Two-Generation Reproduction Study of Ammonium Perchlorate in Drinking Water in Rats Evaluates Thyroid Toxicity

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Perchlorate is an inorganic ion that has recently been detected in drinking water supplies throughout the country, but little is known about its effects on reproductive function. This two-generation reproductive study examines the effects of ammonium perchlorate on the male and female reproductive systems in rats, and on the growth and development of offspring. Adult Sprague-Dawley rats (30/sex/group) were given continuous access to ammonium perchlorate in their drinking water at doses of 0, 0.3, 3.0, and 30.0 mg/kg-day. F1 generation rats were given the same ammonium perchlorate doses as their respective P1 generation sires and dams beginning at weaning and continuing through the day of sacrifice. Standard reproductive parameters were evaluated; blood was collected for determination of serum thyroid-stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) levels. Histopathological examination was conducted on major tissues, including the thyroid. No significant changes in developmental parameters were observed. In the F1 generation adult rats, relative thyroid weights were significantly increased in all dose groups for female rats and in the 3.0 and 30.0 mg/kg-day dose groups for male rats. Histopathologic changes in the thyroid consisted of hypertrophy and hyperplasia that increased in incidence and severity in a dose-related manner. Dose-related, statistically significant changes in TSH and T_4 or T_3 occurred at doses higher than those that resulted in changes in thyroid weight and thyroid histopathology, 30 mg/kg-day. Thus, perchlorate is not a reproductive toxicant in rats when administered in the drinking water at doses up to 30 mg/kg-day, but it can affect the thyroid at doses $\geq$3 mg/kg-day. Based on these findings, 0.3 mg/kg-day is identified to be the no-observable-adverse-effect level (NOAEL) for this study.

Keywords Perchlorate, Rat, Reproduction, Thyroid

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Perchlorate compounds have been widely used as solid rocket propellants and ignitable sources in munitions and fireworks. Perchlorates are also a laboratory waste by-product of perchloric acid. Perchlorate salts, particularly potassium perchlorate, have been used therapeutically to treat hyperthyroidism resulting from Graves' disease and amiodorone-induced thyrotoxicosis. Recent advances in analytical chemistry for perchlorate have revealed that perchlorate is present in the public drinking water supply in several areas in California (see http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/perchl/perchl.htm) and also present in Lake Mead in Nevada (U.S. Environmental Protection Agency [EPA] 1998). The current detection limit for perchlorate is 4 ppb. Perchlorate has been detected in Lake Mead and the Colorado at levels of 4 to 16 ppb and has been detected in 38 California public water supply wells at concentrations greater than the provisional action level of 18 ppb (U.S. EPA 1998). Therefore, State and Federal regulatory agencies must conduct risk assessments on perchlorate in order to develop drinking water standards.

The perchlorate ion is a tetrahedron with four oxygen molecules at the corners and a chlorine atom at the center. The perchlorate ion, with a partial molal ionic volume of 44.5 at 25°C, is of a similar ionic size to iodide with a partial molal ionic volume of 36.7 ml/mol (Wolff 1998). Perchlorate competitively inhibits the active transport of iodide into the thyroid and also stimulates the discharge of unorganified iodide from the thyroid (Saito et al. 1983; Wolff 1998). Perchlorate was used until the mid-1960s at doses of 600 to 1200 mg/day to treat people who had hyperthyroidism because of Graves' disease (Connell 1981; Crooks and Wayne 1960; Godley and Stanbury 1954; Morgans and Trotter 1960; Stanbury and Wyngaarden 1952); however, very little data suitable for risk assessment purposes are available. Many studies have examined the effects of perchlorate in Graves' patients but few have studied the effects in normal humans. The studies that were conducted in normal humans did

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not look at long-term exposure to perchlorate. Long-term studies in animals clearly show thyroid toxicity at high doses; but generally these studies did not examine targets other than the thyroid. No two-generation reproductive study in any species has been conducted prior to this study. In summary, the perchlorate database defines well the mechanisms by which perchlorate acts on the thyroid but provides little information on the dose-response of perchlorate or on the likely effects in normal humans after chronic exposure to low doses.

In 1997, Toxicology Excellence for Risk Assessment (*TERA*) convened an independent peer review panel that evaluated the suitability of the perchlorate database for developing a reference dose (RfD) for chronic environmental exposure by the oral route. Overall, the panel concluded that the database for perchlorate was insufficient to support the development of a RfD. Several major questions were left unanswered by the existing data including the shape of the dose-response curve in humans, the effects of perchlorate after long-term exposure, and the possibility of effects in organs or systems other than the thyroid. The panel recommended that additional studies of perchlorate be conducted. As a result of this recommendation, a second panel of risk assessors, perchlorate experts, and thyroid experts was convened in order to develop a prioritized list of toxicological studies critical for the development of a RfD (see http://www.tera.org/peer). That panel recommended the following studies: developmental neurotoxicity, 90-day rat toxicity, rabbit developmental toxicity, two-generation reproductive toxicity, mutagenicity/genotoxicity, immunotoxicity, and kinetics to be conducted under current U.S. EPA guidelines.

The purpose of this two-generation reproductive study was to provide information concerning the effects of ammonium perchlorate on the male and female reproductive systems in rats, including gonadal function, estrous cycles, mating behavior, conception, parturition, lactation, weaning, and the growth and development of offspring. The study was also designed to provide information about effects on neonatal morbidity, mortality and preliminary data on potential prenatal developmental toxicity, as well as to serve as a guide for use in the design of subsequent tests.

MATERIALS AND METHODS[1]

Animals

Crl:CD(SD)IGS BR VAF/Plus (Sprague-Dawley) rats were supplied by Charles River Laboratories, Inc. (Portage, MI).

P Generation

Thirty rats per sex were randomly assigned per dose group. Rats, approximately 38 and 39 days old on arrival, were individually housed except during the cohabitation and 21-day postpartum periods. Beginning no later than day of gestation (DG) 20, female rats were individually housed in nesting boxes. All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources 1996).

F1 and F2 Generation Pups/Rats

After weaning, the F1 generation rats were individually housed before and after cohabitation. The same type of caging and housing schedule was used as described for the P generation rats. On day of lactation (DL) 21, 30 male and 30 female F1 generation pups per group were randomly selected, resulting in a total of 240 rats (120 per sex) chosen for continued evaluation. At least one male and one female pup per litter, when possible, were selected.

Test Material

Ammonium perchlorate (CAS No. 7790-98-9), 99.8% purity, was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI, lot 03907LF). Test formulations of ammonium perchlorate in deionized water were prepared weekly at concentrations that yielded target doses of 0, 0.3, 3.0, and 30.0 mg/kg-day. Perchlorate stock solution at a concentration of 50 mg/ml was prepared, then each week appropriate dilutions of the stock solution were made to deliver the target dose, based on actual measured body weight and water consumption from the previous week. Water consumption was measured by weighing the water bottles. The concentrations of the ammonium perchlorate dosing solutions were verified by ion chromatographic analysis to be within an acceptable range of $\pm 10\%$. Adult P and F1 generation rats were given continuous access to test material in their drinking water. Separate doses for the F1 generation were estimated beginning at weaning; water concentrations for target doses were estimated as for the parental generation. F1 and F2 generation pups may have been exposed in utero during gestation and via maternal milk and maternal water during the postpartum period.

Observations

P Generation

Male and female rats were observed for viability at least twice each day of the study. The rats were examined daily for clinical observations, body weights and feed and water consumption.

Estrous cycling for the P generation female rats was evaluated daily beginning 21 days before the scheduled cohabitation period and continuing until evidence of mating. This was considered to be DG 0. The female rats were evaluated for duration of gestation (DG 0 to the day the first pup was delivered) and litter size (live and dead pups and live pups only). Each litter was examined daily for pup viability. Maternal behavior and litters were evaluated on DLs 1, 4, 7, 14, and 21.

Female rats that did not deliver a litter were sacrificed on DG 25 and examined for pregnancy status by staining with

[1]The study was conducted in compliance with Good Laboratory Practice (GLP) regulations of the U.S. Environmental Protection Agency (TSCA/FIFRA), the Organization for Economic Cooperation and Development (OECD), and the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF).

10% ammonium sulfide to confirm the absence of implantation sites.

A gross necropsy was conducted at terminal sacrifice of all P generation rats. The adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, and thyroids following fixation were weighed. Histopathologic examination by a board certified veterinary pathologist was performed on the aorta, bone marrow, cervix/vagina, esophagus, eyes, femur, large and small intestine, lungs and trachea, lymph nodes, mammary gland, pancreas, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, stomach, thymus, thyroid, urinary bladder, uterus, and Zymbal's gland and gross lesions from the control and high-dose groups. In addition, the thyroid from all low-dose and intermediate-dose male and female rats was examined microscopically.

Hormone Analysis

Blood was collected from the inferior vena cava from control and exposed rats at the time of sacrifice. Serum was immediately frozen on dry ice and sent for analysis to Ani-Lytics, Inc. (Gaithersburg, MD) for determination of serum thyroid-stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) levels. Throughout the sacrifice procedures, every effort was made to avoid inducing stress in the rats because this could affect scheduled hormone evaluations. Assays for T_3, T_4, and TSH were performed using radioimmunoassay (RIA) kits according to manufacturer's standard procedures. Samples and standards were run in triplicate. Assay kits from the same batch number and with the same expiration date were used for all thyroid hormone or TSH measurements for each animal. Tracer (^{125}I) radioactivity was measured with a gamma counter (Packer Instrument Co., Meriden, CT). Sources of the RIA kits and antiserum/antibody were: (1) T_3 RIA assay kits were purchased from Diagnostic Products Corp. (Los Angeles, CA) and canine T_3 antibody coated tubes were used; (2) T_4 RIA assay kits were purchased from from Diagnostic Products Corp. (Los Angeles, CA) and T_4 antibody coated tubes were used; (3) TSH RIA assay kits were purchased from Amersham Corp. (Arlington Heights, IL) and lyophilized rabbit anti-rat TSH serum and Amerlex-M second antibody (donkey anti-rabbit serum coated onto magnetized polymer particles containing sodium azide) were both used.

Sperm Analysis

A sample from the left cauda epididymis was used for evaluation of sperm motility using the Hamilton Thorne Computer Assisted Sperm Analyzer (CASA). A suspension prepared from the remaining portion of the left cauda epididymis was used to morphologically examine approximately 200 sperm per rat at 400× to 1000× magnification. Morphological end points analyzed consisted predominantly of head and tail abnormalities. The suspension remaining after the preparation of slides for morphology for each rat was homogenized to determine cauda epididymal sperm concentration (sperm per gram of tissue weight). The left testis was homogenized after weighing (both before and after removal of the tunica albuginea). Ten fields were analyzed to determine testicular spermatid concentration (spermatids per gram of tissue weight).

F1 Generation Pups/Rats and F2 Generation Litters

Day 1 of lactation (postpartum) was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam).

Each litter was evaluated for viability at least twice each day of the 21-day postpartum period. Dead pups observed at these times were removed from the nesting box. When not precluded by autolysis or cannibalization by the dam, any pup found dead was necropsied and examined for the cause of death and gross lesions. Rats were also observed for clinical observations and effects of the test substance daily during the postweaning period.

The pups present in each litter were counted once each day. Physical signs (including gross external physical anomalies) were recorded for the pups once each day during the preweaning and postweaning periods. Pup body weights and observations of nursing behavior were recorded along with the sex of the pups on DLs 1 (birth), 4, 7, 14, and 21. In addition, body weights and feed and water consumption were recorded for F1 generation adult rats.

Female rats were evaluated for the age of vaginal patency beginning on DL 28 and male rats were evaluated for the age of preputial separation beginning on DL 39. The cohabitation period for F1 generation rats consisted of a maximum of 14 days. Estrous cycling, mating performance, duration of gestation, fertility parameters, maternal behavior, and litter data were recorded as described above for the P generation dams and litters.

Gross necropsy, blood collection for thyroid hormone analysis, histopathology, and analysis of sperm parameters were conducted on F1 generation adult rats as was described for the P generation.

F2 generation pups were sacrificed on DL 21. At least three pups/sex/litter were necropsied and examined for gross lesions, including a single cross-section of the head for apparent hydrocephaly. Histopathological evaluation was performed on adrenal glands, brain, thyroid/parathyroid, liver, spleen, kidneys, and thymus. The brain, thymus, and spleen were also weighed. Following fixation, thyroids were carefully trimmed and weighed, and sent to Research Pathology Services, Inc., for histopathological evaluation. Whole blood samples, pooled by sex per litter, collected into serum separator tubes, and centrifuged, were sent for analysis of TSH, T_3, and T_4 levels as previously cited.

Statistical Analyses

Clinical observations and other proportion data were analyzed using the variance test for homogeneity of the binomial distribution. Continuous data (e.g., body weights, body weight changes, feed consumption data, organ weights, duration of gestation, litter averages for pup body weights, percent male

pups, pup viability, and cumulative survival) were analyzed using Bartlett's test of homogeneity of variances and the analysis of variance, when appropriate (i.e., when Bartlett's test was not significant [$p > .05$]). If the analysis of variance was significant ($p \leq .05$), Dunnett's test was used to identify the statistical significance of the individual groups. If the analysis of variance was not appropriate (i.e., when Bartlett's test was significant [$p \leq .05$]), the Kruskal-Wallis test was used, when 75% or fewer ties were present; when more than 75% ties were present, Fisher's Exact Test was used. In cases where the Kruskal-Wallis test was statistically significant ($p \leq .05$), Dunn's method of multiple comparisons was used to identify the statistical significance of the individual groups. All other natural delivery data involving discrete data were evaluated using the Kruskal-Wallis test procedures previously described.

RESULTS

P Generation Rats

Consumed Doses

The average actual consumed daily doses data are summarized in Table 1. Water analyses indicated that adequate concentrations of ammonium perchlorate in water were achieved, within ±10% of target concentration, in order for rats to receive the target doses. For the parental generation, the actual consumed doses met or exceeded target doses. Doses consumed by females during gestation exceeded target doses 33% to 34% in all dose groups. The excess over target doses was attributed to the higher water consumption values of dams during pregnancy.

Mortality and Clinical Observations

There were no deaths, abortions, or premature deliveries attributed to exposure to ammonium perchlorate. No clinical observations were considered related to the ammonium perchlorate exposure because the incidences were not dose-dependent and the observations that were noted commonly occur in this laboratory with this strain of rat. (Data not shown.)

Water and Feed Consumption Values

Absolute (g/day) and relative (g/kg/day) water consumption values for male rats in both 0.3 and 30.0 mg/kg-day dose groups were significantly reduced ($p \leq .05$), compared to the control group. These reductions in water consumption were considered treatment-related because they were dose-dependent and occurred over the entire exposure period. In contrast, average maternal absolute and relative water consumption values during the precohabitation, gestation, and lactation periods were comparable among the four groups. (Data not shown.)

Absolute (g/day) and relative (g/kg/day) feed consumption values for the male and female rats were generally unaffected by doses of ammonium perchlorate as high as 30.0 mg/kg-day. (Data not shown.)

Necropsy, Terminal Body Weights, and Organ Weights

No necropsy observations were considered to be treatment-related.

Average body weights for male or female rats (during the precohabitation, gestation, and lactation periods) were comparable among the four exposure groups through DS 134 (Table 2). Absolute thyroid weights for the male rats were increased in all three treated groups in a dose-dependent manner; the increases were significant in the 3.0 and 30.0 mg/kg-day target dose groups. Absolute thyroid weights for the female rats were significantly increased in the 30.0 mg/kg-day dose group, compared to the control group. The ratios of thyroid weight to terminal body weight and to the absolute brain weight were significantly increased in the 30.0 mg/kg-day dose group for both the male and female rats. Absolute and relative weights for the other organs collected at necropsy were comparable to control values. (Data not shown.)

Male Mating, Fertility and Sperm Evaluation (Table 3)

Mating and fertility parameters in male P generation rats were unaffected by exposure to ammonium perchlorate at doses up to 30 mg/kg-day. No statistically significant or dose-dependent differences occurred in sperm motility, sperm count, sperm density, or morphology by doses of ammonium perchlorate as high as 30.0 mg/kg-day.

Female Estrous Cyclicity, Mating, Fertility, Natural Delivery, and Litter Observations (Table 4)

Estrous cycling, mating, and fertility parameters in P generation females were unaffected by doses as high as 30 mg/kg-day. There were no differences between controls and treated groups in the number of estrous stages per 14 days or the number of rats with normal length of estrous cycles.

Natural delivery observations were unaffected by exposure to ammonium perchlorate doses as high as 30.0 mg/kg-day. The gestation index was 100% in each group. The number of dams delivering litters, the duration of gestation, averages for implantations, live litter sizes and stillbirths, dams with stillborn pups, viability and lactation indices, sex ratios, and pup body weights were comparable among the four groups and did not significantly differ. No dams had all pups die before DL 21.

The lactation index was significantly increased in the 3.0 ($p \leq .05$) and 30.0 ($p \leq .01$) mg/kg-day dose groups compared to the control group. These increases were not considered treatment-related because the expected effect of a toxicant would be a decrease, rather than an increase, in pup survival.

Histopathology (Table 5)

Exposure-related histomorphologic changes were observed only in the thyroid gland. These changes occurred in both males and females and were primarily hypertrophy and hyperplasia of the thyroid follicular epithelium. In many of the affected thyroids there were increased numbers of small follicles (hyperplasia) and these follicles had enlarged (hypertrophied) follicular

TABLE 1
Actual consumed dosages of ammonium perchlorate (mg/kg-day)

	Target dose group			
	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30.0 mg/kg-day
P generation				
Male				
Terminal body weight (g)	631 ± 64	645 ± 63	664 ± 53	636 ± 55
Average water consumption (g/day)	42 ± 6	40 ± 5	42 ± 6	38 ± 4**
Average actual consumed dose	0 ± 0.0 (100%)[a]	0.3 ± 0.0[b] (100%)	3.1 ± 0.4 (103%)	30.8 ± 4.2 (103%)
Female				
Precohabitation				
Body weight (g)	289 ± 28	294 ± 31	286 ± 26	288 ± 20
Average water consumption (g/day)	37 ± 9	34 ± 9	36 ± 7	34 ± 7
Average actual consumed dose	0 ± 0.0 (100%)	0.3 ± 0.1 (100%)	3.2 ± 0.6 (107%)	30.0 ± 6.4 (100%)
Gestation				
Body weight (g)	458 ± 41	451 ± 40	451 ± 35	455 ± 35
Average water consumption (g/day)	54 ± 8	49 ± 10	53 ± 11	55 ± 14
Average actual consumed dose	0 ± 0.0 (100%)	0.4 ± 0.1 (133%)	4.0 ± 0.8 (133%)	40.2 ± 10.3 (134%)
Lactation				
Body weight (g)	352 ± 33	359 ± 23	361 ± 27	353 ± 24
Average water consumption (g/day)	76 ± 15	82 ± 15	85 ± 16	81 ± 16
Average actual consumed dose	0 ± 0.0 (100%)	0.3 ± 0.1 (100%)	3.3 ± 0.6 (110%)	31.6 ± 6.8 (105%)
F1 generation				
Male				
Terminal body weight (g)	617 ± 76	665 ± 58	653 ± 74	638 ± 49
Average water consumption (g/day)	43 ± 6	41 ± 5	43 ± 6	42 ± 6
Average actual consumed dose	0 ± 0.0 (100%)	0.3 ± 0.0 (100%)	2.7 ± 0.4 (90%)	26.5 ± 3.7 (88%)
Female				
Precohabitation				
Body weight (g)	286 ± 26	299 ± 21	300 ± 29	301 ± 28
Average water consumption (g/day)	34 ± 5	33 ± 6	35 ± 7	37 ± 6
Average actual consumed dose	0 ± 0.0 (100%)	0.3 ± 0.1 (100%)	2.8 ± 0.6 (93%)	27.8 ± 4.8 (93%)
Gestation				
Body weight (g)	441 ± 20	459 ± 33	459 ± 43	456 ± 36
Average water consumption (g/day)	61 ± 11	56 ± 11	58 ± 11	61 ± 10
Average actual consumed dose	0 ± 0.0 (100%)	0.2 ± 0.0 (67%)	3.0 ± 0.6 (100%)	28.3 ± 5.8 (94%)
Lactation				
Body weight (g)	354 ± 19	361 ± 23	368 ± 21	367 ± 21
Average water consumption (g/day)	90 ± 14	90 ± 11	84 ± 13	85 ± 14
Average actual consumed dose	0 ± 0.0 (100%)	0.4 ± 0.0 (133%)	3.6 ± 0.7 (120%)	34.7 ± 6.2 (116%)

[a]Actual doses expressed as percent target dose.
[b]Mean ± *SD*.
**Significantly different from control, $p \leq .01$.

TABLE 2
Terminal body and organ weights (g)[a]

	Target dose group			
	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30.0 mg/kg-day
P generation				
Male				
Absolute thyroid weight	0.039 ± 0.004	0.041 ± 0.006	0.043 ± 0.005*	0.051 ± 0.007*
Relative thyroid weight[b]	6.249 ± 0.735	6.404 ± 0.956	6.431 ± 0.667	8.088 ± 1.325**
Ratio of thyroid weight to brain weight	1.67 ± 0.19	1.72 ± 0.29	1.81 ± 0.22	2.17 ± 0.33**
Female				
Absolute thyroid weight	0.024 ± 0.004	0.025 ± 0.004	0.025 ± 0.004	0.030 ± 0.004**
Relative thyroid weight[b]	6.882 ± 1.233	6.970 ± 1.080	6.904 ± 1.162	8.505 ± 1.370**
Ratio of thyroid weight to brain weight	1.10 ± 0.19	1.15 ± 0.17	1.13 ± 0.21	1.37 ± 0.20**
F1 generation pups				
Male				
Thyroid weight	0.008 ± 0.002	0.008 ± 0.002	0.008 ± 0.002	0.010 ± 0.002**
Spleen weight	0.13 ± 0.04	0.14 ± 0.04	0.15 ± 0.06	0.15 ± 0.05
Female				
Thyroid weight	0.008 ± 0.002	0.009 ± 0.002	0.008 ± 0.002	0.010 ± 0.002**
Spleen weight	0.13 ± 0.06	0.15 ± 0.05	0.14 ± 0.07	0.15 ± 0.05*
F1 generation adults				
Male				
Absolute thyroid weight	0.036 ± 0.005	0.041 ± 0.009	0.044 ± 0.005**	0.063 ± 0.012**
Relative thyroid weight[b]	5.935 ± 0.840	6.116 ± 1.092	6.705 ± 0.600**	9.888 ± 1.823**
Ratio of thyroid weight to brain weight	1.52 ± 0.21	1.68 ± 0.37	1.77 ± 0.17**	2.64 ± 0.52**
Female				
Absolute thyroid weight	0.022 ± 0.003	0.025 ± 0.004*	0.028 ± 0.004**	0.033 ± 0.005**
Relative thyroid weight[b]	6.279 ± 0.917	7.032 ± 1.224*	7.707 ± 1.172**	9.018 ± 1.333**
Ratio of thyroid weight to brain weight	1.01 ± 0.14	1.13 ± 0.18*	1.28 ± 0.19**	1.51 ± 0.23**
F2 generation pups				
Male				
Thyroid weight	0.009 ± 0.002	0.008 ± 0.004*	0.010 ± 0.005	0.009 ± 0.002
Female				
Thyroid weight	0.008 ± 0.002	0.008 ± 0.003	0.010 ± 0.005	0.009 ± 0.002*

[a] All organ weights reported as the mean ± *SD*.
[b] Thyroid weight relative to body weight, multiplied by 100.
*Significantly different from the control group value ($p \leq .05$).
**Significantly different from the control group value ($p \leq .01$).

epithelial cells. In the thyroids with a moderate or marked hyperplasia/hypertrophy, there was a decrease or complete absence of visible colloid in the affected follicles.

The degree of the thyroid change ranged from minimal to marked and generally occurred in an exposure-related fashion. The incidence of hyperplasia/ hypertrophy was significantly increased ($p \leq .05$) in the 3.0 and 30.0 mg/kg-day dose groups. An increased incidence of the hyperplasia/hypertrophy, albeit not significant, also occurred in the 0.3 mg/kg-day dose group and was judged to be exposure-related.

All other microscopic changes observed in the other organs and tissues specified for examination from the male and female rats exposed to 30.0 mg/kg-day of ammonium perchlorate were considered to have occurred spontaneously and be typical of those that occur in rodent reproductive studies. Therefore, all other pathologic changes were unrelated to exposure.

Thyroid and Pituitary Hormone Analyses (Table 5)

For the P generation adult male rats, a significant increase ($p \leq .05$) in TSH levels and a concomitant significant decrease ($p \leq .05$) in T_4 values occurred in the 30.0 mg/kg-day dose group, as compared to the control group value. Significant increases ($p \leq .05$) in serum T_3 levels occurred in the 0.3 and 3.0 mg/kg-day dose groups, as compared to the control group

TABLE 3
Male mating, fertility, and sperm observations

Observation	P generation				F1 generation			
	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30 mg/kg-day	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30 mg/kg-day
Days in cohabitation[a]	3.6 ± 3.2	4.5 ± 4.5	3.1 ± 2.4	3.2 ± 2.8	2.9 ± 2.8	3.2 ± 2.6	2.9 ± 1.6	2.4 ± 1.2
Rats that mated[b]	30 (100%)	26 (87%)**	29 (97%)	29 (100%)	29 (97%)	28 (97%)	30 (100%)	29 (100%)
Fertility index[c]	28/30 (93%)	22/26 (85%)	26/29 (90%)	24/29 (83%)	21/29 (72%)	27/28 (96%)**	28/30 (93%)**	27/29 (93%)**
Preputial separation[a,d]	—	—	—	—	46 ± 3	45 ± 2	45 ± 2	46 ± 2
Sperm density[a]	823 ± 285	856 ± 299	770 ± 261	892 ± 264	1544 ± 521	1572 ± 536	1461 ± 439	1373 ± 445
Spermatid count[a]	34 ± 11	33 ± 11	35 ± 14	33 ± 10	37 ± 16	36 ± 14	33 ± 9	30 ± 12
Spermatid concentration[a]	2.0 ± 0.6	1.9 ± 0.6	2.0 ± 0.8	1.9 ± 0.6	2.1 ± 0.9	2.1 ± 0.8	1.9 ± 0.5	1.7 ± 0.8
Spermatid density[a]	116 ± 37	109 ± 34	116 ± 43	113 ± 32	125 ± 44	117 ± 46	109 ± 29	98 ± 41

[a]Mean ± *SD*.
[b]Number examined (*N*).
[c]*N*/*N*.
[d]Average days postpartum that prepuce was observed to be separated.
**Significantly different from the control group value ($p \leq .01$).

TABLE 4
Female estrous cyclicity, mating, fertility, natural delivery, and litter observations

	P generation				F1 generation			
Observation	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30 mg/kg-day	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30 mg/kg-day
Estrous stages/14 days[a]	5.3 ± 0.8	5.2 ± 1.5	5.5 ± 0.9	5.3 ± 1.0	5.0 ± 0.8	4.8 ± 0.8	4.9 ± 0.7	4.9 ± 1.0
Vagina patent [a,d]	—	—	—	—	32.5 ± 2.5	32.0 ± 1.7	30.9 ± 1.5*	31.4 ± 1.7
Duration of gestation[a]	22.8 ± 0.4	22.5 ± 0.5	22.7 ± 0.5	22.8 ± 0.5	22.6 ± 0.5	22.8 ± 0.4	22.8 ± 0.5	22.8 ± 0.4
Implantation sites per delivered litter[b]	15.8 ± 3.1	15.8 ± 3.6	15.0 ± 3.2	15.0 ± 4.1	15.5 ± 3.3	16.2 ± 1.9	15.8 ± 2.9	15.4 ± 3.3
Dams with stillborn pups[b]	3 (11%)	2 (9%)[g]	1 (4%)	2 (8%)	1 (5%)	1 (4%)	4 (14%)	5 (20%)
Dams with no liveborn pups[b]	0	0	0	0	0	0	0	0
Liveborn pups delivered[a]	14.8 ± 3.1	14.7 ± 3.6	14.0 ± 3.3	13.8 ± 3.8	14.3 ± 3.4	15.4 ± 2.0	14.5 ± 3.0	14.2 ± 4.0
Percentage of liveborn and stillborn pups	98.8/1.0	98.8/1.2	99.4/0.3	99.1/0.9	99.6/0.3	99.8/0.2	98.8/1.2	97.5**/2.5**
Viability index[c,e]	405/416 (97%)	318/324 (98%)	346/350 (99%)	325/332 (98%)	280/286 (98%)	410/417 (98%)	399/406 (98%)	346/355 (98%)
Lactation index[c,f]	390/405 (96%)	303/318 (95%)	340/346 (98%)*	321/325 (98%)**	272/280 (97%)	400/410 (98%)	381/399 (96%)	333/346 (96%)
Surviving pups/litter, PND 1[a]	14.8 ± 3.1	14.7 ± 3.6	14.0 ± 3.3	13.8 ± 3.8	14.3 ± 3.4	15.4 ± 2.0	14.5 ± 3.0	14.2 ± 4.0
Surviving pups/litter, DL 21[a]	13.9 ± 3.0	13.8 ± 3.1	13.6 ± 3.2	13.4 ± 3.7	13.6 ± 3.3	14.8 ± 1.8	13.6 ± 3.0	13.3 ± 3.6
Live litter size, DL 1[a]	14.7 ± 3.0	14.6 ± 3.6	14.0 ± 3.3	13.8 ± 3.9	14.2 ± 3.5	15.4 ± 1.9	14.4 ± 2.9	14.1 ± 4.1
Live litter size, DL 21[a]	13.9 ± 3.0	13.8 ± 3.1	13.6 ± 3.2	13.4 ± 3.7	13.6 ± 3.3	14.8 ± 1.8	13.6 ± 3.0	13.3 ± 3.6
Pup weight/litter (g), DL 1[a]	6.6 ± 0.7	6.4 ± 0.6	6.7 ± 0.6	6.6 ± 0.6	6.6 ± 0.6	6.5 ± 0.6	6.5 ± 0.6	6.5 ± 0.4
Pup weight/litter (g), DL 21[a]	37.9 ± 7.4	39.5 ± 6.9	40.5 ± 9.6	39.9 ± 5.9	40.1 ± 7.8	37.0 ± 6.1	38.7 ± 7.4	39.5 ± 9.4

[a] Mean ± *SD*.
[b] Number examined (*N*).
[c] *N*/*N*.
[d] Average day postpartum that the vagina was patent.
[e] Number of live pups on DL 4/number of live pups on DL 1.
[f] Number of live pups on DL 21/number of live pups on DL 4.
[g] One dam was moribund sacrificed on GD 9.
*Significantly different from the control group value ($p \leq .05$).
**Significantly different from the control group value ($p \leq .01$).

TABLE 5
Summary of thyroid effects observed in P, F1, and F2 generations

Effect	Male				Female			
	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30.0 mg/kg-day	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30.0 mg/kg-day
	Follicular cell hyperplasia/hypertrophy (incidence/number thyroids examined)							
P adult								
Minimal	0/30	2/30	1/30	0/30	2/28	4/21	4/26	0/24
Mild	1/30	5/30	5/30	0/30	1/28	3/21	8/26	0/24
Moderate	1/30	2/30	11/30	1/30	1/28	3/21	8/26	2/24
Marked	0/30	0/30	8/30	29/30	0/28	0/21	0/26	22/24
Total	2/30	9/30	25/30*	30/30*	4/28	10/21	20/26*	24/24*
F1 pup								
Minimal	2/28	3/22	3/25	3/23	1/28	3/22	9/24	0/23
Mild	1/28	1/22	10/25	8/23	2/28	2/22	4/24	3/23
Moderate	0/28	0/22	1/25	10/23	0/28	0/22	3/24	13/23
Marked	0/28	0/22	0/25	2/23	0/28	0/22	0/24	7/23
Total	3/28	4/22	14/25	23/23*	3/28	5/22	16/24*	23/23*
F1 adult								
Minimal	0/30	0/30	3/30	0/30	4/20	5/27	6/27	1/25
Mild	3/30	4/30	5/30	0/30	2/20	1/27	6/27	2/25
Moderate	2/30	4/30	5/30	3/30	0/20	0/27	1/27	10/25
Marked	0/30	0/30	6/30	23/30	0/20	0/27	0/27	11/25
Total	5/30	8/30	19/30*	26/30*	6/20	6/27	13/27	24/25*
F2 pup								
Minimal	1/20	0/27	10/28	1/25	1/20	3/27	6/28	2/25
Mild	0/20	2/27	6/28	1/25	0/20	0/27	10/28	3/25
Moderate	0/20	0/27	1/28	14/25	0/20	0/27	2/28	15/25
Marked	0/20	0/27	0/28	6/25	0/20	0/27	0/28	5/25
Total	1/20	2/27	17/28*	22/25*	1/20	3/27	18/28*	25/25**

(Continued on next page)

TABLE 5
Summary of thyroid effects observed in P, F1, and F2 generations *(Continued)*

Effect	Male				Female			
	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30.0 mg/kg-day	0 mg/kg-day	0.3 mg/kg-day	3.0 mg/kg-day	30.0 mg/kg-day
				Hormone levels (mean ± *SD*)				
P adult (*N*)	29	30	30	29	29	30	29	30
T_3 (ng/dl)	72.6 ± 11.2	87.4 ± 16.3*	88.4 ± 18.6*	78.6 ± 14.4	57.8 ± 28.2	64.8 ± 29.3	56.4 ± 14.0	60.4 ± 22.0
T_4 (μg/dl)	4.64 ± 0.58	4.73 ± 0.82	4.74 ± 0.79	3.58 ± 0.86*	2.13 ± 0.68	2.90 ± 0.04*	2.92 ± 0.84*	2.42 ± 0.79
TSH (ng/ml)	1.53 ± 0.96	1.35 ± 0.64	1.49 ± 0.82	3.87 ± 3.50*	2.05 ± 0.87	2.21 ± 0.99	1.99 ± 0.77	2.17 ± 0.74
F1 pup (*N*)	27	21	25	23	28	22	25	23
T_3 (ng/dl)	105.9 ± 10.0	111.2 ± 16.4	109.8 ± 15.7	107.4 ± 16.1	106.0 ± 13.1	109.9 ± 13.1	109.3 ± 13.6	97.6 ± 11.0*
T_4 (μg/dl)	4.40 ± 1.01	4.62 ± 0.98	4.53 ± 0.79	4.52 ± 1.09	4.27 ± 1.02	4.86 ± 0.95*	4.32 ± 0.78	3.91 ± 0.98
TSH (ng/ml)	1.24 ± 0.45	0.94 ± 0.34*	0.88 ± 0.25*	1.27 ± 0.38	1.12 ± 0.51	1.19 ± 0.35	1.14 ± 0.38	1.30 ± 0.35
F1 adult (*N*)	30	30	30	30	30	29	30	29
T_3 (ng/dl)	82.5 ± 8.7	81.3 ± 15.4	83.2 ± 16.5	83.0 ± 13.4	61.5 ± 25.2	51.2 ± 21.9	53.4 ± 19.6	56.8 ± 18.9
T_4 (μg/dl)	3.78 ± 0.55	4.21 ± 0.86*	4.20 ± 0.87*	2.78 ± 0.72**	2.22 ± 1.03	2.03 ± 0.84	2.27 ± 1.05	2.13 ± 0.86
TSH (ng/ml)	2.51 ± 1.01	2.16 ± 1.04	2.30 ± 1.73	5.18 ± 2.52**	1.62 ± 1.01	1.22 ± 0.66	1.65 ± 0.88	2.12 ± 0.69*
F2 pup (*N*)	20	26	28	25	20	26	28	25
T_3 (ng/dl)	106.3 ± 18.3	108.0 ± 14.6	119.5 ± 20.1*	107.1 ± 21.4	108.4 ± 21.1	107.4 ± 13.0	107.9 ± 20.7	98.8 ± 24.0
T_4 (μg/dl)	3.2 ± 0.84	3.3 ± 0.86	3.8 ± 0.88	3.4 ± 0.80	3.4 ± 0.72	3.3 ± 0.79	4.2 ± 1.0*	3.8 ± 0.83
TSH (ng/ml)	0.82 ± 0.19	0.88 ± 0.26	0.95 ± 0.28	0.96 ± 0.21	0.94 ± 0.28	0.91 ± 0.29	0.96 ± 0.22	0.97 ± 0.22

*Significantly different from the carrier group value ($p \leq .05$).
**Significantly different from the carrier group value ($p \leq .01$).

value. Thyroid and pituitary hormone levels in the female P generation rats were unaffected by exposure to ammonium perchlorate as high as 30.0 mg/kg-day.

F1 Generation Pups

Pup Clinical and Necropsy Observations

All clinical and necropsy observations in the F1 generation pups were considered unrelated to ammonium perchlorate exposure (data not shown).

Organ Weights (Table 2)

The weight of the thyroid was significantly increased ($p \leq .05$) for both male and female pups in the 30.0 mg/kg-day dose group. The weight of the spleen was slightly increased in all treated groups (107.7% to 115.4% of the control group value), but the increase was only significant ($p \leq .05$) in the 30.0 mg/kg-day dose group female pups. The weights of all other tissues (brain and thymus) were comparable among the four exposure groups for both male and female pups.

Histopathology in F1 Pups (Table 5)

Exposure-related histomorphologic changes were observed in the thyroid gland of F1 generation male and female pups. These changes were primarily hypertrophy and hyperplasia of the thyroid follicular epithelium. Similar to the observations in P adults, in many of the affected thyroids there were increased numbers of small follicles (hyperplasia) and these follicles had enlarged (hypertrophied) follicular epithelial cells. In the thyroids with a moderate or marked hyperplasia/hypertrophy, there was a decrease or complete absence of visible colloid in the affected follicles.

The degree of the thyroid change ranged from minimal to marked and generally occurred in an exposure-related fashion. The incidence of hyperplasia/hypertrophy was significantly increased ($p \leq .05$) in the 30.0 mg/kg-day dose group for both the male and female pups and in the 3.0 mg/kg-day dose group for the female pups. An increased incidence and severity of hyperplasia/hypertrophy, albeit not significant, also occurred in the 3.0 mg/kg-day dose group in the male pups and was considered to be exposure-related. The incidence in the 0.3 mg/kg-day dose group F1 generation male and female pups was comparable to the control group.

All other microscopic changes observed in the other organs and tissues specified for examination were considered to have occurred spontaneously.

Thyroid and Pituitary Hormone Analyses (Table 5)

Serum T_3 level was significantly reduced ($p \leq .05$) for the female pups in the 30.0 mg/kg-day dose group, compared to the control group value. This reduction in serum T_3 level is considered an effect of ammonium perchlorate exposure because the reduction was exposure dependent and ammonium perchlorate is known to cause a decrease in serum T_3 production (Siglin et al. 2000). TSH levels were significantly reduced ($p \leq .05$) for male pups in the 0.3 and 3.0 mg/kg-day dose groups, compared to the control group value. Serum T_4 values were significantly increased ($p \leq .05$) for female pups in the 0.3 mg/kg/day dose group.

F1 Generation Adult Rats

Consumed Doses

The average actual consumed daily doses data are summarized in Table 1. For the F1 generation, actual doses were 88% to 100% of target for males and 67% to 100% of target for females during all periods, except lactation. During lactation, F1 females were exposed to 116% to 133% of target doses.

Mortality and Clinical Observations

There were no deaths, abortions, or premature deliveries. All clinical observations that occurred in the F1 generation male and female rats were considered unrelated to the test substance. (Data not shown.)

Water and Feed Consumption

Absolute and relative water and feed consumption values for F1 generation male and female (during precohabitation, gestation, and lactation periods) rats were unaffected by exposures up to 30.0 mg/kg-day of ammonium perchlorate. (Data not shown.)

Necropsy, Terminal Body Weights and Organ Weights (Table 2)

No necropsy observations for the male and female rats were considered related to the test substance. Terminal body weights for the male F1 generation rats were significantly increased in the 0.3 mg/kg-day dose group, a non–dose-dependent event, unrelated to ammonium perchlorate exposure (Table 2). Terminal body weights for the female rats were unaffected by the test substance.

Thyroid weights and the ratios of the thyroid weight to the terminal body weight and to the brain weight were significantly increased for the male rats in the 3.0 and 30.0 mg/kg-day dose groups (Table 2), and for the female rats in the 0.3, 3.0, and 30.0 mg/kg-day dose groups. These increases in absolute and relative thyroid weights are considered treatment-related because they were dose-dependent.

Mating, Fertility, Sexual Maturation, and Sperm Evaluation (Table 3)

Mating and fertility in F1 male rats were comparable between control and dose groups. Doses of ammonium perchlorate as high as 30.0 mg/kg-day did not affect the average day of preputial separation for the F1 male rats. The fertility index and the numbers of rats pregnant per rats in cohabitation were significantly increased in the 0.3, 3.0, and 30.0 mg/kg-day dose groups, compared to the control group. This increase in fertility of the male rats exposed to ammonium perchlorate was considered unrelated to treatment because the values were not dose-dependent and were probably due to the lower fertility in the control group

used for the statistical comparison. No statistically significant or dose-dependent differences occurred; sperm motility, count, morphology, and density were unaffected by doses of ammonium perchlorate as high as 30.0 mg/kg-day.

Mating, Fertility, Sexual Maturation, Natural Delivery and Litter Observations (Table 4)

Estrous cycling observations were comparable among the treated female rats prior to cohabitation. There were no differences between controls and treated groups in the number of estrous stages per 14 days or the number of rats with normal length of estrous cycles. There was no effect on the number of days in cohabitation or the number of rats mating during the first or second week of cohabitation. The average day of vaginal patency for the treated F1 female rats was comparable to control rats.

Observations in natural delivery and lactation were unaffected by treatment. The percentages of liveborn pups and stillborn pups were significantly decreased and increased, respectively, in the 30.0 mg/kg-day dose group. These changes are considered unrelated to ammonium perchlorate treatment because the average number of liveborn and stillborn pups per litter were comparable in the four exposure groups.

Histopathology (Table 5)

Exposure-related histomorphologic changes were observed in the thyroid gland of the adult male and female F1 generation rats. These changes were primarily hypertrophy and hyperplasia of the thyroid follicular epithelium and were similar to that seen in the thyroid of the P generation. The incidence of hyperplasia/ hypertrophy was significantly increased ($p \leq .05$) in male rats from the 3.0 and 30.0 mg/kg-day dose groups and in female rats from the 30.0 mg/kg-day dose group.

All the microscopic changes observed in the other organs and tissues specified for examination from the F1 generation male and female parental rats were considered to have occurred spontaneously.

Thyroid and Pituitary Hormone Analyses (Table 5)

For the F1 generation adult male and female rats, a significant increase in serum TSH levels occurred in the 30.0 mg/kg-day dose group, as compared to the control group value. A concomitant significant decrease in serum T_4 values occurred in male rats in the same dose group. Significant increases in serum T_4 levels occurred in male rats in the 0.3 and 3.0 mg/kg-day dose groups, as compared to the control group value. These increases in serum T_4 levels are not considered to be treatment related.

F2 Generation Pups

Necropsy, Organ Weights

All clinical and necropsy observations in the F2 generation pups were considered unrelated to ammonium perchlorate treatment. (Data not shown.) Thyroid weight was significantly increased for the female F2 generation pups in the 30.0 mg/kg-day dose group (Table 2). The weights of all other organs were unaffected by exposures to ammonium perchlorate as high as 30.0 mg/kg-day.

Histopathology, and Thyroid and Pituitary Hormone Analyses (Table 5)

Exposure-related histomorphologic changes were observed in the thyroid gland of male and female F2 generation pups. The incidence of hyperplasia/ hypertrophy was significantly increased in the 3.0 and 30.0 mg/kg-day dose groups. The incidence in the 0.3 mg/kg-day dose group is considered comparable to the control group. Thyroid and pituitary hormone levels in the F2 generation pups were unaffected by parental exposure to doses of ammonium perchlorate as high as 30.0 mg/kg-day.

DISCUSSION

Perchlorate ion is not a reproductive toxicant in the rat at doses as high as 30 mg/kg-day. In both the P and F1 adult rats, there were no deaths, abortions, or premature deliveries attributed to perchlorate exposure. Doses as high as 30 mg/kg-day had no affect on any sperm parameters in either P or F1 generation adult male rats nor on mating or fertility parameters in either P or F1 generation adult female rats. This included estrous cyclicity, fertility index, number of days in cohabitation, and number of rats mated. In addition, sexual maturation of both male and female F1 generation rats is comparable among treated and control groups.

Natural delivery and litter observations for both F1 and F2 generation pups were comparable among the treated and control groups. Doses as high as 30 mg/kg-day had no effect on the gestation index, the number of dams delivering litters, the duration of gestation, the average number of implantations, the average number of live pups, the viability and lactation indices, the sex ratios, or the pup body weights.

This study confirms the findings of earlier, although incomplete, reproductive studies of perchlorate that the thyroid is the target organ for perchlorate and that in utero exposure can affect the thyroids in neonatal animals. Perchlorate has been administered in drinking water to both pregnant rats (Brown-Grant and Sherwood 1971) and pregnant guinea pigs (Postel 1957). In rats, the number of implantation sites per dams was comparable between treated and control groups, and the authors concluded that perchlorate treatment had no significant effect on blastocyst survival or the ability to implant under conditions delaying implantation (Brown-Grant and Sherwood 1971). In contrast, it was observed that perchlorate exposure during gestation resulted in increased thyroid weights in both dams and fetuses compared with controls. Thyroid enlargement was also observed in the fetuses of guinea pigs that received perchlorate in drinking water during gestation days 21 to 48; the fetuses were not examined for other developmental effects (Postel 1957).

Recently, developmental and reproductive studies have been conducted in deer mice (*Peromyscus maniculatus*) (Roots et

2000; Thuett et al. 2000). Exposures were 0, 1 nM, 1 μM and 1 mM ammonium perchlorate in the drinking water to 10 paired mice/group. These exposures are roughly equivalent to doses of 0.00003, 0.03, and 32 mg/kg-day, respectively, assuming default body weights and water consumption values for B6C3F1 mice. Therefore, the high-dose group in Roots et al. (2000) and Thuett et al. (2000) is equivalent to the high-dose group tested in this study. In the reproductive study, pup body weights were reduced on most observation days in the 1mM group and this exposure group also had an increased length of time from cohabitation to parturition and a 10% reduction in conception rate, compared to control values. In the developmental study, all dose groups had significantly increased or decreased specific organ weights on postnatal day 21. The authors concluded that ammonium perchlorate appears to affect impregnation and the ability to maintain pregnancy, and may result in organ-specific developmental toxicity. Roots et al. (2000) and Thuett et al. (2000) observed effects that were not observed in the present study or in a rabbit developmental toxicity study (York et al. 2001) at similar doses. With the exception of the rats in our study being exposed before cohabitation, the protocol of the two studies was similar. Deer mice are an unfamiliar species, we are not aware of their typical responses in laboratory toxicity studies and are not prepared to comment on the difference in the results between the two studies.

In the present study, perchlorate exposure caused statistically significant, dose-dependent changes in thyroid weight, histopathology, and hormone levels in P, F1, and F2 generation rats. Relative thyroid weights (the ratio of thyroid weight to body weight) were significantly increased in the 30 mg/kg-day dose group for both males and females in the P generation and for F2 generation pups. However in the F1 generation adult rats, relative thyroid weights were significantly increased in all dose groups for female rats and in the 3.0 and 30.0 mg/kg-day dose groups for male rats. Thus, the F1 generation adults appear to be the most sensitive group to thyroid weight alterations; this group also had the longest continuous exposure to perchlorate.

All three generations developed histopathologic thyroid changes that are typical of perchlorate exposure. The changes consisted of hypertrophy and hyperplasia of the follicular epithelium that increased in incidence and severity in a dose-related manner. There appears to be an association between thyroid hyperplasia and hypertrophy in that hyperplasia did not occur without hypertrophy. This association may suggest a mechanism of toxicity for ammonium perchlorate on the thyroid that is sequential, because continued hyperplasia is also known to be necessary for tumor development at one end of the spectrum of toxicity for this chemical, and increased TSH levels are needed for the development of hypertrophy at the other end.

The lowest dose at which statistically significant increases in incidence of hypertrophy/hyperplasia occurred is at the 3.0 mg/kg-day dose group of male and female P generation rats, the female F1 generation pups, the male F1 generation adults rats, and the male and female F2 generation pups. In male and female P generation adults, an exposure-related increase in incidence was observed in the 0.3 mg/kg-day dose group, although this increase was not statistically significant.

Dose-related changes in TSH, T_4, and T_3 occurred at doses higher than those resulting in changes in thyroid weight and thyroid histopathology. These changes were inconsistent among the different generations, males and females, and ages of animals. In adult animals (P males, but not females, and all F1 adults), statistically significant, exposure-related increases in TSH and concomitant decreases in T_4 were observed at 30 mg/kg- day. No adult rats in either generation responded to perchlorate exposure with the expected decrease in T_3 at any dose level. In general, perchlorate exposure had no effect on the thyroid hormones of pups in either generation. In the F1 generation female pups, T_3 was statistically significantly decreased in the 30 mg/kg-day group. However, the biological significance of this observation is questionable, because no changes were observed in TSH and T_4.

One striking feature of the hormone analyses from this study is the high degree of variability in the data and the fact that in many of the dose groups, hormone changes were in the opposite direction from predicted. However, these inconsistencies were also observed in other recently completed bioassays of perchlorate (summarized in U.S. EPA 1998), prompting an investigation into whether the variability, both between the different laboratories conducting analyses and within each laboratory, was due to the analytical procedure. Three laboratories, including the laboratory that performed the hormone analysis for this study, measured TSH, T_3, T_4 in standards and unknown serum samples using RIA test kits obtained from the same source and lot number (Narayanan and Mattie 2000). Each laboratory followed the test kit manufacturer's procedure for conducting the hormone analyses. This investigation confirmed that both variability between laboratories and within laboratories was within reasonable limits and suggests that the variability observed in this study is not the result of inappropriate analytical procedure.

The hormone data from this study suggest that our understanding of how perchlorate affects thyroid homeostasis is still incomplete. Thyroid homeostasis is a dynamic, cyclical condition. The data sets collected in this study are snapshots in time, and individual animals can all be in a different phase of the cycle, leading to the high variability observed. Ongoing research into the pharmacokinetics of perchlorate and iodide inhibition provides some additional insight into the observed results (Fisher 2000). The classical response to iodide inhibition involves the decrease of T_3/T_4 and the increase of TSH. However, upregulation by TSH can cause the system to overshoot, resulting in T_3/T_4 levels higher than controls if there is sufficient iodine available, even under conditions of substantial iodine uptake inhibition (Fisher 2000). In addition, there are data to suggest that perchlorate may be affecting thyroid homeostasis extrathyroidally as well. Perchlorate has been shown to displace protein-bound T_4 in the serum, resulting in an increase in serum free T_4 (Fisher 2000). This increase in serum free T_4

in turn downregulates TSH, resulting in hormone levels equal to or lower than control values. The pattern of hormone levels from this study, particularly for the 0.3 and 3 mg/kg-day dose groups, is consistent with this phenomenon; although because free T_4 was not measured, it is not possible to confirm this hypothesis.

Defining the difference between adaptive and adverse effects when the thyroid is the target organ is difficult because the thyroid-pituitary axis is a dynamic system capable of considerable compensation in order to maintain homeostasis. In 1998, a peer review panel convened by U.S. EPA to evaluate a risk assessment of perchlorate concluded that thyroid hypertrophy alone was not considered to be an adverse effect of perchlorate (U.S. EPA 1999). In addition, the panel concluded that data available at the time did not suggest that thyroid hypertrophy was correlated with an adverse effect, such as hyperplasia. The panel recommended that a Pathology Working Group (PWG) be convened to review the thyroid histopathology from all perchlorate studies, including the two-generation study reported here, and establish clear dose-response curves for hypertrophy and hyperplasia separately. The PWG report has been completed and is available on the website of U.S. EPA National Center for Environmental Assessment (NCEA, www.epa.gov/ncea/perch.htm).

The PWG evaluated the incidence of decreased colloid, follicular cell hypertrophy, and follicular cell hyperplasia separately. In the P generation, significantly increased[2] incidence of follicular cell hyperplasia was only observed in the high-dose female rats, but not in any other treated group. In the F1 generation pups, significantly increased incidence of follicular cell hyperplasia was observed in the high-dose females and the mid- and high-dose males. Interestingly, significantly increased incidence of hyperplasia was not observed in any treated group of F1 adult male or female rats. Nor was an increased incidence of hyperplasia observed in either sex of the F2 generation pups. However, two F1 adult male rats in the 30 mg/kg-day dose group developed thyroid adenomas.

It has been suggested that effects in the thyroid are not considered adverse until thyroid hyperplasia accompanied by alterations in thyroid hormone levels and increased thyroid weight has been observed. Observation of either altered thyroid hormone levels, increased thyroid weight, or thyroid hypertrophy alone suggest only that thyroid is behaving normally to maintain homeostasis (Capen 1999). Following these lines of thinking, the 3.0 mg/kg-day dose level is considered to be the lowest-observable-adverse-effect level (LOAEL) based on the observation of increased thyroid weight (F1 adult rats) and increased incidence of thyroid hyperplasia (F1 adult male rats). Although significantly increased relative thyroid weight was observed in the F1 female adults at the 0.3 mg/kg-day dose level, these animals did not also demonstrate altered hormones or thyroid hyperplasia. Therefore, 0.3 mg/kg-day is considered to be the no-observable-adverse-effect level (NOAEL).

An unexpected result of this study is that increased thyroid weight and thyroid hypertrophy/hyperplasia occurred at lower doses than those required to cause an increase in TSH or a decrease in T_4. One reasonable explanation is that hormone levels had peaked at an earlier time point in the study and then equilibrated to normal at the time of experimental animal sacrifice.

In summary, perchlorate is not a reproductive toxicant in rats when administered in the drinking water at doses up to 30 mg/kg-day. The thyroid appears to be the only target organ for perchlorate; oral administration resulted in increased thyroid weight and thyroid hyperplasia at doses of 3.0 mg/kg-day and higher. Based on these findings, 0.3 mg/kg-day was determined to be the NOAEL for this study.

[2]Fisher Exact Test conducted by Toxicology Excellence for Risk Assessment, not the PWG.

REFERENCES

Brown-Grant, K., and M. R. Sherwood. 1971. Viability of the rat blastoc following the oral administration of potassium perchlorate or potassium iodi to the mother. *J. Reprod. Fertil.* 27:265–267.

Capen, C. 1999. Personal Communication to *TERA* staff.

Connell, J. M. C. 1981. Long-term use of potassium perchlorate. *Postgrad. M J.* 57:516–517.

Crooks, J., and E. J. Wayne. 1960. A comparison of potassium perchlora methylthiouracil, and carbimazole in the treatment of thyrotoxicosis. *Lan* 20:401–404.

Fisher, J. 2000. Personal Communication from Dr. Jeffery Fisher, University Georgia, to Joan Dollarhide on September 4, 2000.

Godley, A. F., and J. B. Stanbury. 1954. Preliminary experience in the treatm of hyperthyroidism with potassium perchlorate. *J. Clin. Endocrinol. Me* 14:70–78.

Institute of Laboratory Animal Resources. 1996. *Guide for the care and us laboratory animals.* Washington, DC: National Academy Press.

Morgans, M. E., and W. R. Trotter. 1960. Potassium perchlorate in thyrotox sis. *BMJ* 8:1086–1087.

Narayanan, L., and D. Mattie. 2000. Personal communication. Thyroid horm and TSH co-laboratory study.

Postel, S. 1957. Placental transfer of perchlorate and triiodothyroxine in guinea pig. *Endocrinology* 60:53–66.

Roots, E. H., K. A. Thuett, B. A. Gentles, R. J. Kendall, and E. E. S 2000. Gestational through post-lactational exposure of ammonium per rate to deer mice (peromyscus maniculatus). Abstract 1386. *Toxicologi* 296.

Saito, K., K. Yamamoto, T. Takai, and S. Yoshida. 1983. Inhibition of i accumulation by perchlorate and thiocyanate in a model of the thyroid i transport system. *Acta Endocrinol. (Copenh).* 104:456–461.

Siglin J. C., D. R. Mattie, D. E. Dodd, P. K. Hildebrandt, and W. H. I 2000. A 90-day drinking water toxicity study in rats of the environm contaminant ammonium perchlorate. *Toxicol. Sci.* 57:61–74.

Stanbury, J. B., and J. B. Wyngaarden. 1952. Effect of perchlorate on the l thyroid gland. *Metabolism* 1:533–539.

Thuett, K. A., E. H. Roots, B. A. Gentles, R. J. Kendall, and E. E. 2000. Developmental toxicity of ammonium perchlorate administered in drinking water to deer mice (peromyscus maniculatus). Abstract *Toxicologist* 54:296.

Toxicology Excellence for Risk Assessement *(TERA).* 2000. Peer rev perchlorate. Online at: http://www.tera.org/perchlorate.

U.S. Environmental Protection Agency (U.S. EPA). 1998. Perchlorate e mental contamination: Toxicological review and risk characterizatio on emerging information. External review draft. NCEA-1-0503. Was

DC. U.S. EPA. Office of Research and Development. December 31, 1998. (http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/perchl/perchl.htm)

U.S. EPA. 1999. Perchlorate Peer Review Workshop report. EPA Contract Number 68-W98-085. Washington, DC. U.S. EPA. Office of Solid Waste. May 31, 1999.

Wolff, J. 1998. Perchlorate and the thyroid gland. *Pharmacol. Rev.* 60:89–105.

York, R. G., W. R. Brown, M. F. Girard, and J. S. Dollarhide. 2001. Oral (drinking water) developmental toxicity study of ammonium perchlorate in New Zealand white rabbits. *Int. J. Toxicol.* 20:199–205.