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—Full Paper—

Effects of Diesel Exhaust Particles on the Male Reproductive System in Strains of Mice with Different Aryl Hydrocarbon Receptor Responsiveness

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Abstract. Diesel exhaust particles (DEPs) contain polycyclic aromatic hydrocarbons (PAH) that bind to aryl hydrocarbon receptors (AhRs) and decrease sperm production. Since it is not clear if AhR mediates DEP toxicity, we investigated the effect of DEPs in four strains of mice that have different AhR responsiveness. We treated BALB/c, C57BL/6, ICR and DBA/2 mice with DEP suspensions and compared their toxicity in each strain. In both the vehicle- and DEP-treated groups, ethoxyresorufin-O-deethylase (EROD) activity, as an indirect index of AhR activity, was increased in the order of BALB/c > C57BL/6 > ICR > DBA/2. Only BALB/c and C57BL/6 mice had significantly lower daily sperm production (DSP) than vehicle-treated mice. All strains exhibited increased sperm abnormalities. In particular, the C57BL/6, ICR and DBA/2 mice exhibited significantly increased abnormalities. A significant correlation was found between EROD activity and DSP or incidence of morphologically abnormal sperm. These data suggest that DEP toxicity may affect the male reproductive system in an AhR-dependent manner.

Key words: Cytochrome P450 1A1, Diesel exhaust particle, Epididymis, Sperm, Testis

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Diesel exhaust particles (DEPs) are particulate matter from diesel exhaust (DE) containing many toxic compounds that can cause pulmonary cancer, allergic rhinitis and bronchial asthma [1, 2]. It has been reported that DEPs also contain endocrine disrupters, such as polyaromatic hydrocarbons (PAHs) [1], dioxin derivatives [3, 4] and nitrophenols [5–9].

DEPs and DE have also been reported to cause male reproductive toxicity. DEPs decrease sperm production in mice [10] and rats [11], while DE increases serum concentrations of testosterone and the weight of the accessory glands in rats [12]. Furthermore, the number of sperm and Sertoli cells decreases in mature rats exposed to DE as fetuses [13]. We hypothesized that the male reproductive toxicity of DEPs may depend on PAHs. We previously reported that DEPs can activate aryl hydrocarbon receptors (AhR) in an *in vitro* system using an Ah-Immunoassay kit [14]. In addition, DEP-treated mice showed decreased daily sperm

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production (DSP) and increased plasma concentrations of testosterone and hepatic ethoxyresorufin-O-deethylase (EROD) activity, which is an indirect index of AhR activity [15]. However, the relationship between spermatogenesis and AhR activity *in vivo* remains unknown.

The present study was designed to determine whether or not the effect of DEPs on spermatids in the testis and epididymis is dependent on AhRs. Four strains of mice, namely, BALB/c, C57BL/6, ICR and DBA/2 mice, were treated with DEPs. BALB/c and C57BL/6 mice have high AhR responsiveness, while DBA/2 mice have low responsiveness [16–19]. ICR mice are not inbred but instead are outbred. It is also well known that the ICR colony has a variety of AhR SNPs [19], and the AhR responsiveness of ICR mice is unknown. Therefore, hepatic EROD activity was measured after DEP treatment to confirm the height of AhR responsiveness in the four strains of mice. EROD activity represents the catalytic activity of cytochrome P4501A isoforms and is an indirect index of AhR activity [20]. Toxicity toward spermatids in the testis and epididymis was also determined for each strain, and the relationship between spermatogenesis and AhR was investigated.

Materials and Methods

Chemicals and DEPs

NADPH and fluorescamine were purchased from Wako Pure Chemical (Osaka, Japan). Resorufin and ethoxy resorufin were obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA). DEPs were collected and a suspension was prepared according to the method published by Sagai [21].

Animals and DEP injection

Six-week-old BALB/c, C57BL/6, ICR and DBA/2 male mice were purchased from Japan Clea (Tokyo, Japan). The mice were allowed to adapt for 1 week in an environmentally controlled room at 23 ± 2 C with 55 ± 7 % humidity and a 12 h/12 h light/dark cycle. CE-2 commercial diet (Japan Clea) and water were given *ad libitum*. After 1 week of adaptation, the mice were divided into two groups: DEP-treated groups and a vehicle-treated group for each strain (n=4–5 for each group). The mice in the DEP-treated groups received 74 μg of the DEP suspension (0.2 ml of 0.37 DEP mg/ml), which was injected into the dorsal subcutaneous layer 10 times over a period of 5 weeks. In the vehicle-treated groups, 0.2 ml of PBST was injected in the same manner. In our small pilot study, 24.7, 74.0 or 220 μg of DEP suspension was used per mouse. DSP decreased in mice treated with more than 74.0 μg/mouse DEP. Therefore, a concentration of 74 μg/mouse was used in the present study. Two weeks after the last treatment, mice at 13 weeks of age were weighed and euthanized under deep ethyl ether anesthesia. The testes, epididymides and liver were collected from each animal and weighed. Testes were stored at –80 C until being assayed for daily sperm production (DSP). Epididymides were used immediately for the analysis of sperm viability and morphology. Livers were stored at –80 C until the assay for ethoxyresorufin-O-deethylase (EROD) activity.

The study was carried out in accordance with the Guidelines for Animal Experimentations, Aomori University of Health and Welfare.

Measurement of hepatic ethoxyresorufin-O-deethylase (EROD) activity

Livers were homogenized in 0.15 M KCl using a Polytron homogenizer. Microsomes were isolated by ultracentrifugation (105,000 × g, 60 min), and ethoxyresorufin-O-deethylase (EROD) activity was measured using the procedure of Kennedy *et al*. [22].

Sperm analysis

DSP was determined by the procedure of Joyce *et al*. [23] and Yoshida *et al*. [10]. The left testis was homogenized for 30 seconds in 5 ml of 10 mM PBS containing 0.05% Triton X-100 using a Polytron homogenizer. Aliquots of the suspensions were placed in a hematocytometer chamber, and the number of step 14–16 spermatids per testis was counted. Developing spermatids spend 4.84 days in step 14–16 during spermatogenesis in mice [10, 23]. Thus, the spermatid count was divided by 4.84 to obtain the DSP. Sperm viability was monitored using vital staining with eosin-aniline blue. Sperm suspensions were stained with 0.5% eosin Y and 0.5% aniline blue mix solution for 10 sec and smeared. The numbers of live (colorless) and dead (red) sperm were counted. Sperm morphology was evaluated by the procedure of Wyrobek *et al*. [24] and Watanabe *et al*. [25] with a minor modification.

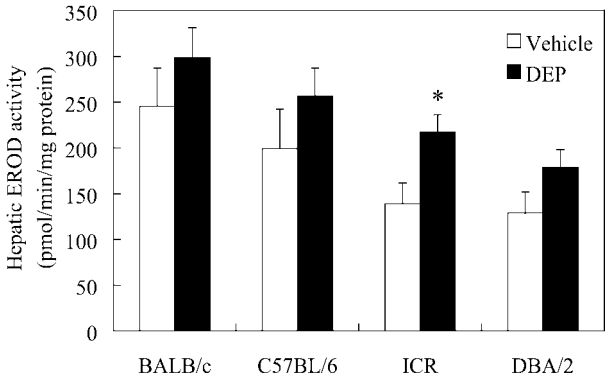

Fig. 1. Effects of diesel exhaust particles (DEPs) on hepatic ethoxyresorufin-O-deethylase (EROD) activity in the four strains of mice. AhR responsiveness was assessed for all four strains of mice by measuring hepatic EROD activity 2 weeks after the last dose of DEPs. Values represent means ± SEM (n=4–5).
* P<0.05 *vs.* the vehicle group for the same strain.

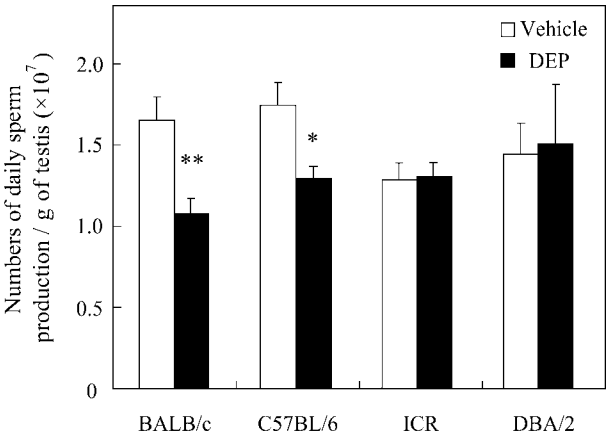

Fig. 2. Effects of diesel exhaust particles (DEPs) on daily sperm production in all four strains of mice. Values represent means ± SEM (n=4–5). *P<0.05 *vs.* the vehicle group for the same strain.

Sperm smears were made with a suspension collected immediately from the left cauda epididymis in 4 ml of physiological saline at 37 C. The sperm preparations were stained with 1% eosin Y solution and examined under a light microscope. Abnormalities of sperm shape were classified according to the criteria described by Watanabe *et al*. [25]; that is, they were classified according to head, neck, midpiece and tail abnormalities. Four hundred sperm from each mouse were examined.

Measurement of plasma concentrations of testosterone

Plasma concentrations of testosterone were measured using a Testosterone EIA Kit (Cayman Chemical, Ann Arbor, MI, USA).

Statistical analysis

All data are expressed as means ± SEM. Differences between the DEP- and vehicle-treated groups were evaluated by the Student's *t*-test. Pearson's partial correlation was used to examine the relationships among hepatic EROD activity, DSP, sperm viability rate, incidence of morphological abnormal sperm and plasma concentrations of testosterone. A P-value<0.05 was considered statistically significant.

Results

DEPs had no effect on body weights or the weights of the testes and epididymides (both absolute and relative to body weight) in any of the strains (data not shown).

The hepatic EROD activities of the four strains of mice treated with DEPs are shown in Fig. 1. Hepatic EROD activity decreased in the order of BALB/c > C57BL/6 > ICR > DBA/2 in the DEP- and vehicle-treated groups of all the mouse strains. Only the EROD activity of the DEP-treated group of DBA/2 mice was significantly higher than the vehicle group. The EROD activity of the DEP-treated groups of the other strains tended to be higher than the vehicle-treated groups, although the differences were not statistically significant.

The DSP results of the four strains of mice treated with DEPs are shown in Fig. 2. DSP was significantly lower in the DEP-treated groups of BALB/c and C57BL/6 mice compared with the vehicle-treated groups, although there were no significant changes in the ICR and DBA/2 mice.

The sperm viability rates of the four strains of mice treated with DEPs are shown in Fig. 3. In three out of the four strains of mice, specifically the BALB/c, C57BL/6 and ICR mice, the sperm viability rate was significantly lower in the DEP-treated groups than in the vehicle-treated groups. There was no significant difference in the sperm viability

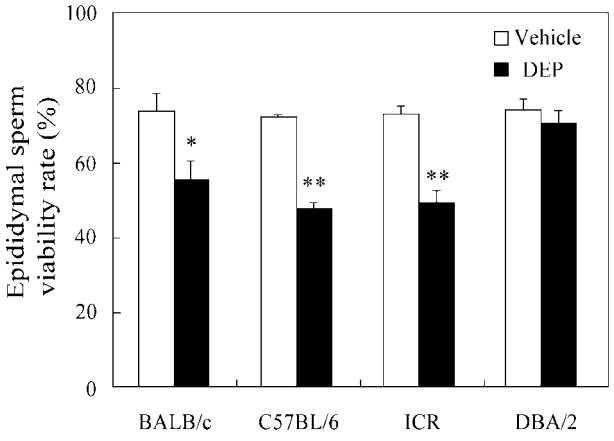

Fig. 3. Effects of diesel exhaust particles (DEPs) on the epididymal sperm viability rate of all four strains of mice. Values represent means ± SEM (n=4–5). *P<0.05 *vs.* the vehicle group for the same strain.

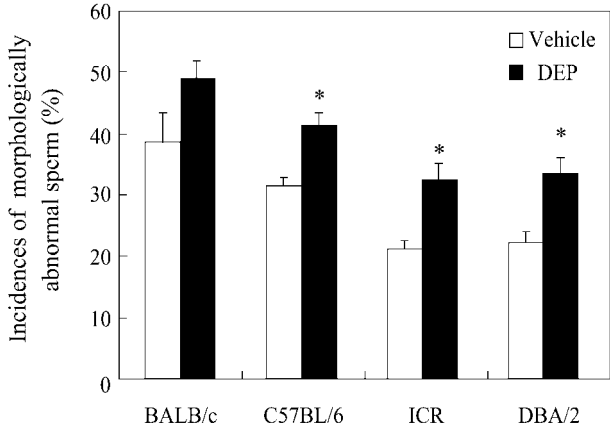

Fig. 4. Effects of diesel exhaust particles (DEPs) on the incidences of morphological abnormalities of sperm in the epididymis of all four strains of mice. Values represent means ± SEM (n=4–5). *P<0.05 *vs.* the vehicle group for the same strain.

rates of the DBA/2 mice.

The incidences of morphological abnormalities of sperm in the four strains of mice treated with DEPs are shown in Fig. 4. All strains showed increased sperm abnormalities. In particular, the C57BL/6, ICR and DBA/2 mice showed a significant increase in the number of abnormalities. The plasma concentrations of testosterone of the four strains of mice treated with DEPs are shown in Fig. 5. The plasma concentration of testosterone in the DEP-

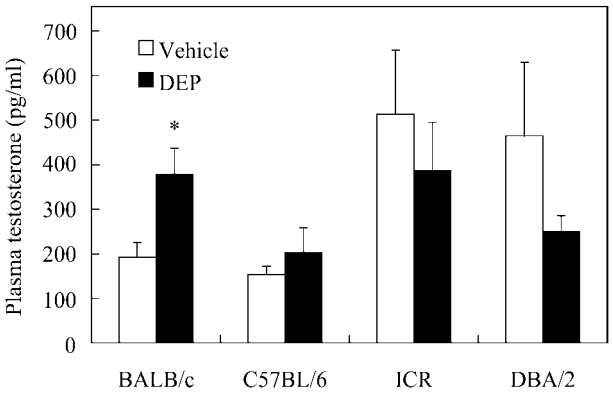

Fig. 5. Effects of diesel exhaust particles (DEPs) on the plasma concentrations of testosterone in all four strains of mice. Values represent means ± SEM (n=4–5). *P<0.05 *vs.* the vehicle group for the same strain.

treated group of BALB/c mice was significantly higher than that in the vehicle-treated group. There was no significant difference in the other three strains of mice.

The correlations among hepatic EROD activity, DSP, sperm viability rate, incidence of morphological abnormalities of sperm and plasma concentrations of testosterone in the four strains of mice treated with DEPs are shown in Table 1 and Fig. 6. Hepatic EROD activity was strongly correlated with DSP and the incidence of morphologically abnormal sperm. A clear negative correlation was observed between hepatic EROD activity and DSP (Fig. 6A), whereas a clear positive correlation was observed between hepatic EROD activity and the incidence of morphologically abnormal sperm (Fig. 6B) in all four strains of mice treated with DEPs, respectively. There was no statistically significant correlation among the measurements for the four strains of vehicle mice.

Discussion

In the present study, we examined whether the negative effects of DEPs on spermatids in the testis and epididymis were dependent on AhR in four strains of mice. Our results clearly demonstrated that DEPs decrease daily sperm production and increase the incidence of abnormal sperm.

DEPs are major air pollutants in large cities and are toxic when inhaled. Following inhalation, toxic compounds in DEPs may be transported to various

Table 1. Correlations among hepatic ethoxyresorufin-O-deethylase activity (EROD), daily sperm production (DSP), sperm viability rate (viability), incidence of morphological abnormalities of sperm (abnormality) and plasma concentrations of testosterone (testosterone) in the four strains of DEP-treated mice

		DSP	Viability	Abnormality	Testosterone
EROD	P	–0.593	–0.196	0.539	0.234
	r	0.008	0.396	0.022	0.383
DSP	P		0.029	–0.040	–0.283
	r		0.906	0.875	0.308
Viability	P			–0.425	0.095
	r			0.062	0.717
Abnormality	P				0.051
	r				0.850

P–values calculated by Pearson's correlation test.

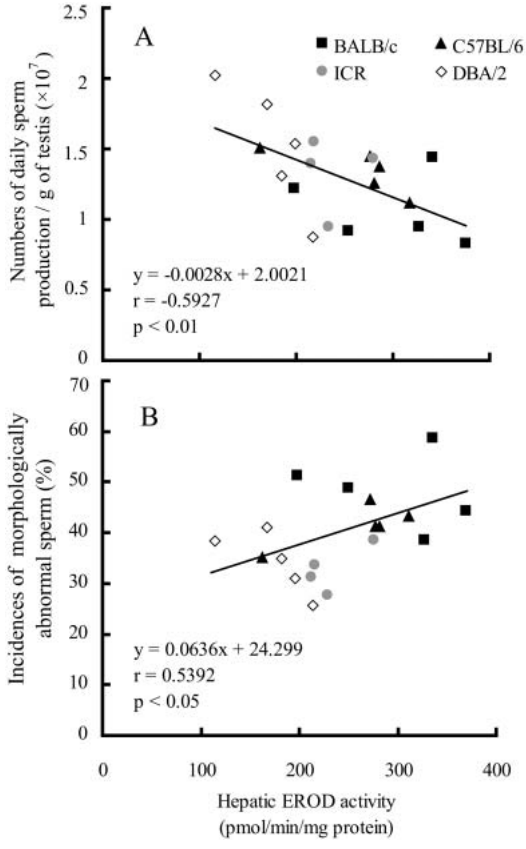

Fig. 6. Correlations among hepatic EROD activity, (A) daily sperm production and (B) incidence of morphological abnormalities of sperm. Formulae are based on all samples from the DEP-treated groups.

organs through blood vessels. In this study, DEP toxicity was examined by dorsal subcutaneous treatment with a DEP suspension. Following subcutaneous treatment, the toxic compounds may also be transported from the dorsal subcutaneous tissue to various organs through blood vessels. The subcutaneous route was an inevitable selection due to a technical problem in the present study.

DEPs had no effect on body weights or the weights of testes and epididymides (both absolute and relative to body weight) in any of the strains. It has been reported that inhalation of diesel exhaust alters body weight, but not testis and epididymis weight, in mice [10]. The negative effect of DEPs on body or reproductive organ weights may depend on the dosage. It is reasonable that reproductive organ weights might remain unchanged because the plasma concentration of testosterone was not decreased.

As previously discussed, BALB/c and C57BL/6 mice have high AhR responsiveness, while DBA/2 mice have low responsiveness [16–19]. AhR is a ligand-activated transcription factor [26] that upregulates CYP1A1 expression, which has EROD activity [27]. Thus, hepatic EROD activity is an indirect endpoint for AhR responsiveness. We examined hepatic EROD activity in the four strains of mice treated with vehicle, because the AhR responsiveness of ICR mice was unknown. Based on the EROD activity, we show here that ICR mice have intermediate AhR responsiveness. Accordingly, AhR responsiveness decreased in the order BALB/c > C57BL/6 > ICR > DBA/2 vehicle-treated mice. When these four strains of mice were treated with DEPs, hepatic EROD activity was increased compared with that in vehicle-treated mice. Therefore, this was indicated that DEPs upregulate CYP1A1 expression, and as a result, hepatic EROD

activity increased.

Testosterone is a crucial hormone for spermatogenesis. It had been reported that both 3-methyl-4-nitrophenol (PNMC) [7, 28] and 4-nitrophenol (PNP) [29], which are components of DEPs, have antiandrogenic activity, and they significantly decrease the plasma concentration of testosterone in rats [7, 28]. DE inhalation from birth to 3 months does not affect plasma testosterone in rats [13], but inhalation for 8 months significantly increases plasma testosterone in adult rats [12]. In the present study, DEPs were given to adult mice. In the BALB/c mice, the plasma concentrations of testosterone in the DEP-treated group were significantly higher than those in the vehicle-treated group. In the C57BL/6 mice, the plasma concentrations of testosterone were also higher, but this difference was not significant. These two strains have high AhR responsiveness. However, in the ICR and DBA/2 mice, which have middle and low AhR responsiveness, respectively, the plasma concentrations of testosterone in the DEP-treated groups were lower than those in the vehicle-treated groups, but the differences were not significant. The present results suggest that DEPs do not have androgenic activity, but instead, have antiandrogenic activity in BALB/c and C57BL/6 mice. Therefore, the LH level may increase due to feedback regulation resulting from the antiandrogenic activity of DEP. As a result, the testis is stimulated and the testosterone level is increased by more than is required in an AhR-dependent manner.

Furthermore, we also examined the correlations among our results. EROD activity exhibited negative correlation with DSP/g testis and positive correlation with the incidence of morphologically abnormal sperm in the DEP-treated groups. In the vehicle groups, EROD activity showed no correlation with DSP/g testis or the incidence of morphologically abnormal sperm (data not shown). Since it has been reported that both liver and testis CYP1A1 are induced by PAH in mice [30], hepatic EROD activity reflects testicular activity. The male reproductive toxicity induced by DEPs may correlate with AhR activity. Fukuzawa *et al*. reported that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is an AhR ligand and a DEP [1, 4], suppresses expression of the P450scc and LH receptor genes in the testis in an AhR-dependent manner [31]. Revel *et al*. reported that Benzo(a)pyrene (BaP), which is an AhR ligand and a component of DEP [1], induces sperm apoptosis in the seminiferous tubules of mice. However, resveratrol, which is a flavonoid and AhR antagonist [32], protects sperm from the apoptosis caused by BaP [33]. Consequently, AhR may participate in male reproductive toxicity, and DEPs may be endocrine disrupters because they include a number of AhR ligands.

In conclusion, DEP toxicity towards the male reproductive system may occur in an AhR-dependent manner because the DSP/g of testis and incidence of morphologically abnormal sperm were correlated with AhR activity.

References

1. **McClellan RO**. Health effects of exposure to diesel exhaust particles. *Annu Rev Pharmacol Toxicol* 1987; 27: 279–300.
2. **Sagai M, Ichinose T**. Health effect of diesel exhaust and diesel exhaust particles. *In*: Fuchs J, Packer L (eds.), Environmental Stressors in Health and Disease, 1st ed, New York: Marcel Dekker; 2001: 157–184.
3. **Clunies-Ross C, Stanmore BR, Millar GJ**. Dioxins in diesel exhaust. *Nature* 1996; 381: 379.
4. **Miyabara Y, Hashimoto S, Sagai M, Morita M**. PCDDs and PCDFs in vehicle exhaust particles in Japan. *Chemosphere* 1999; 39: 143–150.
5. **Taneda S, Mori Y, Kamata K, Hayashi H, Furuta C, Li C, Seki K, Sakushima A, Yoshino S, Yamaki K, Watanabe G, Taya K, Suzuki AK**. Estrogenic and anti-androgenic activity of nitrophenols in diesel exhaust particles (DEP). *Biol Pharm Bull* 2004; 27: 835–837.
6. **Furuta C, Suzuki AK, Taneda S, Kamata K, Hayashi H, Mori Y, Li C, Watanabe G, Taya K**. Estrogenic activities of nitrophenols in diesel exhaust particles. *Biol Reprod* 2004; 70: 1527–1533.
7. **Li C, Takahashi S, Taneda S, Furuta C, Watanabe G, Suzuki AK, Taya K**. Impairment of testicular function in adult male Japanese quail (*Coturnix japonica*) after a single administration of 3-methyl-4-nitrophenol in diesel exhaust particles. *J Endocrinol* 2006; 189: 555–564.
8. **Li C, Taneda S, Suzuki AK, Furuta C, Watanabe G,**

Taya K. Effects of 3-methyl-4-nitrophenol in diesel exhaust particles on the regulation of testicular function in immature male rats. *J Androl* 2006; 28: 252–258.

9. **Li C, Takahashi S, Taneda S, Furuta C, Watanabe G, Suzuki AK, Taya K**. Effects of 3-methyl-4-nitrophenol in diesel exhaust particles on the regulation of reproductive function in immature female Japanese quail (*Coturnix japonica*). *J Reprod Dev* 2007; 53: 673–678.
10. **Yoshida S, Sagai M, Oshio S, Umeda T, Ihara T, Sugamata M, Sugawara I, Takeda K**. Exposure to diesel exhaust affects the male reproductive system of mice. *Int J Androl* 1999; 22: 307–315.
11. **Watanabe N, Oonuki Y**. Inhalation of diesel engine exhaust affects spermatogenesis in growing male rats. *Environ Health Perspect* 1999; 107: 539–544.
12. **Tsukue N, Toda N, Tsubone H, Sagai M, Jin WZ, Watanabe G, Taya K, Birumachi J, Suzuki AK**. Diesel exhaust (DE) affects the regulation of testicular function in male Fischer 344 rats. *J Toxicol Environ Health A* 2001; 63: 115–126.
13. **Watanabe N**. Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses. *Toxicol Lett* 2005; 155: 51–58.
14. **Izawa H, Watanabe G, Taya K, Sagai M**. Inhibitory effects of foods and polyphenols on the activation of the aryl hydrocarbon receptor induced by diesel exhaust particles. *Environment Sci* 2007; 14: 149–156.
15. **Izawa H, Kohara M, Watanabe G, Taya K, Sagai M**. Diesel exhaust particle toxicity on spermatogenesis in the mouse is arylhydrocarbon receptor dependent. *J Reprod Dev* 2007; 53: 1069–1078.
16. **Pohjanvirta R, Tuomisto J**. Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals: effects, mechanisms, and animal models. *Pharmacol Rev* 1994; 46: 483–549.
17. **Poland A, Glover E**. Characterization and strain distribution pattern of the murine Ah receptor specified by the Ahd and Ahb-3 alleles. *Mol Pharmacol* 1990; 38: 306–312.
18. **Zacharova LY, Gulyaeva LF, Lyakhovich VV, Mikhailova ON, Timofeeva OA, Filipenko ML, Kaledin VI**. Cytochrome P4501A1 and 1A2 gene expression in the liver of 3-methylcholanthrene- and o-aminoazotoluene-treated mice: a comparison between PAH-responsive and PAH-nonresponsive strains. *Toxicol Sci* 2003; 73: 108–113.
19. **Thomas RS, Penn SG, Holden K, Bradfield CA, Rank DR**. Sequence variation and phylogenetic history of the mouse Ahr gene. *Pharmacogenetics* 2002; 12: 151–163.
20. **Burke MD, Prough RA, Mayer RT**. Characteristics of a microsomal cytochrome P-448-mediated reaction. Ethoxyresorufin O-de-ethylation. *Drug Metab Dispos* 1977; 5: 1–8.
21. **Sagai M, Saito H, Ichinose T, Kodama M, Mori Y**. Biological effects of diesel exhaust particles. I. *In vitro* production of superoxide and *in vivo* toxicity in mouse. *Free Radic Biol Med* 1993; 14: 37–47.
22. **Kennedy SW, Jones SP**. Simultaneous measurement of cytochrome P4501A catalytic activity and total protein concentration with a fluorescence plate reader. *Anal Biochem* 1994; 222: 217–223.
23. **Joyce KL, Porcelli J, Cooke PS**. Neonatal goitrogen treatment increases adult testis size and sperm production in the mouse. *J Androl* 1993; 14: 448–455.
24. **Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW, Jr Letz G, Malling HV, Topham JC, Whorton MD**. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res* 1983; 115: 1–72.
25. **Watanabe T, Endo A**. Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. *Mutat Res* 1991; 262: 93–99.
26. **Hankinson O**. The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol* 1995; 35: 307–340.
27. **Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, Dalton TP**. Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem Pharmacol* 2000; 59: 65–85.
28. **Li C, Taneda S, Suzuki AK, Furuta C, Watanabe G, Taya K**. Anti-androgenic activity of 3-methyl-4-nitrophenol in diesel exhaust particles. *Eur J Pharmacol* 2006; 543: 194–199.
29. **Li C, Taneda S, Suzuki AK, Furuta C, Watanabe G, Taya K**. Estrogenic and anti-androgenic activities of 4-nitrophenol in diesel exhaust particles. *Toxicol Appl Pharmacol* 2006; 217: 1–6.
30. **Shimada T, Sugie A, Shindo M, Nakajima T, Azuma E, Hashimoto M, Inoue K**. Tissue-specific induction of cytochromes P450 1A1 and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered C57BL/6J mice of arylhydrocarbon receptor gene. *Toxicol Appl Pharmacol* 2003; 187: 1–10.
31. **Fukuzawa NH, Ohsako S, Wu Q, Sakaue M, Fujii-Kuriyama Y, Baba T, Tohyama C**. Testicular cytochrome P450scc and LHR as possible targets of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the mouse. *Mol Cell Endocrinol* 2004; 221: 87–96.
32. **Casper RF, Quesne M, Rogers IM, Shirota T, Jolivet A, Milgrom E, Savouret JF**. Resveratrol has antagonist activity on the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. *Mol Pharmacol* 1999; 56: 784–790.
33. **Revel A, Raanani H, Younglai E, Xu J, Han R, Savouret JF, Casper RF**. Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo(a)pyrene. *Reprod Toxicol* 2001; 15: 479–486.