Letter

Positive and negative ions by air purifier have no effects on embryo-fetal development in rats

Dai Yamamoto, Kiyoshi Wako, Yukari Sato, Mayumi Fujishiro, Ikuo Matsuura
and Yasuyuki Ohnishi

*Nonclinical Research Center, Drug Development Service Segment, LSI Medience Corporation
14-1 Sunayama, Kamisu-shi, Ibaraki 314-0255, Japan*

(Received March 6, 2014; Accepted April 24, 2014)

ABSTRACT — Air purifiers, which release positive and negative ions generated by an electric discharge into the air, have been widely used in common households. In this study, the developmental toxicity potential of the ionized air containing positive and negative ions was evaluated in SD rats [CrI:CD(SD)] following whole-body inhalation to obtain preliminary information for the definitive study. Two groups of 10 pregnant female rats were exposed to the ionized air at concentrations of 0 and 7,000,000 ions/cm³ for 6 hr per day from Days 6 to 19 of gestation. All dams underwent a cesarean section on Day 20 of gestation and their fetuses were examined externally, viscerally, and skeletally for morphological changes. The ionized air had no effects on dams in terms of clinical signs, body weight, food consumption, gravid uterine weights, corrected body weight by gravid uterine weight, or necropsy findings. In addition, there were no effects on the maintenance of pregnancy, including abortion or premature delivery. No exposure-related changes were detected in the number of corpora lutea, implantations, dead embryos, or live fetuses, implantation loss, live fetal weights, sex ratio, or placental weight or features. Fetal examination revealed no external, visceral, or skeletal anomalies or variations caused by the ionized air, nor were there any changes in degree of ossification. Although this study did not fully adhere to the current guidelines because of a smaller number of animals per group, it was suggested that the ionized air has no maternal toxicity or embryo-fetal toxicity in rats.

Key words: Positive and negative ions, Whole-body inhalation, Embryo-fetal development toxicity

INTRODUCTION

The air purifier, which releases positive and negative ions generated by an electric discharge into the air, has been widely used in common households since the device was created (Nishikawa and Nojima, 2001; Nojima and Nishikawa, 2002). Air containing positive and negative ions have the ability such as purification of atmosphere and deodorization and inactivation of bacteria, mold, and other allergens (Nishikawa and Yagi, 2006; Kawamoto *et al.*, 2006). It has been recognized that the ionized air causes a remarkable decrease in allergenicities of atomized Japanese cedar pollen (Kawamoto *et al.*, 2006). In addition, it has been reported that the ionized air improves skin moisture and comfort sensation (Nishitani *et al.*, 2010) and was recently shown to suppress the symptoms of atopic dermatitis in the model mice (Hiramoto *et al.*,

2011). Based on the above studies, the air purifier is used more widely in not only common households, but also in offices and public facilities such as hospitals.

The effectiveness of air containing positive and negative ions is well known as described above. On the other hand, there are few toxicological data concerning ionized air except for a report proving that there is no potential to induce DNA damage in lung cells of rats or mice (Takasawa *et al.*, 2011), although it has been reported that the bacteria inactivation by ions was induced by fragmentation of the surface membrane proteins (Digel *et al.*, 2005; Nishikawa, 2013). It is generally assumed that the results of toxicity studies using animals are relevant to human health. Associated with the expanded use of air purifiers in various areas, there is also an increase in the chance for pregnant females being exposed to ions generated from these devices. Reproductive and developmen-

tal toxicity studies in animal models, therefore, play an important part in overall toxicology, but there is no information concerning the effects on embryo-fetal development. This study was conducted to obtain preliminary information for assessing the safety of air containing positive and negative ions on dams and embryo-fetal development when exposed by whole-body inhalation to pregnant rats.

MATERIALS AND METHODS

Test atmosphere

Air containing positive and negative ions generated by electric discharge devices for air purifiers (Nishikawa and Nojima, 2001; Nojima and Nishikawa, 2002) developed by Sharp Corporation (Osaka, Japan) is defined here as "ionized air (IONA)". The IONA is generated into the air as follows. First, water and oxygen molecules are decomposed by applying positive and negative high voltages to each discharge electrode of the ion generation devices, and the devices generate positive hydrogen ions (H^+) and negative oxygen ions (O_2^-). Then, each ion is surrounded by water molecules like a bunch of grapes to maintain stability. Accordingly, $[(H_2O)_m H]^+$ and $[(H_2O)_n O_2]^-$ as positive and negative ions, respectively, with "m" and "n" as natural numbers, are cluster-shaped and released into the air. The IONA are known as PlasmaCluster Ions® (Nojima and Nishikawa, 2002; Nishikawa and Yagi, 2006; Kawamoto *et al.*, 2006).

Animals were exposed to IONA by whole-body inhalation at concentrations of 0 (control) and 7,000,000 ions/cm³ for 6 hr a day from implantation to the day before cesarean section (Days 6 to 19 of gestation). The high-exposure concentration of IONA was set at the maximum feasible concentration, which is 280- to 1,000-fold higher than that usually applied to humans (7,000 to 25,000 ions/cm³). The exposure period was set in accordance with the OECD Guidelines for the Testing of Chemicals (OECD TG 412, 2009; OECD TG414, 2001). IONA concentrations were determined using an ion-counter (KEC-990, Kyoritsu Electronics Co., Ltd., Kawasaki, Japan, measuring range: 100 to 19,990,000 ions/cm³) just before and after the exposure throughout the exposure period.

Animals and husbandry

Male and female Crl:CD(SD) rats were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). The animals were 12 and 11 weeks old upon receipt, respectively. After at least 5 days of quarantine and acclimation, male and female rats were cohabited overnight on a one-to-one basis. Vaginal smears were col-

lected the next morning of the cohabitation, and examined for the presence of sperm. Copulation was confirmed by the presence of a vaginal plug and/or sperm in the vaginal smear. The copulated females were defined as pregnant animals (hereafter referred to as dams), and the day on which evidence of copulation was found was designated as Day 0 of gestation (GD 0). On each day of mating, dams were assigned to the control and IONA groups by the stratified-by-weight randomization method based on the body weights on GD 0, to get almost the same mean body weight for each group. Each group consisted of 10 dams.

Animals were housed individually, except for the cohabitation period, in a polycarbonate cage with hardwood bedding in an air-conditioned room (set at $22 \pm 3^\circ C$ and $55 \pm 20\%$ relative humidity) with artificial lightning from 7:00 to 19:00 and ventilation providing all fresh air. Food (CRF-1: Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given *ad libitum*.

All procedures involving animals were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at the test facility and in accordance with the guidance of the animal experiments issued by IACUC. The facility has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Whole-body inhalation exposure to IONA

Each group of dams was exposed in an approximately 1.6 m³ stainless and glass whole-body inhalation chamber (Tokiwa Kagaku Kikai Co., Ltd., Tokyo, Japan). Each chamber was dedicated to one exposure group. The chambers were operated under a full ventilation system at an air flow of 15 air changes per hour (ventilation volume: 24 m³ per hour). Environmental conditions within the chambers were monitored and maintained at temperatures between $21^\circ C$ and $23^\circ C$ and relative humidity between 50% and 57%. The oxygen concentration was 20.9% and 21.1%.

The whole-body inhalation exposure was conducted to dams housed in a wire mesh cage, which was composed of 5 rooms (partitioned into 2 sub-rooms, 2 dams per room) from GD 6 to 19. The IONA generation device was constructed from 1 ion generator and 1 fan for 1 room, and each device was placed just above each room. The air speed of the fan was set approximately 1.5 m/sec (maximum air speed: approximately 2.3 m/sec) in the cage. In the chamber for the 0 ions/cm³ group, the device without the ion generator was placed in the same manner.

Examination of dams, embryos and fetuses

The clinical signs of all dams were observed twice a day (before and after exposure) from GD 6 to 19 and once a day during the other periods. They were weighed on GD 0, 6, 9, 12, 15, 17, and 20 and the body weight gain was calculated based on the body weight on GD 6. Food consumption was measured at the same intervals as the body weight. All dams were euthanized by exsanguination from the abdominal aorta under anesthesia with an intravenous injection of thiopental sodium into the tail vein on GD 20. The thoracic and abdominal organs/tissues were examined macroscopically.

In the examination of embryos and fetuses, the ovary and uterus of dams were removed and the gravid uterus weight was recorded. The number of corpora lutea, implantations, early resorptions, late resorptions, dead fetuses, and live fetuses were examined. Live fetuses were euthanized by exsanguination from the umbilical artery and vein in the amniotic fluid before being removed from the uterus. The fetuses were sexed and examined for external anomalies including those in the oral cavity, and they were weighed individually. The placentae were observed macroscopically and weighed individually. About half of the fetuses of each dam were fixed in a formalin-acetic acid mixed solution and examined for the visceral anomalies in the head by Wilson's method (Wilson, 1965) and in the neck, thorax, and abdomen by the microdissection method (Nishimura, 1974) under a stereoscopic microscope. The other half were stained with alizarin red S (Staples and Schnell, 1964) to examine the skeletal abnormalities (anomalies and variations) and degrees of ossification including the number of ossified sternebrae and sacral/caudal vertebral bodies under a stereoscopic microscope.

Statistical analysis

Fetal data were analyzed on the basis of litter mean values, except for the sex ratio. For the comparison of the metric data between the 0 and 7,000,000 ions/cm³ groups, the homogeneity of variance was tested by the F test, and

when the variance was homogeneous, Student's t test was performed for the statistical comparison; when heterogeneous, the Aspin-Welch t test was used. For some of the data such as implantation loss, embryo-fetal mortality, and incidence of fetus with abnormality, Wilcoxon's rank sum test was used to compare with the other metric data. The count data were analyzed by the χ^2 test. The significance level of the F test was 5% and those of the other analyses were 1 or 5% (two tailed).

RESULTS AND DISCUSSION

Each concentration of positive and negative ions in the IONA group was 7,000,000 ions/cm³ or more and was comparable between pre- and post-exposure. Additionally, the coefficient variance (C.V.) values of daily minimum and maximum concentrations were below 10% (Table 1). It was therefore judged that the pregnant animals were properly exposed to the homogenous IONA from GD 6 to 19.

As for the effects on dams, no abnormalities in clinical signs were noted in any animals. The body weight and food consumption in the IONA group were comparable to those in the control group, as were gravid uterine weight and corrected body weight of dams (Table 2). In addition, there were no IONA-related changes in necropsy findings (data not shown), nor were there any changes suggesting effects on the maintenance of pregnancy or reproductive function of dams such as abortion or premature delivery.

As for the effects on embryo-fetal development, no changes attributable to the IONA exposure were observed in the number of corpora lutea, implantations, dead embryos/fetuses, live fetuses, implantation loss, sex ratio, live fetal weights, placental features, or placental weights (Table 3). In the fetal examination, no IONA-related anomalies were detected in external, visceral, or skeletal features. Some visceral or skeletal variations such as dilatation of the renal pelvis, cervical rib, and short supernumerary rib were observed in the control and IONA groups. However, they were judged to be incidental, because they

Table 1. Actual concentration of cluster ions in IONA exposed group

Type of ion		Actual conc. (ions/cm ³) ^a		
		Mean $\pm$ S.D.	Min. to Max.	C.V. (%) ^b
Pre-exposure	Positive	8,550,000 $\pm$ 500,000	7,130,000 to 9,290,000	0.0 to 9.2
	Negative	8,320,000 $\pm$ 409,000	7,560,000 to 9,300,000	0.0 to 6.3
Post-exposure	Positive	8,690,000 $\pm$ 270,000	8,370,000 to 9,180,000	2.7 to 5.7
	Negative	8,270,000 $\pm$ 166,000	8,060,000 to 8,560,000	3.2 to 7.7

^a The actual concentrations were calculated from daily mean concentrations of 5 devices.

^b Daily coefficient variation of ion concentration.

Table 2. Effects on dams by IONA

Observations	Group (concentration; ions/cm ³)	
	Control (0)	IONA (7,000,000)
Number of dams	10	10
Number of dams with abnormality	0	0
Body weight (g)		
Day 0	263.66 ± 13.68 ^a	266.51 ± 13.47
Day 6	303.10 ± 14.75	306.66 ± 11.54
Day 9	314.10 ± 15.72	316.53 ± 9.47
Day 12	332.35 ± 16.28	334.65 ± 9.04
Day 15	346.95 ± 17.25	351.24 ± 8.37
Day 17	369.72 ± 17.36	374.13 ± 10.37
Day 20	416.17 ± 21.50	423.45 ± 12.81
Body weight gain (g)		
Day 6 to 9	11.00 ± 5.74	9.87 ± 3.78
Day 6 to 12	29.25 ± 8.57	27.99 ± 7.02
Day 6 to 15	43.85 ± 8.48	44.58 ± 7.93
Day 6 to 17	66.62 ± 9.09	67.47 ± 8.26
Day 6 to 20	113.07 ± 14.17	116.79 ± 14.63
Food consumption (g/day)		
Day 0 to 6	22.37 ± 1.39	22.32 ± 1.18
Day 6 to 9	22.49 ± 1.27	21.98 ± 1.32
Day 9 to 12	23.82 ± 1.93	24.01 ± 1.67
Day 12 to 15	24.35 ± 1.69	24.50 ± 1.63
Day 15 to 17	24.57 ± 1.73	24.94 ± 1.99
Day 17 to 20	25.96 ± 1.74	26.97 ± 1.70
Gravid uterine weight (g)	73.634 ± 12.306	79.335 ± 11.945
Corrected body weight (g) ^b	342.54 ± 18.47	344.12 ± 8.19

^a Mean ± S.D.^b Body weight on GD 20 – gravid uterine weight**Table 3.** Effects on embryo-fetal development by IONA

Observations	Group (concentration; ions/cm ³)	
	Control (0)	IONA (7,000,000)
Number of dams	10	10
Number of corpora lutea	15.70 ± 1.16 ^a	15.70 ± 2.11
Number of implantations	14.30 ± 2.50	15.30 ± 2.16
Pre-implantations loss (%)	9.14 ± 13.79	2.52 ± 4.41
Post-implantations loss (%)	7.25 ± 8.39	7.32 ± 10.59
Early resorptions (%)	7.25 ± 8.39	7.32 ± 10.59
Late resorptions (%)	0.00 ± 0.00	0.00 ± 0.00
Dead fetuses (%)	0.00 ± 0.00	0.00 ± 0.00
Number of live fetuses per dam	13.20 ± 2.35	14.10 ± 2.13
Sex ratio (Male/Female)	1.07	0.96
Fetal body weight (g)		
Male	3.605 ± 0.238	3.656 ± 0.208
Female	3.459 ± 0.232	3.416 ± 0.146
Placental body weight (g)		
Male	0.468 ± 0.076	0.468 ± 0.054
Female	0.447 ± 0.054	0.442 ± 0.034
Placental anomaly		
Fusion	1(0.67)/1(10.0) ^b	0

^a Mean ± S.D.^b Number of fetuses/litters with abnormality (%)

No effects of positive and negative ions on embryo-fetal development in rats

Table 4. Effects on morphological examinations in fetuses by IONA

Observations	Group (concentration; ions/cm ³)	
	Control (0)	IONA (7,000,000)
Number of dams	10	10
External examination		
Number of fetuses examined	132	141
Anomaly	1(0.83)/1(10.0) ^a	0
Thread-like tail	1(1) ^b	0
Visceral examination		
Number of fetuses examined	64	68
Anomaly	3(4.17)/2(20.0)	1(2.00)/1(10.0)
Ventricular septal defect	3 (2)	1 (1)
Variation	4(6.19)/3(30.0)	2(3.33)/1(10.0)
Thymic remnant in neck	1 (1)	0
Dilatation of renal pelvis	3 (2)	2 (1)
Skeletal examination		
Number of fetuses examined	68	73
Anomaly	1(1.67)/1(10.0)	0
Absence of lumbar vertebra	1 (1) [#]	0
Absence of sacral vertebra	1 (1) [#]	0
Absence of caudal vertebra	1 (1) [#]	0
Variation	4(6.67)/4(40.0)	7(10.22)/6(60.0)
Splitting of ossification center of lumbar vertebral body	1 (1)	0
Cervical rib	1 (1)	1 (1)
Short supernumerary rib	2 (2)	6 (5)
Degree of ossification		
Sternebrae	5.573 ± 0.374 ^c	5.787 ± 0.292
Sacral/caudal vertebrae	7.802 ± 0.454	7.789 ± 0.307

^a Number of fetuses/litters with any abnormality (%)^b Number of fetuses with abnormality (litter)^c Mean ± S.D.[#], observed in the fetus with thread-like tail

are seen spontaneously in rats of this strain (Nakatsuka *et al.*, 1997) and no significant differences were observed in their incidence, fetuses with abnormalities, or dams with abnormal fetuses between the control and IONA groups (Table 4). In the degree of ossification, all indices in the IONA group were comparable to those in the control group, with no significant differences (Table 4).

As mentioned above, the ionized air containing positive and negative ions had no effects on dams, including the maintenance of pregnancy or reproductive function, nor were there any embryo-fetal lethality, developmental delay/impairment, or teratogenicity even at the concentrations 280- to 1,000-fold higher than that normally used in humans. Although this study did not fully adhere to the current guidelines because of a smaller number of animals per group, it was suggested that the ionized air has no maternal toxicity or embryo-fetal toxicity in rats.

REFERENCES

- Digel, I., Temiz Artmann, A., Nishikawa, K., Cook, M., Kurulgan, E. and Artmann, G.M. (2005): Bactericidal effects of plasma-generated cluster ions. *Med. Biol. Eng. Comput.*, **43**, 800-807.
- Hiramoto, K., Orita, K., Yamate, Y., Sato, E.F., Okano, H., Nishikawa, K. and Inoue, M. (2011): Plasma cluster ions decrease the antigenicity of mite allergens and suppress atopic dermatitis in NC/Nga mice. *Arch. Dermatol. Res.*, **303**, 367-370.
- Kawamoto, S., Oshita, M., Fukuoka, N., Shigeta, S., Aki, T., Hayashi, T., Nishikawa, K. and Ono, K. (2006): Decrease in the allergenicity of Japanese cedar pollen allergen by treatment with positive and negative cluster ions. *Int. Arch. Allergy Immunol.*, **141**, 313-321.
- Nakatsuka, T., Horimoto, M., Ito, M., Matsubara, Y., Akaike, M. and Ariyuki, F. (1997): Japan pharmaceutical manufacturers association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. *Cong. Anom.*, **37**, 47-138.
- Nishikawa, K. and Nojima, H. (2001): Air purification effect of positively and negatively charged ions generated by discharge plasma at atmospheric pressure. *Jpn. J. Appl. Phys.*, **40**, L835-L837.

- Nishikawa, K. and Yagi, H. (2006): Bactericidal effects of positively and negatively charged cluster ions generated by plasma discharge at atmospheric pressure. Proc. UT Sympo. NanoBio. Integration (NANO-BIO-TOKYO), 337-340.
- Nishikawa, K. (2013): Air purification technology of positively and negatively charged cluster ions by plasma discharge at atmospheric pressure. J. Plasma Fusion Res., **89**, 164-168.
- Nishitani, M., Sugino, T., Kadowaki, T., Sakikawa, N., Nishikawa, K. and Kajimoto, O. (2010): Effects of plasma cluster ions on moisturizing skin and increasing comfort sensation (in Japanese). Jpn. J. Compl. Alternative Med., **7**, 113-116.
- Nishimura, K. (1974): A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses (in Japanese). Cong. Anom., **14**, 23-40.
- Nojima, H. and Nishikawa, K. (2002): Air purification technology using ions generated by discharge plasma at atmospheric pressure (in Japanese). J. Inst. Electrostat. Jpn., **26**, 153-157.
- Staples, R.E. and Schnell, V.L. (1964): Refinements in rapid clearing technic in the KOH-alizarin red S method for fetal bone. Stain Technol., **39**, 61-63.
- Takasawa, H., Wako, K., Sato, Y., Takashima, R., Hattori, A., Kawabata, M., Tsuchitani, M., Wako, Y., Yamada, N. and Hamada, S. (2011): Air containing ions generated by electric discharge has no potential to induce DNA damage in lung cells in rats and mice. Genes and Environment., **33**, 96-102.
- Wilson, J.G. (1965): Methods for administering agents and detecting malformations in experimental animals. In: Wilson, J.G. and Warkany, J. eds, Teratology: Principles and techniques, The University of Chicago Press, 262-277.