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Fetotoxic Effects of Exposure to the Vapor of Organic Solvents from a Synthetic Adhesive in Mice

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Synthetic adhesives are widely used in various industries as well as at home. Adhesives usually contain several organic solvents which easily vaporize. Exposure can cause aplastic anemia and polyneuropathy in adults (Harada 1961; Iida et al. 1969; Cianchetti et al. 1976). Chronic glue sniffing results in aplastic anemia, polyneuropathy, and muscular atrophy (Powars 1965; Gonzalez and Downey 1972; Suzuki et al. 1974; Shirabe et al. 1974).

Inhalation of the solvent contained in adhesives, such as n-hexane, toluene, xylene, and benzene by pregnant animals can decrease the number of live fetuses and retard fetal growth (Green et al. 1978; Hudak and Ungvary 1978; Marks et al. 1980; Ungvary et al. 1980; Shigeta et al. 1982). In humans, the risk of spontaneous abortion is increased in workers exposed to organic solvents (Lindbohm et al. 1990; Ng et al. 1992). However, information is still limited about the effects of exposure to organic solvents vaporized from adhesives on fetuses.

In the present study, female mice were exposed throughout pregnancy to organic solvents vaporized from an adhesive to clarify the effects of the inhalation on progeny.

MATERIALS AND METHODS

Female ddY mice were housed individually in aluminum pan cages (15 cm X 15 cm X 11 cm) under a 12L12D photoperiod (lights on 0600h) and temperature at 20–22 °C. Mice were mated with a proven male of the same strain; the day on which vaginal plugs were found was designated day 0 of pregnancy. Food and water were given *ad libitum* except for the inhalation period during which they were withheld.

Animals were exposed daily for 60 minutes around 0800h from days 0 to 17 of pregnancy to organic solvents vaporized from 18.0, 22.5, 27.0, 31.5, or 36.0 g of a commercial adhesive

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popularly used by home owners in Japan. The adhesive consisted of 30% synthetic rubber, and 70% organic solvents by weight. The constituents in the phase of organic solvents are shown in Table 1. Inhalation was performed as following: Five animals were transferred to a wire mesh cage (25 X 17 X 13 cm) within a chamber (0.144 m^3) containing two to four aluminum sheets (30 X 12 cm), according to the dose, coated equally on the surface with 13.5 g, at maximum, of the adhesive. Animals in the control group were similarly manipulated and exposed to room air, not to the solvents.

Body weight was recorded daily. After the exposure on day 17 of pregnancy, mice were autopsied, and their uteri removed to observe the number of implantation sites and fetuses. Sex and external malformations of fetuses were noted, and fetal and placental weights measured.

To determine the concentration of each solvent in the air of each chamber, 50 ml of the air was collected at 15, 30, 45 and 60 min from a chamber containing 18.0, 22.5, and 27.0 g of adhesive. Concentration was measured by gas chromatography, using n-heptane as an internal standard. Briefly, we used a Shimadzu (Kyoto, Japan) Model GC-9A gas chromatograph equipped with a hydrogen flame ionization detector and a glass column (3 m X 3 mm I.D.) packed with 25% PEG 1500 on 60-80 mesh Shimalite. Gas chromatographic conditions were the following: injection port temperature at 160 °C, column temperature at 40 °C for 2.5 min, increasing at the rate of 40 °C/min for the following 1.5 min, and 100 °C afterwards, and gas flow or pressure rate at 50 ml/min of nitrogen, 0.6 kg/cm^2 of hydrogen, and 0.5 kg/cm^2 of air.

Student's t test was used for statistical analysis between the groups.

Table 1. The percentage by the weight of each constituent in organic solvent phase of the adhesive.

Substance	Percentage
Cyclohexane	52.4%
Acetone	22.0%
Isopropyl Acetate	10.2%
n-Hexane	9.8%
Methyl Cyclohexane	2.2%
3-Methyl Pentane	1.4%
2,3-Dimethyl Butane +2-Methyl Pentane[1]	1.2%
Toluene	0.7%

Constituents were analyzed based on the method by Naruse (1984).
1; 2,3-Dimethyl butane and 2-methyl pentane showed one peak overlapped on the chromatogram.

RESULTS AND DISCUSSION

The concentration of constituents detected in air of the chamber is shown in Figure 1. A peak was obtained 15 min after exposure, and the level gradually decreased afterwards. The value of each substance increased in a dose-dependent manner; highest in 27.0 g of adhesive, and lowest in 18.0 g. Peak concentrations of cyclohexane, acetone, n-hexane, and toluene were higher, even in the exposure of 18.0 g of adhesive, than the allowable values (TWA) recommended by Japan Association of Industrial Health (1992). The peaks of cyclohexane, acetone and n-hexane were 20-200 times higher than the allowable value in the exposure of 27.0 g of adhesive. The inhalation of organic solvents at the concentration as shown in the present study could seldom occur in the usual situation, such as in workplaces. However, considering the safety factors (usually 1/100), the concentration in the present study would not be too high to extrapolate the results to our daily life. Also, persons who are sniffing glues have been suggested to inhale n-hexane at the concentration as high as 44000 ppm (about 155 mg/L, Gonzalez and Downey 1972), which value was much greater than that in this study.

All animals died by day 5 of pregnancy in the groups exposed to the vapor from 31.5 and 36.0 g of the adhesive (Table 2). Fifty percent of animals exposed to the solvents from 27.0 g of adhesive was alive on day 17 of pregnancy, while no mice died in groups exposed to less than 27.0 g of adhesive.

Nine out of 15 mice that survived in the group exposed to 27.0 g of the adhesive had implantation sites, and 5 animals possessed live fetuses on day 17 of pregnancy (Table 2). All mice had implantation sites and fetuses in groups exposed to the vapor from 22.5 and 18.0 g of adhesive, as well as controls. Chi square test revealed a significant difference in the distribution of number of dams with or without alive fetuses among survived animals between the groups exposed to 27.0 g of adhesive and to room air ($X^2=7.726$, $P<0.01$).

Table 3 shows the average number of implantation sites and fetuses in litters preserved until day 17 of pregnancy. Number of implantation sites was 50% less in mice exposed to 27.0 g of adhesive than other groups; the average number of fetuses was also significantly reduced.

Number of animals which had implantation sites and that of implantation sites were smaller in the group exposed to the vapor from 27.0 g of adhesive than other groups (Tables 2 and 3). This finding may have suggested that these dams lost their embryos in early pregnancy, such as before or around the time of implantation. However, in these animals, about a half of the implanted embryos seemed to be resorbed after implantation (Table 3). Therefore, we suggest that the fate of embryos was influenced before and after implantation by

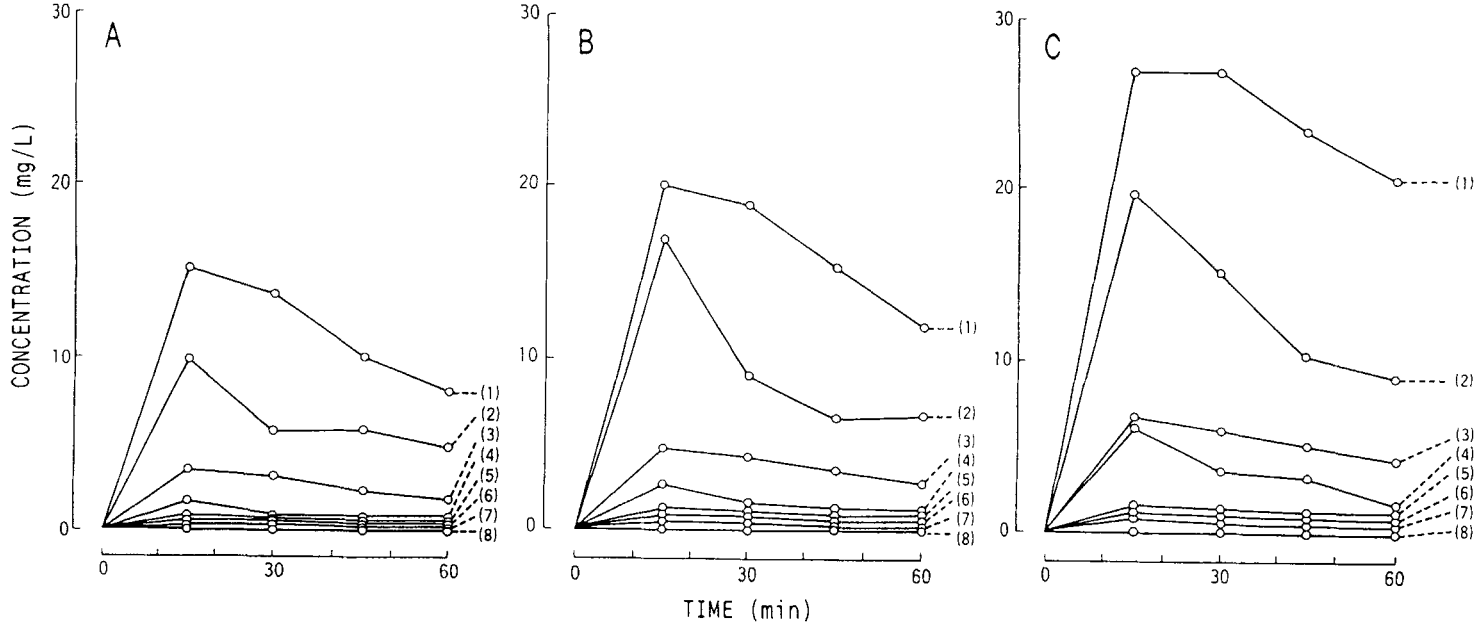

Figure 1. Changes in concentration of solvents in the air of chamber. Panels A, B, and C indicate the average value of 2 or 3 measurements in the exposure to 18.0, 22.5, and 27.0 g of adhesive, respectively. Numerals represent cyclohexane (1), acetone (2), n-hexane (3), isopropylacetate (4), methylcyclopentane (5), 3-methylpentane (6), 2,3-dimethylbuthane and 2-methylpentane (7), and toluene (8).

Table 2. The number of pregnant mice exposed to 0 - 37 grams of adhesive that survived, contained uterine implantation sites and had viable fetuses.

Exposure Group	Number Exposed	Number Survived	Number with implantation sites	Number with live fetuses
37.0 g	8	0	0	0
31.5 g	8	0	0	0
27.0 g	30	15	9	5
22.5 g	7	7	7	7
18.0 g	8	8	8	8
Control	9	9	9	9

Table 3. The average number of implamntation sites and fetuses per pregnant dams on day 17 of pregnancy, as influenced by amount of adhesive exposure.

Group	Implantation sites	Fetuses
27.0 g	6.0±2.0**	2.6±1.1**
22.5 g	12.2±2.2	10.2±2.7
18.0 g	12.2±2.3	11.7±2.0
Control	12.5±2.5	11.7±2.0

Mean±S.D. Number of dams is 5, 7, 8, and 9 in the groups exposed to 27.0, 22.5 and 18.0 g, and of control, respectively.
** : Significant difference ($P<0.01$) from other groups.

Table 4. Mean weight of fetuses and placentas as inflenced by amount of adhesive exposure.

Group	No. of samples	Fetal weight (mg)	Placental weight (mg)
27.0 g	13	822±202**	97±14*
22.5 g	72	1006±182**	98±13**
18.0 g	94	1106±191	104±15
Control	106	1143±107	104±11

* and ** : Significant difference from control at $p<0.05$ and $P<0.01$, respectively.

inhalation of organic solvents from the adhesive.

Fetal growth was suppressed in proportion to the exposure dose (Table 4). The weight of fetuses was 28% and 12% smaller in the groups exposed to 27.0 and 22.5 g of adhesive, respectively, than controls ($P<0.01$). Placental weight was also significantly reduced in these groups, although the dose-response relationship was not always apparent.

Weight gain was suppressed in dams in the groups of 27.0 and 22.5 g adhesive exposure, especially during later part of pregnancy (data not shown). Body weight of mice exposed to 27.0 and 22.5 g of adhesive was 30% and 13%, respectively, less than control mice on day 17 of gestation ($P<0.01$), which suggested that a shortage in food intake in exposed dams might result in the fetal retardation.

External malformations in fetuses by the inhalation of organic solvents vaporized from the adhesive were not apparent.

The substance(s) and mechanisms causing the reproductive aberrations observed in this study are not known at present. Toluene may have been at least partly responsible for the present results. Exposure to toluene has been documented to cause a higher incidence of abortion or resorption of fetuses in humans and animals (Shigeta et al. 1982; Donald et al. 1991; Ng et al. 1992). Other solvents contained in the adhesive have not been reported to cause lethal effect on fetuses (Hudak and Ungvary 1978; Marks et al. 1980), nor investigated. Tatrai et al. (1980) reported that embryotoxic effects were different in animals with mixed exposure to benzene and toluene than from inhalation of a single solvent. Since animals were exposed to a mixture of organic solvents in this study, only toluene could not cause the fetotoxicity.

As in the present study, fetal growth retardation has been observed in many previous studies in which inhalation of organic solvents was performed in pregnant animals (Green et al. 1978; Marks et al. 1980; Ungvary et al. 1980; Shigeta et al. 1982; Mirkova et al. 1983).

Finally, the present study demonstrated that exposure to organic solvents vaporized from an adhesive causes fetal lethality at higher doses, and suppresses fetal growth in a dose-related manner. Although the results were obtained under the condition of higher concentration of solvents, it would be possible, considering the safety factors, to apply the present results to our daily life. Therefore, this study suggests that pregnant women should be well cared for, when they are exposed to the organic solvents somewhere like workplaces, as well as habitually sniffing glues.

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