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Subchronic inhalation toxicity study of diethylbenzene in Wistar rats

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Abstract

Diethylbenzene (DEB) is a colorless flammable liquid composed of a benzene ring and two ethyl substituents. DEBs mostly exist as a mixture of isomers and are mainly used as intermediates and solvents occupationally. Workers may be exposed to DEB inhalation during their occupational activities including manufacturing or processing of materials; however, limited data are available on the risk assessment of DEB mixtures. In this study, male and female Wistar rats were exposed to vapors of a DEB mixture for 13-weeks (6 hr/day, 5 days/week) at concentrations of 0, 40, 80, and 160 ppm in a whole-body inhalation chamber. Clinical signs, mean body weight, food consumption, bronchoalveolar lavage fluid (BALF), hematology, blood biochemistry, gross findings, organ weights, and microscopic findings were examined to determine the toxicity of DEB mixture. The exposure concentrations in chambers were 39.48 ± 1.13 ppm, 80.43 ± 2.06 ppm, and 160.20 ± 4.42 ppm for the low, medium, and high dose groups, respectively. No changes related to the test substance were observed, including changes in clinical observation, body weight, food consumption, BALF and blood analysis, necropsy findings, absolute and relative organ weights or histopathological analysis. Based on these results, the NOAEC (no-observed-adverse-effect-concentration) of DEB was defined as 160 ppm under the study conditions.

Keywords: inhalation; toxicity; diethylbenzene; no-observed-adverse-effect-concentration; occupational exposure

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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Ethics Approval
This study was approved by the Institutional Animal Care and Use Committee of the Inhalation Research Center, Occupational Safety and Health Research Institute, and all experiments

INTRODUCTION

Diethylbenzene (DEB, CAS No. 25340-17-4) is an almost colorless and flammable liquid with an aromatic odor. DEB is a byproduct of the synthesis of ethylbenzene from ethylene and benzene and contains a benzene ring and two ethyl substituents. It is primarily manufactured as a mixture of isomers (ortho: 1,2-; meta: 1,3-; and para: 1,4-) [1]. It is widely used in the production of synthetic rubber and resins as an intermediate or solvent in powderless etching [2], and is manufactured and/or imported in the European Economic area about 10 tonnes per year [3].

There are no data on DEB toxicity in humans, including skin or respiratory irritation, sensitization, or reported cases of occupational exposure. There is a high risk of exposure to workers through inhalation or skin absorption, with an estimated 60% of DEB is being absorbed through the respiratory tract, particularly in cases involving structurally similar chemicals [1]. There are

were conducted according to the established institutional animal care and use protocol (Approval No. IACUC-2203).

few studies on the DEB mixtures because of the ratio difference between the three isomers; however, several studies on the toxic effects of each isomer in animals are available.

1,2-DEB is known to be the most toxic among isomers and causes central and peripheral neurotoxicity such as limb weakness associated with nerve fiber changes [4, 5]. Decreased leukocyte and lymphocyte counts were observed in rats exposed to the isomer mixture, and even though neurotoxic effect was not observed at the highest concentration (252 ppm), the researchers reported 10% of the highest concentration as no-observed-adverse-effect-concentration (NOAEC) of clinical neurotoxicity [1, 6]. Neither *in vitro* nor *in vivo* genotoxicity has been observed for the DEB isomer mixture [3]. Depending on the use of the substance to be manufactured, DEB is mainly exposed through the route of inhalation; however, there are limited data on repeated inhalation exposure, which was performed recently, compared to the oral exposure of animals. In addition, limited animal toxicity studies based on the OECD guidelines have been conducted. Therefore, we performed a 13-week repeated toxicity study to determine the toxic effects of DEB by inhalation exposure, according to the OECD Test Guideline No. 413, in accordance with Good Laboratory Practice (GLP) [7].

MATERIALS AND METHODS

Experimental animals and ethics

Five-week-old specific-pathogen-free Wistar rats (40 males and 40 females) were obtained from SPF Biotechnology (Beijing, China) and acclimatized for two week in polysulfone cages (up to three animals of the same sex). The animal room was maintained at 22 ± 3°C, 50 ± 20% relative humidity, and a 12-hr light/dark cycle. Male and female rats weighed 235–275 g and 167–197 g, respectively, before exposure. The whole-body inhalation chambers (WITC-14M, HCT, Icheon, Korea) that were maintained at 22 ± 3°C and 50 ± 20% relative humidity with individual multi-compartment stainless steel wire mesh cages (W240 × L1200 × H200) were used for exposure. The chambers were vented 10–15 times per hour, and the oxygen concentration was maintained at least 19% during the exposure period. Animals were housed in polysulfone cages after the initial exposure and fed an animal diet (Teklad certified irradiated global 18% protein Rodent Diet 2918C, Envigo RMS, Indianapolis, IN, USA) and filtered water *ad libitum*. We also provided wooden chewing sticks to each rat as an environmental enrichment item. All animal care and inhalation studies were performed under GLP conditions, and the procedures were in accordance with the National Institutes Guide for the Care and Use of Laboratory Animals [8]. This study was approved by the Institutional Animal Care and Use Committee of the Inhalation Research Center, Occupational Safety and Health Research Institute, and all experiments were conducted according to the established institutional animal care and use protocol (Approval No. IACUC-2203).

Test material and exposure conditions

DEB (mixture of isomers; 99.2% purity) was purchased from Alfa Aesar (Thermo Fisher Scientific, Waltham, MA, USA), and the ratio of isomer was unknown. The vaporized mate-

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rial was generated using a liquid-vapor generator (LVG-04-A, HCT), and the concentrations of the materials were analyzed using a gas chromatograph (Model No. TRACE1310, Thermo Fisher Scientific). The environmental conditions in the chambers were monitored every 30 min (Model No. ITC Manager, HCT).

Subchronic toxicity assessment design

The OECD guidelines (TG413) and the previously published method of Cho were used for the 13-week repeated inhalation study of DEB with modifications [9, 10]. A preliminary 28-day inhalation toxicity study was performed to determine the exposure concentration and showed no toxicological effect at a maximum concentration of 160 ppm, and a 90-day test was planned with reference to this results (unpublished). Male and female rats were randomly divided into four groups according to sex, with each group consisting of ten animals. Each group was exposed to 0, 40, 80, or 160 ppm DEB for 6 hr/day, 5 days/week, for 13 weeks. Concentrations of low (40 ppm), medium (80 ppm), and high (160 ppm) exposure groups were selected. All animals were observed once or twice daily (before and after exposure) to confirm detailed clinical signs, and body weight data were collected once or twice a week. Food consumption was measured once a week. At the end of the study, all the rats were anesthetized with isoflurane (I-Fran liquid, Hana Pharm, Hwaseong, Korea) and euthanized via exsanguination of the abdominal aorta and vein.

Blood analyses

Blood samples were collected from the abdominal aorta before euthanasia. The samples were collected in tubes containing an anticoagulant (ethylenediaminetetraacetic acid or sodium citrate) for hematological parameters, or in serum-separating tubes for biochemical examination. The anti-coagulant tubes containing sodium citrate and serum separating tubes were centrifuged at 3,000 × g at 4°C for 10 min to separate the serum. Hematological or biochemical parameters were examined using blood cell analyzer (ADIVA 2120i, Siemens Diagnostics, Tarrytown, NY, USA), coagulation analyzer (ACL Elite Systems, Instrumentation Laboratory, Bedford, MA, USA), or blood chemistry analyzer (TBA-120FR, Toshiba, Tokyo, Japan): white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count, reticulocyte (RET) count, differential WBC count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), activated partial thromboplastin time (APTT), prothrombin time (PT), sodium (Na), potassium (K), chloride (Cl), total protein (TP), albumin (ALB), creatinine (CREA), blood urea nitrogen (BUN), glucose (GLU), calcium (Ca), inorganic phosphorus (IP), total bilirubin (TBIL), total cholesterol (TCHO), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and albumin/globulin (A/G) ratio.

Bronchoalveolar lavage fluid examination

Bronchoalveolar lavage fluid (BALF) samples were collected immediately after euthanasia.

The samples were collected from the right-sided lung by lavage with phosphate buffered saline and centrifuged at 3,000 × g at 4°C for 10 min to separate the cells and supernatant. Biochemical parameters including TP, ALB and lactate dehydrogenase (LDH), were examined using a blood chemistry analyzer, and total cells were counted using an automatic cell analyzer (NC-200, ChemoMetec, Allerod, Denmark). At least 400 cells, including alveolar macrophages, monocytes, lymphocytes, neutrophils, and eosinophils, were counted under a light microscope (Axio Scope, Carl Zeiss, Oberkochen, Germany).

Organ weights, gross, and histopathological parameters

During necropsy, the adrenal glands, brain, epididymis, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus, and uterus were examined and weighed. And the following tissues were also examined and collected in 10% neutral buffered formalin or Davison's fixative (for the testes and eyes/optic nerves) for the histopathologic examination: aorta, bone marrow, cecum, colon, duodenum, esophagus, eyes/optic nerves, femur, gallbladder, harderian glands, ileum, jejunum, larynx, lung (left lobe only), lymph nodes (tracheobronchial and mesenteric), mammary gland, nasopharyngeal tissue, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands (submandibular, sublingual, and parotid), sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, sternum, stifle joint, stomach, teeth, thyroid, tongue, urinary bladder, and vagina. According to the OECD TG413, all of the fixed tissues from control and high exposure groups of male and female rats were paraffin-embedded, 4 μm thickness-sectioned, stained with hematoxylin and eosin (Automatic Stainer, DAKO, Glostrup, Denmark), and examined under a light microscope (DM3000, Leica, Wetzlar, Germany). However, histopathologic examinations from low and medium concentrations were not performed in consideration of the effect of the test substance.

Statistical analysis

Data are expressed as mean ± S.D. Analysis of parameters, including body weight, food consumption, organ weights, and hematological or blood biochemical data was performed using the Pristima® system (Version 7.1.0, Xybion, Princeton, NJ, USA). Data were checked for homogeneity using Levene's test and analyzed using with one-way analysis of variance (Dunnett's multiple test) or Kruskal–Wallis (Dunn rank sum test). Differences were considered significant at $p<0.05$ or $p<0.01$.

RESULTS

Concentration in exposure chambers

The analytical concentrations of DEB in chambers during the exposure period for the low, medium, and high exposure groups were 39.48 ± 1.13 ppm, 80.43 ± 2.06 ppm, and 160.20 ± 4.42 ppm, respectively.

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Clinical signs, body weight, and food consumption changes

No animals died or unusual behavioral or clinical signs were observed during the study period (data not shown). In addition, there were no significant changes in the body weight (Fig. 1) or food consumption (data not shown) of male and female rats during the study.

Hematology, blood biochemistry, and bronchoalveolar lavage fluid examination

The PT level was significantly increased in the medium- and high-exposure groups in male rats compared to that in control males (medium: $p<0.01$; high: $p<0.05$). Additionally, ALP levels in the male high-exposure group were significantly high ($p<0.05$). No significant differences were observed in other hematological or biochemical parameters between the control and experimental groups (Tables 1, 2, 3, and 4). In addition, there were no significant differences in BALF data between the control and experimental groups (Table 5).

Organ weights and histopathological examination

The absolute organ weights or organ weights relative to the whole-body weight were investigated (Tables 6 and 7). In terms of absolute organ weights, no significant change was found between the experimental groups and the control group. The kidney weight in the low group was significantly lower than that in the control in regard to the relative organ weights. Gross findings were observed in the lungs, spleen, testes, and vagina (Table 8), and were identified by microscopic examination as alveolar macrophage aggregation in the lung, unclassified sarcoma in the spleen, seminiferous tubular dilatation in the testes, and cysts (s) in the vagina. Microscopic findings were also observed in the heart, liver, lungs, nasal cavity, pancreas, pituitary, testes, and thyroid vagina in the control and high exposure group of rats (Table 9).

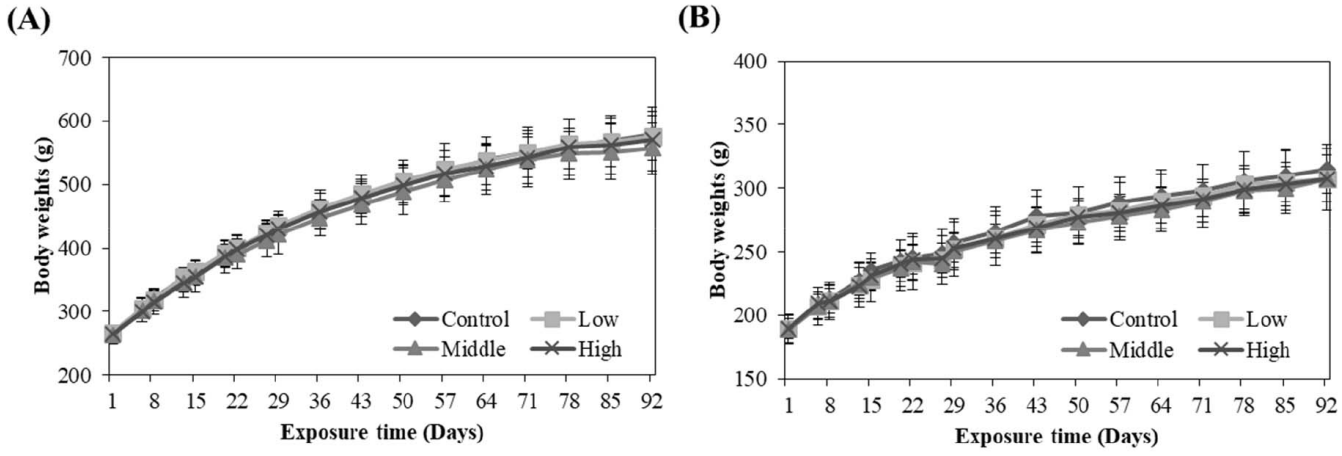

Fig. 1. Body weight changes in male (A) and female (B) Wistar rats. The animals were exposured to diethylbenzene (0–160 ppm) by inhalation during 90 days. The body weights present as the mean ± S.D. (n = 10/group).

Table 1. Summary of hematological data in male rats inhaling diethylbenzene for 90 days (n = 10)

Parameters	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
WBC (× 10^3/μL)	5.232 ± 1.4371	5.23 ± 1.0705	5.818 ± 1.1856	6.079 ± 1.506
RBC (× 10^6/μL)	8.817 ± 0.3198	8.504 ± 0.2251	8.719 ± 0.273	8.631 ± 0.2518
HGB (g/dL)	14.61 ± 0.534	14.43 ± 0.38	14.59 ± 0.463	14.48 ± 0.319
HCT (%)	43.71 ± 1.859	42.83 ± 1.12	43.8 ± 1.359	43.47 ± 1.106
MCV (fL)	49.55 ± 1.396	50.41 ± 1.603	50.22 ± 1.025	50.43 ± 1.578
MCH (pg)	16.57 ± 0.517	16.97 ± 0.648	16.74 ± 0.532	16.79 ± 0.599
MCHC (g/dL)	33.43 ± 0.579	33.67 ± 0.419	33.33 ± 0.485	33.29 ± 0.504
PLT (× 10^3/μL)	879.9 ± 346.5	1,014.5 ± 120.31	910 ± 262.3	1,026.7 ± 84.71
NEU% (%)	24.38 ± 7.3	20.82 ± 3.707	22.51 ± 9.948	20.16 ± 3.311
LYM% (%)	70.53 ± 7.694	74.07 ± 3.811	71.86 ± 9.558	74.68 ± 3.633
MON% (%)	2.52 ± 0.745	2.43 ± 0.748	2.5 ± 0.897	2.19 ± 0.53
EOS% (%)	1.67 ± 0.706	1.49 ± 0.407	1.83 ± 0.726	1.93 ± 0.517
BAS% (%)	0.21 ± 0.099	0.17 ± 0.082	0.22 ± 0.063	0.21 ± 0.074
NEUA (× 10^3/μL)	1.23 ± 0.419	1.096 ± 0.3614	1.314 ± 0.6451	1.206 ± 0.2847
LYMA (× 10^3/μL)	3.74 ± 1.2199	3.868 ± 0.7686	4.176 ± 1.0253	4.561 ± 1.2814
MONA (× 10^3/μL)	0.129 ± 0.0479	0.122 ± 0.0305	0.142 ± 0.0492	0.132 ± 0.0382
EOSA (× 10^3/μL)	0.085 ± 0.035	0.079 ± 0.0351	0.108 ± 0.0457	0.114 ± 0.036
BASA (× 10^3/μL)	0.012 ± 0.0079	0.01 ± 0.0047	0.013 ± 0.0048	0.014 ± 0.007
RET% (%)	2.018 ± 0.2126	2.187 ± 0.3717	2.251 ± 0.2593	2.369 ± 0.5667
RETA (× 10^9/μL)	177.52 ± 14.933	185.84 ± 31.11	196.4 ± 24.496	204.23 ± 48.818
APTT (sec)	13.26 ± 1.643	14.74 ± 3.133	15.65 ± 3.267	14.23 ± 2.365
PT (sec)	12 ± 0.76	12.1 ± 0.69	12.9 ± 0.58$^+$	12.7 ± 0.47*

Data are expressed as the mean ± S.D.

* Dunnett's LSD test significant at the 0.05 level; $^+$ Dunnett's LSD test significant at the 0.01 level.

n, number of animals examined; WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; NEU%, neutrophil relative; LYM%, lymphocyte relative; MON%, monocyte relative; EOS%, eosinophil relative; BAS%, basophil relative; NEUA, neutrophil absolute; LYMA, lymphocyte absolute; MONA, monocyte absolute; EOSA, eosinophil absolute; BASA, basophil absolute; RET%, reticulocyte relative; RETA, reticulocyte absolute; APTT, activated partial thromboplastin time; PT, prothrombin time.

DISCUSSION

DEB is a by-product of the synthesis of ethylbenzene from ethylene and benzene. It occurs as a mixture of three isomers and the ratio of each isomer varies according to the production process [11]. In the 1950s, the ratio of 1,2-DEB in the mixture was known to be about 25%, and studies in the 1990s showed that 6%–10% of 1,2-DEB was included in the mixture [1, 6, 12, 13].

In the animal toxicity studies, the LC_{50} of DEB mixture (25% 1,2-DEB, 40% 1,3-DEB, and 35% 1,4-DEB) was reported to be 2,100 ppm or higher after 7-hr single inhalation exposure. Several clinical signs, such as nasal irritation and dizziness were observed, and decreased body weight and blue-colored organs were noted [3]. In another study, the exposure of male and female SD rats to DEB by inhalation at 0, 34, 110, and 252 ppm resulted in decreased leukocyte

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Table 2. Summary of hematological data in female rats inhaling diethylbenzene for 90 days (n = 10)

Parameters	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
WBC (× 10^3/μL)	2.74 ± 0.9464	2.643 ± 0.4128	3.086 ± 0.8166	3.1 ± 0.651
RBC (× 10^6/μL)	7.482 ± 0.3503	7.522 ± 0.2402	7.749 ± 0.4451	7.568 ± 0.2164
HGB (g/dL)	13.48 ± 0.503	13.51 ± 0.476	13.95 ± 0.624	13.59 ± 0.506
HCT (%)	40.07 ± 1.606	39.84 ± 0.999	41.64 ± 1.788	40.4 ± 1.642
MCV (fL)	53.57 ± 1.277	53 ± 1.031	53.78 ± 1.694	53.38 ± 0.95
MCH (pg)	18.00 ± 0.492	17.97 ± 0.56	18.02 ± 0.600	17.96 ± 0.309
MCHC (g/dL)	33.64 ± 0.369	33.89 ± 0.611	33.51 ± 0.412	33.63 ± 0.35
PLT (× 10^3/μL)	935.2 ± 99.77	1,037.2 ± 99.15	1,003.7 ± 218.25	986.6 ± 60.91
NEU% (%)	20.33 ± 5.572	18.74 ± 5.05	20.68 ± 8.960	16.14 ± 4.222
LYM% (%)	73.66 ± 6.715	75.02 ± 5.939	73.04 ± 9.171	78 ± 5.057
MON% (%)	2.79 ± 0.867	3.00 ± 0.745	2.98 ± 0.716	2.73 ± 1.456
EOS% (%)	2.03 ± 0.533	2.07 ± 0.512	1.95 ± 0.762	1.69 ± 0.925
BAS% (%)	0.15 ± 0.085	0.14 ± 0.073	0.12 ± 0.042	0.12 ± 0.083
NEUA (× 10^3/μL)	0.554 ± 0.237	0.482 ± 0.1049	0.598 ± 0.1938	0.487 ± 0.1082
LYMA (× 10^3/μL)	2.019 ± 0.7307	1.999 ± 0.4023	2.289 ± 0.8014	2.431 ± 0.6243
MONA (× 10^3/μL)	0.079 ± 0.0407	0.079 ± 0.0226	0.094 ± 0.0443	0.086 ± 0.0456
EOSA (× 10^3/μL)	0.055 ± 0.0212	0.052 ± 0.012	0.061 ± 0.0288	0.05 ± 0.025
BASA (× 10^3/μL)	0.003 ± 0.0048	0.003 ± 0.005	0.001 ± 0.0032	0.004 ± 0.0053
RET% (%)	2.193 ± 0.428	2.252 ± 0.3009	2.147 ± 0.5907	2.17 ± 0.4717
RETA (× 10^9/μL)	163.65 ± 30.315	169.24 ± 22.426	165.41 ± 42.104	164.41 ± 37.168
APTT (sec)	16.9 ± 1.781	18.1 ± 0.814	17.94 ± 1.861	18.78 ± 0.931
PT (sec)	10.8 ± 0.59	11 ± 0.48	11.4 ± 1.1	11.4 ± 0.61

Data are expressed mean ± S.D.

n, number of animals examined; WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; NEU%, neutrophil relative; LYM%, lymphocyte relative; MON%, monocyte relative; EOS%, eosinophil relative; BAS%, basophil relative; NEUA, neutrophil absolute; LYMA, lymphocyte absolute; MONA, monocyte absolute; EOSA, eosinophil absolute; BASA, basophil absolute; RET%, reticulocyte relative; RETA, reticulocyte absolute; APTT, activated partial thromboplastin time; PT, prothrombin time.

levels in both sexes above 110 ppm. At a concentration of 252 ppm, bluish-colored organs and reduced body weight were identified, and there was a decrease in biochemical parameters, including ALT, AST, and CREA kinase activity in females. There were no adverse histological findings or neurotoxicity, and the NOAEC was determined to 34 ppm [1, 3]. In addition, the inhaled DEB mixture affected the action potential of peripheral nerves via decreased motor and sensory conduction and increased the amplitude of sensory action in SD rats exposed to 500 ppm for 18-weeks [12]. In addition, inhalation exposure to a DEB mixture reduces the levels of WBCs and lymphocytes [1, 6].

Among the results of several studies on the toxicity of DEB isomers, 1,2-DEB is known to depress the central nervous system, and 1,2-Diacetylbenzenes, are metabolites of 1,2-DEB, have shown clear peripheral neurotoxicity [14, 15]. An oral administration of 1,2-DEB to rats at a dose of 1,000 mg/kg caused liver weight gain and centrilobular hypertrophy related with inductioin of hepatic enzymes and resulted in increased thyroid weight and changes in thyroid

Table 3. Summary of blood biochemistry data in male rats inhaling diethylbenzene for 90 days (n = 10)

Parameters	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
Na (mmol/L)	147.42 ± 4.56	149.95 ± 4.155	143.05 ± 18.055	149.48 ± 3.021
K (mmol/L)	5.252 ± 0.3827	5.38 ± 0.2043	5.201 ± 0.43	5.297 ± 0.3325
Cl (mmol/L)	106.45 ± 3.682	109.24 ± 3.23	104.37 ± 12.805	108.34 ± 2.147
TP (g/dL)	6.04 ± 0.366	6.02 ± 0.297	6.08 ± 0.644	6.11 ± 0.42
ALB (g/dL)	3.95 ± 0.196	3.98 ± 0.204	3.93 ± 0.313	3.92 ± 0.22
CREA (mg/dL)	0.384 ± 0.0222	0.415 ± 0.0299	0.409 ± 0.0458	0.402 ± 0.0349
BUN (mg/dL)	17.47 ± 2.874	19.68 ± 1.737	17.93 ± 1.941	19.05 ± 2.32
GLU (mg/dL)	149.79 ± 35.882	152.86 ± 20.589	165.73 ± 38.972	167.59 ± 24.634
Ca (mg/dL)	9.55 ± 0.334	9.55 ± 0.36	9.5 ± 0.316	9.68 ± 0.429
IP (mg/dL)	5.8 ± 0.516	6.2 ± 0.476	6.23 ± 0.574	6.36 ± 0.687
TBIL (mg/dL)	–0.005 ± 0.0207	0.005 ± 0.0151	–0.017 ± 0.0216	–0.018 ± 0.0244
TCHO (mg/dL)	84.37 ± 14.67	96.69 ± 12.187	96.79 ± 20.897	88.69 ± 15.305
TG (mg/dL)	71.78 ± 31.735	55.39 ± 19.925	47.91 ± 14.067	59.44 ± 18.51
AST (IU/L)	150.09 ± 37.835	52.8 ± 15.618	148.96 ± 56.9	132.92 ± 14.409
ALT (IU/L)	42.91 ± 7.243	186.35 ± 64.471	47.55 ± 9.92	48.05 ± 9.594
ALP (IU/L)	274.23 ± 57.055	325.66 ± 71.903	302.62 ± 83.337	388.10 ± 122.569*
A/G ratio	1.92 ± 0.199	1.95 ± 0.246	1.86 ± 0.171	1.79 ± 0.11

Data are expressed as the mean ± S.D.
* Dunnett's LSD test significant at the 0.05 level.
n, number of animals examined; Na, sodium; K, potassium; Cl, chloride; TP, total protein; ALB, albumin; CREA, creatinine; BUN, blood urea nitrogen; GLU, glucose; Ca, calcium; IP, inorganic phosphorus; TBIL, total bilirubin; TCHO, total cholesterol; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; A/G ratio, albumin/globulin ratio.

Table 4. Summary of blood biochemistry data in female rats inhaling diethylbenzene for 90 days (n = 10)

Parameters	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
Na (mmol/L)	148.58 ± 3.446	149.67 ± 2.76	148.2 ± 0.937	149.23 ± 1.794
K (mmol/L)	4.141 ± 0.3743	4.436 ± 0.4323	4.294 ± 0.408	4.397 ± 0.2867
Cl (mmol/L)	110.54 ± 3.795	110.33 ± 2.277	109.74 ± 1.952	110.03 ± 1.946
TP (g/dL)	6.8 ± 0.512	6.96 ± 0.381	6.92 ± 0.225	6.72 ± 0.463
ALB (g/dL)	4.93 ± 0.347	5.11 ± 0.298	4.97 ± 0.183	4.91 ± 0.395
CREA (mg/dL)	0.506 ± 0.0288	0.477 ± 0.0381	0.492 ± 0.0547	0.499 ± 0.059
BUN (mg/dL)	19.52 ± 3.803	18.83 ± 1.703	20.65 ± 5.16	20.27 ± 4.999
GLU (mg/dL)	156.9 ± 22.547	159.78 ± 24.865	164.05 ± 27.899	174.14 ± 23.706
Ca (mg/dL)	9.9 ± 0.306	10.12 ± 0.273	10.13 ± 0.221	9.99 ± 0.372
IP (mg/dL)	4.79 ± 0.698	5.07 ± 0.82	5.1 ± 1.011	5.17 ± 1.02
TBIL (mg/dL)	0.029 ± 0.0228	0.05 ± 0.0278	0.028 ± 0.0132	0.027 ± 0.0212
TCHO (mg/dL)	102.09 ± 32.35	108.6 ± 17.884	109.24 ± 15.919	109.43 ± 23.045
TG (mg/dL)	51.41 ± 27.341	54.77 ± 22.325	69.49 ± 23.27	55.92 ± 23.403
AST (IU/L)	131.43 ± 102.464	133.18 ± 43.149	103.72 ± 22.677	174.52 ± 71.06
ALT (IU/L)	52.54 ± 23.967	44.46 ± 12.564	48.9 ± 11.351	58.09 ± 17.349
ALP (IU/L)	193.37 ± 70.115	251.92 ± 161.945	237.13 ± 104.98	237.4 ± 109.837
A/G ratio	2.65 ± 0.207	2.77 ± 0.2	2.56 ± 0.241	2.72 ± 0.239

Data are expressed as the mean ± S.D.
n, number of animals examined; Na, odium; K, potassium; Cl, chloride; TP, total protein; ALB, albumin; CREA, creatinine; BUN, blood urea nitrogen; GLU, glucose; Ca, calcium; IP, inorganic phosphorus; TBIL, total bilirubin; TCHO, total cholesterol; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; A/G ratio, albumin/globulin ratio.

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Table 5. Summary of bronchoalveolar lavage fluid data in rats inhaling diethylbenzene for 90 days (n = 5)

Parameters	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
Male				
TC (cells/mL)	734,200 ± 434,704	792,200 ± 338,021	687,000 ± 504,003	975,600 ± 278,974
MAC% (%)	97.7936 ± 2	98.8439 ± 0	99.0122 ± 1	98.1659 ± 1
LYM% (%)	0.3419 ± 0	0.3725 ± 0	0.2122 ± 0	0.6854 ± 0
NEU% (%)	1.3824 ± 2	0.7442 ± 0	0.5522 ± 1	1.0738 ± 1
EOS% (%)	0.4821 ± 1	0.0394 ± 0	0.2233 ± 0	0.0749 ± 0
MACA (cells/mL)	721,593.4 ± 434,455	783,280.6 ± 335,412	677,579 ± 490,157	957,281.2 ± 272,820
LYMA (cells/mL)	3,499.8 ± 4,692	3,285.2 ± 3,187	1,728.4 ± 3,016	6,251 ± 1,885
NEUA (cells/mL)	6,462.83 ± 8,117	5,328.23 ± 2,315	5,694.21 ± 9,108	11,146.6 ± 11,320
EOSA (cells/mL)	2,644.07 ± 2,321	305.91 ± 684	1,998.37 ± 2,825	921.35 ± 2,060
LDH (IU/L)	71.76 ± 40	80.94 ± 43	92.88 ± 57	69.54 ± 47
TP (g/dL)	0.0156 ± 0	0.0152 ± 0	0.0226 ± 0	0.019 ± 0
ALB (g/dL)	0.007 ± 0	0.0084 ± 0	0.0114 ± 0	0.0064 ± 0
Female				
TC (cells/mL)	437,000 ± 153,747	471,800 ± 86,912	417,600 ± 216,571	406,000 ± 218,628
MAC% (%)	99.1772 ± 1	99.0613 ± 1	85.9168 ± 14	98.3978 ± 2
LYM% (%)	0 ± 0	0.129 ± 0	0.1306 ± 0	0 ± 0
NEU% (%)	0.3757 ± 0	0.5805 ± 1	13.6525 ± 14	1.1676 ± 2
EOS% (%)	0.4471 ± 1	0.2291 ± 0	0.3001 ± 0	0.1864 ± 0
MACA (cells/mL)	433,203.8 ± 152,423	467,834.8 ± 89,516	376,708.4 ± 218,130	397,040.2 ± 207,251
LYMA (cells/mL)	0 ± 0	472.2 ± 1,056	690.8 ± 1,020	0 ± 0
NEUA (cells/mL)	1,881.78 ± 2,148	2,549.46 ± 2,743	39,140.63 ± 29,375	7,345.56 ± 12,677
EOSA (cells/mL)	1,914.32 ± 2,796	943.34 ± 934	1,060.17 ± 1,042	956.91 ± 1,460
LDH (IU/L)	84.84 ± 21	45.48 ± 8	52.64 ± 13	52.92 ± 9
TP (g/dL)	0.0276 ± 0	0.0128 ± 0	0.0134 ± 0	0.016 ± 0
ALB (g/dL)	0.0106 ± 0	0.0072 ± 0	0.0076 ± 0	0.0076 ± 0

Data are expressed as the mean ± S.D.

n, number of animals examined; TC, total cells; MAC%, alveolar macrophage relative; LYM%, lymphocyte relative; NEU%, neutrophil relative; EOS%, eosinophil relative; MACA, alveolar macrophage absolute; LYMA, lymphocyte absolute; NEUA, neutrophil absolute; EOSA, eosinophil absolute; LDH, lactate dehydrogenase; TP, total protein; ALB, albumin.

hormone levels [1].

Regarding the health effect of workers, despite the fact that the DEB substance has light soluble in water and modulate vapor pressure, this substance can be volatile from water or soil to the atmosphere and exposed to workers as a route of inhalation [3]. Therefore, we conducted a 13-week inhalation toxicity study in accordance with the OECD Guidelines (TG 413) to confirm the toxicity of the DEB mixture. The results of this study indicated that the inhalation of 0, 40, 80, or 160 ppm DEB in Wistar rats did not result in toxicity. Despite the consideration of higher concentrations of exposure by references and the preliminary test, the maximum test concentration was adjusted due to the structure of the chamber and the characteristics of the test material. Hematological examination showed a significant increase in PT in the medium- and high-exposure groups; however, no dose-dependent changes in other parameters or organs

Table 6. Summary of absolute organ weight data in rats inhaling diethylbenzene for 90 days (n = 10)

Organs (g)	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
Male				
Adrenal glands	0.0588 ± 0.00473	0.0601 ± 0.01184	0.0527 ± 0.0106	0.0555 ± 0.01229
Brain	2.0917 ± 0.10405	2.0891 ± 0.08539	2.0457 ± 0.09041	2.0899 ± 0.08758
Epididymides	1.5725 ± 0.13997	1.4585 ± 0.08497	1.4619 ± 0.13024	1.5218 ± 0.12653
Heart	1.4519 ± 0.11511	1.4488 ± 0.09532	1.344 ± 0.12741	1.3856 ± 0.1415
Kidneys	3.4528 ± 0.30324	3.206 ± 0.14593	3.3262 ± 0.27257	3.4775 ± 0.32525
Liver	14.5399 ± 0.90226	14.245 ± 1.27354	13.8062 ± 1.69652	14.7038 ± 1.6898
Lung	0.7356 ± 0.12172	0.7047 ± 0.0624	0.7262 ± 0.07621	0.7449 ± 0.07664
Spleen	1.0301 ± 0.17322	0.9974 ± 0.09512	0.9655 ± 0.10179	1.0473 ± 0.17922
Testes	3.792 ± 0.32464	3.7898 ± 0.53555	3.5297 ± 0.44182	3.8188 ± 0.31786
Thymus	0.3674 ± 0.04833	0.3512 ± 0.07437	0.3329 ± 0.04882	0.3479 ± 0.03877
Female				
Adrenal glands	0.0635 ± 0.01219	0.065 ± 0.00645	0.0608 ± 0.00732	0.0642 ± 0.00838
Brain	1.9297 ± 0.08218	1.975 ± 0.05988	1.9461 ± 0.0457	1.9538 ± 0.07729
Heart	0.8957 ± 0.07737	0.8889 ± 0.09421	0.8552 ± 0.09261	0.8759 ± 0.0856
Kidneys	1.888 ± 0.13662	1.8508 ± 0.18423	1.89 ± 0.08911	1.9879 ± 0.19474
Liver	8.8245 ± 1.27524	8.3293 ± 0.75785	8.6293 ± 1.04305	8.9003 ± 0.93689
Lung	0.5559 ± 0.0365	0.5263 ± 0.04167	0.5583 ± 0.03857	0.5382 ± 0.04932
Ovaries	0.1091 ± 0.01818	0.1012 ± 0.02264	0.1064 ± 0.01893	0.1057 ± 0.01701
Spleen	0.5908 ± 0.05493	0.5953 ± 0.11658	0.5819 ± 0.04356	0.6051 ± 0.05022
Thymus	0.283 ± 0.08271	0.2276 ± 0.04225	0.2642 ± 0.05614	0.2705 ± 0.04523
Uterus	0.8653 ± 0.21703	0.8974 ± 0.21898	0.8164 ± 0.19991	0.8683 ± 0.32513

Data are expressed as the mean ± S.D.
n, number of animals examined.

related to blood clotting function were identified. In the histopathological analysis, some lesions were found in the heart, liver, lung, nasal cavity, pancreas, pituitary, testes, thyroid, and vagina, but these findings were considered to be incidental or spontaneous, as they represented low severity and frequency and were commonly observed in similarly aged rats as background lesions. Therefore, the NOAEC of DEB was 160 ppm under the study conditions. However, additional studies will be needed to investigate the toxic effect of DEB such as neurotoxicity.

We conducted 13-week inhalation toxicity studies with vaporized DEB at a maximum concentration of 160 ppm in male and female Wistar rats, in accordance with the GLP and OECD guidelines. No toxic effects were observed on body weight, food consumption, BALF, blood, or gross or histopathological analyses. Based on these results, the NOAEC for DEB was determined to be 160 ppm.

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Table 7. Summary of relative organ weight data in rats inhaling diethylbenzene for 90 days (n = 10)

Organs (%)	Groups			
	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
Male				
Adrenal glands	0.0104 ± 0.00095	0.0106 ± 0.00205	0.0097 ± 0.00169	0.0099 ± 0.00181
Brain	0.3702 ± 0.02368	0.3684 ± 0.02462	0.3773 ± 0.0267	0.3751 ± 0.031
Epididymides	0.2784 ± 0.02783	0.2574 ± 0.02353	0.269 ± 0.0201	0.2729 ± 0.02685
Heart	0.2566 ± 0.01781	0.255 ± 0.01458	0.2467 ± 0.0100	0.2472 ± 0.01353
Kidneys	0.6099 ± 0.04355	0.5652 ± 0.0369*	0.611 ± 0.01994	0.6215 ± 0.04359
Liver	2.569 ± 0.09974	2.5058 ± 0.1827	2.5307 ± 0.17815	2.6207 ± 0.17052
Lung	0.1302 ± 0.02234	0.1244 ± 0.01409	0.1335 ± 0.0112	0.133 ± 0.00759
Spleen	0.183 ± 0.03525	0.176 ± 0.01983	0.1779 ± 0.01981	0.1864 ± 0.02227
Testes	0.6711 ± 0.06296	0.6698 ± 0.11129	0.6499 ± 0.08108	0.6832 ± 0.0476
Thymus	0.0649 ± 0.0077	0.0621 ± 0.01376	0.0615 ± 0.01042	0.0625 ± 0.00878
Female				
Adrenal glands	0.0208 ± 0.00393	0.0216 ± 0.0023	0.0201 ± 0.00225	0.0215 ± 0.00278
Brain	0.6321 ± 0.03148	0.6556 ± 0.04446	0.6465 ± 0.04283	0.6558 ± 0.04322
Heart	0.2932 ± 0.02339	0.2934 ± 0.0146	0.283 ± 0.02332	0.293 ± 0.0207
Kidneys	0.6175 ± 0.03091	0.6113 ± 0.02568	0.6279 ± 0.05103	0.6661 ± 0.06205
Liver	2.891 ± 0.43007	2.7557 ± 0.18567	2.8588 ± 0.31933	2.9791 ± 0.25669
Lung	0.1823 ± 0.0157	0.1742 ± 0.01009	0.185 ± 0.01121	0.1805 ± 0.01768
Ovaries	0.0358 ± 0.00617	0.0337 ± 0.00813	0.0353 ± 0.00594	0.0354 ± 0.00568
Spleen	0.1934 ± 0.01717	0.1967 ± 0.03446	0.1926 ± 0.00704	0.2028 ± 0.01588
Thymus	0.0928 ± 0.02763	0.0759 ± 0.01674	0.0876 ± 0.01861	0.0909 ± 0.01662
Uterus	0.2867 ± 0.08935	0.2968 ± 0.06851	0.2706 ± 0.06434	0.2907 ± 0.10959

Data are expressed as the mean ± S.D.

* Dunnett's LSD test significant at the 0.05 level.

n, number of animals examined.

Table 8. Summary of gross findings in rats inhaling diethylbenzene for 90 days (n = 10)

Sex	Male				Female			
Group	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)	Control	Low (40 ppm)	Middle (80 ppm)	High (160 ppm)
Organ								
Lung								
Spot	0	0	0	1	0	1	0	0
Spleen								
Nodule	1	0	0	0	0	0	0	0
Testes								
Increased size	0	1	0	0	-	-	-	-
Vagina								
Nodule	-	-	-	-	0	1	0	0

n, number of animals examined.

Table 9. Summary of histopathological findings in rats inhaling diethylbenzene for 90 days

Sex		Male			Female		
Group		Control	Middle (80 ppm)	High (160 ppm)	Control	Low (40 ppm)	High (160 ppm)
n		10	1	10	10	2	10
Organ							
Heart							
Cardiomyopathy	1[a]	3	-	1	0	-	0
	Total	3	-	1	0	-	0
Liver							
Infiltration, mononuclear cell	1[a]	0	-	1	1	-	1
	Total	0	-	1	1	-	1
Lung							
Aggregates, alveolar macrophage	1	5	-	3	2	0	1
	2[a]	0	-	2	1	1	0
	Total	5	-	5	3	1	1
Crystals, hematoidin	1	1	-	1	0	0	0
	Total	1	-	1	0	0	0
Hyperplasia, bronchiolo-alveolar	1[a]	1	-	2	0	0	0
	Total	1	-		0	0	0
Infiltration, mononuclear cell	1[a]	0	-	1	0	0	0
	Total	0	-	1	0	0	0
Nasal cavity							
Hyperplasia, mucous cell	1	1	-	2	0	-	0
	Total	1	-	2	0	-	0
Pancreas							
Atrophy, acinar cell	1	1	-	1	0	-	0
	2	1	-	0	0	-	0
	Total	2	-	1	0	-	0
Infiltration, mixed cell	1	0	-	0	0	-	1
	Total	0	-	0	0	-	1
Inflammation, granulomatous	1	0	-	1	0	-	0
	2	1	-	0	0	-	0
	Total	1	-	1	0	-	0
Pigmentation	1	1	-	0	0	-	0
	Total	1	-	0	0	-	0
Vacuolation, acinar cell	2	0	-	0	0	-	1
	Total	0	-	0	0	-	1
Pituitary							
Cyst, pars distalis	1	0	-	1	0	-	0
	Total	0	-	1	0	-	0
Spleen							
Extramedullary hematopoiesis	1	0	-	1	1	-	2
	Total	0	-	1	1	-	2
Sarcoma, NOS	Total	1	-	0	0	-	0
Testes							
Dilation, seminiferous tubules	2	0	1	0	-	-	-
	Total	0	1	0	-	-	-

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Table 9. Continued

Sex		Male			Female		
Group		Control	Middle (80 ppm)	High (160 ppm)	Control	Low (40 ppm)	High (160 ppm)
n		10	1	10	10	2	10
Thyroids							
Adenoma, C-cell	Total	0	-	0	0	-	1
Ectopic tissue, thymus	1	1	-	0	0	-	0
	Total	1	-	0	0	-	0
Ultimobranchial cyst	1	2	-	3	0	-	2
	Total	2	-	3	0	-	2
Vagina							
Cyst	1	-	-	-	0	1	0
	Total	-	-	-	0	1	0

[a] Severity; 1, Minimal; 2, Mild.

n, number of animals examined; NOS, not otherwise specified; -, not applicable.

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