32.3±5.6*	34.8±5.6
p-Nitrophenol hydroxylase	Control	0.37± 0.01	0.25± 0.01	n.d.	n.d.
(nmol/min per mg-protein)	PCE	0.15± 0.004***	n.d.***	n.d.	n.d.
	1,1,1-TCA	0.13± 0.05 **	0.06± 0.02***	n.d.	n.d.
Erythromycin *N*-demethylase	Control	0.64± 0.03	0.18± 0.01	n.d.	n.d.
(nmol/min per mg-protein)	PCE	0.49± 0.02 **	0.08± 0.03*	n.d.	n.d.
	1,1,1-TCA	0.74± 0.11	0.33± 0.04*	n.d.	n.d.
Co-administration					
Ethoxyresorufin *O*-deethylase	PB	182.7 ±14.6	210.3 ± 6.6	n.d.	n.d.
(pmol/min per mg-protein)	PCE+PB	254.4 ±54.4	75.7 ±16.8§§§	n.d.	n.d.
	1,1,1-TCA+PB	222.4 ±39.0	126.0 ± 7.3§§§	n.d.	n.d.
Pentoxyresorufin *O*-dealkylase	PB	633.5 ±51.8	241.9 ± 6.9	42.7±1.3	29.0±3.3
(pmol/min per mg-protein)	PCE+PB	64.9 ±26.8§§§	7.5 ± 3.1§§§	n.d.§§§	n.d.§§§
	1,1,1-TCA+PB	923.9 ±96.9§	252.9 ±24.4	21.6±1.7§§§	16.6±1.2§§
p-Nitrophenol hydroxylase	PB	0.71± 0.27	0.77± 0.14	n.d.	n.d.
(nmol/min per mg-protein)	PCE+PB	0.31± 0.02§§§	0.24± 0.03§	n.d.	n.d.
	1,1,1-TCA+PB	0.66± 0.04	0.60± 0.03	n.d.	n.d.
Erythromycin *N*-demethylase	PB	0.77± 0.03	0.24± 0.01	n.d.	n.d.
(nmol/min per mg-protein)	PCE+PB	0.61± 0.03§§	0.23± 0.03	n.d.	n.d.
	1,1,1-TCA+PB	1.03± 0.11	0.34± 0.04	n.d.	n.d.

Rats were given a daily i.p. injection of trichloroethylene (1 g/kg) and/or phenobarbital (80 mg/kg) for 3 d. a) Data are shown as the mean± S.E. for 4–6 individual rats with statistically significant differences compared with the control group or PB-treated group at $p<0.05$(*), (§), $p<0.01$(**), (§§), and $p<0.001$(***), (§§§). n.d.: not detected. (EROD, PROD<1 pmol/min/mg-protein, PNPH, EMND<0.1 nmol/min/mg-protein)

rats than in female rats.

When compared with the control group, a lower pulmonary PROD was observed in both VHH-treated groups, especially in the PCE-treated group where PROD activity could no longer detected. In marked contrast, the detection of hepatic PROD was confined to PCE-treated male animals. Hepatic PNPH was suppressed by both PCE- and 1,1,1-TCA-treatments, with a sex difference being observed in the PCE-treated group; PNPH was below the level of detection in female rats whereas it was reduced to approximately 60% of the control in male rats. The response of EMND was different in the PCE-and 1,1,1-TCA-treated animals, being suppressed and enhanced, respectively, with no regard to the sex.

Combination Effects of PCE and 1,1,1-TCA with PB on CYP Isoform-Specific Monooxygenase Activity

The results for the combined treatments between individual VHHs and PB are shown in the lower part of Table 2. As previously described,[21] PB alone induced hepatic PROD without affecting this activity in the lung.

The hepatic EROD, several-fold enhanced in the presence of PB, was suppressed to 35% and 60% of the activity produced by PB-monotreatment by PCE and 1,1,1-TCA, respectively, in the case of females. However, there were potentiative effects on male EROD. In the PB-treated rats, hepatic PROD and PNPH were significantly reduced in the presence of PCE. For example, the residual hepatic PROD activity was only 10% in male rats and 3% in female rats. However, 1,1,1-TCA enhanced the PROD activity by 50% in male rats. The response of pulmonary PROD to both VHHs in the PB-cotreatment experiments was more sensitive to suppression than that observed in the case of VHH-monotreatment.

Effects of PCE and 1,1,1-TCA on the Expression of P450 Isoform Apoproteins

The CYP isoform-apoproteins were detected by immunoblotting under various conditions and the results for CYP2B and 2E1 are shown in Figs. 1 and 2, respectively. For monotreatment, an increased amount of hepatic CYP2B-apoprotein was observed in the presence of PCE only for male rats in good agreement with the effect of PCE on EROD activity. PCE and 1,1,1-TCA suppressed the expression of pulmonary CYP2B-apoprotein to the same extent, although PCE was far more efficient in suppressing enzyme activity. The increased amount of CYP2E1-apoprotein detected by immunoblotting following treatment with VHHs was not accompanied by enhanced PNPH activity.

DISCUSSION

In this study, the experimental animals were treated with PCE or 1,1,1-TCA, i.p. at 1 g/kg body weight/d for 3 d, to investigate the role of CYP isoforms in the toxicity of VHHs in lung and liver. The treatment conditions adopted were those used in previous papers comparing the effects of TCE.[21,28]

Most of the administered PCE and 1,1,1-TCA, as well as TCE was excreted in the urine in unchanged form while part, was metabolized to trichloroacetic acid (TCAA) and trichloroethanol (TCOH) with the help of CYP isoforms (first phase enzymes), accompanied by second phase enzymes, prior to urinary excretion. The exposure levels of PCE, TCE and 1,1,1-TCA correlated well with the urinary levels of TCAA and TCOH.[1]

It was found that hepatic microsomal fractions metabolized PCE to TCAA either in the free or phospholipid-bound form in the presence of NADPH (oxidative pathway), with metabolic intermediates causing hepatotoxicity. When PCE was incubated with hepatic microsomes and cytosol in the presence of glutathione (GSH), however, *S*-(1,2,2-trichlorovinyl)glutathion (TCVG) was recovered (conjugative pathway). The hepatic TCVG formation, renal metabolism to TCVC, and a cleavage of TCVC by β-lyase resulted in the formation of mutagenic intermediates responsible for the nephrocarcinogenicity of PCE.[29] It has been shown that the absorption ratios of inhaled VHHs in rats were, in descending order, TCE>PCE>1,1,1-TCA.[30] Although there have been some reports of the effects of PCE and 1,1,1-TCA on hepatic CYP isoforms, there is nothing on pulmonary CYPs.

In hepatic microsomes, the increase in CYP1A and decrease in CYP2E1 resulting from PCE-treatment as shown in Table 2, were in good agreement with the previous findings for TCE.[21] However, hepatic CYP2B was undetectable with-

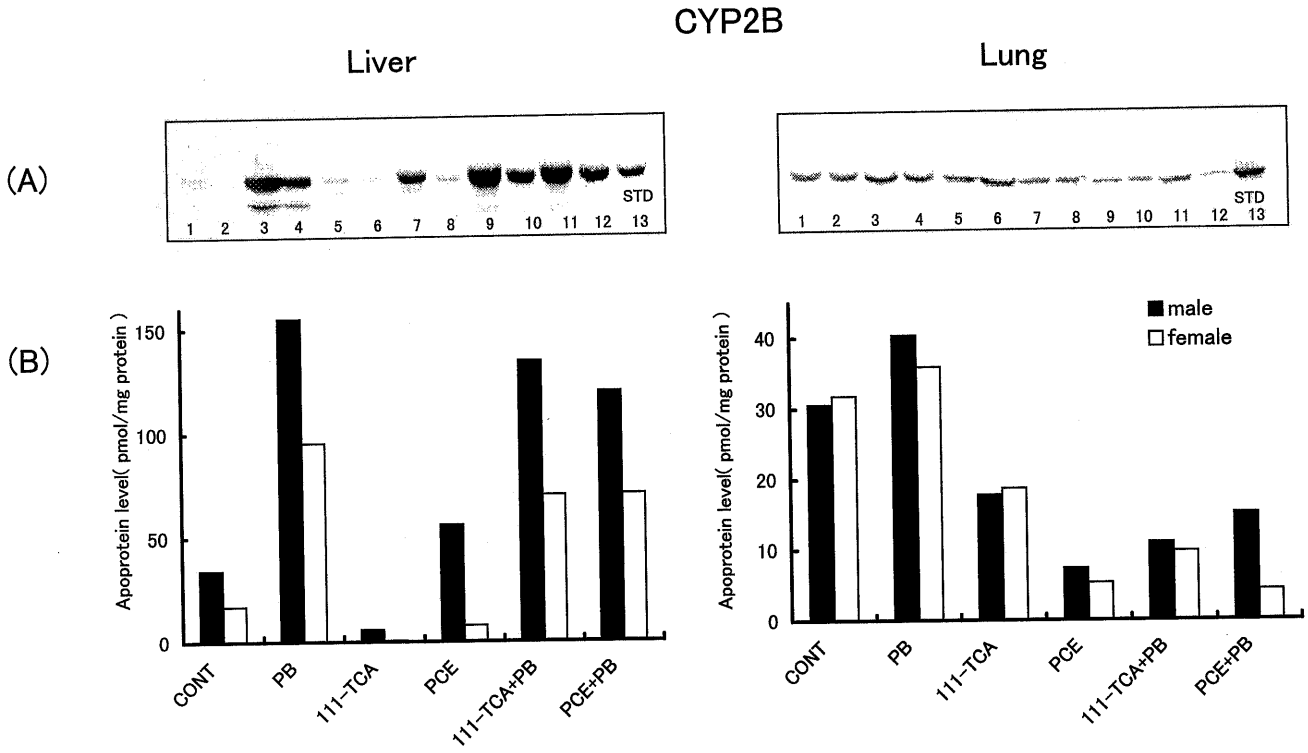

Fig. 1. Comparison of CYP2B-Apoprotein Expression between Lung and Liver

(A) Western immunoblots. Each lane was loaded with a mixture of microsomal samples prepared from 5 individual animals (50 μg as protein). Lane 1, male control; lane 2, female control; lane 3, male PB-treatment; lane 4, female PB-treatment; lane 5, male 1,1,1-TCA-treatment; lane 6, female 1,1,1-TCA-treatment; lane 7, male PCE-treatment; lane 8, female PCE-treatment; lane 9, male 1,1,1-TCA and PB-treatment; lane 10, female 1,1,1-TCA and PB-treatment; lane 11, male PCE and PB-treatment; lane 12, female PCE and PB-treatment; lane 13, standard microsome. (B) Readings from Western blots. CONT, corn oil-treated control; PB, PB-treatment; 111-TCA, 1,1,1-TCA-treatment; PCE, PCE-treatment; 111-TCA+PB, 1,1,1-TCA and PB-treatment; PCE+PB, PCE and PB-treatment.

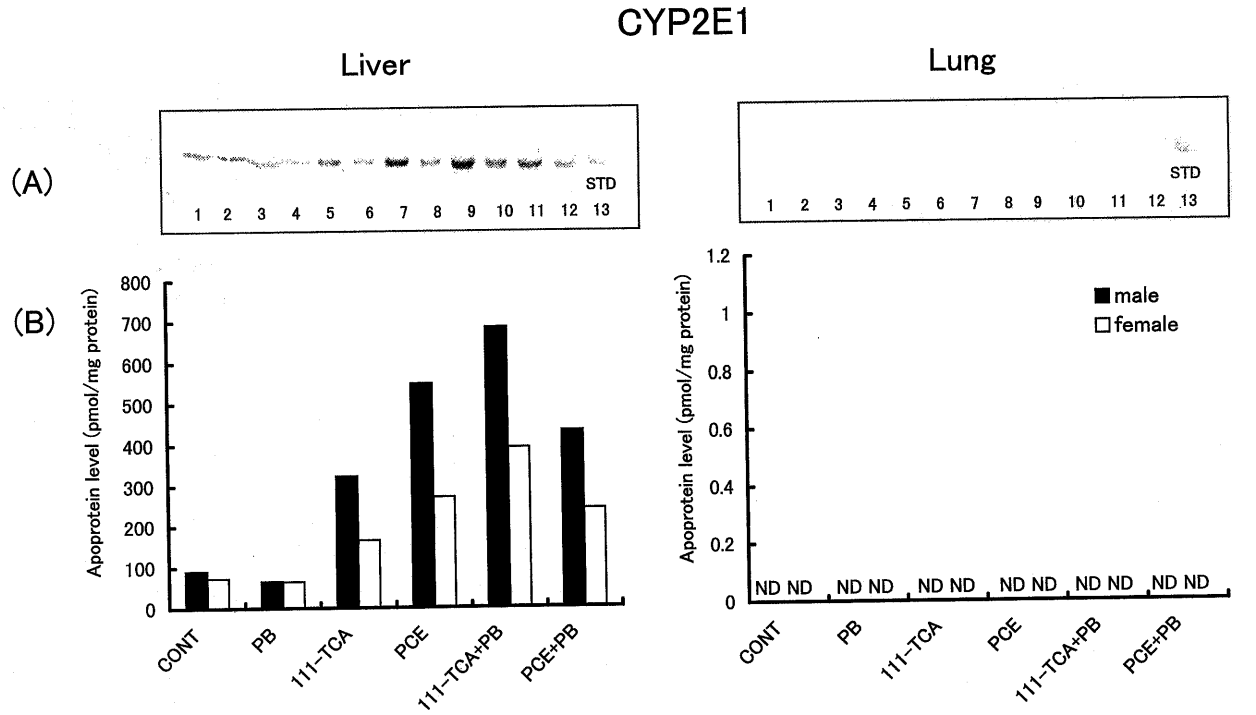

Fig. 2. Comparison of CYP2E1-Apoprotein Expression between Lung and Liver

(A) Western immunoblots. Each lane was loaded with a mixture of microsomal samples prepared from 5 individual animals (50 μg as protein). Lane 1, male control; lane 2, female control; lane 3, male PB-treatment; lane 4, female PB-treatment; lane 5, male 1,1,1-TCA-treatment; lane 6, female 1,1,1-TCA-treatment; lane 7, male PCE-treatment; lane 8, female PCE-treatment; lane 9, male 1,1,1-TCA and PB-treatment; lane 10, female 1,1,1-TCA and PB-treatment; lane 11, male PCE and PB-treatment; lane 12, female PCE and PB-treatment; lane 13, standard microsome. (B) Readings from Western blots. CONT, corn oil-treated control; PB, PB-treatment; 111-TCA, 1,1,1-TCA-treatment; PCE, PCE-treatment; 111-TCA+PB, 1,1,1-TCA and PB-treatment; PCE+PB, PCE and PB-treatment.

out PCE-treatment.

With regard to pulmonary CYP2B, the enzyme activity was below the level of detection following treatment with PCE compared with a *ca.* 50 % reduction in the case of TCE and an increase in female rats treated with 1,1,1-TCA (Table 2). In general, the effects of PCE on CYP activity resembled those of TCE but not 1,1,1-TCA, being attributable in part to a structural difference between polychlorinated ethylenes and ethanes, namely, the possibility of forming ethylene epoxide intermediates.

Hanioka *et al.* studied the effects of PCE on hepatic CYPs in male rats,[31] reporting similar behavior of CYP1A and CYP2B to that obtained in this study. In the case of CYP2E1 and CYP3A, however, their results were quite different from ours, probably due to a difference in the organ effective concentrations of PCE between oral and i.p. dosings. The sex difference in the CYP responses was another feature of the present study. In general, CYPs from female rats are more sensitive to the suppressive effect of VHHs compared with those from male rats which tend to show inductive responses, implying the existence of hormonal control in CYP-responses to VHHs.

As previously reported,[21] the expression of CYP2B in the control rats was very different in lung and liver; pulmonary CYP2B was expressed constitutively while hepatic enzyme showed an obvious induction-type phenotype. When treated with PCE, minimal induction of hepatic CYP2B was observed exclusively in male rats, whereas pulmonary CYP2B fell in both male and female rats. This could be accounted for by the organ difference either in the CYP2B subtypes or in the control mechanisms of the common subtype between lung and liver. As lung is not a primary metabolizing organ, pulmonary CYPs are at low levels compared with the hepatic enzymes and, furthermore, they are refractory to xenobiotic-dependent induction. Beside CYP isoforms, the second phase enzymes in lung are at lower levels than those in liver. The metabolism of many kinds of xenobiotics by the oxidative pathway, catalyzed by the CYP isoforms in collaboration with second phase enzymes, is not always detoxifying. The accumulation of metabolically activated intermediates is, furthermore, accelerated by induction of CYP isoforms by xenobiotics. Taking all theses fact into account, lung would be more susceptible to xenobiotic toxicity than liver and the CYP functions must be suppressed in response to xenobiotics to minimize such toxicity.

It is well known that combinations of certain chemicals can cause one-sided or mutual interference by metabolic mechanisms. Murayama *et al.* reported that PB-mediated induction of CYP2B was suppressed to control levels by coadministered mephobarbital, and this occurred at the transcriptional level.[32] Therefore, we tested the effect of combination of PB and PCE or 1,1,1-TCA on the expression of CYP isoforms, as in the case of combination of PB and TCE,[21] although the mechanism of PB-mediated induction has not yet been elucidated in detail. In this study, PB had no marked effect on CYP isoforms in lung. In contrast, PB induced CYP2B and enhanced CYP1A and 2E1 to different degrees in liver. When coadministered with PB, PCE suppressed 90% of the PB-inducible hepatic CYP2B, and more than 50% of the PB-monotreatment level of CYP2E1. Complete suppression of pulmonary CYP2B by PCE was also found in PB-cotreated rats. 1,1,1-TCA potentiated PB-dependent hepatic CYP2B but suppressed the same enzyme from the lung. The amount of pulmonary CYP2B apoprotein was reduced markedly by PCE and to a lesser extent by 1,1,1-TCA. The reduction in CYP2B was in good agreement with the reduced apoprotein content except for the case of female rats treated with 1,1,1-TCA. In rats cotreated with PB, the expression of CYP2B apoprotein was suppressed in parallel with the reduction in CYP2B. It is noteworthy that the accumulation of hepatic CYP2E1 apoprotein was in striking contrast to the reduction in enzyme activity. It has been shown that CYP2E1 is controlled transcriptionally and post-translationally by phosphorylation. Oesh-Bartlomowicz *et al.* reported that the phosphorylation of Ser-129 in CYP2E1, catalyzed by db-cAMP in rat hepatocyte culture, resulted in the inactivation of enzyme activity without being accompanied by a reduction in enzyme protein.[33] Furthermore, chlorinated ethylenes, such as TCE and PCE, are known to work as suicide substrates for CYP2E1 by covalently binding to the catalytic site of enzymes, although they may be inducers of CYP2E1.[34] Therefore, our results are probably due to the combined effects of VHHs at transcriptional and post-translational levels. In conclusion, three VHHs,

PCE, TCE and 1,1,1-TCA, suppressed the expression of CYP isoforms with some sex and organ specificity in the descending order of PCE> TCE>1,1,1-TCA, as shown typically by the results obtained with pulmonary and PB-dependent hepatic CYP2B.

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