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EVALUATION OF THE EMBRYOTOXICITY OF HYDRAZINE IN RATS

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



ROGER C. INMAN, Colonel, USAF
Chief

Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Hydrazine (Hz) was evaluated for embryotoxic and teratogenic potential in rats. Pregnant Fischer 344 (F-344) rats were treated with 0, 2.5, 5.0, and 10.0 mg Hz/kg ip on gestation days 6-15. Dose-related embryolethality and maternal toxicity were observed at the two higher doses. Pregnant F-344 rats were treated with 10.0 mg Hz/kg ip on gestation days 7-9, 10-12, or 13-15. The		

prenatal period most susceptible to H₂ toxicity was days 7-9. Embryo lethality and an increase in the incidence of anomalies, but not major malformations, were observed in this group. Pregnant F-344 rats were percutaneously treated with 0, 5.0, and 50.0 mg H₂/kg on gestation day 9. The higher dose produced a high incidence of embryo lethality. Pregnant F-344 rats were treated with 10.0 mg H₂/kg ip on gestation days 7-9. Pups were evaluated for postnatal effects of prenatal H₂ treatment. An increase in perinatal mortality was observed, but none of the developmental parameters monitored including weight gain, ear detachment, incisor eruption, eye opening, surface righting, cliff avoidance, forward motion, and swimming ability were adversely affected. The principal toxic effect of prenatal H₂ treatment was embryo lethality which was observed at doses which produced maternal toxicity.

PREFACE

This research was performed in the Toxicology Branch, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory, from May 1978 through January 1982. It was performed in support of project 6302 "Occupational and Environmental Hazards in Air Force Operations"; task 630208, "Toxicology of Aerospace Fuels"; work unit 63020804, "Chronic Toxicology of Hydrazine Strategic Missile Fuels".

The authors acknowledge the technical assistance of Sgt T. Whittaker and SSgt P. Chambers.

Evaluation of the Embryotoxicity of Hydrazine in Rats¹

INTRODUCTION

Hydrazine (N_2H_2) is a potent reducing agent with wide application in various industrial processes. It is used by the Air Force either as the neat material or in combination with other compounds as a missile propellant.

The toxic properties of hydrazine (Hz) have been recognized since 1887 when Curtis described the effects of inhaled Hz vapors (Clark, 1953). Since then the biological effects of Hz have been widely studied, and a complete review of the published literature can be found in the Hz occupational exposure criteria document published by NIOSH (1978). Most of these studies have dealt with the toxicity or carcinogenic potential of Hz exposure; a few have described its embryotoxicity. Lee and Aleyassine (1970) treated pregnant rats with 8.0 mg Hz/kg/day for 10 days. Fetal survival rate and fetal body weight of the Hz treated litters were found to be less than of unexposed controls, but no gross malformations were observed. Lyng et al. (1980) treated pregnant mice with various doses of Hz and examined the 17-day litters. A dose-related increase in fetal abnormalities and embryonic death was observed. Despite the paucity of definitive studies, Hz is one of the chemicals regarded as a special hazard to the fetus (Rawls, 1980).

The potential embryotoxicity associated with Hz exposure during the handling of propellant materials is an important area of concern since women in the Air Force now fill many roles formerly held solely by men, and the majority of these women are of childbearing age.

The purpose of this study was to evaluate further the potential embryotoxicity of Hz in rodents.

¹ - Portions of this work were presented at the 1980 Annual Meeting of the Society of Toxicology, Abstract No. 61.

MATERIALS AND METHODS

Experiment 1a

Rats dosed on days 6-15

Virgin female Fischer 344 rats² were housed in plastic cages containing wood chip bedding in a room maintained at 70-76° F with a 12 hour light cycle. The rats received food³ and water ad libitum. The females were placed with fertile males of the same stock overnight and checked for presence of sperm by vaginal wash the next morning. The day on which sperm was found was designated day 0 of pregnancy. The pregnant rats were weighed daily. Hydrazine⁴ was diluted with physiologic saline and administered by ip injections at concentrations of 2.5, 5.0 and 10.0 mg/kg daily on days 6 through 15 of gestation. Rats serving as negative controls were injected with an equivalent volume of physiologic saline. The pregnant females were sacrificed on day 20 and the fetuses delivered by caesarean section. The number and placement of fetuses and resorption sites were recorded. Fetuses were removed, weighed, sexed, and examined for external abnormalities. About 2/3 of each litter were fixed in Bouin's solution and the remainder in absolute ethanol. Fetuses fixed in Bouin's solution were serially sectioned with a razorblade and examined under a dissection microscope for soft tissue abnormalities (Wilson and Warkany, 1965). Fetuses fixed in ethanol were cleared in KOH, stained with Alizarin Red S, and examined for skeletal abnormalities (Dawson, 1926). Measured data were analyzed for statistical significance by the Student's t method, and are listed as mean $\pm$ standard error (SE). Incidence data were analyzed with Fisher's exact test. The level of significance chosen for all tests was $p \leq 0.05$.

Experiment 1b

Rats dosed for 3 day periods

Because the high incidence of early embryonic death found in experiment 1a precluded examination of Hz effects on later embryonic development it was decided to dose pregnant rats during one of three shorter injection periods (days 7-9, days 10-12, days 13-15) and determine the incidence of resorptions and fetal abnormalities. A dose of 10.0 mg Hz/kg/day was administered. The experiment was carried out as described for experiment 1a.

Experiment 2

Percutaneous Hz treatment

Pregnant rats were treated percutaneously with doses of either 0, 5.0, or 50.0 mg Hz/kg on gestation day 9. Hair was clipped from the flank with a No. 40 blade and Hz applied to the area. The exposed area was then covered with a 2.5 cm square patch of plastic held in place with adhesive tape. Thirty minutes post-application the patch was removed and the Hz-treated area

2 - Charles River Breeding Laboratories, Wilmington, MA.

3 - Ralston Purina Company, St. Louis, MO.

4 - Eastman, Rochester, NY. (< 95%)

was rinsed with dilute hypochlorite solution and H₂O. The animals were returned to their respective cages and the remainder of the experiment was carried out as described for experiment 1a.

Experiment 3 Postnatal evaluation

Pregnant rats (10/group) were injected ip with 10.0 mg H₂/kg or physiologic saline (controls) on gestation days 7 through 9. They were placed in individual cages on day 20 and held for the duration of the experiment. Litters were examined following parturition for abnormalities and perinatal death. On postparturition day 1 litters were sexed, weighed, and adjusted to a litter size of 5 pups by sacrifice and crossfostering of pups. Crossfostering produced 4 groups of pups: controls; H₂ treated; H₂ treated crossfostered to control dams, and control crossfostered to H₂ treated dams. Pups were also weighed on postnatal days 7, 14, and 21. Evaluation of postnatal development included bilateral pinna detachment, lower incisor eruption, and eye opening. Time to occurrence of surface righting, cliff avoidance, forward motion, and swimming were also determined. The righting reflex was evaluated by placing the pups on their backs on a textured surface and observing them for 20 seconds. The criterion was met when the pup had turned over and placed all four limbs in a weight-bearing position. Forward motion was evaluated by placing the pups on a flat textured surface at eye level, and eliciting movement. The criterion was met when both head and body were simultaneously free of the surface while the animal was moving forward. Cliff avoidance was evaluated by placing the pups on an elevated edge with the noses and forepaws just over the precipice. The criterion was met when the pups completely withdrew from the edge by moving either backwards or sideways away from the edge within 20 seconds. Swimming was evaluated by immersing the pups and releasing them, allowing them to rise to the surface and begin swimming. The criterion was met when the pups paddled with the front limbs sufficiently well to keep the nares continuously above water. Trials were performed on each pup twice daily between 8 to 11 am until the criterion was met. Analyses of variance were performed on the postnatal development data with the Newman Keuls post test used to evaluate individual groups in the event of significant F ratios (Winer, 1971).

RESULTS

Experiment 1a Rats dosed on days 6-15

A significant dose-related increase in the number of resorptions/litter occurred at Hz doses of 5.0 mg/kg or greater (Table 1). With the exception of one litter which had no resorptions and six viable fetuses, all litters exposed to 10.0 mg Hz/kg were totally resorbed. Most of the resorptions were early with only metrial glands remaining. Slight but significant decreases in weights occurred in the 20-day 5.0 mg Hz/kg treated fetuses (Table 1). No significant increase in the incidence of fetal abnormalities were seen after dosing with Hz (Table 1). However, only one litter could be examined at the 10.0 mg Hz/kg dose because of the high incidence of resorptions. A dose-related reduction in weight gain was found in the Hz treated pregnant females (Figure 1). The rats in the 5.0 mg Hz/kg group gained weight throughout the treatment period but the weight gains were less than controls. Rats dosed with 10.0 mg Hz/kg began to lose weight after the first dose, and weight loss continued through the 10 day dose regimen. Body weight began to increase after the dose regimen was completed.

TABLE 1. EFFECT OF HYDRAZINE TREATMENT ON LITTER PARAMETERS

PARAMETER	HZ DOSE (MG/KG) ^A			
	0	2.5	5.0	10.0
NUMBER OF LITTERS	27	17	19	6
IMPLANTS/LITTER ^B	8.2 ± 0.6	8.1 ± 0.7	6.5 ± 0.7	7.0 ± 1.9
RESORPTION/LITTER ^B	1.5 ± 0.4	1.8 ± 0.4	3.3 ± 0.7 ^C	6.0 ± 2.3 ^C
NUMBER OF LITTERS MORE THAN 50% RESORBED	4	1	10	5
FETAL WEIGHT ^B (GRAMS)	3.1 ± 0.04	3.1 ± 0.04	2.9 ± 0.1 ^C	3.1 ± 0.3
INCIDENCE OF ABNORMALITIES LITTER (FETUSES) EXAMINED	27(181)	17(107)	15(60)	1(6)
LITTER (FETUSES) EFFECTED	8(11)	4(5)	7(8)	1(3)
MAJOR MALFORMATIONS	7 ^{E,F}	2 ^E	4 ^G	0
ANOMALIES ^D	6	3	4	3

A INTRAPERITONEAL INJECTION GIVEN ON GESTATION DAYS 6-15

B MEAN S.E.

C SIGNIFICANTLY DIFFERENT FROM CONTROL $P \leq 0.05$

D ANOMALIES CONSISTED OF SUPERNUMERARY RIBS, FUSED RIBS, DELAYED OSSIFICATION, MODERATE HYDRONEPHROSIS, MODERATE DILATION OF BRAIN VENTRICLES AND OTHER SIMILAR BUT LESS FREQUENTLY OCCURRING ABNORMALITIES

E MAJOR MALFORMATION WAS ANOPHTHALMIA

F THREE FETUSES WITH ANOPHTHALMIA WERE FOUND IN ONE LITTER

G MAJOR MALFORMATIONS WERE ANOPHTHALMIA (2) RIGHT SIDE AORTA (1) AND MONORCHID (1)

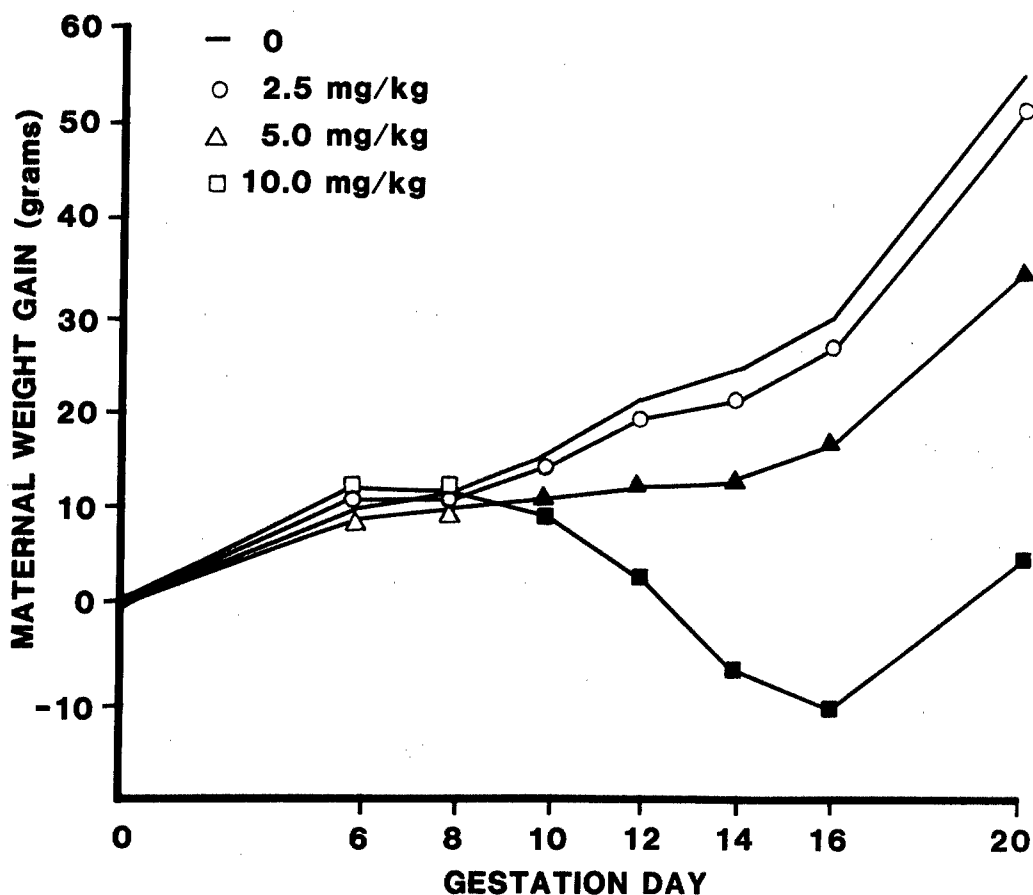


Figure 1. Effect of hydrazine on maternal weight gain in pregnant rats injected ip on gestation days 6-15. Solid symbols represent significance ($p \leq 0.05$) control versus test group.

Experiment 1b

Rats dosed for 3 day periods

The most susceptible period for the effects of HZ was during gestation days 7 through 9 (Table 2). The incidence of resorptions was significantly higher in this group than in either the control or litters treated with HZ during the later stages of gestation. The fetal weights were significantly reduced for both the days 7 through 9 treatment group and days 13 through 15 treatment group. The incidence of fetal abnormalities was significantly increased in the group treated with HZ on gestation days 7 through 9 (Table 2). Maternal weight loss was similar to that observed in the group injected with 10.0 mg HZ/kg on days 6 through 15. All 3 groups lost weight during the 3-day treatment period and gained weight after the dose regimen ended.

TABLE 2. EFFECT OF HYDRAZINE EXPOSURE PERIOD ON LITTER PARAMETERS

PARAMETER	EXPOSURE PERIOD (GESTATION DAYS) ^A			
	CONTROL (6-15) ^C	7-9	10-12	13-15
NUMBER OF LITTERS	27	11	1	10
IMPLANTS/LITTER ^B	8.2 ± 0.6	7.5 ± 1.1	8.9 ± 1.0	7.7 ± 1.4
RESORPTIONS/LITTER ^B	1.5 ± 0.4	6.1 ± 1.1 ^D	0.8 ± 0.4	1.0 ± 0.3
NUMBER OF LITTERS MORE THAN 50% RESORBED	4	8	0	0
FETAL WEIGHT ^B (GRAMS)	3.1 ± 0.4	2.7 ± 0.1 ^D	3.1 ± 0.1	2.9 ± 0.6 ^D
INCIDENCE OF ABNORMALITIES LITTERS (FETUSES) EXAMINED	27(181)	8(16)	10(81)	10(67)
LITTERS (FETUSES) EFFECTED	8(11)	6 ^D (8)	4(4)	4(4)
MAJOR MALFORMATIONS	7	0	2 ^E	0
ANOMALIES	6	8 ^F	2	4

^A ALL GROUPS RECEIVED 10.0 MG HZ/KG

^B MEAN S.E.

^C REPEATED FROM TABLE 1

^D SIGNIFICANTLY DIFFERENT FROM CONTROL $P \leq 0.05$

^E MAJOR MALFORMATIONS CONSISTED OF ANOPHTHALMIA AND ADRENAL AGENESIS

^F ANOMALIES DETECTED IN THE 7-9 DAY TREATMENT GROUP WERE SUPERNUMERARY RIBS (2) MODERATE HYDRONEPHROSIS (2) AND MODERATE HYDROCEPHALUS (4).

Experiment 2

Percutaneous Hz treatment

The incidence of resorptions was significantly increased in the rats treated with 50.0 mg Hz/kg (Table 3). The incidence of fetal abnormalities was not significantly elevated in either Hz treatment group. A circumscribed area of epidermal necrosis about 3 mm in diameter occurred in the pregnant females treated with 50.0 mg Hz/kg, while the group treated with 5.0 mg Hz/kg had an inconsistently necrotized area, usually less than 1 mm in diameter. The 50.0 mg Hz/kg treatment group also exhibited moderate CNS depression for several hours post treatment. In addition, the 50.0 mg Hz/kg treatment group exhibited a loss of weight 24 hours post treatment (-11.0 ± 0.8 gm), while the 5.0 mg Hz/kg group ($+0.9 \pm 0.9$ gm) and controls ($+2.1 \pm 0.5$ gm) gained weight.

TABLE 3. EFFECT OF PERCUTANEOUS HYDRAZINE TREATMENT ON LITTER PARAMETERS

PARAMETER	HZ DOSE (MG/KG) ^A		
	0	5	50
NUMBER OF LITTERS	11	13	12
IMPLANTS/LITTER ^B	8.3 ± 0.9	9.2 ± 0.8	9.6 ± 0.7
RESORPTIONS/LITTER ^B	0.3 ± 0.1	0.9 ± 0.3	9.4 ± 0.8
NUMBER OF LITTERS MORE THAN 50% RESORBED	0	0	12 ^{C,D}
FETAL WEIGHT ^B (GRAMS)	3.0 ± 0.03	3.0 ± 0.03	2.4 ^E
INCIDENCE OF ABNORMALITIES LITTERS (FETUSES) EXAMINED	11(87)	13(108)	2(2) ^E
LITTERS (FETUSES) EFFECTED	1(1)	3(3)	1(1)
MAJOR MALFORMATIONS	2 ^F	2 ^G	0
ANOMALIES	1	2	1

A HZ TREATED ON DAY 9

B MEAN S.E.

C SIGNIFICANTLY DIFFERENT FROM CONTROL $P \leq 0.05$

D TEN OUT OF TWELVE LITTERS WERE COMPLETELY RESORBED

E ONLY 2 PUPS WERE AVAILABLE FOR EXAMINATION

F MAJOR MALFORMATIONS WERE ANOPHTHALMIA AND SEVERE HYDROCEPHALUS BOTH OCCURRING IN THE SAME PUP.

G MAJOR MALFORMATIONS WERE CLEFT PALATE AND ANOPHTHALMIA BOTH OCCURRING IN THE SAME PUP.

Experiment 3

Postnatal evaluation

Postnatal survivability and litter size were decreased following HZ treatment. One half of the HZ treated litters had a perinatal mortality rate exceeding 25% while only 1 control litter had a perinatal mortality this high. The mean litter size was 4.7 ± 0.2 for HZ treated litters and 8.9 ± 0.1 for the control litters. The control pups were consistently heavier than the other groups throughout the 3 week evaluation period (Figure 2). The HZ-treated pups had the lowest day 1 weight while the control pups crossfostered to HZ treated dams had the lowest mean weight while the control pups crossfostered to HZ treated dams had the lowest mean weight on days 7, 14, and 21. However, no significant differences were found between groups with respect to postnatal weight gains. In addition, no significant differences in developmental parameter times were found between groups although the control group required the least time to achieve the criteria for 4 of the 7 parameters (Table 4).

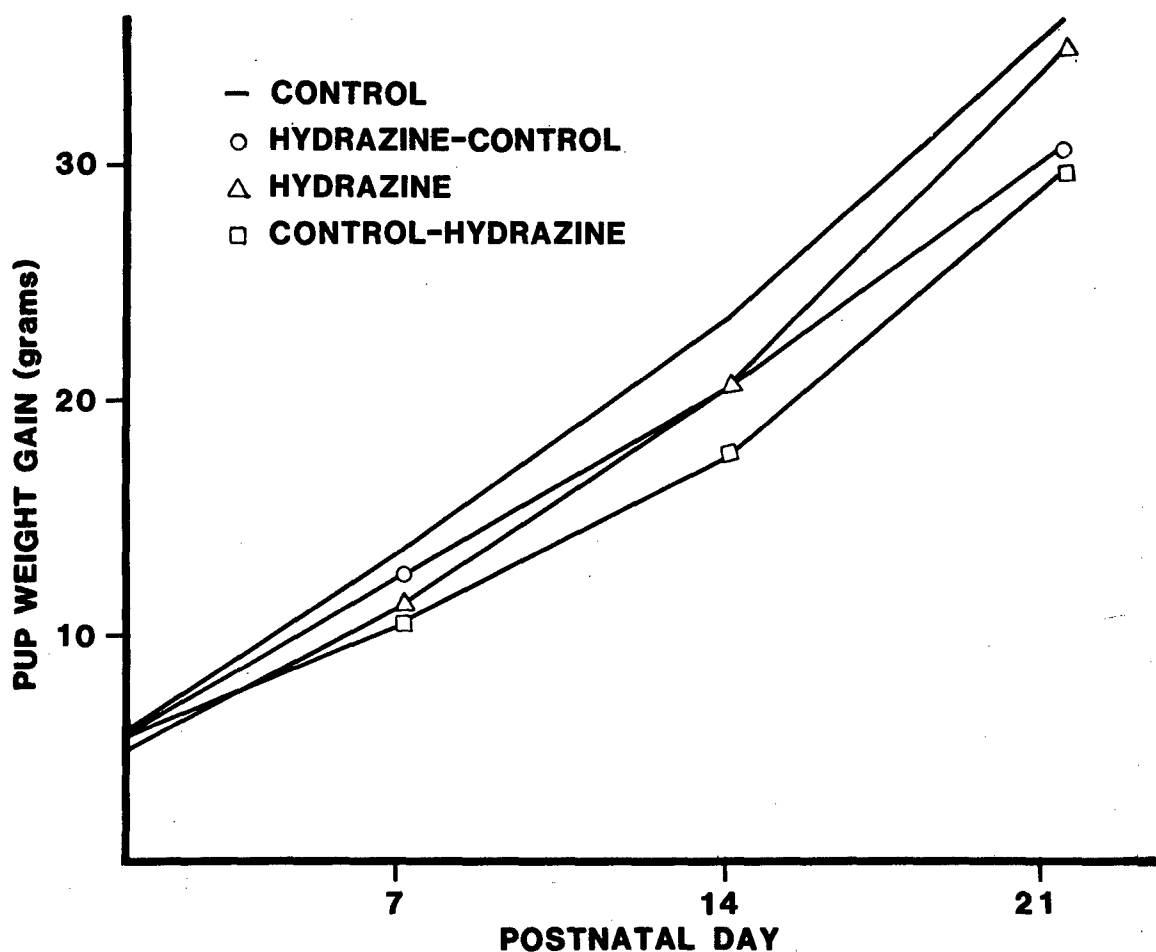


Figure 2. Effect of ip hydrazine treatment of pregnant rats during gestation days 7-9 on postnatal weight gain of pups. The control group contained 39 pups, hydrazine treated group contained 27 pups, hydrazine treated pups crossfostered to control dams (Hydrazine-Control) 9 pups, and the control pups crossfostered to hydrazine treated dams (Control-Hydrazine) 10 pups. A significant difference in weight gain ($p \leq 0.05$) was found within groups by analysis of variance but not between individual groups by Newman Keuls for days 7 and 14.

TABLE 4. EFFECT OF HYDRAZINE TREATMENT ON POSTNATAL DEVELOPMENT

PARAMETER	TREATMENT GROUP			
	CONTROL	CONT-HzA	HZ-CONTB	HZ
	23♂ 16♀	4♂ 5♀	5♂ 5♀	13♂ 14♀
NUMBER OF PUPS	39	9	10	27
PINNA DETACHMENT ^{C,D}	2.8 ± 0.02	2.9 ± 0.03	3.0 ± 0.0	3.0 ± 0.02
INCISOR ERUPTION (LOWER) ^{C,D}	9.2 ± 0.02	9.6 ± 0.07	9.5 ± 0.06	10.1 ± 0.02
EYE OPENING ^{C,D}	16.3 ± 0.03	16.8 ± 0.03	17.1 ± 0.06	16.5 ± 0.02
SURFACE RIGHTING ^{C,D}	2.0 ± 0.03	4.4 ± 0.3	2.5 ± 0.3	2.2 ± 0.04
CLIFF AVOIDANCE ^{C,D}	4.7 ± 0.06	4.8 ± 0.2	4.3 ± 0.2	5.9 ± 0.1
FORWARD MOTION ^{C,D}	6.9 ± 0.02	6.6 ± 0.1	6.4 ± 0.1	6.4 ± 0.06
SWIMMING ^{C,D}	6.1 ± 0.03	5.7 ± 0.1	5.7 ± 0.1	6.1 ± 0.04

A CONTROL PUPS CROSSFOSTERED TO HZ TREATED DAMS

B HZ TREATED PUPS CROSSFOSTERED TO CONTROL DAMS

C MEAN DAY S.F. PARAMETER CRITERIA WERE MET

D NO SIGNIFICANT DIFFERENCES FOUND WITHIN GROUPS BY ANOVA ($P \leq 0.05$)

DISCUSSION

These results demonstrated that H₂ was embryotoxic in the rat. The embryotoxicity was manifested as a dose-related embryoletality, with about 50% mortality produced at a daily dose of 5.0 mg H₂/kg and 90% mortality at a daily dose of 10.0 mg H₂/kg. No effect was observed at a dose of 2.5 mg/kg. Another significant observation indicative of embryotoxicity was the reduced mean weight of the 20-day fetuses treated with 5.0 mg H₂/kg. The lack of a significantly reduced fetal weight for the 10.0 mg H₂/kg treated fetuses can be explained by the fact that the only surviving fetuses came from one unusual litter which seemed to be unaffected by the H₂ treatment (Table 1).

The developing embryo is apparently more susceptible to the toxic effects of H₂ during early organogenesis than late organogenesis. The incidence of resorptions was greatly increased following treatment of pregnant females on days 7 through 9 of gestation compared to the incidence of resorptions following treatment on days 10 through 12 or days 13 through 15 of gestation. Two other observations indicative of increased susceptibility to H₂ in early organogenesis are the reduced weight of the 20-day fetuses treated with H₂ on gestation days 7 through 9 and the increased incidence of fetal abnormalities in this group. It should be emphasized that the majority of these fetal abnormalities were of an anomalous nature rather than being major malformations. This is somewhat similar to the results reported by Lyng et al. (1980) where the incidence of fetal abnormalities in the mouse was significantly increased for H₂-treated litters, and the principal types of abnormalities were also of the anomalous type (supernumerary ribs and hydronephrosis). The mean weight of the 20-day fetuses treated with H₂ on gestation days 13 through 15 was also significantly reduced indicating fetotoxicity due to H₂. This finding supports the report by Lee and Aleyassine (1970) that H₂ is fetotoxic.

We consider the high incidence of embryonic mortality produced by percutaneous H₂ treatment of 50.0 mg/kg on gestation day 9 to be a highly significant but not surprising finding since H₂ is well absorbed percutaneously (Keller et al., 1982). A marked 24 hour weight loss and depression in this H₂ treatment group also indicates maternal toxicity was present at this dose.

Evidence for deleterious postnatal effects of embryonic H₂ exposure was ambiguous. Although there was an increase in perinatal deaths, no significant effects of H₂ were found in postnatal development tests.

Although these data do not indicate the need for a lower H₂ TLV for women of childbearing age, they do imply that the occasional accidental H₂ spill which leads to a short-term, high-level H₂ exposure may pose a greater risk for the embryo than for adults receiving the H₂ exposure.

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