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TERATOLOGIC EVALUATION OF A MODEL PERFLUORINATED ACID, NDFDA

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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

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The teratogenic effect of a model perfluorinated acid, nonadecafluorodecanoic acid (NDFDA), was evaluated in Fischer 344 rats. Rats were given 0, 5, 10, or 15 mg/kg NDFDA by gavage on Day 9 of gestation. Rats were also treated with 0, 10, 20, or 40 mg/kg NDFDA by gavage on Day 12 of gestation. There were no significant differences between treatments and controls at 5, 10, or 15 mg/kg/Day 9 gestation and 10 or 20 mg/kg/Day 12 of gestation. There was a statistically significant decrease in

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maternal body weight gain, corpora lutea, resorptions, and mean fetal body weight at 30 mg/kg/Day 12 of gestation. There were no significant differences between experimentals and control animals in terms of soft tissue or skeletal abnormalities at any dosage level.

PREFACE

This research was performed in the Toxicology Branch, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory from June 1980 through August 1980. It was performed in support of Project 6302,"Occupational and Environmental Toxic Hazards in Air Force Operations;" Task 630201, "Toxicology of Conventional Propellants, Industrial Chemicals and Materials," Work Unit 63020104, "Teratogenic Screening of Air Force Chemicals." It was performed as a Summer Faculty Research Project by Dr. Inez Bacon.

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INTRODUCTION

Nonadecafluorodecanoic acid (NDFDA), a model perfluorinated acid, is structurally related to a surfactant agent used in fire retardant foams by the Air Force. The available literature on the toxicity of NDFDA is limited to an "in house" study at the Air Force Aerospace Medical Research Laboratory, Toxic Hazards Division, Wright-Patterson AFB, Ohio. NDFDA was cited as causing histopathological damage to the thymus, bone marrow, stomach, mesentery, liver, and testes in the male rats (Andersen, 1979)*. These results showed the effect exposure to NDFDA may exhibit in Air Force men, but did not depict the potential hazard in Air Force women. In view of the need for an evaluation of the teratogenic and/or embryotoxic potential of NDFDA, the present study was conducted.

In this study, groups of pregnant rats were exposed to various dose levels of NDFDA by gavage, and various parameters were evaluated in order to assess the potential teratogenicity during gestation.

METHODS AND MATERIALS

NDFDA, purity 98%, was used in this study. Fischer 344 rats were housed in plastic cages in a room with controlled temperature (70 - 76 F) and light cycle (12 h light and dark). The animals were maintained on Ralston Purina Laboratory Chow and tap water ad libitum. The rats were allowed to acclimate one week to their environment before use. The day on which sperm was observed in a vaginal smear was considered Day 0 of gestation.

NDFDA was given in propylene glycol and water (50:50) at a dose volume of 2 ml/kg body weight. Pregnant rats were given 5, 10, or 15 mg/kg NDFDA once on Day 9 of gestation and 10, 20, 30 or 40 mg/kg on Day 12 of gestation. Control rats were given the vehicle. These dose levels were selected on the basis of a preliminary study in which severe weight loss and maternal death occurred in females dosed with 20 mg/kg NDFDA on Day 9 of gestation. Females dosed with the same concentration on Day 12 of gestation remained viable.

The animals were observed daily from Day 9 or 12 of gestation until sacrifice or indications of toxicity from NDFDA. Maternal body weights were recorded daily. Dams were sacrificed by halothane inhalation on Day 20 of gestation.

Using the method of Olson and Back (1978), the numbers of corpora lutea, implantations, live and/or dead and resorbed fetuses were noted. The position of fetuses in the uterus, sex of fetuses, external examinations, and weights were also recorded. After being weighed, one-half (1/2) of the fetuses from each litter were fixed in absolute ethyl alcohol for subsequent clearing and staining with Alizarin Red S (Dawson, 1926) to permit examination for skeletal abnormalities. The remaining fetuses were fixed in Bouin's solution and sectioned according to the method of Wilson and Warkany (1965) in order to

*Andersen, M. E. (1979), Personal communication

detect internal malformations of the soft tissue.

The incidences of implantations per female, fetal resorptions, viable fetuses, body weights, skeletal and soft tissue abnormalities were evaluated by the t-test. In all cases, the level of significance chosen was $p < 0.05$.

RESULTS

Administration of 30 mg/kg/Day 12 of gestation produced significant maternal toxicity in rats (decreased body weight gain) (Table 2). At 20 mg/kg/Day 9 gestation, 6 of 6 females died. In comparison, no maternal deaths were observed among rats administered 10, 20, or 30 mg/kg/Day 12 of gestation. Day 9 controls and tests were not significantly different (Table 1). There was a significant difference in corpora lutea, resorptions, and live fetuses per litter for dams treated with 30 mg/kg NDFDA/Day 12 of gestation (Table 2).

TABLE 1

REPRODUCTIVE FINDINGS IN RATS TREATED WITH NDFDA ON DAY 9 OF GESTATION

	NDFDA (Mg/Kg) DAYS 9 - 20)			
	Vehicle control	5	10	15
Maternal weight gain(g)	54.0 ± 9.3	52.7 ± 12	57.1 ± 15.4	56.1 ± 16.6
Number of litters	23	15	19	19
Corpora lutea / dam[a]	10.7 ± 1.2	11.1 ± 1.8	11.4 ± 1.5	11.2 ± 1.5
Implantations / dam[a]	9.5 ± 2.0	9.3 ± 3.2	10.0 ± 2.7	10.7 ± 2.7
Resorptions / litter[a]	0.4 ± 0.7	0.7 ± 1.0	1.1 ± 2.3	0.8 ± 1.7
Number of litters with resorptions	7	6	8	7
Live fetuses / litter[a]	9.1 ± 1.9	8.7 ± 2.9	9.1 ± 3.4	9.9 ± 3.3
Fetal body weight(g)[b]	3.3 ± 0.1	3.1 ± 0.2	3.2 ± 0.4	3.1 ± 0.4

[a] Mean ± SD.

[b] Mean of litter mean ± SD.

However, there was no sigificant difference between control and experimental implantation sites. The mean fetal body weight from 30 mg/kg/Day 12 dams were significantly lower than the control values ($p < 0.002$). Three of 7 dams treated with 40 mg/kg NDFDA/Day 12 of gestation died on Day 16 of gestation. The remaining 4 dams showed a significant decrease in maternal body weight (mean = -32.50 ± 14.43 S.D., $0.0005 < p < 0.001$, Mann-Whitney U test). Fetuses from these dams had a mean fetal body weight of 1.77 + 0.74 S.D. ($0.0005 < p < 0.001$, Mann-Whitney U test).

TABLE 2

REPRODUCTIVE FINDINGS IN RATS TREATED WITH NDFDA ON DAY 12 OF GESTATION

	NDFDA (Mg/Kg) DAYS 12 - 20			
	Vehicle control	10	20	30
Maternal weight gain(g)	48.5 ± 7.0	52.3 ± 5.5	46.0 ± 15.3	29.5 ± 16.1[c,f]
Number of litters	18	16	23	20
Corpora lutea / dam[a]	11.8 ± 1.6	11.3 ± 1.1	11.8 ± 1.4	10.8 ± 1.2[d,f]
Implantations / dam[a]	10.5 ± 2.0	10.8 ± 1.1	10.2 ± 2.2	9.6 ± 2.3
Resorptions / litter[a]	0.2 ± 0.4	0.4 ± 0.6	0.2 ± 0.4	1.0 ± 1.4[d,f]
Number of litters with resorptions	3	6	5	9
Live fetuses / litter[a]	10.3 ± 2.0	10.4 ± 1.1	9.8 ± 2.3	8.4 ± 2.6[c,f]
Fetal body weight(g)[b]	3.2 ± 0.2	3.3 ± 0.1	3.1 ± 0.2	2.9 ± 0.4[e,f]

[a] Mean ± SD.

[b] Mean of litter mean ± SD.

[c] Significantly different from the control value by the t-test, $p<0.001$.

[d] Significantly different from the control value by the t-test, $p<0.02$.

[e] Significantly different from the control value by the t-test, $p<0.002$.

[f] S^2 is a pooled estimate of the population variance (σ^2), based on the s of both samples combined.

Malformations observed among the litters of rats given NDFDA did not occur at an incidence significantly different from that of the control rats (Table 3). The only malformation observed in the experimental litters of which was not observed among either of the control groups was one case of microphthalmia at 5 mg/kg/Day 9 of gestation.

TABLE 3

INCIDENCE OF FETAL MALFORMATIONS AMONG FETUSES FROM RATS GIVEN NDFDA

	NDFDA (Mg/Kg) DAYS 9 - 20				NDFDA (Mg/Kg) DAYS 12 - 20			
	Vehicle control	5	10	15	Vehicle control	10	20	30
External examination[a]	209(23)	130(15)	172(19)	188(19)	186(18)	166(16)	225(23)	169(20)
Soft tissue examination[a]	105(12)	65(7)	86(10)	94(9)	93(9)	83(8)	113(11)	84(10)
Skeletal examination[a]	104(11)	65(8)	86(9)	94(10)	93(9)	83(8)	112(12)	85(10)
External examination[b] microphthalmia	0(0)	1(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Soft tissue examination[b]								
undescended testis	1(1)	1(1)	0(0)	0(0)	1(1)	0(0)	0(0)	4(2)
displacement of testis	0(0)	1(1)	0(0)	0(0)	1(1)	0(0)	0(0)	0) 0)
Skeletal examination[b]								
bipartite sternebrae	0(0)	0(0)	0(0)	1(1)	4(3)	2(1)	2(2)	4(4)
split centrum	4(2)	3(2)	0(0)	0(0)	0(0)	0(0)	3(3)	2(2)
Total malformed	5(3)	6(5)	0(0)	1(1)	6(5)	2(1)	5(5)	10(8)

[a] Number of fetuses (number of litters) examined.

[b] Number of fetuses (number of litters) affected.

DISCUSSION

Evidence of teratogenicity was not observed in fetuses of dams administered NDFDA by gavage on Day 9 of gestation. Toxic effects in fetuses occurred only in the presence of dose-related maternal toxicity (less maternal body weight gain at 30 mg/kg/Day 12 of gestation). There was a significant decrease in the mean fetal body weight among the litters of rats treated with 30 mg/kg/Day 12 of gestation. There was also an increase in resorptions and a corresponding decrease in the number of live fetuses per litter. There was no significant difference between control and experimental soft tissue or skeletal abnormalities at any dosage level. Butcher et al. (1972) reported that some compounds, when administered at the critical stage of organogenesis in doses which do not cause malformations, can induce postnatal toxic effects and lasting behavioral changes in the offspring. This hypothesis was also supported by Tanimura (1976) and Frohberg (1975 and 1977).

In contrast to maternal death of dams treated with 20 mg/kg NDFDA on Day 9 of gestation, maternal death did not occur among dams treated with 10, 20, or 30 mg/kg NDFDA on Day 12 of gestation. The reason for this phenomenon is not clear; however, differences in the effects may be due to differences in the amount of maternal plasma proteins and/or other changes as a result of hormonal or enzymatic activity at that stage of pregnancy.

Since fetal toxicity paralleled maternal toxicity, it might be useful to conduct postnatal studies to determine whether NDFDA causes behavioral changes in pups.

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