Evaluation of the Subchronic, Reproductive, and Developmental Toxicity of a Fluoroalkylethyl Surfactant

S.A. MacKenzie, E. Mylchreest, S.M. Munley, J.C. Stadler, J.F. Hansen, and N.E. Everds
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Abstract

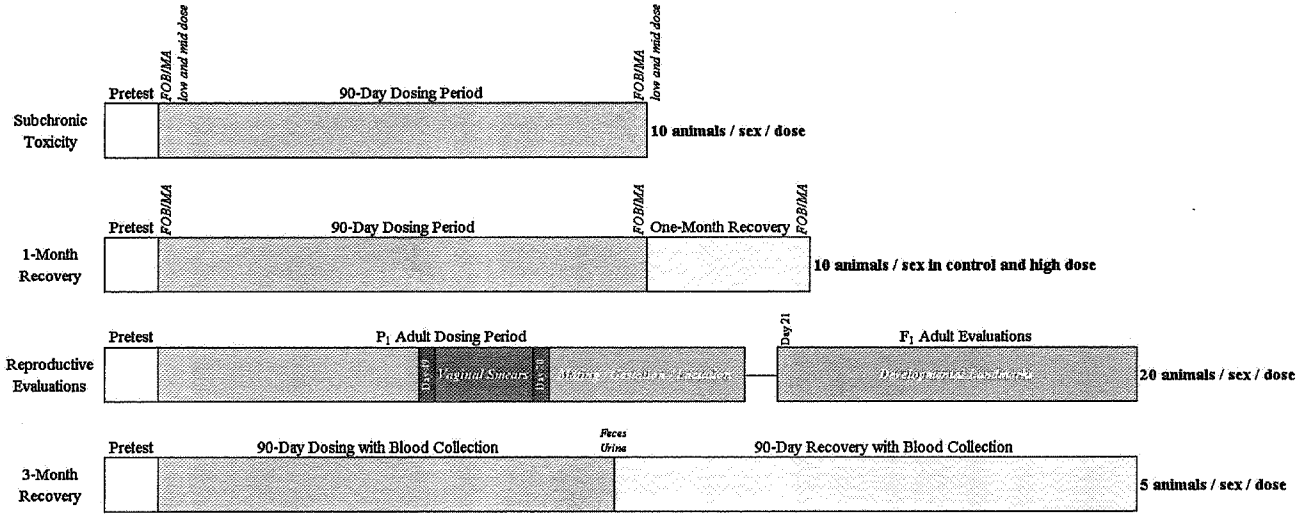
The objective of these studies was to evaluate the subchronic, reproductive, and developmental toxicity of a fluoroalkylethyl ethoxylate surfactant in rats. Test substance was administered by gavage at 0, 25, 100, or 500 mg/kg/day (90-day subchronic toxicity and one-generation reproduction), and at 0, 50, 100, or 400 mg/kg/day on gestation days 6-20 (developmental toxicity). The NOEL for subchronic toxicity was 25 mg/kg/day based on clinical signs, reduced body weight and nutritional parameters, thyroid follicular hypertrophy, and chronic progressive nephropathy at ≥100 mg/kg/day. Other adverse effects at 500 mg/kg/day were reduced red blood cell mass, increased liver enzyme activity, and reduced grip strength. Non-adverse liver hypertrophy and splenic effects were observed at ≥25 and ≥100 mg/kg/day, respectively. Except for effects on the thyroid at 500 mg/kg/day and kidney at ≥100 mg/kg/day, other effects were reversible after a 1- or 3-month recovery. No NOEL was determined for reproductive toxicity, based on reduced fertility in P_1 rats at ≥25 mg/kg/day, which was of similar magnitude at all dose levels. Body weights, food efficiency, and number of implantation sites were reduced in P_1 rats at 500 mg/kg/day, and viability and weights were reduced in F_1 pups at ≥100 mg/kg/day. The reduced pup viability was relatively less severe and required much higher doses than the structurally related perfluorooctane sulfonate. Red blood cell and spleen effects were similar to those observed with ethylene glycol ethers, but there were no developmental or testicular effects as seen with glycol ethers. The mechanism for reduced fertility is not known. In the developmental toxicity study, the NOELs were 50 and 100 mg/kg/day for maternal and fetal toxicity, respectively, based on reduced maternal body weight and nutritional parameters, and clinical signs at ≥100 mg/kg/day and reduced fetal weight and increased fetal variations at 400 mg/kg/day. No fetal malformations were observed.

Introduction

The objective of these studies was to evaluate the subchronic, reproductive, and developmental toxicity of a fluoroalkylethyl ethoxylate surfactant in rats. The test substance was administered by gavage, the route selected as the most efficient way to administer an accurate dose. In a two-month rangefinder study, rats demonstrated body weight loss when exposed to 1000 mg/kg/day and male rats demonstrated reduced body weight gain when exposed to 500 mg/kg/day. Blood fluorine levels plateaued within 2-3 weeks of dosing. The high dose of 500 mg/kg/day for the 90-day study was expected to produce toxicity without excessive mortality. The low dose of 25 mg/kg/day was expected to be the no-observed-effect level, while the 100 mg/kg/day dose was expected to induce minimal or no toxicity. The doses for the developmental toxicity study of 0, 50, 100, and 400 mg/kg/day were selected based on a pilot study in which maternal and fetal toxicity were observed at $\geq$400 mg/kg/day but no maternal or fetal toxicity were observed at $\leq$100 mg/kg/day.

Study Design

90-Day and One-Generation Reproduction Study



- Body weights, detailed clinical signs, and food consumption were determined weekly throughout the 90-day exposure and 1-month recovery periods.
- Clinical pathology, gross and microscopic pathology, and biochemical evaluations were performed at the end of the 90-day exposure, 1-month recovery, and 3-month recovery periods.
- Reproductive evaluations were performed prior to mating and during the mating, gestation, and lactation periods. Developmental landmarks and gross pathology was also assessed on F_1 adults.

Developmental Toxicity Study

- Groups of time-mated rats (22/dose) received oral gavage dose once daily over gestation days 6-20.
- Body weight, food consumption, and clinical observations data were collected.
- Dams were euthanized on gestation day 21 and examined for gross external and visceral alterations.
- Uteri were weighed and dissected and uterine contents were examined.
- Fetuses were removed, weighed, sexed, and examined for external, visceral, head, and skeletal alterations.

Figure 1: Mean Body Weights of Male Rats

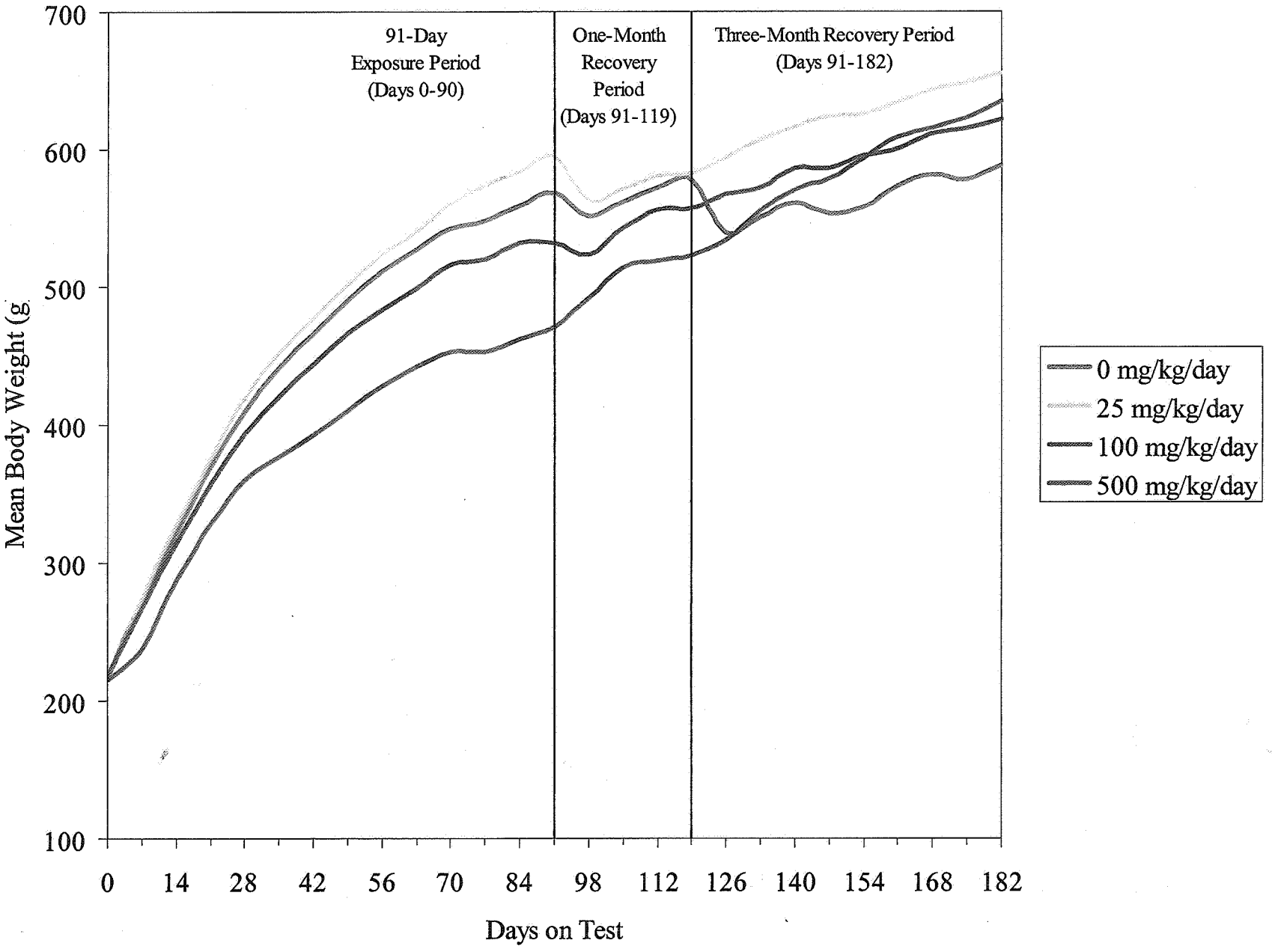


Figure 2: Mean Body Weights of Female Rats

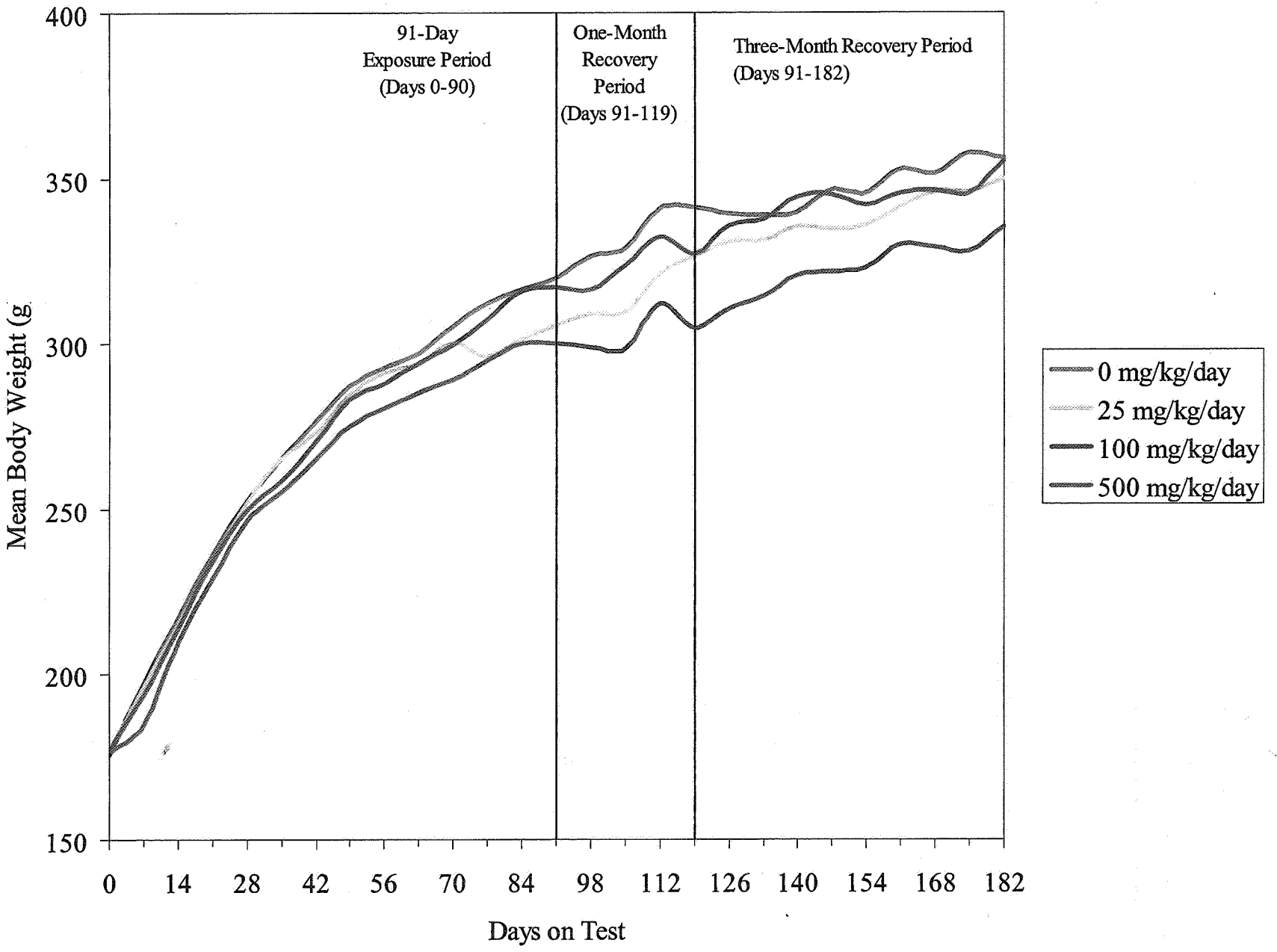


TABLE 1

CLINICAL PATHOLOGY DATA

		Males			Females		
	Dose (mg/kg/day):	25	100	500	25	100	500
RBC (x10^6/µL)	end-of-dosing	100%	98%	103%	97%	***91%***	***89%***
	1-month recovery	ND	ND	97%	ND	ND	98%
	3-month recovery	99%	98%	97%	100%	99%	101%
HGB (g/dL)	end-of-dosing	99%	***95%***	***92%***	97%	***90%***	***85%***
	1-month recovery	ND	ND	***95%***	ND	ND	97%
	3-month recovery	101%	98%	99%	101%	94%	97%
HCT (%)	end-of-dosing	99%	96%	***94%***	98%	***97%***	***96%***
	1-month recovery	ND	ND	96%	ND	ND	97%
	3-month recovery	100%	99%	100%	104%	96%	100%
SDH (U/L)	end-of-dosing	113%	124%	130%	89%	68%	75%
	1-month recovery	ND	ND	***219%***	ND	ND	112%
	3-month recovery	157%	160%	***221%***	86%	120%	131%
ALT (U/L)	end-of-dosing	109%	112%	***152%***	76%	61%	73%
	1-month recovery	ND	ND	***129%***	ND	ND	***55%***
	3-month recovery	156%	147%	***194%***	83%	94%	109%
Plasma F (µg/mL)	end-of-dosing	200%	***200%***	***500%***	100%	200%	***500%***
	1-month recovery	ND	ND	100%	ND	ND	***NC***
	3-month recovery	100%	100%	100%	100%	100%	100%
Urine F (µg)	end-of-dosing	418%	***1164%***	***5228%***	431%	***1080%***	***5295%***
	1-month recovery	ND	ND	***1214%***	ND	ND	***714%***
	3-month recovery	132%	211%	459%	146%	168%	***266%***
Urine Vol. (mL)	end-of-dosing	134%	85%	***368%***	194%	166%	***351%***
	1-month recovery	ND	ND	123%	ND	ND	63%
	3-month recovery	77%	78%	111%	139%	135%	131%

Values are percent of control. Those in ***green bold italics*** are statistically significant.
ND = No data
NC = Not calculable as control value was zero.

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RBC (x10^6/µL)	end-of-dosing	100	98	103	97	***91***	***89***
	1-month recovery	ND	ND	97	ND	ND	98
	3-month recovery	99	98	97	100	99	101
HGB (g/dL)	end-of-dosing	99	***95***	***92***	97	***90***	***85***
	1-month recovery	ND	ND	***95***	ND	ND	97
	3-month recovery	101	98	99	101	94	97
HCT (%)	end-of-dosing	99	96	***94***	98	***97***	***96***
	1-month recovery	ND	ND	96	ND	ND	97
	3-month recovery	100	99	100	104	96	100
SDH (U/L)	end-of-dosing	113	124	130	89	68	75
	1-month recovery	ND	ND	***219***	ND	ND	112
	3-month recovery	157	160	***221***	86	120	131
ALT (U/L)	end-of-dosing	109	112	***152***	76	61	73
	1-month recovery	ND	ND	***129***	ND	ND	***55***
	3-month recovery	156	147	***194***	83	94	109
Plasma F (µg/mL)	end-of-dosing	200	***200***	***500***	100	200	***500***
	1-month recovery	ND	ND	100	ND	ND	***NC***
	3-month recovery	100	100	100	100	100	100
Urine F (µg)	end-of-dosing	418	***1164***	***5228***	431	***1080***	***5295***
	1-month recovery	ND	ND	***1214***	ND	ND	***714***
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	1-month recovery	ND	ND	123	ND	ND	63
	3-month recovery	77	78	111	139	135	131

Values are percent of control. Those in ***green bold italics*** are statistically significant.
ND = No data
NC = Not calculable as control value was zero.

TABLE 2

HISTOPATHOLOGY DATA

	Males				Females			
Dose (mg/kg/day):	0	25	100	500	0	25	100	500
End-of-Dosing								
Thyroid								
Hypertrophy, Follicular	0/11	1/10	***9/11***	***11/12***	0/10	0/10	***2/10***	***9/10***
Alteration, Colloid	6/11	10/10	***10/11***	***11/12***	3/10	***6/10***	***10/10***	***10/10***
Kidney								
Nephropathy, Chronic Progressive	5/11	2/10	5/11	***7/12***	2/10	2/10	***5/10***	***5/10***
Liver								
Hypertrophy, Centrilobular	1/11	***5/10***	***10/11***	***12/12***	0/10	0/10	***9/10***	***10/10***
Spleen								
Hematopoiesis	0/11	1/10	***8/11***	***10/12***	0/10	1/10	***6/10***	***7/10***
Pigment	0/11	0/10	***4/11***	***6/12***	1/10	0/10	0/10	***10/10***
1-Month Recovery								
Thyroid								
Hypertrophy, Follicular	0/10	ND	ND	***8/10***	0/10	ND	ND	***6/10***
Alteration, Colloid	6/10	ND	ND	***10/10***	1/10	ND	ND	***10/10***
Kidney								
Nephropathy, Chronic Progressive	3/10	ND	ND	***8/10***	0/10	ND	ND	***8/10***
Liver								
Hypertrophy, Centrilobular	0/10	ND	ND	***7/10***	0/10	ND	ND	1/10
Spleen								
Hematopoiesis	0/10	ND	ND	***8/10***	0/10	ND	ND	1/10
Pigment	0/10	ND	ND	***7/10***	0/10	ND	ND	0/10
3-Month Recovery								
Thyroid								
Hypertrophy, Follicular	0/4	0/5	0/4	***4/5***	0/4	0/5	0/4	0/4
Alteration, Colloid	1/4	***5/5***	***4/4***	***5/5***	1/4	***3/5***	***4/4***	***3/4***
Kidney								
Nephropathy, Chronic Progressive	4/4	4/5	3/4	2/5	2/4	2/5	***4/5***	***4/5***
Liver								
Hypertrophy, Centrilobular	0/4	0/5	0/4	0/5	0/4	0/5	0/5	0/5
Spleen								
Hematopoiesis	0/4	1/5	1/4	0/5	0/4	0/5	0/5	0/5
Pigment	0/4	0/5	0/4	0/5	0/4	0/5	***2/5***	***2/5***

Values in ***green bold italics*** are statistically significant.

Neurotoxicology Effects

- Reduced forelimb and hindlimb grip strength in male rats at 500 mg/kg/day, attributed to reduced body weight.
- No effects considered to represent neurotoxicity.

Reproduction Evaluation

- Reduced body weight parameters at 500 mg/kg/day – P_1 males and females
- Reduced food efficiency at 500 mg/kg/day – P_1 females
- No effect on P_1 generation sperm parameters
- Increased P_1 female estrous cycle length at all dose levels
- Reduced P_1 fertility at all dose levels
- No effect on P_1 generation mating or gestation length
- Reduced P_1 uterine implantation sites and litter size at 500 mg/kg/day
- Reduced F_1 lactation pup survival parameters and body weights at 100 and 500 mg/kg/day

TABLE 3

P_1 GENERATION REPRODUCTIVE PARAMETERS

Dose (mg/kg/day):	0	25	100	500
Percent of Days in Estrus	32	31	34	29
Percent of Days in Diestrus	60	59	60	60
Percent of Days in Proestrus	7	10	6	10
Mean Cycle Length (days)	4.3	***4.7***	***4.9***	***4.8***
Mean Precoital Interval (days)	3.4	3.3	2.8	3.1
Mating Index (%) (number copulated/cohoused)	100.0	100.0	95.0	100.0
Fertility Index (%) (number delivered/copulated)	85.0	**55.0**	**57.9**	**65.0**
Gestation Length (days)	22.6	22.4	22.5	22.9
Number of Implantation Sites	15.3	14.1	14.8	***10.7***
Implantation Efficiency (%)[a]	90.0	93.2	88.6	86.0
Sperm Motility (% motile)	92.8	91.7	93.4	91.1
Sperm Morphology (% normal)	98.3	98.3	98.9	97.7
Epididymal Sperm (millions)				
Per cauda	318.4	***278.8***	***291.1***	***279.4***
Per gram Cauda	1180.2	979.2	969.0	1062.1
Testicular Spermatids (millions)				
Per Testis	126.5	128.8	128.2	***137.0***
Per gram Testis	83.3	93.7	87.4	83.4

a Number of pups born/number of implantation sites X 100.

Values in ***green bold italics*** are statistically significant.
Values in **blue bold** are not statistically significant but considered test substance-related.

TABLE 4

F_1 GENERATION LITTERS

Dose (mg/kg/day)	0	25	100	500
N	17	11	11	13
Mean Number of Pups/Litter				
Born	14.3	13.7	13.4	***10.3***
Born Alive	14.3	13.5	13.1	***9.4***
Day 4 Preculling	14.1	12.8	12.9	***7.5***
Day 4 Postculling	8.0	7.9	8.0	***6.3***
Day 7	8.0	7.9	7.7	***4.8***
Day 14	8.0	7.9	***6.3***	***1.1***
Day 21	8.0	7.9	***6.1***	***0.8***
Survival (%)				
Sex Ratio (males)	0.48	0.58	0.50	0.46
Gestation Index[a]	100.0	100.0	100.0	92.3
Mean % Born Alive	100.0	98.8	98.1	***86.7***
0-4 Day Viability	97.6	98.5	98.9	82.8
Lactation Index[b]	100.0	98.2	***76.1***	***11.4***
Litter Survival[c]	100.0	100.0	90.9	***25.0***
Mean Pup Weights (grams)				
Day 0	6.9	6.8	6.7	***6.2***
Day 4 Preculling	11.5	12.2	***10.8***	***8.2***
Day 4 Postculling	11.5	12.3	***10.8***	***8.2***
Day 7	18.4	18.9	***15.9***	***9.9***
Day 14	38.0	37.7	***35.7***	***21.3***
Day 21	62.6	59.9	58.6	***35.9***

a Percent litters delivered having at least one live pup.
b Mean percent survival from Day 4 Postculling to Day 21.
c Percent litters born with at least one pup alive on Day 21.

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TABLE 5

DEVELOPMENTAL TOXICITY STUDY RESULTS

Dose (mg/kg/day):	0	25	100	500
Maternal BWG (g) (GD 6-21)	153.1(20.3)	144.5(21.4)	***133.3(19.8)***	***85.2(19.7)***
Fetal weight (g)	5.50(0.28)	5.49(0.26)	5.40(0.39)	***4.76(0.29)***
Supernumerary ribs (litters affected/examined)	6/22	6/21	10/22	***12/21***
Skull, retarded ossification (litters affected/examined)	10/22	9/21	12/22	***16/21***

Values in ***green bold italics*** are statistically significant.

Discussion

- Exposure to the test substance produced reversible decrements in body weight and nutritional parameters and non-specific clinical signs of toxicity.
- No evidence of neurotoxicity was observed. Reductions in grip strength were attributed to lower body weight.
- Liver, thyroid, kidney, and red blood cells were identified as target organs, based on clinical and anatomic pathology evaluations. Effects were generally mild and all adverse effects demonstrated reversibility, except chronic progressive nephropathy in females.
- The reduction in fertility index and mean number of implantation sites, in the absence a reduction in implantation efficiency (an indicator of *in utero* post-implantation loss) suggests pre-implantation embryonic loss.
- The reduction in pup viability and survival may be due to the postnatal manifestation of a developmental effect due to *in utero* exposure, maternal factors (insufficient milk supply, neglect), or result from postnatal toxicity from lactational transfer to the nursing pups.
- In the developmental toxicity study, the test substance did not produce fetal malformations. There was no evidence of increased sensitivity of the fetus to the test substance.

Conclusions

- NOEL for Subchronic Toxicity: The no-observed-effect level (NOEL) for males and females was 25 mg/kg/day, based on decrements in body weight and nutritional parameters, clinical signs of toxicity, and thyroid and kidney histopathology, all observed at 100 mg/kg/day.
- NOEL for Reproductive Toxicity: There was no NOEL for the reproductive toxicity parameters evaluated under the conditions of this study; this was based on effects at all dose levels on the fertility index in the P_1 generation.
- NOEL for Developmental Toxicity: The maternal NOEL was 50 mg/kg/day, based on reduced maternal body weight and nutritional parameters. The fetal NOEL was 100 mg/kg/day, based on reduced fetal weight and increased fetal variations. No fetal malformations were observed.

Acknowledgments

We wish to thank the following individuals for their technical assistance:

- Linda A. Malley for neuropathology evaluations
- Primary Technician: Nita B. Baker
- Poster Preparation: Maryanne M. Wilford

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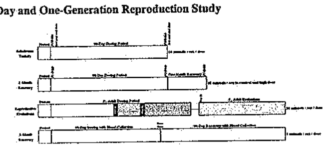

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Developmental Toxicity Study

- Groups of time-mated rats (22/dose) received oral gavage dose once daily over gestation days 6-20.
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- Dams were euthanized on gestation day 21 and examined for gross external and visceral alterations.
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Results

Figure 1 - Mean Body Weights of Male Rats

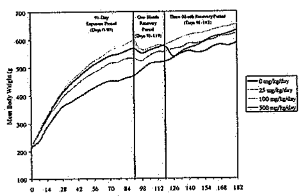

Figure 2 - Mean Body Weights of Female Rats

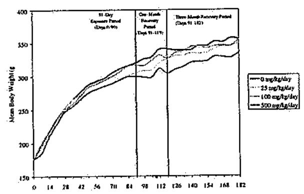

Table 1 - Clinical Pathology Data

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	Dose (mg/kg/day):	25	100	500	25	100	500
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	1-month recovery	ND	ND	97	ND	ND	98
	3-month recovery	99	98	97	100	99	101
HGB (g/dL)	end-of-dosing	99	*95*	*92*	97	*90*	*85*
	1-month recovery	ND	ND	*95*	ND	ND	97
	3-month recovery	101	98	99	101	94	97
HCT (%)	end-of-dosing	99	96	*94*	98	*97*	*96*
	1-month recovery	ND	ND	96	ND	ND	97
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	1-month recovery	ND	ND	123	ND	ND	63
	3-month recovery	77	78	111	139	135	131

Values are percent of control. Those in *green bold italics* are statistically significant.
ND = No data
NC = Not calculable as control value was zero.

Table 2 - Histopathology Data

	Males				Females			
Dose (mg/kg/day):	0	25	100	500	0	25	100	500
End-of-Dosing								
Thyroid								
Hypertrophy, Follicular	0/11	1/10	*9/11*	*11/12*	0/10	0/10	*2/10*	*9/10*
Alteration, Colloid	6/11	10/10	*10/11*	*11/12*	3/10	*6/10*	*10/10*	*10/10*
Kidney								
Nephropathy, Chronic Progressive	5/11	3/10	5/11	*7/12*	2/10	2/10	*5/10*	*5/10*
Liver								
Hypertrophy, Centrilobular	1/11	*5/10*	*10/11*	*12/12*	0/10	0/10	*9/10*	*10/10*
Spleen								
Hematopoiesis	0/11	1/10	*8/11*	*10/12*	0/10	1/10	*6/10*	*7/10*
Pigment	0/11	0/10	*4/11*	*6/12*	1/10	0/10	0/10	*10/10*
1-Month Recovery								
Thyroid								
Hypertrophy, Follicular	0/10	ND	ND	*8/10*	0/10	ND	ND	*6/10*
Alteration, Colloid	6/10	ND	ND	*10/10*	1/10	ND	ND	*10/10*
Kidney								
Nephropathy, Chronic Progressive	3/10	ND	ND	*8/10*	0/10	ND	ND	*8/10*
Liver								
Hypertrophy, Centrilobular	0/10	ND	ND	*7/10*	0/10	ND	ND	1/10
Spleen								
Hematopoiesis	0/10	ND	ND	*8/10*	0/10	ND	ND	1/10
Pigment	0/10	ND	ND	*7/10*	0/10	ND	ND	0/10
3-Month Recovery								
Thyroid								
Hypertrophy, Follicular	0/4	0/5	0/4	*4/5*	0/4	0/5	0/4	0/4
Alteration, Colloid	1/4	*5/5*	*4/4*	5/5	1/4	*3/5*	*4/4*	*3/4*
Kidney								
Nephropathy, Chronic Progressive	*4/4*	4/5	3/4	2/5	2/4	2/5	*4/5*	*4/5*
Liver								
Hypertrophy, Centrilobular	0/4	0/5	0/4	0/5	0/4	0/5	0/5	0/5
Spleen								
Hematopoiesis	0/4	1/5	1/4	0/5	0/4	0/5	0/5	0/5
Pigment	0/4	0/5	0/4	0/5	0/4	0/5	*2/5*	*2/5*

Values in *green bold italics* are considered test substance-related, adverse findings.

Neurotoxicology Effects

- Reduced forelimb and hindlimb grip strength in male rats at 500 mg/kg/day, attributed to reduced body weight.
- No effects considered to represent neurotoxicity.

Reproduction Evaluation

- Reduced body weight parameters at 500 mg/kg/day – P_1 males and females
- Reduced food efficiency at 500 mg/kg/day – P_1 females
- No effect on P_1 generation sperm parameters
- Increased P_1 female estrous cycle length at all dose levels
- Reduced P_1 fertility at all dose levels
- No effect on P_1 generation mating or gestation length
- Reduced P_1 uterine implantation sites and litter size at 500 mg/kg/day
- Reduced F_1 lactation pup survival parameters and body weights at 100 and 500 mg/kg/day

Table 3 - P_1 Generation Reproductive Parameters

Dose (mg/kg/day):	0	25	100	500
Percent of Days in Estrus	32	31	34	29
Percent of Days in Diestrus	60	59	60	60
Percent of Days in Proestrus	7	10	6	10
Mean Cycle Length (days)	4.3	[illegible]	[illegible]	[illegible]
Mean Precoital Interval (days)	3.4	3.3	2.8	3.1
Mating Index (%) (number copulated/cohoused)	100.0	100.0	95.0	100.0
Fertility Index (%) (number delivered/copulated)	85.0	55.0	57.9	65.0
Gestation Length (days)	22.6	22.4	22.5	22.9
Number of Implantation Sites	15.3	14.1	14.8	[illegible]
Implantation Efficiency (%)[a]	90.0	93.2	88.6	86.0
Sperm Motility (% motile)	92.8	91.7	93.4	91.1
Sperm Morphology (% normal)	98.3	98.3	98.9	97.7
Epididymal Sperm (millions)				
Per cauda	318.4	[illegible]	[illegible]	[illegible]
Per gram Cauda	1180.2	979.2	969.0	1062.1
Testicular Spermatids (millions)				
Per Testis	126.5	128.8	128.2	[illegible]
Per gram Testis	83.3	93.7	87.4	83.4

a Number of pups born/number of implantation sites X 100.

Values in [illegible] are statistically significant.
Values in blue bold are not statistically significant but considered test substance-related.

Table 4 - F_1 Generation Litters

Dose (mg/kg/day)	0	25	100	500
N	17	11	11	13
Mean Number of Pups/Litter				
Born	14.3	13.7	13.4	*10.3*
Born Alive	14.3	13.5	13.1	*9.4*
Day 4 Preculling	14.1	12.8	12.9	*7.5*
Day 4 Postculling	8.0	7.9	8.0	*6.3*
Day 7	8.0	7.9	7.7	*4.8*
Day 14	8.0	7.9	*6.3*	*1.1*
Day 21	8.0	7.9	*6.1*	*0.8*
Survival (%)				
Sex Ratio (males)	0.48	0.58	0.50	0.46
Gestation Index[a]	100.0	100.0	100.0	92.3
Mean % Born Alive	100.0	98.8	98.1	*86.7*
0-4 Day Viability	97.6	98.5	98.9	82.8
Lactation Index[b]	100.0	98.2	*76.1*	*11.4*
Litter Survival[c]	100.0	100.0	90.9	*25.0*
Mean Pup Weights (grams)				
Day 0	6.9	6.8	6.7	*6.2*
Day 4 Preculling	11.5	12.2	*10.8*	*8.2*
Day 4 Postculling	11.5	12.3	*10.8*	*8.2*
Day 7	18.4	18.9	*15.9*	*9.9*
Day 14	38.0	37.7	*35.7*	*24.3*
Day 21	62.6	59.9	58.6	*33.9*

a Percent litters delivered having at least one live pup.
b Mean percent survival from Day 4 Postculling to Day 21.
c Percent litters born with at least one pup alive on Day 21.

Values in [illegible] are statistically significant.

Table 5 - Developmental Toxicity Study Results

Dose (mg/kg/day):	0	25	100	500
Maternal BWG (g) (GD 6-21)	153.1(20.3)	144.5(21.4)	*133.3(19.8)*	*85.2(19.7)*
Fetal weight (g)	5.50(0.28)	5.49(0.26)	5.40(0.39)	*4.76(0.29)*
Supernumerary ribs (litters affected/examined)	6/22	6/21	10/22	*12/21*
Skull, retarded ossification (litters affected/examined)	10/22	9/21	12/22	*16/21*

Values in *green bold italics* are statistically significant.

Discussion

- Exposure to the test substance produced reversible decrements in body weight and nutritional parameters and non-specific clinical signs of toxicity.
- No evidence of neurotoxicity was observed. Reductions in grip strength were attributed to lower body weight.
- Liver, thyroid, kidney, and red blood cells were identified as target organs, based on clinical and anatomic pathology evaluations. Effects were generally mild and all adverse effects demonstrated reversibility, except chronic progressive nephropathy in females.
- The reduction in fertility index and mean number of implantation sites, in the absence of a reduction in implantation efficiency (an indicator of in utero post-implantation loss) suggests pre-implantation embryonic loss.
- The reduction in pup viability and survival may be due to the postnatal manifestation of a developmental effect due to in utero exposure, maternal factors (insufficient milk supply, neglect), or result from postnatal toxicity from lactational transfer to the nursing pups.
- In the developmental toxicity study, the test substance did not produce fetal malformations. There was no evidence of increased sensitivity of the fetus to the test substance.

Conclusions

- NOEL for Subchronic Toxicity: The no-observed-effect level (NOEL) for males and females was 25 mg/kg/day, based on decrements in body weight and nutritional parameters, clinical signs of toxicity, and thyroid and kidney histopathology, all observed at 100 mg/kg/day.
- NOEL for Reproductive Toxicity: There was no NOEL for the reproductive toxicity parameters evaluated under the conditions of this study; this was based on effects at all dose levels on the fertility index in the P_1 generation.
- NOEL for Developmental Toxicity: The maternal NOEL was 50 mg/kg/day, based on reduced maternal body weight and nutritional parameters. The fetal NOEL was 100 mg/kg/day, based on reduced fetal weight and increased fetal variations. No fetal malformations were observed.

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