

A 90-Day Toxicological Evaluation of Compound 1080 (Sodium Monofluoroacetate) in Sprague-Dawley Rats

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Received January 10, 2002; accepted July 9, 2002

Groups of Sprague-Dawley rats received sodium monofluoroacetate (Compound 1080) at 0.025, 0.075, and 0.25 mg/kg by oral gavage once daily for 90 days and were then euthanized. The control and 0.25 mg/kg/day groups included additional rats of each sex that were treated for 90 days, then maintained without treatment for a further 56-day recovery period. Microscopic changes were restricted to the testes and the heart, and were seen only in males dosed with 1080 at 0.25 mg/kg/day and included severe hypospermia in the epididymides, severe degeneration of the seminiferous tubules of the testes, and cardiomyopathy. Sperm evaluation indicated severe decreases in all three sperm parameters evaluated (concentration, % motile, and % abnormal) at 0.25 mg/kg/day. There were no microscopic changes or 1080-related effects on sperm parameters at 0.025 and 0.075 mg/kg/day. The no observable effects level (NOEL) for rats administered 1080 via oral gavage for 90 days was 0.075 mg/kg/day. The lowest observable effects level (LOEL) dose was 0.25 mg/kg/day. After dosing with the LOEL dose of 0.25 mg/kg/day, mean concentrations of 1080 in rat plasma were 0.26 $\mu\text{g/ml}$ at 1 h and 0.076 $\mu\text{g/ml}$ at 12 h. Rat urine collected from the same animals contained 0.059 $\mu\text{g/ml}$.

Key Words: sodium monofluoroacetate (1080); 90-day exposure; no observable effect level (NOEL); cardiomyopathy; testicular degeneration.

Regulatory toxicology studies are usually conducted before the launch of new drugs or pesticides. Alternatively, they may be conducted on established products, such as compound 1080 (sodium monofluoroacetate), to update toxicology data generated before new standards and data requirements became commonplace. Compound 1080 is widely used in New Zealand and Australia as a vertebrate pesticide. Early investigations and toxicology studies on 1080 and its mode of action as an inhibitor of the Krebs's cycle have been extensively reviewed elsewhere (Eisler, 1995; Seawright and Eason, 1994).

Before the suite of regulatory toxicology studies was commissioned by New Zealand agencies, including the study in rats reported in this document, a battery of regulatory studies had been completed in the USA because 1080 is used there in

livestock protection collars. This included 15 studies on product chemistry, five studies on wildlife hazards, and three studies relating to human health. The results from these studies, which related to skin and eye irritation and transdermal absorption and toxicity of 1080, have been summarized elsewhere (Fagerstone *et al.*, 1994).

In response to demand for clarification of the potential risk from chronic, low-level exposure to 1080 and to support regulatory assessments of risk in the use of 1080 as a vertebrate pesticide, the first phase of new (New Zealand-directed) regulatory toxicology studies was completed in 1998. Results of three different complementary toxicity studies indicate that 1080 is not mutagenic, and therefore, is not anticipated to be a carcinogen (Eason *et al.*, 1999). Results of developmental toxicology studies indicate that 1080 causes developmental defects in rats when pregnant females are exposed to relatively high doses (0.33 and 0.75 mg/kg) on a daily basis during the period of organogenesis from Day 6 through to Day 17 of gestation. The NOEL for developmental effects was 0.1 mg/kg/day, based on observations of bent ribs at 0.33 mg/kg/day (Eason *et al.*, 1999).

After completion of this study we identified a similar unpublished 90-day study in rats (Wolfe, 1988) that we had not been aware of. In this article, we compare our findings with those of Wolfe (1988), with earlier findings relating to the sublethal effects of 1080 in animals and with results of the teratogenicity study by Eason *et al.* (1999). The current study differs from all previous studies of the sublethal effects of 1080 in animals, as blood and urine analyses for 1080 concentrations have been undertaken so that LOELs and NOELs can be related to 1080 levels in blood and urine as well as the amount of 1080 ingested in mg/kg/day.

Previous studies have focused on either the metabolism and fate of 1080 in animals, or separate experiments on the toxic effects of 1080 (Eason *et al.*, 1994a,b). This study further differs from the study by Wolfe (1988) in that a substantial recovery period was included. Estrous cyclicity and additional assessment on the testes were included as there have been concerns regarding the effects of 1080 on reproductive systems

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in male and female animals (Eason *et al.*, 1999; Shinoda *et al.*, 2000; Sullivan *et al.*, 1979; Wolfe, 1988).

MATERIALS AND METHODS

This study was run in accordance with the United States Environmental Protection Agency Guidelines (U.S. EPA, 1998), which define the number of treatment groups, the number of animals per group, and provide guidelines for the parameters assessed. In addition to the standard procedures outlined in these guidelines, special tests were included to assess the effect of 1080 on known target organs (e.g., testes), recovery groups were included, and 1080 concentration was assessed in the blood and urine.

Animals and treatment. Sixty male and 60 female Sprague-Dawley rats (about six weeks of age) were obtained from Charles River Laboratories, USA, and acclimatized for three weeks at MPI Research, Michigan. Prior to assignment to study groups, the rats were weighed, sexed, and examined for evidence of disease and other physical abnormalities. All rats available for the selection process had body weights that were within $\pm 20\%$ of the mean body weight for each sex. A randomization procedure was used to assign the rats to control and treatment groups, so that there were 20 of each sex in the control group (0 mg/kg/day), 10 of each sex in the 0.025 mg/kg/day dose group, 10 of each sex in the 0.075 mg/kg/day dose group, and 20 of each sex in the 0.25 mg/kg/day dose group. The standard group size (rats/sex/group) recommended by the U.S. EPA (1988) for a 90-day study is 10. An extra 10 rats of each sex in the control and highest 1080 dose group were utilized as recovery subgroups.

The dose levels were selected on the basis of results from the preceding 28-day pilot study. In this pilot study, a NOEL was not determined when doses ranging from 0.2 to 0.8 mg/kg/day were administered, because significant effects on the testes were noted at all dose levels. Hence, for the 90-day study, one dose level was chosen (0.25 mg/kg/day) in which these toxic effects would be manifest. Because there was no indication of a NOEL between 0.2 and 0.8 mg/kg/day, substantially lower doses were selected for the mid- and low-dose groups. The two lower-dose groups were designed to span different degrees of exposure so that a NOEL could be identified in one or both groups. The oral route was chosen as it is one of the potential routes of human exposure to 1080.

Compound 1080 obtained from Tull Chemical Company, USA, was dissolved in HPLC Grade water (Aldrich Chemical Company) and administered by gastric intubation once a day for 90 consecutive days at a dose volume of 10 ml/kg/day. The control group was given water at the same dose volume.

To ensure the correct dose was administered, test article analyses for concentration and stability were undertaken at Landcare Research's toxicology laboratory. Dosing solutions were freshly prepared within each 21-day interval and periodically analyzed for 1080 concentration and stability using a validated gas-chromatography technique.

Following the 90-day exposure period, treatment was discontinued for half the animals in Groups 1 and 4 for a further period of 56 days.

The rats were individually identified with implanted transponders (Biomedic Data Systems, Inc.) and housed individually in suspended, stainless steel wire-mesh cages. Fluorescent lighting was provided for approximately 12 h per day. Temperature and humidity were monitored and maintained at 18–24°C and 36–75%, respectively.

Rodent Diet (certified Rodent Chow® #5002, PMI Nutrition International, St. Louis, MO) and water were freely available to all rats.

General examinations. Standard OECD and U.S. EPA (U.S. EPA, 1998) guidelines were followed in terms of the selection of tissues and procedures, which included comprehensive general observations, blood analyses for hematology and clinical chemistry parameters, and tissue pathology, including gross and microscopic examination, to determine whether 1080 adversely affected any particular target organ. All rats were observed for morbidity, mortality, and evidence of toxic effects. Individual food consumption of each rat was measured and recorded during the study, including the recovery period for Groups 1 and 4. Detailed clinical examinations were conducted weekly during the study and recovery periods.

Individual body weights were measured and recorded for all rats two days after receipt, weekly during the study, and recovery periods, and before postmortem examination at the end of the treatment or recovery periods. Food consumption was not measured. Ophthalmoscopic examinations were conducted before dosing and after 90 days of treatment to determine whether or not 1080 caused ocular toxicity.

Estrous cyclicity. To evaluate potential effects of 1080 on the estrous cycle, all females were evaluated during the last three weeks of the 90-day exposure period. Each female received a vaginal lavage daily and the stage of estrus was determined by microscopic examination of the cell types present.

Sperm evaluation. The testes are known to be a target organ for 1080-induced toxicity (Sullivan *et al.*, 1979). Because of this, the testes were the focus for observations. A section of the right vas deferens was used for videotaping a prepared sperm sample for automated evaluation of sperm motility utilizing the Hamilton-Thorne Computer-Assisted Semen Analysis (CASA) System. The right cauda epididymis was separated, weighed, and used for manual (visual) assessment of sperm concentration. Slides were prepared for assessment of sperm morphology from sperm concentration preparations.

Clinical pathology: Hematology and clinical chemistry studies. Blood samples (3–4 ml) were collected by cardiac puncture after carbon dioxide anesthesia at termination of study and at the end of the 56-day recovery period. Whole blood was used for hematological examination and separate subsamples were centrifuged and plasma retained for clinical chemistry analyses.

Hematological parameters determined included leukocyte count, erythrocyte count, hemoglobin, hematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), and platelet numbers assessed on whole blood samples using a Baker 9000 hematological counter. Differential leukocyte counts were assessed by light microscopy and prothrombin time (PT) using an MLA Electra 900.

Clinical chemistry parameters determined included blood levels of sodium, potassium, chloride, calcium, phosphorus, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatinine, total protein, albumin, globulin, and glucose determined using an automated Olympus AU600.

Urine and plasma for 1080 concentration. On the day before euthanasia (at least 16 h before), 5 rats of each sex from each group were placed in stainless steel metabolism cages to collect urine samples. The animals had free access to water, but were fasted overnight. The urine samples were frozen at -20°C after collection pending 1080 analyses.

Blood samples (approximately 1.5–2.0 ml) were collected from the orbital plexus for determination of plasma concentration of 1080. Blood samples were collected at 1 and 12 h after dosing on Days 10 and 77, from three male rats per group at each time point for each interval. Samples were placed in tubes containing EDTA. Plasma samples were frozen at approximately -20°C pending analysis. The selection of Day 10 as a sampling point, rather than Day 1, was to allow 1080 concentration to reach steady state equilibrium. Day 77 was selected as a second assessment to evaluate whether a difference in the rate of excretion and metabolism occurred during the course of the study. The 1 h time point was chosen to approximate the likely t-max for 1080 in rats (Eason *et al.*, 1994b).

Frozen samples of urine and plasma were analyzed for 1080 concentration using gas chromatography with ECD detection, following derivatization to form a dichloroanide (Eason *et al.*, 1994a).

Macroscopic examination. All rats were given a complete postmortem examination after euthanasia by carbon dioxide inhalation. Each rat was examined carefully for external abnormalities. The abdominal, thoracic, and cranial cavities were examined for abnormalities, and the organs were removed, examined, and fixed. Organ weights (brain, adrenal, heart, kidney, liver, ovary, or testis) were recorded for all rats at postmortem. Appropriate organ weight ratios were calculated (relative to body and brain weights).

Microscopic examination. A full complement of organs and tissues was prepared and microscopically examined by a veterinary pathologist for all rats

in the control and 0.25 mg/kg/day dose groups. The heart, kidneys, liver, lungs, epididymides (male), and testes (male) were determined to be potential target organs and were microscopically examined in the 0.025 and 0.075 mg/kg/day dose groups. In addition, all gross lesions were microscopically examined in all groups. A 4-step grading system of trace, mild, moderate, and severe was used to define gradable lesions for comparison between treatment groups.

Statistics

Statistical comparisons were made between the control group and each treatment group. If more than one set of comparisons was required, all analyses were conducted separately on each set unless stated otherwise. The sexes were analyzed separately.

By convention in toxicology, an observable effect is one for which there is positive evidence, i.e., one that is statistically significant. Following this convention, significance tests were used to screen results as described below. Where a statistically significant effect was found, typically between the high rate and control, means for lower rates and for associated variables were examined for any trends suggesting physiologically significant effects masked by experimental variability.

Group pair-wise comparisons. For each specified end point (body weight, body weight change, hematology except leukocyte counts, clinical chemistry, absolute and relative to body and brain organ weights, mean estrous cycle time, number of estrous cycles/period, and epididymal sperm concentration) and for all collection intervals, Levene's test (Milliken and Johnson, 1992) was used to assess homogeneity of group variances. If Levene's test was not significant ($p > 0.01$), Dunnett's test (Dunnett, 1955) was used to compare each treatment group with the control group. If Levene's test was significant ($p < 0.01$), comparison with the control group was made using Welch's *t*-test (Welch, 1937) with a Bonferroni correction. Results of all pair-wise comparisons were reported at the 0.05 and 0.01 significance levels.

Log transformation. For data not distributed normally (total and differential leukocyte counts), a log transformation was performed and then analyzed as described above.

Arcsin-square-root transformation. Data comprising percentage values (spermatogenesis parameters; % abnormal and % motility) were transformed using the arcsin of the square root (Steel and Torrie, 1980). Group pair-wise comparisons were used to analyze the transformed percentage values.

RESULTS

Analysis of 1080 Solutions

The 0.025 mg/kg/day dosing solution was found to be stable when stored under refrigeration for 21 days. Dosing solutions used for each group at Weeks 1, 2, 3, 4, 8, and 12, upon analysis, had a mean 1080 concentration nearly identical to the nominal concentrations (recovery of 1080 in the dosing solution was greater than 93% for up to 21 days), indicating that the dosing solutions were accurately prepared.

General Examinations

All animals survived to the end of treatment or recovery, and no general clinical signs relating to 1080 toxicity (e.g., behavior changes and mortality) were noted in the treated groups when compared with controls during the treatment or the recovery periods.

No 1080-related changes in body weight occurred in the treatment groups when compared with controls during the treatment or recovery periods.

TABLE 1
Mean 1080 Concentration (Day 90) in Rat Urine
following Oral Dosing with 1080 solution

Group (<i>n</i> = 3)	Dose in mg/kg/day	1080 concentration ($\mu\text{g/ml} \pm \text{SD}$)
1	0	0
2	0.025	0.006 \pm 0.008
3	0.075	0.032 \pm 0.029
4	0.25	0.059 \pm 0.126

Note. The detection limit of urine was 0.0005 $\mu\text{g/ml}$.

Statistically significant decreases in body weight change were noted in males treated with 0.25 mg/kg/day when compared with controls during Days 77–79 (treatment period) and Days 126–133 (recovery period). However, these changes did not form a consistent pattern and are, therefore, not considered 1080-related. Increase in body weight in females at 0.25 mg/kg/day was statistically different from controls at the Week 17–18 (recovery) interval, but this was not considered to be an adverse or 1080-related effect. Body weight and body weight increase at 0.25 mg/kg/day for all other intervals, including the recovery period, were similar to controls. No statistically significant changes were noted at 0.025 or 0.075 mg/kg/day when compared with controls during the treatment period.

No 1080-related changes in food consumption or food efficiency were noted during the treatment or recovery periods. No 1080-related ophthalmoscopic findings were noted in the 1080-treated groups when compared with controls.

Clinical Pathology

At Day 90, there was a small decrease (approximately 6%) in erythrocyte counts in males at 0.25 mg/kg/day when compared with controls. An association with 1080 administration was not clear, and no differences were noted following the recovery period. Occasionally, other values were statistically different from controls, but these were considered incidental and not related to 1080 administration.

There were no apparent 1080-related alterations in the clinical chemistry parameters evaluated. Occasional values were statistically different from controls, but these were considered incidental and not associated with 1080 administration.

Urine and Plasma 1080 Concentration Studies

The dose levels of 0.025, 0.075, and 0.25 mg/kg/day showed a dose-related, but not a dose-proportional trend, with mean concentrations of 0.006, 0.032, and 0.059 mg/ml in rat urine at higher dose levels (Table 1). However, urine levels of 1080 were variable (noting that these data were not corrected for urine volume or creatinine content, which were not measured). The plasma concentrations 1 and 12 h after dosing on Days 10 and 77 also appeared to increase in a dose-related manner

TABLE 2
1080 Concentration in Rat Plasma at Day 10 and Day 77 following Oral Dosing with 1080 solution

Group (n = 3)	Dose (mg/kg/day)	1080 concentration $\mu\text{g/ml} \pm \text{SD}$			
		Day 10		Day 77	
		1 h	12 h	1 h	12 h
1	0	ND	ND	ND	ND
2	0.025	0.038 \pm 0.014	0.005 ^a	0.034 \pm 0.011	ND
3	0.075	0.088 \pm 0.011	0.021 \pm 0.007	0.084 \pm 0.014	0.023 \pm 0.003
4	0.25	0.234 \pm 0.096	0.069 \pm 0.021	0.283 \pm 0.121	0.083 \pm 0.039

Note. ND = not detected. The detection limit on blood was 0.012 $\mu\text{g/ml}$ (a relatively high detection limit since only small volumes of 0.5 ml were available). Concentration of 1080 in rat plasma at Day 10 and Day 77 was only measured in male animals.

^a One value of 0.014 and two values < LDL, therefore no SD was calculated.

(Table 2). There was no evidence of any difference between the concentrations on Day 10 and Day 77.

Estrous Cyclicity

No 1080-related changes were observed in the treatment groups when compared with controls. The mean cycle length and number of cycles were similar in all groups including the controls.

Sperm Evaluation

Statistically significant changes in sperm parameters were noted at 0.25 mg/kg/day when compared with controls at the end of the treatment period (Table 3). The 56-day recovery period appeared to have no effect on improving sperm parameters seen after 90 days exposure at 0.25 mg/kg/day. The values in male rats in the 0.25 mg/kg/day group after the recovery were, if anything, lower than the values seen at the conclusion of the treatment period (Table 6).

TABLE 3
Summary of Sperm Parameters

Parameter	Dose level (mg/kg/day)			
	0	0.025	0.075	0.25
After 90 days 1080 treatment				
Sperm concentration (No. $\times 10^8/\text{g}$)	8.761	8.659	7.895	2.218*
Sperm motility (%)	94.0	88.0	97.0	0*
Abnormal (%)	1.4	1.4	2.5	99.8*
After 56 days recovery period without exposure to 1080				
Sperm concentration (No. $\times 10^8/\text{g}$)	8.757	—	—	1.569*
Sperm motility (%)	93.0	—	—	0*
Abnormal (%)	6.2	—	—	99.2*

Note. —, not examined. There were only 20 rats allocated to recovery groups, 10 to the control group, and 10 to the high-dose group.

* Significantly different from the control group ($p < 0.01$).

All three sperm parameters were statistically lower than controls in the 0.25 mg/kg/day male rats after both the treatment and recovery periods. Sperm motility was reduced to 0%, more than 99% of all sperm was abnormal, and a reduction of 75% in the concentration of sperm was observed in the 0.25 mg/kg/day dose group at the end of the treatment period. At the end of the 56-day recovery period no improvement occurred in all three parameters. The majority of sperm seen in these male rats appeared to be fragmented, with heads and tails separated. Although sperm were present in samples used to analyze motility, all appeared dead, and no movement was evident. No changes were observed in the male rats dosed with 0.025 or 0.075 mg/kg/day when compared with the control group.

Macroscopic Examination

1080-related macroscopic effects were observed in the epididymides and the testes (Tables 4 and 5). The epididymides were notably small in 3 of 10 males receiving 1080 at 0.25 mg/kg/day, but normal in all other treated groups and the

TABLE 4
Incidence of Macroscopic Observations Relating to Effects on the Testes—Terminal Sacrifice: Male Rats

Tissue observation	0 mg/kg/day (control)	0.025 mg/kg/day	0.075 mg/kg/day	0.25 mg/kg/day
Epididymides				
Small	0	0	0	3
Mild	0	0	0	2
Moderate	0	0	0	1
Testes				
Small	0	0	0	10
Mild	0	0	0	1
Moderate	0	0	0	8
Severe	0	0	0	1

Note. Number of animals examined = 10 for all groups.

TABLE 5
Incidence of Macroscopic Observations Relating to Effects
on the Testes—Recovery Sacrifice: Male Rats

Tissue observation	0 mg/kg/day (control)		0.25 mg/kg/day	
	DOS	SAC	DOS	SAC
Number of animals examined	0	10	0	10
Testes				
Small, mild	0	0	0	9

Note. DOS = died on study, SAC = animals euthanized for scheduled necropsies.

control. The testes were small in all males at 0.25 mg/kg/day, but normal in all other treated groups and the control group. Treatment-related effects were still present in the testes after 56 days of recovery, where the testes were small in 9 of 10 males at 0.25 mg/kg/day, but normal in the control animals.

These macroscopic observations were correlated with 1080-related organ weight changes in the testes and epididymides of male rats following exposure to 0.25 mg/kg/day 1080 for 90 days (Tables 6, 7, 8, and 9). There were also 1080-related changes in heart weight in both males and females at 0.25 mg/kg/day after 90 days.

In male rats dosed at 0.25 mg/kg/day, the heart to body weight-ratio was significantly increased when compared to

controls, while the testes weight, testes to body weight-ratio, and testes to brain weight-ratio were all significantly decreased at 0.25 mg/kg/day when compared with controls. In female rats dosed at 0.25 mg/kg/day, the heart weight, heart to body weight-ratio, and heart to brain weight-ratio were all significantly increased when compared with controls.

Treatment-related organ weight changes in the heart and testes of rats were still evident after 56 days of recovery without 1080 exposure. In male rats, the heart to body weight-ratio was statistically significantly increased when compared to controls at 0.25 mg/kg/day, while the testes weight, testes to body weight-ratio, and testes to brain weight-ratio were all statistically significantly decreased at 0.25 mg/kg/day when compared with the controls. There were no significant organ weight changes in the female rats at 0.25 mg/kg/day after 56 days of recovery when compared with the control group.

Microscopic Examination

1080-related microscopic changes were present in the epididymides and testes in male rats exposed to 0.25 mg/kg/day (Table 10). There was severe hypospermia of the epididymis and severe degeneration of the seminiferous tubules of the testes in all males after exposure to 1080 at 0.25 mg/kg/day for 90 days. There was also an increase in the incidence of cardiomyopathy in males exposed to 0.25 mg/kg/day when compared with the control group. Incidence was 3 out of 10 at the

TABLE 6
Organ Weight Values—90-Day Male Rats

Parameters measured	0 mg/kg/day (control)			0.025 mg/kg/day			0.075 mg/kg/day			0.25 mg/kg/day		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Body weight (g)	504.0	37.2	10	503.0	34.8	10	503.0	44.0	10	492.0	32.5	10
Brain (g)	1.98	0.110	10	2.05	0.096	10	2.11*	0.116	10	1.99	0.097	10
Brain/body weight (% × 10)	3.96	0.352	10	4.09	0.445	10	4.21	0.280	10	4.06	0.364	10
Adrenal (mg)	57.0	5.5	10	52.0	7.6	10	59.0	5.7	10	57.0	8.4	10
Adrenal/body weight (% × 10 ³)	11.4	1.30	10	10.4	1.85	10	11.8	1.37	10	11.5	1.82	10
Adrenal/brain weight (%)	2.90	0.326	10	2.54*	0.320	10	2.80	0.280	10	2.84	0.382	10
Heart (g)	1.79	0.148	10	1.77	0.191	10	1.83	0.172	10	1.95	0.175	10
Heart/body weight (% × 10)	3.55	0.233	10	3.54	0.508	10	3.65	0.228	10	3.96*	0.253	10
Heart/brain weight (% × 10)	9.03	0.941	10	8.67	0.931	10	8.70	0.804	10	9.81	0.999	10
Kidney (g)	4.08	0.647	10	4.11	0.347	10	4.20	0.474	10	4.06	0.345	10
Kidney/body weight (% × 10)	8.08	0.894	10	8.17	0.605	10	8.33	0.443	10	8.25	0.337	10
Kidney/brain weight (% × 10)	2.07	0.379	10	2.02	0.229	10	1.99	0.183	10	2.05	0.228	10
Liver (g)	15.49	2.005	10	15.21	1.505	10	15.36	2.107	10	14.90	1.732	10
Liver/body weight (%)	3.07	0.281	10	3.03	0.275	10	3.05	0.241	10	3.02	0.211	10
Liver/brain weight (% × 10 ⁻²)	7.83	1.193	10	7.44	0.770	10	7.28	0.875	10	7.52	1.129	10
Testis (g)	3.45	0.179	10	3.70*	0.259	10	3.54	0.224	10	1.75**	0.241	9
Testis/body weight (% × 10)	6.87	0.592	10	7.38	0.707	10	7.07	0.650	10	3.57**	0.567	9
Testis/brain weight (% × 10 ⁻²)	1.74	0.114	10	1.81	0.150	10	1.68	0.081	10	0.88**	0.091	9

Note. *n* = number of animals.

* Significantly different from the control group ($p < 0.05$).

** Significantly different from the control group ($p < 0.01$).

TABLE 7
Organ Weight Values—Terminal Sacrifice: Female Rats

Parameters measured	0 mg/kg/day (control)			0.025 mg/kg/day			0.075 mg/kg/day			0.25 mg/kg/day		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Body weight (g)	256.0	10.5	10	264.0	14.7	10	256.0	11.9	10	255.0	16.2	10
Brain (g)	1.89	0.078	10	1.93	0.086	10	1.91	0.069	10	1.88	0.051	10
Brain/body weight (% × 10)	7.43	0.452	10	7.32	0.396	10	7.45	0.425	10	7.41	0.525	10
Adrenal (mg)	77.0	13.5	10	78.0	8.2	10	79.0	8.2	10	73.0	12.6	10
Adrenal/body weight (% × 10 ³)	30.0	5.82	10	29.4	3.27	10	31.1	3.76	10	29.0	5.81	10
Adrenal/brain weight (%)	4.05	0.752	10	4.03	0.489	10	4.17	0.477	10	3.89	0.674	10
Heart (g)	1.05	0.081	10	1.06	0.102	10	1.07	0.076	10	1.17*	0.096	10
Heart/body weight (% × 10)	4.12	0.269	10	4.01	0.253	10	4.16	0.149	10	4.58**	0.310	10
Heart/brain weight (% × 10)	5.58	0.586	10	5.51	0.537	10	5.60	0.452	10	6.20*	0.481	10
Kidney (g)	2.10	0.121	10	2.20	0.161	10	2.23	0.229	10	2.09	0.238	10
Kidney/body weight (% × 10)	8.22	0.325	10	8.35	0.571	10	8.68	0.620	10	8.17	0.603	10
Kidney/brain weight (% × 10)	1.11	0.085	10	1.15	0.104	10	1.17	0.124	10	1.11	0.131	10
Liver (g)	7.88	0.533	10	8.49	0.740	10	8.24	0.860	10	7.74	1.010	10
Liver/body weight (%)	3.08	0.200	10	3.21	0.164	10	3.21	0.211	10	3.03	0.262	10
Liver/brain weight (% × 10 ²)	4.17	0.416	10	4.40	0.331	10	4.33	0.483	10	4.11	0.543	10
Ovary (mg)	123.0	14.8	10	126.0	9.7	10	111.0	12.1	10	114.0	8.1	10
Ovary/body weight (% × 10 ²)	4.82	0.630	10	4.79	0.350	10	4.36	0.593	10	4.48	0.380	10
Ovary/brain weight (%)	6.50	0.820	10	6.56	0.443	10	5.86	0.751	10	6.05	0.405	10

Note. *n* = number of animals.

* Significantly different from the control group ($p < 0.05$).

** Significantly different from the control group ($p < 0.01$).

TABLE 8
Organ Weight Values—Recovery Sacrifice: Male Rats

Parameters measured	0 mg/kg/day (Control)			0.25 mg/kg/day		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Body weight (g)	545.0	53.3	10	538.0	34.7	10
Brain (g)	2.11	0.085	10	2.14	0.112	10
Brain/body weight (% × 10)	3.88	0.308	10	4.01	0.383	10
Adrenal (mg)	55.0	8.4	10	51.0	6.1	10
Adrenal/body weight (% × 10 ³)	10.1	1.09	10	9.5	0.84	10
Adrenal/brain weight (%)	2.62	0.389	10	2.39	0.370	10
Heart (g)	1.76	0.176	10	1.84	0.093	10
Heart/body weight (% × 10)	3.23	0.108	10	3.43*	0.226	10
Heart/brain weight (% × 10)	8.38	0.728	10	8.62	0.757	10
Kidney (g)	4.29	0.564	10	4.33	0.461	10
Kidney/body weight (% × 10)	7.88	0.877	10	8.06	0.779	10
Kidney/brain weight (% × 10)	2.04	0.259	10	2.03	0.260	10
Liver (g)	16.17	2.038	10	15.62	1.531	10
Liver/body weight (%)	2.96	0.149	10	2.90	0.169	10
Liver/brain weight (% × 10 ⁻²)	7.67	0.851	10	7.32	0.975	10
Testis (g)	3.80	0.407	10	1.83**	0.273	10
Testis/body weight (% × 10)	6.99	0.706	10	3.41**	0.428	10
Testis/brain weight (% × 10 ⁻²)	1.80	0.152	10	0.86**	0.137	10

Note. *n* = number of animals.

* Significantly different from the control group ($p < 0.05$).

** Significantly different from the control group ($p < 0.01$).

TABLE 9
Organ Weight Values—Recovery Sacrifice: Female Rats

Parameters measured	0 mg/kg/day (Control)			0.025 mg/kg/day		
	Mean	SD	n	Mean	SD	n
Body weight (g)	287	28.6	9	277	26.3	10
Brain (g)	1.86	0.104	9	1.78	0.071	10
Brain/body weight (% × 10)	6.52	0.587	9	6.47	0.669	10
Adrenal (mg)	79	15.0	9	78	11.8	10
Adrenal/body weight (% × 10 ³)	27.8	5.74	9	28.1	4.41	10
Adrenal/brain weight (%)	4.27	0.840	9	4.34	0.555	10
Heart (g)	1.19	0.159	9	1.14	0.094	10
Heart/body weight (% × 10)	4.14	0.317	9	4.13	0.344	10
Heart/brain weight (% × 10)	6.41	0.803	9	6.41	0.564	10
Kidney (g)	2.34	0.238	9	2.36	0.277	10
Kidney/body weight (% × 10)	8.18	0.690	9	8.51	0.428	10
Kidney/brain weight (% × 10)	1.26	0.138	9	1.33	0.168	10
Liver (g)	9.31	1.593	9	8.45	1.546	10
Liver/body weight (%)	3.24	0.371	9	3.04	0.404	10
Liver/brain weight (% × 10 ⁻²)	5.01	0.786	9	4.76	0.917	10
Ovary (g)	131	20.6	9	140	26.1	10
Ovary/body weight (% × 10)	4.61	0.872	9	5.06	0.825	10
Ovary/brain weight (% × 10 ⁻²)	7.09	1.377	9	7.89	1.527	10

Note. n = number of animals. No statistical significance was observed for the female animals.

end of the recovery period versus 5 out of 10 after 90 days of 1080 exposure.

1080-related microscopic changes were still present in the heart, epididymides, and testes in male rats in the 0.25 mg/kg/day group following the 56 days of recovery. There was hypospermia of the epididymis in 9 of 10 males, and degeneration of the seminiferous tubules of the testes in 10 of 10 males. Both changes were less severe at the end of recovery than at the end of treatment, suggesting a partial reversal for both of these lesions following 56 days of recovery (Table 11).

TABLE 10
Incidence of Microscopic Observations—Terminal Sacrifice:
Male Rats

Tissue observation	0 mg/ kg/day (control)	0.025 mg/ kg/day	0.075 mg/ kg/day	0.25 mg/ kg/day
Epididymides				
Within normal limits	9	10	8	0
Infiltration, mononuclear cell, trace	1	0	2	0
Hypospermia, severe	0	0	0	10
Testes				
Within normal limits	10	10	10	0
Degeneration, severe	0	0	0	10
Heart				
Within normal limits	10	8	10	5
Cardiomyopathy, trace	0	1	0	5
Myocarditis, trace	0	1	0	0

Note. Number of animals examined = 10 for all groups.

DISCUSSION

This study has confirmed that the NOEL for repeat-dose exposure to 1080 is low. In a recently completed teratogenicity assessment in rats, a NOEL of 0.1 mg/kg was derived (Eason *et al.*, 1999). In the present study, after 90 days exposure, a similar NOEL of 0.075 mg/kg was identified. We also conclude that during sublethal exposure to 1080, the most sensitive sites for toxicity are the heart, epididymides, testes, and fetus in rats. However, the absence of changes in body weight, food consumption, hematology, and clinical chemistry in this study indicate that the sublethal toxicity of 1080 is relatively specific to identified organs.

Similar histological changes after exposure to fluorocitrate (the toxic metabolite of 1080) or 1080 itself have been previously reported in rat and lizard testes (Smith *et al.*, 1977; Sullivan *et al.*, 1979; Twigg *et al.*, 1988; Wolfe, 1988), cat brain (Koenig, 1969), rat kidney (McDowell, 1972a,b), and sheep and guinea pig hearts (Schultz *et al.*, 1982; Whitten and Murray, 1963). Previous researchers have suggested that the testes may be the most sensitive organ as morphological damage to the testes occurred in rats exposed to 1080 in their drinking water at doses that caused no morphological changes in the liver and kidneys (Savarie, 1984). In contrast, Whitten and Murray (1963) working with sheep concluded that the heart is the most sensitive organ. Acute multifocal injury to the myocardium occurred after doses as low as 0.11 mg/kg/day for 3–7 days, and lesser effects were also noted at doses as low as 0.055 mg/kg/day and attributed to 1080-induced damage.

This 90-day study has confirmed that the epididymides,

TABLE 11
Incidence of Microscopic Observations—Recovery Sacrifice:
Male Rats

Tissue observation	0 mg/kg/day (control)		0.25 mg/kg/day	
	DOS	SAC	DOS	SAC
Epididymes				
Within normal limits	0	10	0	0
Hyalitis, moderate	0	0	0	1
Hypospermia	0	0	0	9
Mild	0	0	0	6
Moderate	0	0	0	3
Testes				
Within normal limits	0	10	0	0
Degeneration	0	0	0	10
Mild	0	0	0	1
Moderate	0	0	0	3
Severe	0	0	0	6
Heart				
Within normal limits	0	8	0	7
Cardiomyopathy	0	2	0	3
Trace	0	1	0	3
Mild	0	1	0	0

Note. DOS = died on study, SAC = animals euthanized for scheduled necropsies. Number of animals examined = 0 in the DOS group and 10 in the SAC group.

testes, and the heart are target organs for sublethal effects of 1080. Earlier reports suggest that seminiferous tubules degenerated after rats drank water containing 1080, but completely regenerated after treatment had been withdrawn (Sullivan *et al.*, 1979). By contrast, recovery from testicular damage did not occur in our current 90-day study even after 56 days without treatment. Mazzanti (1965) described regressive modifications of the seminiferous tubules caused by sodium monofluoroacetate in albino rats, and described the action of sodium monofluoroacetate as similar to that of fluoroacetamide. Treatment of normal rats with fluoroacetamide also produced atrophic testes (Mazzanti *et al.*, 1968), with observations of morphological changes showing that testicular germinal epithelium was fully regenerated 165 days after treatment. This suggests potential for the reversal of the type of testicular damage described in this study outside a 56-day recovery period.

In the study by Sullivan *et al.* (1979), Sprague-Dawley rats were dosed with 0, 0.07, 0.18, or 0.71 mg/kg/day for 7 days followed by 21 days without treatment. Testicular atrophy and nonreversible tubular degeneration were found in the mid- and high-dose groups. At the lowest dose (0.07 mg/kg/day), it is unclear whether or not there was an effect. This publication reports "the testes were histologically normal, but acute degenerative changes were evident, there being no evidence of spermatocyte depletion or of seminiferous tubular atrophy," which appears to be contradictory. Our interpretation is that

this dose of 0.07 mg/kg/day could be considered close to the borderline of the LOEL and NOEL.

These earlier 1080 studies, particularly the drinking water study in rats (Sullivan *et al.*, 1979) and the multidose study in sheep (Whitten and Murray, 1963), demonstrate that there could be deleterious effects at dose levels lower than the NOEL of 0.075 mg/kg/day determined by the current 90-day rat study. It is likely that the different results are due to different routes of exposure, and interspecies differences in susceptibility between sheep and rats. Other vertebrate pesticides have similar toxicity. For example, in a five-day study in rats, the NOEL for brodifacoum, a rodenticide used throughout the world, was 0.02 mg/kg/day (WHO, 1995).

In addition to the sublethal effect of 1080 previously reported in the literature, we have recently sourced a previously unknown and unpublished 90-day study (Wolfe, 1988) conducted by Hazleton Laboratories in 1988. In this study, Sprague-Dawley rats were dosed with 1080 at doses of 0, 0.05, 0.20, or 0.50 mg/kg/day. The NOEL for 1080 in rats defined by Wolfe was 0.05 mg/kg/day. The LOEL was 0.20 mg/kg/day based on dose-related effects similar to those reported for this study (e.g., absolute and/or relative weight of the heart and spleen, abnormal sperm and decreased size and weight of testes and epididymides in males). The study by Wolfe (1988) did not include 1080 analyses in blood and urine for correlation of toxic effects with exposure levels, and no recovery periods were included.

Our current study and Wolfe's earlier study confirm the principal target organ of concern for sublethal effects from 1080 exposure in rats to be the testes. Oligospermia or aspermia have been associated with exposure of mink to sublethal doses of 0.08 mg/kg/day 1080 for 2 months (Hornshaw *et al.*, 1986), providing further evidence that the sublethal effects of 1080 occur across a range of species. Although no lesions or microscopic effects on sperm abundance or development were observed in starlings fed sublethal doses of 1080 over a four-week period, a reduction in testis weight (although not at statistically significant levels) was reported (Balcomb *et al.*, 1983). These authors suggested a difference in the sensitivity of birds and mammals to the effects of sublethal, chronic doses of 1080 on testes.

In a recent publication on 1080, a single dose at 1.0 mg/kg (4 times higher than the top dose of 0.25 mg/kg/day used in this 90-day study) administered to male Sprague-Dawley rats was associated with testicular toxicity (Shinoda *et al.*, 2000). The study by Shinoda *et al.* highlights the "steep" dose-response curve associated with 1080 toxicity, and that even a single exposure could affect sperm production and viability (heart tissue was not examined).

The current 90-day study has established blood and urine concentrations in rats that can be correlated with LOEL and NOEL doses of 1080 on a mg/kg/day basis. After administration of 0.25 mg/kg/day 1080 for 10 or 77 days, the LOEL dose, correlated with mean urine concentrations of 0.059 $\mu\text{g/ml}$ and

plasma concentrations of 0.234–0.283 $\mu\text{g/ml}$, 1 h after exposure and 0.069–0.089 $\mu\text{g/ml}$, 12 h after exposure. The NOEL dose of 0.075 mg/kg/day correlated with a mean urine concentration of 0.032 $\mu\text{g/ml}$ and a mean plasma concentration of 0.088–0.84 $\mu\text{g/ml}$, 1 h after exposure and 0.021–0.023 $\mu\text{g/ml}$, 12 h after exposure. However, previous studies have established a lower LOEL of 0.20 mg/kg/day (Wolfe, 1988) and 0.07 mg/kg/day (Sullivan *et al.*, 1979). Hence caution is required with regard to emphatically stating that blood and urine concentrations below those detected in rats exposed in this study would not be associated with sublethal effects. Doses below 0.25 mg/kg/day (i.e., 0.20 and 0.07 mg/kg/day) have been associated with toxicity in rats, hence, concentrations of 1080 in blood and urine below those reported in rats receiving 0.25 mg/kg/day could be associated with damage to the testes and the heart.

We defined a higher NOEL than Wolfe (1988) as a result of using different doses. This study involved treatment of rats with 0.025, 0.075, and 0.25 mg/kg/day, whereas in the study by Wolfe (1988) there was a gap between the dose level of 0.05 mg/kg/day and 0.20 mg/kg/day. Based on the results of this study, the NOEL for rats administered 1080 via oral gavage for 90 days was 0.075 mg/kg/day and is similar to the NOEL of 0.1 mg/kg/day established in a teratogenicity study in rats (Eason *et al.*, 1999). Target organs in rats include the testes and the heart. Severe hypospermia, severe degeneration of the seminiferous tubules of the testes, and cardiomyopathy were seen in males at 0.25 mg/kg/day. Sperm physiology was affected by 1080 exposure at 0.25 mg/kg/day and did not return to normal after 56 days cessation of exposure. The potential for reversibility of, or recovery from testicular damage resulting from sublethal exposure to 1080, especially from doses below the LOEL, could be addressed in future studies incorporating longer recovery periods and measurement of additional fertility parameters.

REFERENCES

- Balcomb, R., Bowen, C. A., 2nd, and Williamson, H. O. (1983). Acute and sublethal effects of 1080 on starlings. *Bull. Environ. Contam. Toxicol.* **31**, 692–698.
- Dunnett, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Stat. Assoc.* **56**, 2–64.
- Eason, C. T., Gooneratne, R., Fitzgerald, H., Wright, G., and Frampton, C. (1994a). Persistence of sodium monofluoroacetate in livestock animals and risk to humans. *Hum. Exp. Toxicol.* **13**, 119–122.
- Eason, C. T., Gooneratne, R., and Rammell, C. G. (1994b). A review of the toxicokinetics and toxicodynamics of sodium monofluoroacetate in animals. In *Proceedings of the Science Workshop on 1080* (A. A. Seawright and C. T. Eason, Eds.), pp. 82–89. The Royal Society of New Zealand Miscellaneous Series 28.
- Eason, C. T., Wickstrom, M., Turck, P., and Wright, G. R. G. (1999). A review of recent regulatory and environmental toxicology studies on 1080: Results and implications. *N.Z. J. Ecol.* **23**, 129–137.
- Eisler, R. (1995). Sodium monofluoroacetate (1080) hazards to fish, wildlife, and invertebrates: Synoptic review. U.S. Department of the Interior National Biological Service Biological Report 27, p. 47.
- Fagerstone, K. A., Savarie, P. J., Elias, D. J., and Schafer, E. W., Jr. (1994). Recent regulatory requirements for pesticide registration and the status of compound 1080 studies conducted to meet EPA requirements. In *Proceedings of the Science Workshop on 1080* (A. A. Seawright and C. T. Eason, Eds.), pp. 33–38. The Royal Society of New Zealand Miscellaneous Series 28.
- Hornshaw, T. C., Ringer, R. K., Aulerich, R. J., and Casper, H. H. (1986). Toxicity of sodium monofluoroacetate (compound 1080) to mink and European ferrets. *Environ. Toxicol. Chem.* **5**, 213–223.
- Koenig, H. (1969). Acute axonal dystrophy caused by fluorocitrate: The role of mitochondrial swelling. *Science* **164**, 310–312.
- Mazzanti, L., Lopez, M., and Berti, M. G. (1965). [Atrophy of the testis produced by sodium monofluoroacetate in albino rats.] *Experientia* **21**, 446–447.
- Mazzanti, L., Lopez, M., and Del Tacca, M. (1968). [Regeneration of the atrophic testis by fluoroacetamide.] *Experientia* **24**, 258–259.
- McDowell, E. M. (1972a). Light- and electron-microscopic studies of the rat kidney after administration of inhibitors of the citric acid cycle *in vivo*: Changes in the proximal convoluted tubule during fluorocitrate poisoning. *J. Pathol.* **108**, 303–318.
- McDowell, E. M. (1972b). Light- and electron-microscopic studies of the rat kidney after administration of inhibitors of the citric acid cycle *in vivo*. I. Effects of sodium fluoroacetate on the proximal convoluted tubule. *Am. J. Pathol.* **66**, 513–542.
- Milliken, G. A., and Johnson, D. E. (1992). *Analysis of Messy Data*. Chapman and Hall, London.
- Savarie, P. (1984). Toxic characteristics of fluorocitrate, the toxic metabolite of compound 1080. In *Proceedings of the Eleventh Vertebrate Pest Conference* (D. O. Clark, Ed.), pp. 132–137.
- Seawright, A. A., and Eason, C. T., Eds. (1994). *Proceedings of the Science Workshop on 1080*. The Royal Society of New Zealand Miscellaneous Series 28, p. 178.
- Schultz, R. A., Coetzer, J. A., Kellerman, T. S., and Naude, T. W. (1982). Observations on the clinical, cardiac and histopathological effects of fluoroacetate in sheep. *Onderstepoort J. Vet. Res.* **49**, 237–245.
- Shinoda, K., Mitsumori, K., Uneyama, C., and Uehara, M. (2000). Induction and inhibition of testicular germ cell apoptosis by fluoroacetate in rats. *Arch. Toxicol.* **74**, 33–39.
- Smith, F. A., Gardner, D. E., and Yuile, C. L. (1977). Defluorination of fluoroacetate in the rat. *Life Sci.* **20**, 1131–1138.
- Steel, R. G. D., and Torrie, J. H. (1980). *Principles and Procedures of Statistics. A Biometrical Approach*. McGraw-Hill, New York.
- Sullivan, J. L., Smith, F. A., and Garman, R. H. (1979). Effects of fluoroacetate on the testis of the rat. *J. Reprod. Fertil.* **56**, 201–207.
- Twigg, L. E., King, D. R., and Bradley, A. J. (1988). The effect of sodium monofluoroacetate on plasma testosterone concentration of *Tiliqua rugosa* (Gray). *Comp. Biochem. Phys.* **91**, 343–347.
- U.S. EPA (1998). Health effects test guidelines OPPTS 870-3100 90-day oral toxicity in rodents. United States Environmental Protection Agency. Prevention, Pesticides and Toxic Substances (7101). EPA 712-C-98-199, August 1998.
- Welch, B. L. (1937). The significance of difference between two means when the population variances are unequal. *Biometrika* **29**, 50–62.
- Whitten, J. H., and Murray, L. R. (1963). The chemistry and pathology of Georgina River poisoning. *Aust. Vet. J.* **39**, 168–173.
- WHO (1995). World Health Organisation: Anticoagulant rodenticides. *Environ. Health Criteria* **175**, 97.
- Wolfe, G. (1988). Subchronic toxicity study in rats with sodium monofluoroacetate. Study No. HLA-2399-118. Unpublished study conducted by Hazelton and cited in EPA Reregistration Eligibility Decision (RED) Sodium fluoroacetate.