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Developmental Toxicity Induced by Inhalation Exposure of Pregnant Rats to *N,N*-Dimethylacetamide

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Abstract: Developmental Toxicity Induced by Inhalation Exposure of Pregnant Rats to *N,N*-Dimethylacetamide: Hirokazu Okuda, *et al*. Japan Bioassay Research Center, Japan Industrial Safety and Health Association—Developmental toxicity of *N,N*-dimethylacetamide (DMAC) was examined by exposing pregnant rats by inhalation to DMAC vapor at 0 (control), 100, 300, 450 or 600 ppm (v/v) for 6 h/d during Gestation Days 6 through 19. Fetal body weight and the number of male live fetuses were significantly decreased, along with a tendency of the number of intrauterine deaths to increase. The number of fetuses with visceral and skeletal malformations was significantly increased in the 450 and 600 ppm groups, while the number of fetuses with anasarca as an external malformation was increased at 600 ppm. Observed cardiovascular malformations included ventricular septum defect, persistent truncus arteriosus, malpositioned subclavian branch and retroesophageal subclavian artery. Persistent truncus arteriosus was accompanied by ventricular septal defect (VSD). Incidences of the persistent truncus arteriosus, which was classified as a serious congenital heart disease affecting postnatal survival, were increased at 450 and 600 ppm. Increased liver weights and hepatocellular swelling occurred in the dams exposed to 300 ppm and above, whereas neither hepatocellular necrosis nor increased serum activity of liver transaminases was observed in any of the exposed groups. Maternal body weights were decreased at 450 and 600 ppm. The most sensitive signs of developmental toxicity appeared at the exposure level of 300 ppm which was also the level of slight maternal toxicity. The No-Observed-Adverse-Effect-Level (NOAEL) was determined as 100 ppm for the endpoints of fetal and maternal toxicities. The NOAEL of 100 ppm and the induction of serious cardiovascular malformations occurring at 450 ppm and above were discussed with reference to the existing occupational exposure limit for DMAC.
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Key words: Dimethylacetamide, DMAC, Inhalation, Teratogenicity, Fetotoxicity, Cardiovascular malformation, NOAEL, Rat

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N,N-Dimethylacetamide (DMAC, CAS No. 127–19–5) has been widely used as a solvent for organic syntheses, resins, polymers, crystallization and purification, because of its wide solubility, water miscibility, stability and high boiling and low freezing points. Several studies of human toxic effects have revealed that DMAC causes toxic hepatitis[1, 2] and symptoms and signs indicative of liver involvement[3, 4] among workers exposed to DMAC through inhalation and dermal contact. The liver toxicity, including hepatocellular degeneration and necrosis, was demonstrated by inhalation exposures of dogs, rats and mice to DMAC vapor[5–7]. Besides, reproductive and developmental effects of DMAC have been reported using pregnant rats given DMAC by various routes of administration[8–12]. Those reported effects included abnormalities of cellular development in the embryos after a single intraperitoneal injection of DMAC in rats[8], increased fetal mortality and skeletal malformations after dermal application of DMAC to rats[9], and increased incidences of cardiovascular malformations after administration of DMAC to rats by gavage[10]. However, two inhalation studies[11, 12] failed to demonstrate altered reproductive performance or fetal anomalies in parental exposures of male and female rats up to 300 ppm DMAC[11], and developmental toxicity in the maternal exposure of rats to DMAC at 282 ppm and below[12]. Since inhalation and dermal exposures to DMAC are the principal routes for workers exposed to DMAC, data from whole-body inhalation exposure of animals to DMAC vapor are more relevant to health risk assessment of

DMAC-exposed workers than those from oral and intraperitoneal administrations. An occupational exposure limit (OEL) value of 10 ppm for DMAC has been recommended by the Japan Society for Occupational Health[13], the American Conference of Governmental Industrial Hygienists[14] and Deutsche Forschungsgemeinschaft[15].

The present study was designed to examine the developmental toxicity of DMAC by repeatedly exposing pregnant rats by inhalation to DMAC vapor at 4 different concentrations for a 14-d period from Gestation Day (GD) 6 through 19. The DMAC-induced developmental and maternal effects were discussed with reference to the existing OEL value of 10 ppm for DMAC.

Material and Methods

Chemical

DMAC of reagent grade (purity >99.9%) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The DMAC used in this study was analyzed for its identity and purity by both infrared spectrometry and mass spectrometry and for its stability by gas chromatography. Neither decomposition products nor impurities were detected.

Animal

Male and female Crj:CD(SD)IGS rats were obtained from Charles River Japan Inc. (Kanagawa, Japan) at the age of 10 wk and 9 wk, respectively. After 1-wk quarantine, female rats were paired with males on a 1:1 basis until copulation. The day sperm or a plug was found in the vagina was designated GD 0. Pregnant females were individually housed in stainless-steel wire mesh cages (150 [W] × 270[D] × 176[H] mm) in the inhalation chambers, each of 1.11 m^3 in volume. The temperature, relative humidity and fluorescent lighting were maintained at 23 ± 2°C, 55 ± 20% and a 12-h light (08:00–20:00)/dark (20:00–08:00) cycle. Tap water (filtered and UV-irradiated water) and commercial pellet diet (CRF-1, Oriental Yeast Co., Tokyo, Japan) were given *ad libitum*.

Exposure to DMAC

Pregnant females were divided into five groups of 10 animals each by body weight-stratified randomization, and were exposed by whole-body inhalation (6 h/d) to DMAC vapor at an air concentration of 0 (control), 100, 300, 450, or 600 ppm (v/v) for 6 h/d for GD 6 through 19. The concentrations of 600 and 300 ppm were selected in consideration of DMAC toxicity data[12] in rats: inability of dams to maintain pregnancy at 625 ppm and decreased maternal and fetal growth rates at 282 ppm. The exposure to 450 ppm was carried out in order to examine fetal development in detail. Selection of 100 ppm was based on both the OEL value of 10 ppm for DMAC[13–15] and an uncertainty factor of 10 for extrapolating the rodent data to humans[16]. The inhalation exposure was conducted using the same method and technique as described in a previous report[17]. Chamber concentrations of DMAC were monitored by gas chromatography every 15 min during the 6-h exposure period, and were kept at 101.8 ± 2.7 (mean ± SD), 297.6 ± 3.8, 447.0 ± 5.0 and 594.2 ± 6.5 ppm. A group of 10 pregnant females was exposed to clean air, and served as a control.

Maternal and fetal examinations

Dams were observed daily for clinical signs and mortality, and weighed on GDs 6, 7, 9, 13, 17 and 20. Following diethylether anesthesia, at necropsy on GD 20, blood was taken from the abdominal aorta and examined for AST, ALT and LDH. Maternal liver was dissected for the organ weight and histopathological examinations. The liver was fixed in 10% neutral buffered formalin and embedded in paraffin. Liver sections 5 μm thick were prepared and stained with hematoxylin and eosin (H & E). The uterus was opened and evaluated for the number of live or dead fetuses (including resorptions) and implantation sites. The live fetuses were weighed, sexed and examined for external abnormalities. Approximately one-half of the live fetuses per litter were examined for visceral abnormalities by Nishimura's method[18] after fixation in Bouin's solution. The other half of the live fetuses were fixed in 99.5% ethanol, eviscerated, macerated in 1.5% KOH solution, and stained with alizarin red S for the examination for skeletal abnormalities.

Statistical analysis

Maternal and fetal body weights, maternal liver weights, numbers of implantations, intrauterine deaths and live fetuses, and blood biochemical parameters were analyzed by Dunnett's test. The parameters of developmental toxicity in fetuses were expressed as the litter unit. At first, Bartlett's test was applied to determine whether the variance was homogeneous or not. When the variance was homogeneous, one-way ANOVA was applied. When the variance was not homogeneous, the Kruskal-Wallis rank sum test was performed by arranging all data of the control and exposed groups in descending order. Statistical differences in the means and the rank means among the groups were analyzed by Dunnett's multiple comparison test, and the same multiple comparison test by rank, respectively. Incidences of hepatocellular swelling in dams and external, visceral and skeletal malformations in fetuses were analyzed by Chi-square test. Two-sided analysis with a p-value of 0.05 or 0.01 was performed.

Results

All dams survived to the scheduled necropsy on GD

Table 1. Body weights, blood chemistry, organ weight and histopathology of pregnant rats exposed to *N*,*N*-dimethylacetamide or clean air as a control

	Maternal exposure concentration (ppm)				
	0	100	300	450	600
No. of pregnant rats examined	10	10	10	10	10
Body weight (g)					
GD 6	263 ± 7	260 ± 7	262 ± 15	261 ± 8	259 ± 10
GD 13	301 ± 11	299 ± 14	295 ± 17	290 ± 10	280 ± 14**
GD 20	383 ± 21	379 ± 23	368 ± 26	356 ± 16*	330 ± 30**
Blood biochemistry					
AST (IU/l)	49 ± 9	49 ± 6	50 ± 12	50 ± 8	49 ± 7
ALT (IU/l)	33 ± 6	35 ± 6	34 ± 6	32 ± 6	33 ± 7
LDH (IU/l)	192 ± 122	130 ± 26	127 ± 40	137 ± 36	138 ± 47
Organ weight					
Absolute liver weight (g)	14.3 ± 1.2	14.6 ± 1.3	15.5 ± 1.5	14.9 ± 1.3	14.5 ± 1.3
Relative liver weight (%)	3.73 ± 0.20	3.86 ± 0.29	4.22 ± 0.24**	4.19 ± 0.31**	4.43 ± 0.21**
Histopathology					
No. of rats bearing swelling of centrilobular hepatocytes	0	0	4	10##	7##

All values, except histopathology, are expressed as mean ± S.D. Relative liver weight: liver weight/body weight measured at time of necropsy. * and **: Significantly different from the control group at $p \leq 0.05$ and $p \leq 0.01$ by Dunnett's test, respectively. ## : Significantly different from the control group at $p \leq 0.01$ by Chi-square test.
AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase.

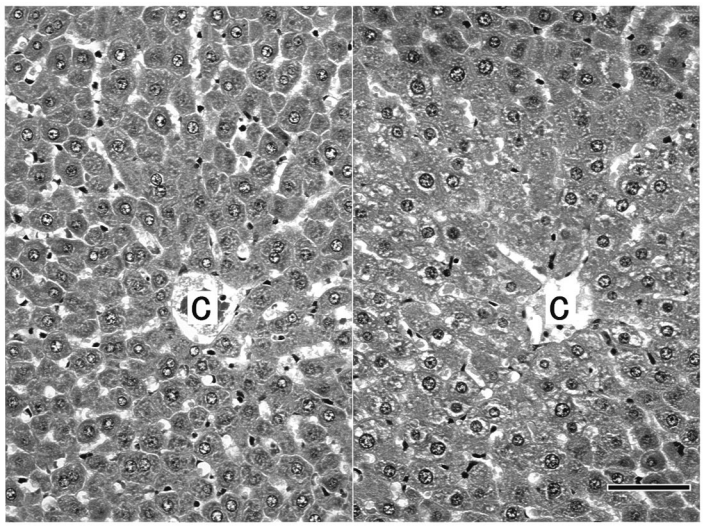

Fig. 1. Swelling of centrilobular hepatocytes in a pregnant rat exposed to 600 ppm DMAC (right), compared with normal centrilobular hepatocytes in a pregnant rat exposed to clean air as a control (left). Hydropic change of the cytoplasm can be seen in the centrilobular area surrounding the central vein (C). H & E stain. Bar indicates 100 μm.

20. No overt clinical sign was observed in any dam during the study. Body weights of dams were decreased with an increase in the exposure concentrations and the repetition of exposure along the time course of pregnancy. Body weights of dams measured on GD 13 and exposed to 600 ppm and those measured on GD 20 and exposed to 450 and 600 ppm were significantly lower than the control (Table 1). Neither AST, ALT nor LDH was significantly increased in any of the exposed dam groups. Relative liver weights were significantly higher in the 300 ppm

Table 2. Viability and body weight of fetuses following maternal exposure to *N,N*-dimethylacetamide or clean air as a control

	Maternal exposure concentration (ppm)				
	0	100	300	450	600
No. of litters examined	10	10	10	10	10
No. of implantations	15.2 ± 1.5	14.5 ± 1.8	14.5 ± 1.1	14.2 ± 0.8	13.3 ± 4.5
No. of intrauterine deaths	1.1 ± 1.2	0.8 ± 0.6	1.5 ± 1.8	1.1 ± 1.1	3.4 ± 5.0
No. of live fetuses					
Males	7.4 ± 1.3	7.8 ± 1.3	6.3 ± 2.9	6.9 ± 1.4	4.0 ± 2.8**
Females	6.7 ± 2.5	5.9 ± 1.6	6.7 ± 1.8	6.2 ± 1.5	5.9 ± 3.1
Fetal body weights (g)					
Males	3.90 ± 0.21	3.89 ± 0.20	3.52 ± 0.21**	3.11 ± 0.19**	2.53 ± 0.26**
Females	3.67 ± 0.16	3.70 ± 0.14	3.36 ± 0.17**	2.89 ± 0.23**	2.46 ± 0.48**

All values are the mean ± S.D on a litter basis. * and **: Significantly different from the control group at $p \leq 0.05$ and $p \leq 0.01$ by Dunnett's test, respectively.

Table 3. External, visceral and skeletal malformations following maternal exposure to *N,N*-dimethylacetamide or clean air as a control

	Maternal exposure concentration (ppm)									
	0		100		300		450		600	
External malformations										
No. of fetuses examined	141	(10)	137	(10)	130	(10)	131	(10)	99	(9)
No. of fetuses with malformations	0	(0)	0	(0)	0	(0)	0	(0)	4#	(3)#
Anasarca	0	(0)	0	(0)	0	(0)	0	(0)	4#	(3)#
Cleft palate	0	(0)	0	(0)	0	(0)	0	(0)	1	(1)
Visceral malformations										
No. of fetuses examined	68	(10)	65	(10)	63	(10)	63	(10)	49	(8)
No. of fetuses with malformations	0	(0)	0	(0)	2	(2)	7##	(6)##	23##	(8)##
Ventricular septal defect	0	(0)	0	(0)	2	(2)	7##	(6)##	22##	(8)##
Persistent truncus arteriosus	0	(0)	0	(0)	0	(0)	2	(1)	12##	(7)##
Malpositioned subclavian branch	0	(0)	0	(0)	0	(0)	0	(0)	4#	(3)#
Retroesophageal subclavian	0	(0)	0	(0)	0	(0)	0	(0)	3#	(3)#
Skeletal malformations										
No. of fetuses examined	73	(10)	72	(10)	67	(10)	68	(10)	50	(9)
No. of fetuses with malformations	0	(0)	0	(0)	0	(0)	4#	(2)	6##	(6)##
Fused exoccipital	0	(0)	0	(0)	0	(0)	0	(0)	4#	(4)#
Fused cervical arch	0	(0)	0	(0)	0	(0)	4#	(2)	2	(2)
Fused rib	0	(0)	0	(0)	0	(0)	0	(0)	2	(2)

and ##: Significantly different from the control group at $p \leq 0.05$ and $p \leq 0.01$ by Chi-square test, respectively. Parentheses indicate the number of litters examined or having fetuses with each malformation.

and higher groups than in the control group, although the absolute liver weight was not increased in any exposed group. Histopathologically, slight liver failure characterized by swelling of the centrilobular hepatocytes without the occurrence of hepatocellular necrosis was noted in the 300 ppm and higher groups of dams (Fig. 1).

The number of male live fetuses was significantly decreased in the 600 ppm group, while the number of female live fetuses was not increased in that group (Table 2). On the other hand, the number of intrauterine deaths tended to increase in the 600 ppm group. A significant and dose-dependent decrease in fetal body weights of both sexes was noted at 300 ppm and above.

The number of fetuses with visceral and skeletal malformations was significantly increased in the 450 and 600 ppm groups. Furthermore, the number of fetuses

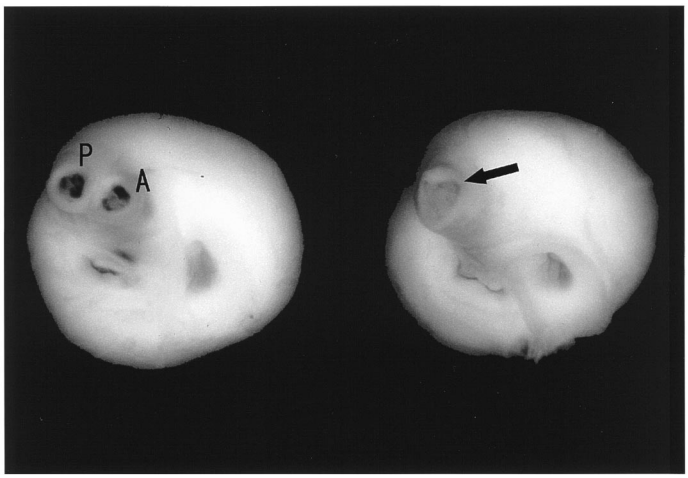

Fig. 2. A serious cardiovascular malformation of persistent truncus arteriosus (arrow) accompanied by VSD in a live fetus whose dam was exposed to 600 ppm DMAC (right), compared with the normal basis cordis in a live fetus whose dam was exposed to clean air as a control (left). Note abnormal division of the aorta (A) and pulmonary artery (P), as indicated by an arrow.

with anasarca as an external malformation was significantly increased only in the 600 ppm group (Table 3). The number of litters having fetuses with cardiovascular malformations was increased in the 450 and 600 ppm groups, while the number of litters having fetuses with external or skeletal malformations was increased in the 600 ppm group. Ventricular septal defect (VSD) was observed in the fetuses from the 300 ppm and higher groups, and the number of fetuses with VSD was significantly increased in the 450 and 600 ppm groups. Persistent truncus arteriosus was noted in the fetuses from the 450 and 600 ppm groups, and the number of fetuses with persistent truncus arteriosus was significantly increased in the 600 ppm group. Significantly increased incidences of malpositioning of the subclavian branch and the retroesophageal subclavian artery were noted in the 600 ppm group. Notably, the persistent truncus arteriosus which was characterized by abnormal division of the aorta and pulmonary artery (Fig. 2) was accompanied by VSD, and these combined cardiovascular malformations were observed in two and twelve fetuses whose dam groups were exposed to 450 and 600 ppm, respectively. In the skeletal observations, the number of fetuses with fused bones (exoccipital, cervical arch or rib) was also significantly increased in the 450 and 600 ppm groups.

Discussion

The developmental effects of DMAC on fetuses whose dams were exposed by inhalation to DMAC vapor during GDs 6 through 19 were characterized by serious cardiovascular malformations, in addition to the external and skeletal malformations, retarded fetal growth rate and a decreased number of male live fetuses. The inhalation exposure of pregnant rats to 600 ppm was found to seriously affect the fetal survival as evidenced by both the decreased number of live male fetuses and the tendency of the number of intrauterine deaths to increase. Therefore, it is suggested that the exposure to 600 ppm DMAC made maintenance of pregnancy difficult and caused fetal death due to serious structural anomalies that would have occurred during the short time period critical to organogenesis. Furthermore, induction of VSD and persistent truncus arteriosus occurring at 450 and 600 ppm was noteworthy among various visceral, skeletal and external malformations. Especially, persistent truncus arteriosus, which was characterized by abnormal division of the aorta and pulmonary artery, is classified as a severe congenital heart disease, and causes cyanosis due to the arterial oxygen-rich blood mixing with oxygen-poor blood of the pulmonary artery after live birth[19]. Therefore, the DMAC-induced cardiovascular malformations of persistent truncus arteriosus would seriously affect the postnatal survival.

The present findings are comparable with those of the 3 studies reported by Johanssen *et al.*[10], Ferenz and Kennedy[11] and Solomon *et al.*[12]. Solomon *et al.*[12] reported that 6-h inhalation exposure of pregnant rats to DMAC vapor at 32, 100 and 282 ppm from GDs 6 through 15 significantly decreased the fetal and maternal body weights at 282 ppm without any fetal malformations, although the ability of dams to maintain pregnancy as

evidenced by complete fetal resorption at 625 ppm was reported to be adversely affected in their preliminary study. Ferenz and Kennedy[11] reported that parental inhalation exposure of rats to up to 300 ppm DMAC did not alter either the mating performance, the fertility of both males and females, gestation length, number of offsprings delivered and the structural anomalies of fetuses, except for missing tails, although the maternal relative liver weight was significantly increased in the 300 ppm-exposed group. However, teratogenicity of DMAC was demonstrated by Johannsen *et al.*[10] who reported that oral administration of DMAC at 65, 160 or 400 mg/kg/d to pregnant rats by gavage on GDs 6 through 19 caused cardiovascular malformations including VSD, common truncus arteriosis, but no ductus arteriosis, at a dose of 400 mg/kg/d. They also observed an increased number of postimplantation losses and the decreased body weights of dams and fetuses at a dose of 400 mg/kg/d. It can be estimated, assuming a lung absorption ratio of DMAC vapor of 100% for a pregnant female rat having a minute volume of 561 ml/min/kg body weight[20], that the amounts of DMAC uptake through 6-h inhalation of air containing 300, 450 and 600 ppm are equivalent to DMAC doses of 214, 321 and 428 mg/kg/d, respectively. Therefore, both the estimated amounts of DMAC uptake through inhalation and the resulting fetal and maternal toxic responses were found to agree well with those induced by the oral administration of DMAC[10], although there was a difference in the route of exposure to DMAC between this study and the study by Johannsen *et al.*[10].

In the present study, the most sensitive endpoint was found to be the decreased fetal body weight for the fetal toxicity and both the increased liver weight and the hepatocellular swelling for the maternal toxicity, all of which occurred at an exposure concentration of 300 ppm. Therefore, the No-Observed-Adverse-Effect-Level (NOAEL) was determined as 100 ppm for both the endpoints of fetal and maternal toxicities of DMAC. The NOAEL value of 100 ppm for DMAC found in this study is in good agreement with that reported by Solomon *et al.*[12]. The maternal hepatotoxicity of DMAC found in this study is thought to be slight, because inhalation exposure of pregnant rats up to 600 ppm induced both hepatocellular swelling and increased liver weight without occurrence of hepatocellular necrosis or the cytolytic release of liver enzymes into serum. One tenth of the NOAEL is equivalent, coincidently, to the existing OEL of 10 ppm for DMAC, assuming only an uncertainty factor of 10 for extrapolating the rodent data to humans[16, 21] without an assumption of another uncertainty factor in the variation of intrahuman susceptibility to toxic responses to DMAC. The German MAK value of 10 ppm for DMAC[15] is marked with the notation of pregnancy risk as "Group C" indicating that there is no reason to fear a risk of damage to the embryo or fetus when MAK and BAT values are observed. It remains unsolved yet, however, whether the NOAEL value of 100 ppm for DMAC provides sufficient evidence for the basis of animal experiments for the existing OEL value of 10 ppm. Further study will be needed to explore causative factors for DMAC-induced developmental toxicity and its relation to maternal toxicity. The present finding of DMAC-induced life-threatening cardiovascular malformations in rat fetuses suggests that due caution should be paid to prevention of short-term exposure of female workers of childbearing age to DMAC vapor, since the time period critical to cardiovascular organogenesis is very short during pregnancy.

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