A 90-Day Gavage Study with One-Generation Reproduction Evaluations in Rats with a Fluoroalkylethanol Mixture

E. Mylchreest, J.C. Stadler, G.T. Makovec, N.E. Everds, and G.S. Ladics

The DuPont Company, Haskell Laboratory for Health and Environmental Sciences, Newark, Delaware, USA

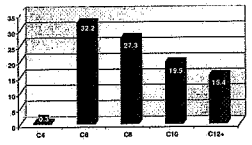
Abstract

The objective of this study was to evaluate the subchronic and reproductive toxicity of a fluoroalkylethanol mixture which is employed as an intermediate in the production of other fluoroorganic compounds utilized as protectants and surfactants. Test material was administered by gavage at dosages of 0, 25, 100, or 250 mg/kg/day. No test substance-related mortality or neurotoxicity occurred in the study. Body weights and/or nutritional parameters were significantly reduced at 100 and 250 mg/kg/day but similar to control after recovery. Broken and absent teeth were observed at 250 mg/kg/day. Microscopic tooth lesions (ameloblast degeneration/disorganization) occurred at 100 and 250 mg/kg/day and persisted after recovery. Plasma and urine fluoride levels were elevated in a dose-dependent manner. Decreased red cell mass parameters occurred at 250 mg/kg/day and persisted after 36 days of recovery. The 100 and 250 mg/kg/day groups had increased ($p < 0.05$) liver weights which persisted in the high dose after recovery and correlated with microscopic hepatocellular hypertrophy in males and females (250 mg/kg/day subchronic animals only). Hepatic β-oxidation was increased in a dose-dependent manner and persisted after recovery at 250 mg/kg/day. Increased kidney weights occurred at 25 (females only), 100 and 250 mg/kg/day which persisted in the high dose after recovery and correlated with microscopic tubular hypertrophy (males only). Thyroid follicular hypertrophy was present at 100 and 250 mg/kg/day but was not present after recovery. The test material contains 2% residual iodides. Litter size at birth and pup weights during lactation were reduced at ≥ 100 mg/kg/day. No other reproductive parameter was affected. There were no adverse effects observed for either subchronic or reproductive toxicity in animals dosed with 25 mg/kg/day. A wide margin of safety exists between potential human exposure levels and the doses which result in adverse effects.

Introduction

The test substance is an intermediate in the production of other fluorotelomer-based products that are used as protectants and surfactants. The test material was administered by gavage, the route selected as the most efficient way to deliver an accurate dosage. Dosage levels for this study were selected based on the toxicity and pharmacokinetic analysis of plasma fluorine concentrations from a range-finding study. Toxicity (i.e., body weight effects or clinical observations) was not observed in rats exposed to 200 mg/kg/day for 35 days, however, plasma fluorine levels approached levels previously seen when mortality occurred. The high-dose level of 250 mg/kg/day 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.

Perfluoroalkylethanol Study Material Composition

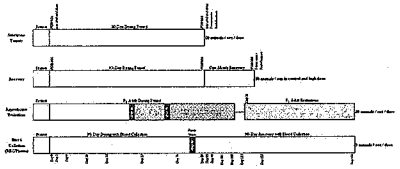


- 94.67% $F(CF_2CF_2)_xCH_2CH_2OH$
- 2.01% $F(CF_2CF_2)_xCH_2CH_2I$
- 5150 ppm Total Elemental Iodine [I]
- 12 ppm free Iodide [I⁻]

Objective

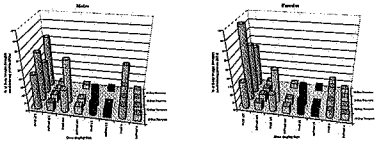
Evaluate the potential subchronic and reproductive toxicity of a fluorotelomer alcohol (a.k.a. perfluoroalkylethanol) mixture in male and female rats. Recovery evaluations were included to investigate the reversibility of any observed toxicological effects.

Study Design



Liver Effects

- Increased liver weights at all dose levels and centrilobular hepatocellular hypertrophy at ≥ 100 mg/kg/day (males), and 250 mg/kg/day (females).
- Increased liver weight (males and females) and hepatocellular hypertrophy (males) persisted after one and three month recovery period at 250 mg/kg/day.
- Increased rate of hepatic β-oxidation in males and females at ≥ 100 mg/kg/day, which persisted after the one- and three-month recovery period at 250 mg/kg/day.



* Statistically significant difference from control ($p < 0.05$) by Dunnett test.
\# Statistically significant difference from control ($p < 0.05$) by Jonckheere-Terpstra trend test.

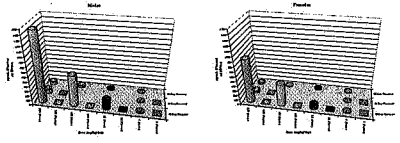
Blood Effects

Plasma Fluoride

- Mildly increased at ≥ 100 mg/kg/day (males and females)
- No increase after one-month recovery

Urine Fluoride

- Increased at ≥ 25 mg/kg/day (males and females)
- Increased at ≥ 100 mg/kg/day (males) and at 250 mg/kg/day (females) after one- and three-month recovery



* Statistically significant difference from control ($p < 0.05$) by Dunnett/Tamhane-Dunnett test.
\# Statistically significant difference from control ($p < 0.05$) by Dunn's test.

- Minimally decreased red cell mass parameters, along with correlative changes in other hematology parameters and red cell morphology at 250 mg/kg/day (males and females). After one month of recovery, mean red cell mass of female rats was similar to control, although some changes were still present in other hematological parameters. Male rats still had decreased red cell mass effects, and associated alterations in other hematological parameters.
- No toxicologically significant changes in clinical chemistry parameters observed at any dose level.

Tooth Effects

- Absent or broken teeth observed clinically at 250 mg/kg/day
- Degeneration/disorganization of enamel organ ameloblast cells (males and females) at ≥ 100 mg/kg/day.
- Ameloblastic degeneration/disorganization persisted with decreased incidence and severity at 250 mg/kg/day (males and females) after a one- and three-month recovery period, and at 100 mg/kg/day (males) after a three-month recovery period.

Upper incisor: male rat. Age-matched control rat orally gavaged with 0.5% aqueous methylcellulose. Note uniform palisading arrangement of enamel organ ameloblast cell layer (between arrows). HE. ~100x.

Upper incisor: male rat orally gavaged with 250 mg/kg/day of fluoroalkylethanol mixture for 90 days. Note degeneration/disorganization of enamel organ ameloblast cell layer (between arrows). HE. ~100x.

Thyroid Effects

- Increased thyroid hypertrophy at ≥ 100 mg/kg/day (males) and 250 mg/kg/day (females).
- No thyroid hypertrophy after one month recovery.

Reproduction Evaluation

There were no compound-related effects on the following parameters:

- Body weights, body weight gains, food consumption and food efficiency in P_1 female rats during gestation and lactation
- Clinical observations in P_1 and F_1 male and female rats
- Estrous cycle and sperm parameters in P_1 rats
- Mating, fertility, gestation length and number of uterine implantation sites in P_1 female rats
- Body weight gain, preputial separation and vaginal opening in F_1 rats

There were compound-related effects on the following parameters:

- Body weights in P_1 male rats (89-91% of control) at 250 mg/kg/day. In the absence of any concomitant reduction in body weight gain this was not considered toxicologically significant.
- Number of pups born, born alive and alive on day 4 at ≥ 100 mg/kg/day (see table).
- Implantation efficiency (# pups born/implants) in P_1 female rats at ≥ 100 mg/kg/day (83.4 and 84.5% respectively compared to 96.6% in the control group) and reflects the reduction in the number of pups born.
- Pup weights during lactation at 250 mg/kg/day (see table).
- Post-weaning body weights in F_1 male and female rats. In the absence of any concomitant reduction in body weight gain, this was considered due to the lower body weight at weaning and was not considered toxicologically significant.

Pup Numbers and Survival: F_1 Generation

Dose (mg/kg/day)	0	25	100	250
N	13	17	14	13
	Mean Number of Pups/Litter			
Born	14.7	13.4	12.4	12.5*
Born Alive	14.6	13.2	11.3	12.1*
Day 4 Preculling	14.6	12.9	10.0*	11.8*
Day 4 Postculling	8.0	7.6	6.3	7.5
Day 7	8.0	7.6	6.3	7.4
Day 14	8.0	7.6	6.3	7.4
Day 21	8.0	7.6	6.3	7.4
Sex Ratio (males)	0.59	0.50	0.50	0.56
	Survival (%)			
Gestation Index[a]	100.0	100.0	92.9	100.0
Mean % Born Alive	99.5	99.2	88.4	92.4
0-4 Day Viability	100.0	97.6	90.6	97.3
Lactation Index[b]	100.0	100.0	100.0	92.3
Litter Survival[c]	100.0	100.0	92.3	92.3

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.

* Statistically significant difference from control ($p < 0.05$) by Jonckheere-Terpstra test.

Statistics were performed on combined pups per litter only.

Pup Weights: F_1 Generation

Dose (mg/kg/day)	0	25	100	250
N	13	17	13	13
	Mean Pup Weights (grams)			
Day 0	6.9 (0.7)[a]	6.7 (0.6)	6.8 (0.7)[b]	6.4 (0.6)
Day 4 Preculling	11.3 (1.5)	11.4 (1.6)	11.9 (1.8)	10.1 (1.7)*
Day 4 Postculling	11.4 (1.6)	11.4 (1.7)	11.9 (1.9)	10.1 (1.7)*
Day 7	18.3 (1.9)	18.0 (2.2)	18.3 (2.4)	15.5 (2.1)*
Day 14	37.9 (3.4)	37.0 (3.0)	36.1 (3.2)	29.5 (3.4)*
Day 21	60.9 (6.9)	60.3 (5.5)	58.3 (4.3)	45.4 (7.7)*

a Standard deviation is reported in parentheses.
b N = 12.

* Statistically significant difference from control ($p < 0.05$) by linear contrast of least square means.

Note: Statistical analyses were conducted on the combined pup weights.

Discussion

The increases in plasma and urine fluoride were considered to be secondary to the administration of a mixture containing fluorinated compounds and/or free fluoride. The presence of fluoride in urine three months after exposure suggests that fluoride-containing compounds are slowly being released from tissue sites. The effects seen in teeth are consistent with elevated levels of fluoride.

Liver effects that included increases in liver weights, peroxisome proliferation as indicated by elevated β-oxidation, and liver hypertrophy are also related to the fluorinated test material.

Thyroid effects may have been due to the presence of iodine in the test material.

Reproductive toxicity occurred at the same dose levels as the subchronic toxicity indicating that protection from subchronic toxicity would likely also protect from the reproductive effects.

Conclusions

NOEL for Subchronic Toxicity: The no-observed -effect level (NOEL) for males and females was 25 mg/kg/day based on adverse decrements in body weight parameters and food efficiency (males only), increased hepatic peroxisomal β-oxidation, and the degeneration and/or disorganization of enamel organ ameloblast cells at 100 mg/kg/day.

NOEL for Reproductive Toxicity: The NOEL for the reproductive evaluations was 25 mg/kg/day based on decreases in the number of pups born, bone alive and the number of pups alive on day 4 of lactation at 100 mg/kg/day.