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Effects of Polycyclic Aromatic Hydrocarbons on Liver and Lung Cytochrome P450s in Mice

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Certain polycyclic aromatic hydrocarbons (PAHs) have been reported to induce cytochrome P450 (CYP) 1A1 and 1A2. In the present study, the effects of six well-known PAHs, such as benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, chrysene, benzo[k]fluorancene and benzo[b]fluorancene, on the activities of hepatic and pulmonary CYP enzymes were investigated in male ICR mice. When mice were treated intraperitoneally with 3, 10 and 30 mg/kg of individual PAHs for 3 consecutive days, the activities of ethoxyresorufin- and methoxyresorufin-O-dealkylases were significantly and differentially induced in both liver and lung. Moreover, other CYP isozyme-associated monooxygenase activities were also induced significantly in liver and lung with characteristic induction profiles. Our present results suggest that individual PAHs might have inductive effects on CYP isozymes, and that the characteristic inductive effects of individual PAHs on certain CYP isozymes would be developed as a marker for determining exposure to certain PAHs.

Key words: Polycyclic aromatic hydrocarbons, Cytochrome P450s, Induction, Liver, Lung, Mice

INTRODUCTION

The risk to humans from air pollutants and their constituent environmental chemicals is an important issue for human health. Many environmental pollutants have the potential to either directly or indirectly react with the genetic material. In particular, polycyclic aromatic hydrocarbons (PAHs), diverse organic compounds containing two or more fused aromatic rings, are widely distributed as environmental contaminants and are always found as a mixture of individual compounds (Kielhorn and Boehnicke, 1998). PAHs are produced not only from incomplete combustion processes or pyrolysis of organic materials, but also in petroleum- and coal-consuming industrial processes, fires, traffic, heating, cooking and tobacco smoke (IARC, 1983). Some of them have well been documented to have toxic, mutagenic, or carcinogenic potentials. Due to their physicochemical properties, PAHs are readily absorbed into the body through the skin, lungs and gastrointestinal tract. The incorporated PAHs are converted to reactive intermediates by drug-metabolizing enzymes, such as cytochrome P450 (CYP)-dependent monooxygenase and epoxide hydrolase. These epoxide intermediates are usually more reactive than the parent compounds and are covalently bound to DNA or to proteins. Otherwise, the intermediates are excreted into the urine in the form of sulfates and glucuronides (Buckly and Lioy, 1992). In these events, oxidation of PAHs by drug-metabolizing enzymes is an initial step in the activation processes, and CYP enzymes play key roles.

CYP enzymes are a group of heme-containing enzymes responsible for the metabolic oxidation of a wide variety of endogenous and exogenous substrates, including steroids, fatty acid, drugs, and environmental toxicants such as chemical carcinogens (Guengerich, 1995; Guengerich and Shimada, 1991). Studies have established that there are multiple CYP enzymes capable of catalyzing activation of a number of environmental procarcinogens to ultimate carcinogenic metabolites (Guengerich, 2000; Guengerich and Shimada, 1998). In particular, CYP 1A1, 1A2 and 1B1

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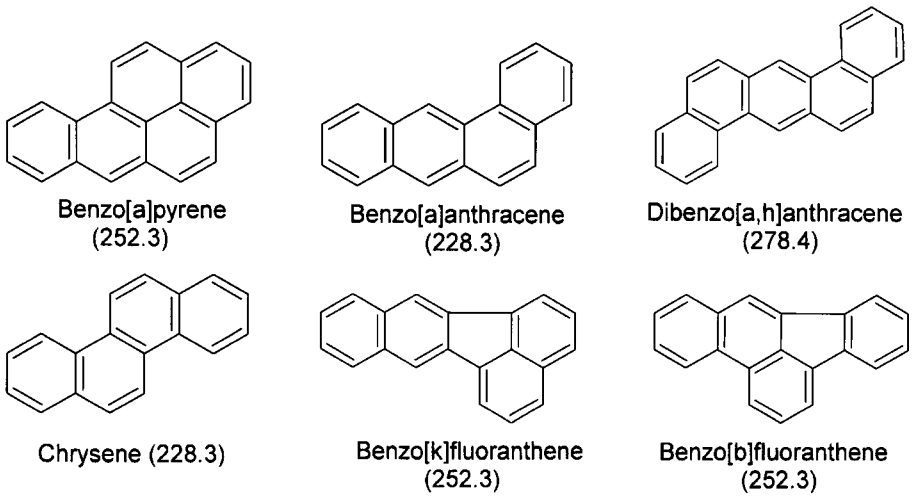

Fig. 1. Chemical structures of PAHs. The numbers in parentheses indicate the molecular weights of individual PAHs.

have been shown to be the major enzymes in the metabolism of potential procarcinogens including many PAHs (Gelboin, 1980; Petry *et al.*, 1996; Nisbet and LaGoy, 1992). The relationship between CYP isozymes and chemical carcinogenesis by PAHs, such as benzo[a]pyrene, has extensively been studied (Dipple *et al.*, 1985; Heidelberger, 1975) In addition, the close correlation of CYP 1A1 and 1A2 inductions with the activation of procarcinogens has also been examined (Conney, 1982). Moreover, a positive correlation of the induction potential of aryl hydrocarbon hydroxylase activity by certain PAHs with the immunosuppression was also reported (White *et al.*, 1985). However, information dealing with the effects of PAHs on activities of other CYP isozymes is relatively limited.

Therefore, in the present studies, we compared effects of six well-known PAHs, such as benzo[a]pyrene (B[a]P), benz[a]anthracene (B[a]A), dibenz[a,h]anthracene (D[a,h]A), chrysene, benzo[k]fluoranthene (B[k]F) and benzo[b] fluoranthene (B[b]F), on activities of hepatic and pulmonary CYP enzymes in the male ICR mice. Six PAHs selected in the present study have been classified in the group of possibly carcinogenic to humans by the United States Environmental Protection Agency (US EPA, 1993). The hepatic and pulmonary CYP enzymes were determined at the same time, because these six PAHs are contaminants in the air, indicating that the primary route of exposure to the PAHs would be through respiration. The structures of six PAHs used were illustrated in Fig. 1. Because B[a]P is a well-known substrate for CYP 1A1 and 1A2, it was used as a reference.

MATERIALS AND METHODS

Chemicals

B[a]P, 7-ethoxyresorufin, methoxyresorufin, 7-benzyloxyresorufin, 7-pentoxyresorufin, *p*-nitrophenol, erythromycin, resorufin, formaldehyde, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADPH were obtained form Sigma Chemical Company (St. Louis, MO, USA). B[a]A, D[a,h]A, chrysene, B[k]F and B[b]F were purchased form Aldrich Chemical Company (Milwaukee, WI). All other chemicals and reagents used in these studies were of the reagent grade of commercially available.

Animals

Specific pathogen-free male ICR mice (20-25 g) were obtained form the Daehan Experimental Animal Center (Eumsung, Korea). The animals received at 4-5 weeks of age were acclimated for at least 1 week prior to the experiments. Upon arrival, the mice were randomized and housed five per cage. All animals were maintained on tap water *ad libitum*. Mice at 6-7 weeks old were used in these studies. The animal quarters were strictly maintained at 23±3°C and 40-60% relative humidity. A 12 h light/dark cycle (07:00-19:00) was used with an intensity of 150-300 Lux.

Animal treatment and S-9 fraction preparation

Each PAH was dissolved or suspended in corn oil and administered intraperitoneally at 3, 10 and 30 mg/kg/10 mL once daily for 3 consecutive days. 30 mg/kg of dose was equivalent to 119 μM/kg for B[a]P, B[k]F and B[b]F, 131 μM/kg for B[a]A and chrysene, and 108 μM/kg for D[a,h]A The doses were selected from a preliminary study using benzo[a]pyrene and a previous report on immunosuppression by PAHs (White *et al.*, 1985). In control animals, corn oil was injected in the same volume and manners as the tested PAHs. Twentyfour hours after the last dose of PAHs, mice were sacrificed by the cervical dislocation and the liver and lung tissues were perfused with ice-cold

0.9% NaCl solution. Liver and lung tissues were excised, quickly rinsed in ice-cold 0.9% NaCl solution, filter paper-dried and then weighed. The S-9 fractions were prepared form the livers and lungs for determining CYP-associated monooxygenase activities. Livers were homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4, using a homogenizer, then centrifuged at 9,000×g for 20 min at 4°C. Aliquots of S-9 fractions were store at -80°C until use. The lungs were homogenized with six volumes of the same buffer. The homogenate was centrifuged and stored in aliquots at -80°C until use. The protein content of S-9 fraction was determined according to the method reported by Lowry *et al.* (1951) using bovine serum albumin as a standard.

Monooxygenase assays

Ethoxyresorufin *O*-deethylase (EROD) activity was determined as described by Blank *et al.* (1987) with a slight modification. The reaction mixture (2 mL) consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 2 mg/mL of bovine serum albumin, 5 mM glucose 6-phosphate, 1 U of glucose 6-phosphate dehydrogenase, 5 μM NADPH, 2.5 μM 7-ethoxyresorufin and an enzyme source. The formation of resorufin was monitored fluorometrically at an excitation maximum of 550 nm and an emission maximum of 585 nm. Methoxyresorufin *O*-demethylase (MROD), benzyloxyresorufin *O*-debenzylase (BROD) and pentoxyresorufin *O*-dependentylase (PROD) activities were determined by the method of Lubet *et al.* (1985) with a slight modification. All reaction components and assay procedures were exactly the same as the EROD assay, except that the substrates were 2.0 μM. *p*-Nitrophenol hydroxylase (PNPH) activity was determined as described by Koop (1986). The reaction mixture (1.0 mL) was composed of 0.1 M potassium phosphate buffer, pH 7.4, containing 100 μM *p*-nitrophenol, 1 mM NADPH and an enzyme source. The amount of 4-nitrocatechol formed was measured spectrophotometrically at 512 nm. Erythromycin *N*-demethylase (ERDM) activity was determined by measuring the amounts of formaldehyde formed, described by Nash (1953). The reaction mixture (1.5 mL) was consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.8 mM NADPH, 5.0 mM $MgCl_2$, 3.0 mM glucose 6-phosphate, 1 U of glucose 6-phosphate dehydrogenase, 7.5 mM semicarbazide hydrochloride, 400 μM erythromycin and an enzyme source. The amount of formaldehyde formed was measured spectrophotometrically at 412 nm.

Statistics

All data were expressed as a mean±S.E. and the Students *t*-test was used to compare the significance of the data obtained. The values significantly different from the vehicle control at $P<0.05$ (*) or $P<0.01$ (**) were represented as asterisks.

RESULTS

In Figs. 2 and 3, the activities of EROD were determined in livers and lungs of male ICR mice, respectively, that received intraperitoneal injections of individual PAHs at the dose levels of 3, 10 and 30 mg/kg of body weight for 3 consecutive days. Hepatic EROD activities were significantly induced by all PAHs. Particularly, B[a]P and B[a]A induced the hepatic EROD activities with a dose dependency up to 3.2- and 6.8-fold, respectively. Other PAHs maximally induced the EROD activities from the lowest dose. In addition, among six PAHs, B[a]P showed the lowest induction potential on EROD. Pulmonary EROD activities were somewhat different from the hepatic activities of EROD. Most PAHs showed the maximum induction of the EROD activity from 3 mg/kg. Interestingly, among six PAHs, the inductive effects of B[a]A and D[a,h]A were profound (i.e., 10.4- and 9.8-fold in maximum, respectively) and B[a]P showed a marginal induction potential (i.e., 1.6-fold in maximum) on pulmonary EROD activities.

In Figs. 4 and 5, the effects of six PAHs on hepatic and pulmonary MROD activities were determined, respectively. B[a]P and B[a]A induced the hepatic MROD activities with a dose dependency up to 5.6- and 3.1-fold, respectively. Most other PAHs showed maximum induction from the lowest dose. Among six PAHs, B[a]P showed the highest induction potential on MROD. However, pulmonary MROD activities were somewhat different from the hepatic activities of MROD. Most PAHs showed the MROD induction from 3 mg/kg. Among six PAHs, B[a]P showed the lowest induction potential (i.e., 1.8-fold in maximum) on pulmonary MROD activities.

In Tables I and II, the effects of six PAHs on CYP 2B-selective BROD and PROD activities were determined, respectively. Most PAHs induced hepatic BROD and PROD activities except B[a]P. Among six PAHs, the inductive effects of B[a]A, D[a,h]A and B[k]F were so remarkable that the PROD activities were induced up to 3.8-, 7.5- and 5.1-fold, respectively. Meanwhile, the inductions of pulmonary PROD and BROD activities by all six PAHs were relatively marginal and did not show a dose dependency.

In Table III, effects of six PAHs on CYP 2E1-selective PNPH activities were investigated. All six PAHs could induce hepatic PNPH activities with different potency. Particularly, the inductive effects of B[k]F and B[b]F were so profound that the PNPH activities were induced up to 4.6- and 4.5-fold, respectively. Meanwhile, the induction of pulmonary PNPH activity by all six PAHs was relatively

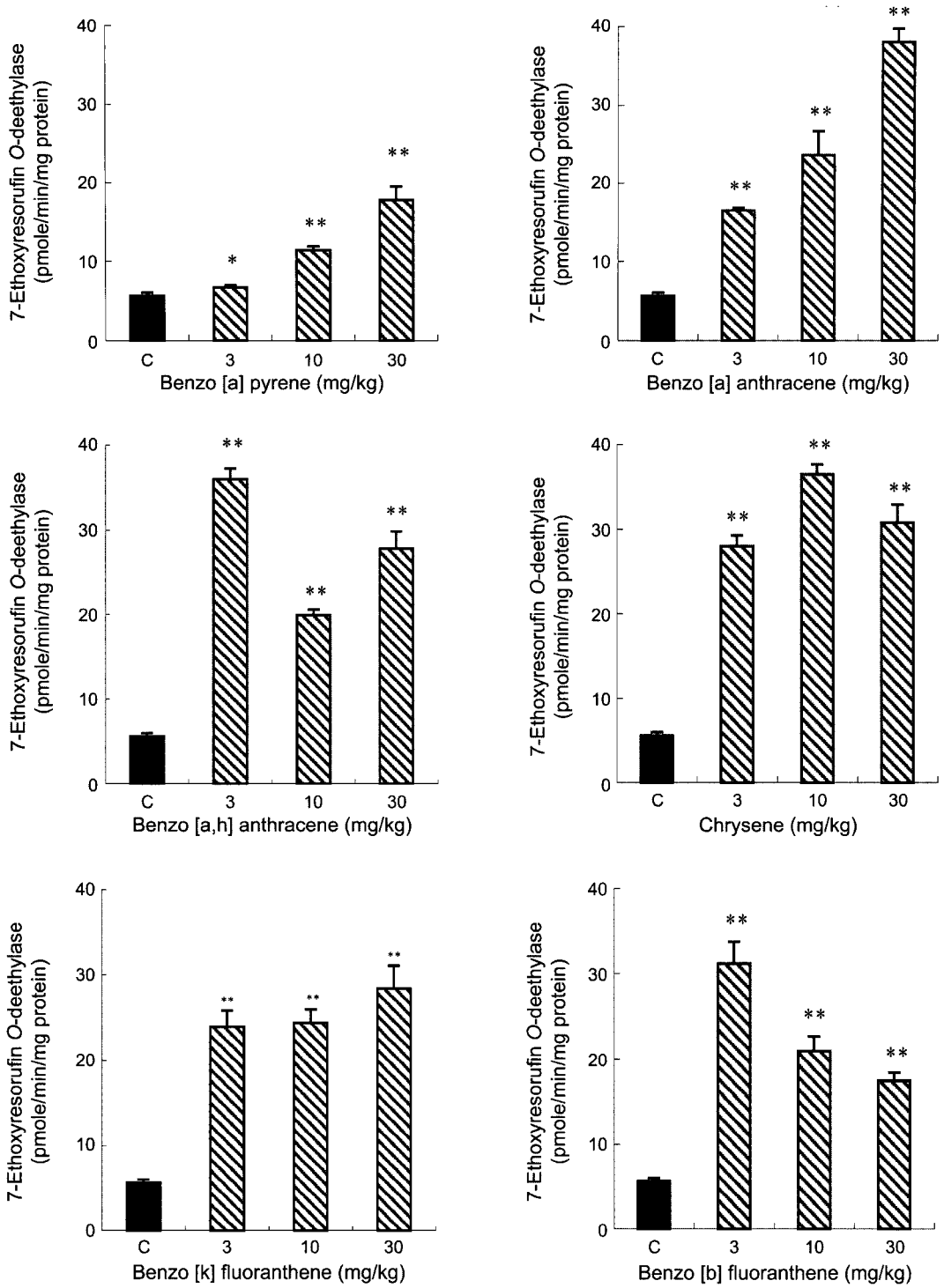

Fig. 2. Effects of six polycyclic aromatic hydrocarbons on activities of 7-ethoxyresorufin *O*-deethylase in liver S-9 fractions. Male ICR mice were treated intraperitoneally with either one of benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, chrysene, benzo[k]fluoranthene and benzo[b]fluorancene in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Each value represents the mean ± S.E. of five animals. The asterisks indicate the values significantly different from the control (C) at $P<0.05$ (*) or $P<0.01$ (**).

marginal.

In Table IV, effects of six PAHs on CYP 3A-selective ERDM activities were investigated. Most PAHs could induce hepatic ERDM activities, except B[a]P and B[a]A. Particularly, the inductive effect of chrysene was most potent from the dose of 3 mg/kg showing 3.8-fold induction. Meanwhile, the induction of pulmonary ERDM activity by all six PAHs was relatively marginal.

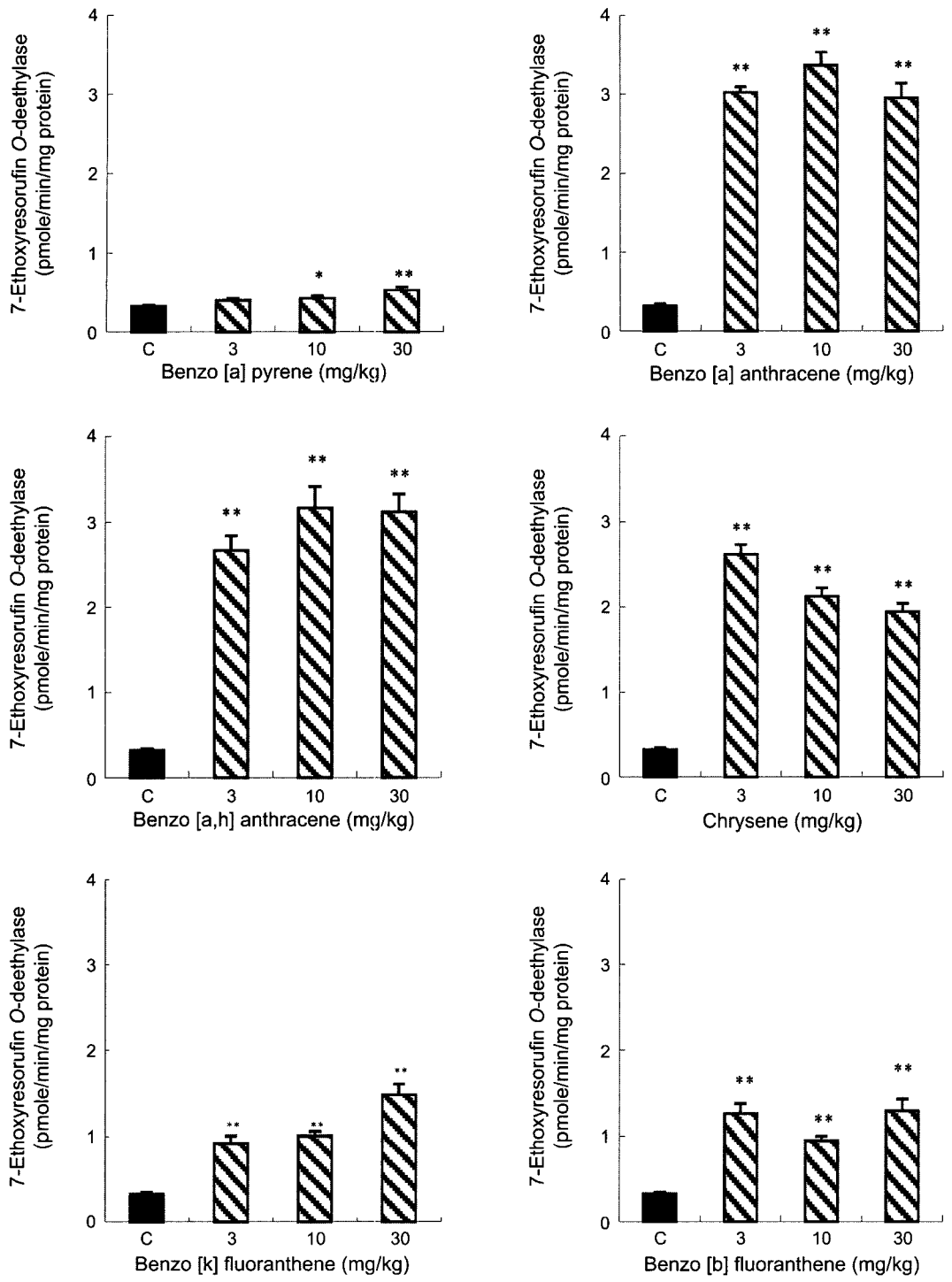

Fig. 3. Effects of six polycyclic aromatic hydrocarbons on activities of 7-ethoxyresorufin *O*-deethylase in lung S-9 fractions. Male ICR mice were treated intraperitoneally with either one of benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, chrysene, benzo[k]fluoranthene and benzo[b]fluorancene in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Each value represents the mean±S.E. of five animals. The asterisks indicate the values significantly different from the control (C) at $P<0.05$ (*) or $P<0.01$ (**).

Taken all together, six PAHs showed characteristic induction profile on CYP enzymes, indicating that certain CYP enzymes might be useful markers for the evaluation of exposure to certain PAHs.

DISCUSSION

PAHs, a group of compounds consisting of at least two fused aromatic rings, are ubiquitous in the environment

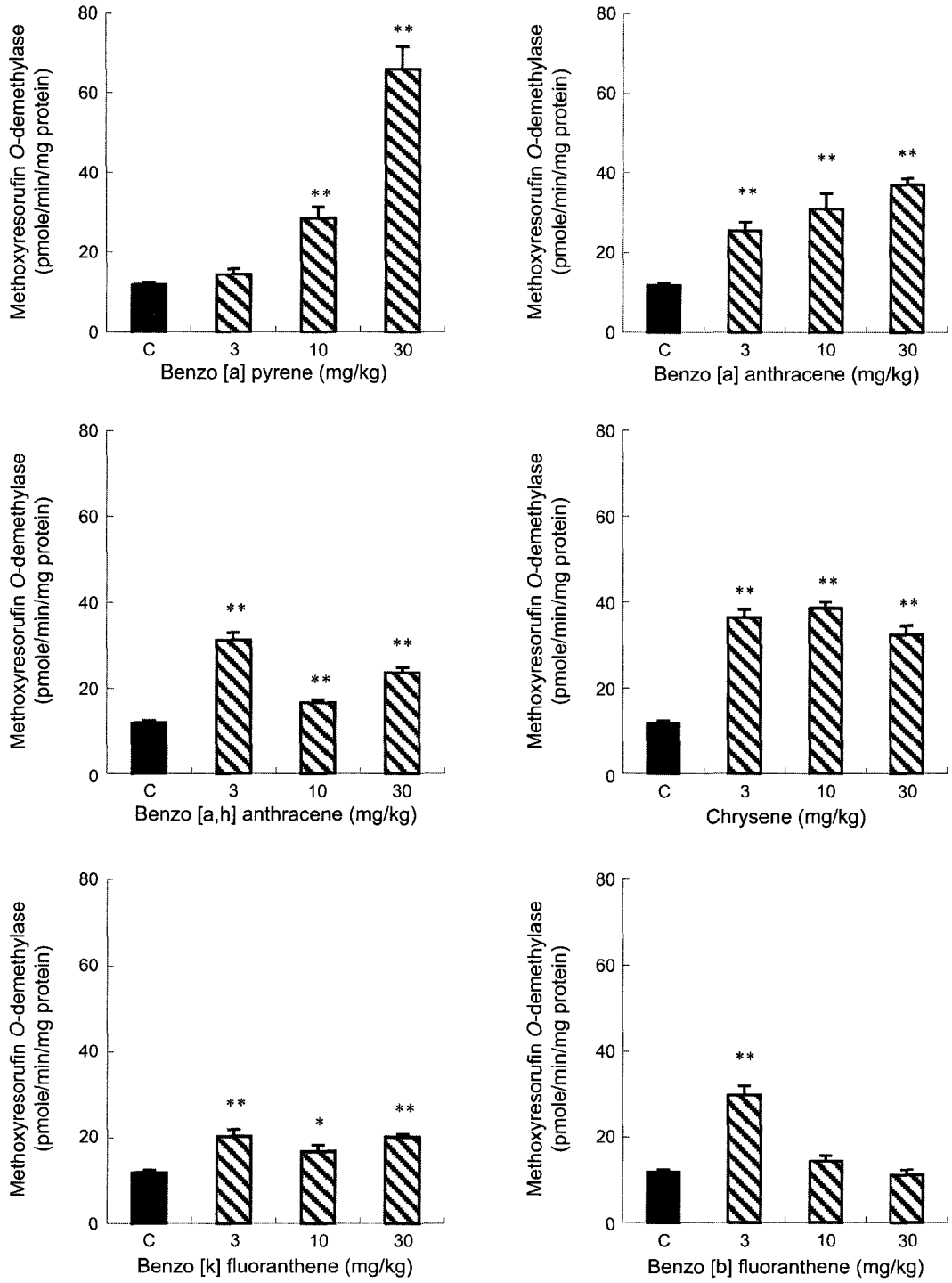

Fig. 4. Effects of six polycyclic aromatic hydrocarbons on activities of methoxyresorufin *O*-demethylase in liver S-9 fractions. Male ICR mice were treated intraperitoneally with either one of benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, chrysene, benzo[k]fluoranthene and benzo[b]fluoranthene in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Each value represents the mean ± S.E. of five animals. The asterisks indicate the values significantly different from the control (C) at $P<0.05$ (*) or $P<0.01$ (**).

and in dietary foodstuffs. Their formation and subsequent deposition by the incomplete combustion of fossil fuels, wood and other organic matters are well documented (US EPA, 1993). In addition, occupational exposure to PAHs occurs in large populations of workers employed in various industries where the pyrolysis processes and the handling of their by-products are prevalent (Menzie *et al.*, 1992). The primary objectives of the present studies were

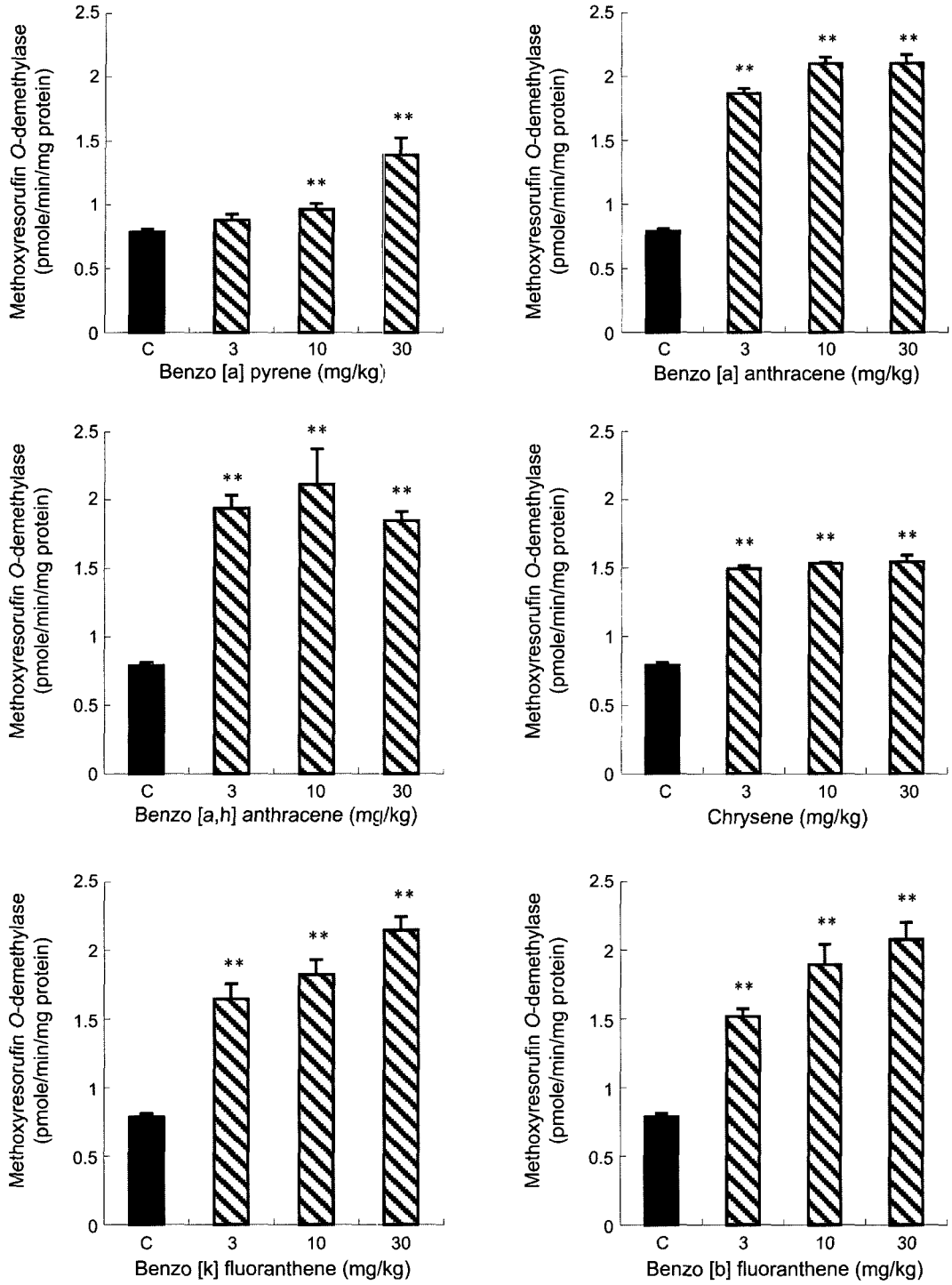

Fig. 5. Effects of six polycyclic aromatic hydrocarbons on activities of methoxyresorufin O-demethylase in lung S-9 fractions. Male ICR mice were treated intraperitoneally with either one of benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, chrysene, benzo[k]fluoranthene and benzo[b]fluorancene in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Each value represents the mean ± S.E. of five animals. The asterisks indicate the values significantly different from the control (C) at $P<0.01$ (**).

two fold: to characterize the effects of PAHs on a variety of CYP enzymes including CYP 1A in livers and lungs; and to investigate certain CYP enzymes as a possible marker for exposure to certain PAHs.

In the present study, effects of six PAHs (i.e., B[a]P, B[a]A, D[a,h]A, chrysene, B[k]F and B[b]F) on activities of hepatic and pulmonary CYP enzymes were investigated in male ICR mice. These six PAHs were selected, because

Table I. Effects of six polycyclic aromatic hydrocarbons on activities of benzyloxyresorufin *O*-debenzylase in liver and lung S-9 fractions

Dose, mg/kg	B[a]P	B[a]A	D[a,h]A	Chrysene	B[k]F	B[b]F
			Liver			
Control	0.40 ± 0.02	0.40 ± 0.02	0.40 ± 0.02	0.40 ± 0.02	0.40 ± 0.02	0.40 ± 0.02
3	0.49 ± 0.05	0.54 ± 0.01**	1.68 ± 0.14**	0.83 ± 0.09**	0.93 ± 0.09**	1.16 ± 0.12**
10	0.46 ± 0.04	0.96 ± 0.09**	0.86 ± 0.07**	1.06 ± 0.07**	0.88 ± 0.11**	0.59 ± 0.11
30	0.49 ± 0.04	1.40 ± 0.12**	1.28 ± 0.21**	1.01 ± 0.03**	0.97 ± 0.08**	0.33 ± 0.04
			Lung			
Control	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02
3	0.41 ± 0.03*	0.32 ± 0.01	0.46 ± 0.02**	0.39 ± 0.02*	0.40 ± 0.02*	0.45 ± 0.03**
10	0.40 ± 0.04	0.31 ± 0.01	0.52 ± 0.06*	0.40 ± 0.01**	0.45 ± 0.02**	0.44 ± 0.04*
30	0.59 ± 0.05**	0.35 ± 0.03	0.41 ± 0.02*	0.42 ± 0.01**	0.51 ± 0.02**	0.44 ± 0.02**

Male ICR mice were treated intraperitoneally with individual PAHs in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Animals of control group were given the corn oil alone. Each value represents the mean ± S.E. of five animals. The asterisks indicate the values significantly different from vehicle-treated control at P<0.05 (*) or P<0.01 (**). The enzyme activity was expressed as pmole resorufin formed/min/mg protein. B[a]P benzo[a]pyrene; B[a]A, benz[a]anthracene; D[a,h]A, dibenz[a,h]anthracene; B[k]F, benzo[k]fluoranthene; B[b]F, benzo[b]fluoranthene.

Table II. Effects of six polycyclic aromatic hydrocarbons on activities of pentoxyresorufin *O*-depentylase in liver and lung S-9 fractions

Dose, mg/kg	B[a]P	B[a]A	D[a,h]A	Chrysene	B[k]F	B[b]F
			Liver			
Control	0.88 ± 0.08	0.88 ± 0.08	0.88 ± 0.08	0.88 ± 0.08	0.88 ± 0.08	0.88 ± 0.08
3	1.17 ± 0.12	1.46 ± 0.03**	6.60 ± 0.66**	1.46 ± 0.10**	3.16 ± 0.16**	3.47 ± 0.27**
10	1.11 ± 0.07	2.55 ± 0.20**	3.90 ± 0.37**	1.97 ± 0.08**	3.23 ± 0.38**	1.79 ± 0.29*
30	1.23 ± 0.13	3.35 ± 0.37**	4.60 ± 0.65**	1.82 ± 0.07**	4.47 ± 0.58**	1.39 ± 0.18*
			Lung			
Control	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.02
3	0.23 ± 0.01	0.22 ± 0.01	0.28 ± 0.01**	0.24 ± 0.01	0.26 ± 0.01*	0.28 ± 0.02*
10	0.24 ± 0.03	0.21 ± 0.01	0.34 ± 0.04*	0.22 ± 0.01	0.29 ± 0.02*	0.28 ± 0.02*
30	0.34 ± 0.02**	0.21 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	0.33 ± 0.02**	0.29 ± 0.01**

Male ICR mice were treated intraperitoneally with individual PAHs in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Animals of control group were given the corn oil alone. Each value represents the mean ± S.E. of five animals. The asterisks indicate the values significantly different from vehicle-treated control at P<0.05 (*) or P<0.01 (**). The enzyme activity was expressed as pmole resorufin formed/min/mg protein. B[a]P, benzo[a]pyrene; B[a]A, benz[a]anthracene; D[a,h]A, dibenz[a,h]anthracene; B[k]F, benzo[k]fluoranthene; B[b]F, benzo[b]fluoranthene.

all of them have been documented in a group of possibly carcinogenic to humans by US EPA (1993), and because these PAHs are widely distributed in the atmospheric environment. Due to the possible route of exposure, the hepatic and pulmonary activities of CYP enzymes were determined at the same time. It was found that the activities of CYP 1A-selective EROD and MROD were significantly induced in both liver and lung tissues of treated mice (Figs. 2 to 5). These results were consistent with not only the reports that CYP 1A1 and 1A2 are the major enzymes in the metabolism of potential procarcinogens such as PAHs (Gelboin, 1980; Petry *et al.*, 1996; Nisbet, 1992), but also the reports that B[a]P, B[k]F, B[b]F, chrysene and B[a]A could induced the EROD activity (Brunstrom *et al.*, 1991; Villeneuve *et al.*, 1995; Willet *et al.*, 1997). In addition, the present results indicated that the pulmonary EROD activities would be an important marker for exposure to B[a]A and D[a,h]A and that the hepatic MROD activities would be an important marker for exposure to B[a]P. In addition, our results showed that B[a]P might have the lowest potential of CYP 1A induction compared to the effects by B[k]F, B[b]F, chrysene and B[a]A, which were in accord with the experimental results by Machala *et al.* (1996).

In the present study, it was also found that hepatic activities of CYP 2B-selective BROD and PROD were significantly induced by most PAHs tested, except B[a]P (Tables I and II). No induction of CYP 2B by B[a]P was

Table III. Effects of six polycyclic aromatic hydrocarbons on activities of *p*-nitrophenol hydroxylase in liver and lung S-9 fractions

Dose, mg/kg	B[a]P	B[a]A	D[a,h]A	Chrysene	B[k]F	B[b]F
			Liver			
Control	29.4 ± 3.08	29.4 ± 3.08	29.4 ± 3.08	29.4 ± 3.08	29.4 ± 3.08	29.4 ± 3.08
3	39.0 ± 3.64	29.5 ± 1.04	152.2 ± 15.21**	59.6 ± 3.78**	104.9 ± 12.10**	76.5 ± 7.06**
10	71.2 ± 4.10**	50.7 ± 1.62**	107.2 ± 14.14**	77.1 ± 0.80**	132.9 ± 4.17**	132.6 ± 16.53**
30	101.7 ± 7.35**	64.2 ± 3.63**	104.9 ± 12.10**	70.4 ± 1.95**	136.1 ± 8.08**	132.9 ± 6.25**
			Lung			
Control	14.1 ± 0.73	14.1 ± 0.73	14.1 ± 0.73	14.1 ± 0.73	14.1 ± 0.73	14.1 ± 0.73
3	11.6 ± 1.25	18.8 ± 1.88**	13.8 ± 1.57	11.6 ± 0.70*	18.2 ± 0.20**	14.6 ± 0.79
10	11.2 ± 0.86*	19.6 ± 1.24**	22.0 ± 1.68**	11.8 ± 1.00	16.4 ± 1.52	13.6 ± 1.17
30	11.6 ± 1.02	19.9 ± 1.32**	13.8 ± 1.16	10.3 ± 0.98*	16.4 ± 1.31	14.1 ± 1.09

Male ICR mice were treated intraperitoneally with individual PAHs in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Animals of control group were given the corn oil alone. Each value represents the mean±S.E. of five animals. The asterisks indicate the val ues significantly different from vehicle-treated control at P<0.05 (*) or P<0.01 (**). The enzyme activity was expressed as pmole 4-nitrocatechol formed/min/mg protein. B[a]P, benzo[a]pyrene; B[a]A, benz[a]anthracene; D[a,h]A, dibenz[a,h]anthracene; B[k]F, benzo[k]fluoranthene; B[b]F, be nzo[b]fluoranthene.

Table IV. Effects of six polycyclic aromatic hydrocarbons on activities of erythromycin *N*-demethylase in liver and lung S-9 fractions

Dose, mg/kg	B[a]P	B[a]A	D[a,h]A	Chrysene	B[k]F	B[b]F
			Liver			
Control	315 ± 12	315 ± 12	315 ± 12	315 ± 12	315 ± 12	315 ± 12
3	271 ± 26	170 ± 21**	598 ± 52**	1193 ± 78**	766 ± 44**	839 ± 76**
10	287 ± 23	222 ± 31*	349 ± 53	808 ± 97**	664 ± 29**	766 ± 64**
30	327 ± 21	197 ± 5**	702 ± 40**	1161 ± 76**	374 ± 75	620 ± 53**
			Lung			
Control	325 ± 21	325 ± 21	325 ± 21	325 ± 21	325 ± 21	325 ± 21
3	270 ± 4*	286 ± 19	250 ± 12*	282 ± 19	249 ± 25*	277 ± 21
10	298 ± 9	369 ± 31	579 ± 58**	287 ± 18	288 ± 14	367 ± 28
30	321 ± 12	369 ± 3	262 ± 13*	293 ± 16	248 ± 19*	330 ± 20

Male ICR mice were treated intraperitoneally with individual PAHs in corn oil at 3, 10 and 30 mg/kg once daily for 3 consecutive days. Animals of control group were given the corn oil alone. Each value represents the mean ± S.E. of five animals. The asterisks indicate the v alues significantly different from vehicle-treated control at P<0.05 (*) or P<0.01 (**). The enzyme activity was expressed as pmole formaldehyde formed/min/mg protein. B[a]P, benzo[a]pyrene; B[a]A, benz[a]anthracene; D[a,h]A, dibenz[a,h]anthracene; B[k]F, benzo[k]fluoranthene; B[b]F, be nzo[b]fluoranthene.

expected at the beginning of these studies, because the metabolism of B[a]P have reportedly not been related to CYP 2B enzymes (Conney *et al.*, 1994; Gelboin, 1980). Meanwhile, the inductive effects of B[a]A, D[a,h]A and B[k]F on the hepatic activities of BROD and PROD were remarkable. Although there have been no reports regarding the role of CYP 2B enzymes in the metabolism of these three PAHs, our present results opened a possibility the hepatic BROD and PROD activities might be a useful marker for the exposure to B[a]A, D[a,h]A and B[k]F. In contrast to the clear induction of BROD and PROD activities in livers, the pulmonary activities of BROD and PROD were modestly induced by certain PAHs (Tables I and II). The discrepancy between the effects of PAHs on hepatic and pulmonary CYP activities could be speculated with several reasons, such as characteristic distribution of PAHs to livers and different regulatory mechanism of CYP expression in two organs. However, further studies are required to elucidate the tissue specificities and regulatory mechanism of CYP 2B induction by PAHs.

Our present results also showed that six PAHs could induce hepatic CYP 2E1-selective PNPH activities with different potency (Table III). Among them, the inductive effects of B[k]F and B[b]F were profound. In Table IV, chrysene could induce hepatic CYP 3A-selective ERDM activities from the dose of 3 mg/kg. Taken together, these results indicated that the hepatic PNPH activity might be a useful marker for the exposure to B[k]F and B[b]F, and

that the hepatic ERDM activity might be a useful marker for the exposure to chrysene.

It is generally accepted that the most PAHs are able to induce CYP 1A enzymes (Brunstrom *et al.*, 1991; Gelboin, 1980; Nisbet, 1992; Petry *et al.*, 1996; Villeneuve *et al.*, 1995; Willett *et al.*, 1997). However, our present results indicated that the six PAHs tested could show characteristic induction profiles on CYP enzymes, indicating a possibility that certain CYP enzymes might be useful markers for the evaluation of exposure to certain PAHs. To use the CYP enzymes mentioned above as a marker for determining the possible exposure to certain PAHs, it is necessary to find a dose-dependent correlation between the induction potential and toxicity induced by certain PAHs. For this reason, studies to investigate a possible correlation between the CYP induction potential of PAHs and the immunotoxic potential of them are currently underway. In this regard, previous reports indicated that PAHs we evaluated were formerly ranked in order of carcinogenic potency as benzo[a]pyrene > dibenz[a,h]anthracene > benz[a]anthracene > chrysene and in order of immunotoxic potency as dibenz[a,h]anthracene > benzo[a]pyrene ≥ benz[a]anthracene > chrysene (White *et al.*, 1985). Therefore, when the immunosuppressive potentials of other PAHs used in the present study are completely studied, it is going to be possible to determine the possible correlation between the CYP induction potential of PAHs and their immunotoxicity.

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