Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in New Zealand White Rabbits

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This developmental toxicity study was conducted to evaluate the embryo-fetal toxicity and teratogenic potential of ammonium perchlorate in New Zealand White [Hra:(NZW)SPF] rabbits. Pregnant rabbits were given continual access to ammonium perchlorate in drinking water at target doses of 0, 0.1, 1.0, 10.0, 30.0, and 100.0 mg/kg-day on gestation days 6 through 28. The actual consumed doses in the study were 0, 0.1, 0.9, 10.4, 30.3, and 102.3 mg/kg-day. The rabbits were sacrificed on gestation day 29, and fetuses were examined for developmental alterations. In addition, blood was collected from does for determination of serum thyroid stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) levels and the thyroid was subjected to histopathologic examination. No maternal deaths were attributed to perchlorate exposure. Ammonium perchlorate as high as 100.0 mg/kg-day did not affect caesarean sectioning or litter parameters studied, and all values were found to be within the historical ranges of the laboratory. The litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, percent dead or resorbed conceptuses, and fetal body weights were comparable and also did not differ significantly in the six dose groups. All placentae appeared normal and no dam had a litter consisting of only resorbed conceptuses. The maternal thyroid was the target organ for ammonium perchlorate in this study. Increased incidence of thyroid follicular hypertrophy was observed in does treated with $\geq$10 mg/kg-day perchlorate and significantly decreased T_4 was observed in does treated with $\geq$30 mg/kg-day. Based on these data, the maternal no-observable-adverse-effect level (NOAEL) for ammonium perchlorate was 1.0 mg/kg-day. The developmental NOAEL for ammonium perchlorate was found to be 100.0 mg/kg-day for rabbits.

Keywords Developmental Toxicity, Perchlorate, Rabbit, Thyroid

Ammonium perchlorate, widely used as solid rocket propellants and ignitable sources in munitions and fireworks, has been detected in a number of public drinking water supplies in the United States (U.S. EPA 1998). Analytical chemistry methods for measuring perchlorate have recently improved, allowing perchlorate to be detected at 4 μg/l (see http://www/dhs.cahwnet.gov/ps/ddwem/chemicals/perchl/perchl_labs.htm). A series of studies are now underway to examine the toxicity of perchlorate to assist state and federal regulatory agencies in developing drinking water guidelines.

Few data suitable for risk assessment purposes have been available on ammonium perchlorate. For a more complete discussion on perchlorate, please refer to the companion paper in this issue (York et al. 2001). The perchlorate ion competitively inhibits the active transport of iodide into the thyroid and also stimulates the discharge of unorganified iodide from the thyroid (Wolff 1998; Saito et al. 1983). For this reason perchlorate salts, particularly potassium perchlorate, were used until the mid-1960s to treat people who had hyperthyroidism because of Graves' disease (Stanbury and Wyngaarden 1952; Godley and Stanbury 1954; Crooks and Wayne 1960; Morgans and Trotter 1960; Connell 1981) and amiodorone-induced thyrotoxicosis. Many studies have examined the effects of perchlorate in Graves' patients; however, few studies have been done in normal humans and these did not look at long-term exposure to perchlorate. In animals, long-term studies clearly show thyroid toxicity at high doses, but these studies generally did not examine targets other than the thyroid (Kessler and Krunkemper 1966; Pajer and Kalisnik 1991). No adequate developmental toxicity study in any species has been conducted to date. The available information on perchlorate defines well the mechanisms by which the perchlorate ion 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.

A joint effort between industry and government has been underway since 1997 to develop data needed to derive a reference dose (RfD) for chronic oral exposure to perchlorate. A panel of risk assessors, perchlorate experts, and thyroid experts

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was convened to recommend the types of studies needed to derive an RfD for perchlorate (see the Toxicology Excellence for Risk Assessment [*TERA*] Internet site http://www.ter.org/peer for notes of this panel discussion). The panel recommended that the following studies be conducted by current U.S. EPA guidelines: developmental neurotoxicity, 90-day rat toxicity (Siglin et al. 2000), rabbit developmental toxicity, two-generation reproductive toxicity (York et al. 2001), mutagenicity/genotoxicity, immunotoxicity, and kinetics.

The purpose of this study was to evaluate the embryo-fetal toxicity and teratogenic potential of ammonium perchlorate administered orally in drinking water to pregnant rabbits.

MATERIALS AND METHODS[1]

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 at least weekly, stored refrigerated, and dosage solutions were brought to room temperature prior to use. Test drinking solutions were adjusted to concentrations that yielded target doses of 0 (control), 0.1, 1.0, 10.0, 30.0, and 100.0 mg/kg-day based on actual weekly water consumption. During the exposure period, rabbits were given continual access to either deionized water (control group) or ammonium perchlorate in deionized water (test groups) as drinking water.

Animals

Naturally bred female New Zealand White [Hra:(NZW)SPF] rabbits were supplied by Covance Research Products, Inc. (Denver, PA). One hundred and fifty rabbits were randomly assigned to one of six dose groups, 25 rabbits per dose group, based on body weight. Rabbits were provided 150 g feed (Certified Rabbit Chow #5322) pre-exposure and 180 g during the exposure period (days of gestation [DGs] 6 to 28). The New Zealand White rabbit was selected because it has been demonstrated to be sensitive to developmental toxins and is one nonrodent mammalian species that is accepted and widely used throughout the industry for nonclinical studies of developmental toxicity. 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).

Experimental Design

Testing was performed by Argus Research Laboratories, Inc., in Horsham, Pennsylvania, USA. The ammonium perchlorate was considered 100% active for the purpose of dose calculations. Doses of 0, 0.1, 1.0, 10.0, 30.0, and 100.0 mg/kg-day were selected on the basis of a dosage-range study (Argus Research Laboratories, Inc., Study 1416-002P). In that study, thyroid histopathology was evident in New Zealand White rabbits at doses of 20, 50, and 100 mg/kg-day. T_3, T_4, and TSH blood levels were reduced at all doses and three malformed fetuses from three litters in the 20 mg/kg-day dose group were observed at gross external examination.

Observations

All rabbits were observed for viability at least twice daily and for general appearance at least once during the pre-exposure period. The rabbits were also examined daily during the exposure period (DGs 6 to 28) and on the day of sacrifice (DG 29) for clinical observations of effects of the ammonium perchlorate, abortions, premature deliveries, and deaths. Body weights, feed, and water consumption values were recorded daily.

On DG 29 rabbits were anesthetized via an intravenous injection of sodium pentobarbital and blood was collected from the inferior vena cava and blood samples were shipped (frozen on dry ice) to AniLytics, Inc. (Gaithersburg, MD) for determination of TSH, T_3, and T_4 levels. Throughout the sacrifice procedures and sample collection every effort was made to avoid inducing stress in the rabbits, because this could affect scheduled hormone evaluations.

Gross necropsy of the thoracic, abdominal, and pelvic viscera of each rabbit was performed and gravid uterine weights were recorded. A section of the trachea containing the thyroids/parathyroids of all rabbits were excised. Following fixation, the thyroid/parathyroid tissue samples were carefully trimmed, weighed, and evaluated histologically by a board-certified veterinary pathologist.

The number of corpora lutea in each ovary was recorded. The uterus was excised and examined for pregnancy, number and distribution of implantations, early and late resorptions, and live and dead fetuses. Each caesarean-delivered fetus was weighed and examined for gross external alterations. All fetuses were examined by dissection and the brain was examined *in situ* for approximately one-half of the fetuses in each litter. The remaining fetuses in each litter were decapitated and the heads were examined using Wilson's sectioning technique. All fetuses were examined for skeletal and cartilaginous alterations.

Statistical Analyses

Clinical observation and other proportion data were analyzed using the variance test for homogeneity of the binomial distribution. Continuous data (e.g., maternal body weights, body weight changes, feed and water consumption values, and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, fetal anomaly data, fetal ossification site data, and thyroid hormone levels) were analyzed using Bartlett's test of homogeneity of variances and the analysis of variance, when appropriate (i.e., Bartlett's test was not significant [$p > .05$]). If

[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).

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., Bartlett's test was significant [$p \leq .05$]), the Kruskal-Wallis test was used, when less than or equal to 75% ties were present; when more than 75% ties were present, Fisher's Exact Test was used. In cases in which 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. Count data obtained at caesarean sectioning were evaluated using the procedures previously described for the Kruskal-Wallis test.

RESULTS

Maternal Observations

Actual consumed doses of ammonium perchlorate on DGs 6 to 19 were 0, 0.1, 1.0, 10.8, 33.9, and 114.2 mg/kg-day, corresponding to 100%, 100%, 108%, 113%, and 114% of the target, respectively. During DGs 19 to 29, actual consumed doses of ammonium perchlorate were 0, 0.1, 0.8, 10.0, 26.7, and 90.4 mg/kg-day, corresponding to 100%, 80%, 100%, 89%, 100%, and 90% of target, respectively. Therefore, average actual consumed doses for the entire period of gestation were 0, 0.1, 0.9, 10.4, 30.3, and 102.3 mg/kg-day. There were no deaths attributed to the ammonium perchlorate. Two does in the 1.0 mg/kg-day dose group aborted on DG 28 and were sacrificed. Both abortions are considered unrelated to the ammonium perchlorate because the incidences were not dose-dependent. One dam in the 100.0 mg/kg-day dose group prematurely delivered on DG 27. Because rabbits normally deliver on DG 31 and the pups appeared to be full term (they had fur and were nursing), it is assumed that this rabbit had been incorrectly identified and shipped by the supplier on the wrong day of gestation. All other does survived to scheduled sacrifice.

Clinical observations (data not shown) included localized alopecia, ungroomed coat, scant, soft or liquid feces, and a red perivaginal, perinasal, or perioral substance. All these observations were considered unrelated to the ammonium perchlorate because the incidences were not dose-dependent; the observation was associated with abortion of a litter, and/or the observations are commonly seen in rabbits in the laboratory environment.

The maternal body weights in the control group were consistently lower than the exposed groups over the gestation period (Figure 1). There were, however, no statistically significant differences in average maternal body weights, gravid uterine weights, body weight gains, or corrected DG 29 body weights (DG 29 body weight minus the gravid uterine weight) among exposure groups during gestation (data not shown).

There were no statistically significant differences in average terminal body weights, absolute thyroid weights, or ratio of thyroid weight to terminal body weight (data not shown). The only adverse necropsy observation was a mottled liver that occurred in the 1.0 mg/kg-day dose group doe that had aborted.

Microscopic examination of the dams' thyroid glands revealed hypertrophy of the follicular epithelium in the rabbits in the 10.0, 30.0, and 100.0 mg/kg-day exposure groups that was

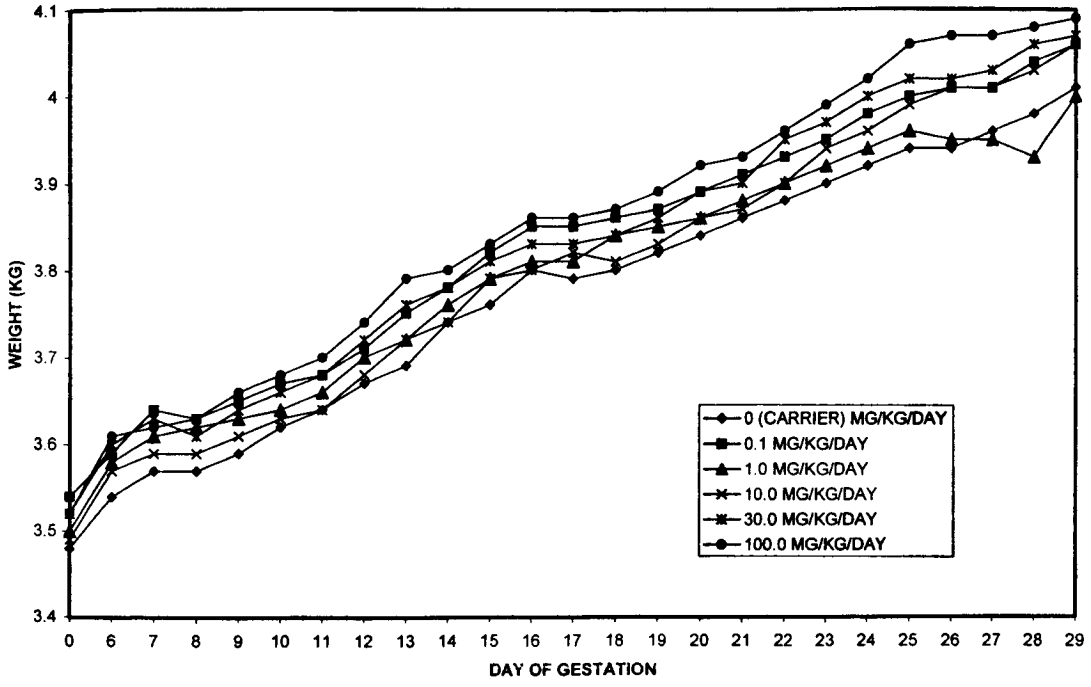

FIGURE 1
Maternal body weights.

TABLE 1
Summary of maternal thyroid effects of ammonium perchlorate in rabbits

Effect	0 mg/kg-day	0.1 mg/kg-day	1.0 mg/kg-day	10.0 mg/kg-day	30.0 mg/kg-day	100.0 mg/kg-day
			Thyroid histopathology			
Number examined	25	25	25	25	25	25
Number normal	12	13	10	6	7	4*
Hypertrophy, follicular epithelium						
Minimal	0	0	0	4	3	10*
Mild	0	0	0	3	6*	2
Moderate	0	0	0	0	4	4
Total incidence, all grades	0	0	0	7*	13*	16*
			Thyroid hormones			
Number examined	22	24	23[a]	24	24	23[b]
Serum T_3 (ng/dl)	138 ± 43	134 ± 34	110 ± 37	128 ± 33	124 ± 43	126 ± 51
Serum T_4 (μg/dl)	1.92 ± 0.9	1.99 ± 0.8	1.47 ± 0.8	1.52 ± 0.5	1.44 ± 0.7*	1.18 ± 0.6*
Serum TSH (ng/ml)	2.11 ± 0.3	2.04 ± 0.3	1.97 ± 0.5	2.00 ± 0.3	1.94 ± 0.2	2.09 ± 0.4

[a]Excludes values for does that aborted.
[b]Excludes values for a doe which delivered on day 27 of gestation; the mating date was incorrectly identified (presumed day 31 of gestation).
*Significantly different from the control group value ($p \leq .05$).

considered treatment related (Table 1). No treatment-related microscopic changes were observed in the thyroid gland of any the rabbits offered 0.1 or 1.0 mg/kg-day of ammonium perchlorate. In the affected thyroids, there was an increased height or enlargement (hypertrophy) of the follicular epithelium, occasionally resulting in a decrease in the lumen of the follicles, which contained pale and occasionally vacuolated colloid.

T_3, T_4, and TSH results for dams are summarized in Table 1. Serum T_4 levels appeared to decrease in an exposure-related manner. There was a statistically significant decrease ($p < .05$) in serum T_4 levels at the 30.0 and 100.0 mg/kg-day target doses, compared to the control group values. The levels of serum T_4 in the 1.0 and 10.0 mg/kg-day dose groups were also reduced, but not significantly, and the serum T_4 level in the 0.1 mg/kg-day dose group was comparable to the control group value. There were no statistically significant differences in serum T_3 and TSH blood levels among the six exposure groups. The significant decrease in serum T_4 levels at the 30.0 and 100.0 mg/kg-day target doses was considered treatment-related because it corresponded to the hypertrophy of the follicular epithelium of the thyroid glands seen in the 30.0 and 100.0 mg/kg-day target dose groups.

Caesarean Sectioning and Litter Observations

Caesarean sectioning observations were based on 22, 24, 23, 24, 24, and 23 pregnant does that survived to DG 29 in the 0, 0.1, 1.0, 10.0, 30.0, and 100.0 mg/kg-day dose groups, respectively.

Exposures to ammonium perchlorate as high as 100.0 mg/kg-day in the drinking water did not affect DG 29 caesarean sectioning or litter parameters. All values were within laboratory historical ranges. The litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, percent dead or resorbed conceptuses, and fetal body weights were comparable and did not differ significantly among the six exposure groups (Table 2). All placentae appeared normal and no dam had a litter consisting of only resorbed conceptuses.

Fetal alternation evaluations were based on 180, 184, 196, 195, 189, and 206 live fetuses in the six exposure groups, respectively. Each fetus was examined for gross external alterations, soft tissue alterations, skeletal alterations, and fetal ossification sites. Fetal alterations are defined as malformations (irreversible changes that spontaneously occur at low incidence) or variations (reversible delays or accelerations in development that commonly occur in this species/strain).

As shown in Table 2, no fetal alterations are attributable to exposure to ammonium perchlorate at doses up to 100.0 mg/kg-day. The control and treated groups were comparable for the number of litters that had at least one fetus with any alteration, the number of fetuses with any alteration, or the percent of fetuses with any alteration/litter. All alterations observed are determined to be unrelated to ammonium perchlorate because the incidences were not exposure-dependent; the observation occurred in only one or two high-exposure group fetuses; or the incidences are within the averages observed historically at the laboratory.

Fetal gross external alterations were rarely observed and were comparable between control and treated groups. Only six fetuses had gross external alterations: three from the control group litters, two from the 1.0 mg/kg-day group litters, and one from a 100 mg/kg-day group litter. Alterations included umbilical hernia, abdominal distension, right forepaw flexed downward, shortened digits, short snout, protruding tongue, absent nares

TABLE 2

Summary of caesarean sectioning observations and fetal alterations in rabbits exposed to ammonium perchlorate

Observation (historical control mean value)[a]		0 mg/ kg-day	0.1 mg/ kg-day	1.0 mg/ kg-day	10.0 mg/ kg-day	30.0 mg/ kg-day	100.0 mg/ kg-day
Litters evaluated	*N*	22	24	23	24	24	23
Corpora lutea (9.7)	Mean ± SD	9.0 ± 2.3	9.5 ± 1.8	9.3 ± 2.1	9.2 ± 2.0	9.8 ± 2.0	10.2 ± 1.8
Implantations (8.9)	Mean ± SD	8.4 ± 2.4	8.3 ± 2.1	8.7 ± 2.1	8.6 ± 2.3	8.2 ± 2.1	9.3 ± 2.1
Fetuses evaluated	*N*	180	185[b]	196	195	189	206
Litter size (8.5)	Mean ± SD	8.2 ± 2.5	7.7 ± 1.9	8.5 ± 1.7	8.1 ± 2.2	7.9 ± 2.0	9.0 ± 2.0
Does with any resorptions (25%)	*N* (%)	3 (13.6)	8 (33.3)	2 (8.7)	8 (33.3)	5 (20.8)	5 (21.7)
% Dead or resorbed conceptuses/litter (4.1)	Mean ± SD	3.2 ± 9.4	6.8 ± 10.0	1.3 ± 4.7	6.7 ± 12.1	3.2 ± 7.2	3.3 ± 6.6
Live fetal body weight (g)/litter (43.8)	Mean ± SD	45.5 ± 5.71	44.82 ± 5.29	44.31 ± 4.53	45.27 ± 4.38	46.09 ± 5.12	43.74 ± 4.64
Litters with fetuses with any alteration observed	*N* (%)	15 (68.2)	13 (54.2)	11 (47.8)	13 (54.2)	13 (54.2)	16 (69.5)
Fetuses with any alteration observed	*N* (%)	35 (19.4)	24 (13.0)	19 (9.7)	25 (12.8)	22 (11.6)	29 (14.1)
% Fetuses with any alteration/litter	Mean ± SD	19.8 ± 21.5	13.5 ± 15.3	9.3 ± 11.9	16.0 ± 22.7	11.1 ± 13.4	14.0 ± 11.9

[a]Historical control data collected from June 1995–1997 from 68 studies composed of 830 rabbits pregnant at caesarean sectioning on day 29 of gestation.
[b]One dead fetus was observed in this dose group, which was excluded from group averages and statistical analyses.

and papillae, a fleshy protrusion in the center of the head, a skin closure defect on the head, and depressed eye bulges.

Fetal soft tissue alterations were sporadic among the six exposure groups. Except for folded retina described below, soft tissue malformations or variations were observed in four fetuses in the control group litters, two fetuses in the 0.1 mg/kg-day group litters, three fetuses in the 1.0 mg/kg-day group litters, two fetuses in the 30 mg/kg-day group litters, and five fetuses in the 100 mg/kg-day group litters. The observed alterations included small brain with no apparent lateral or third ventricles, close-set and disorganized eyes, absent nasal passages, enlarged heart and liver, absent ventricular septum and a common truncus, protruding intestines, dilation of the lateral ventricles in the brain, absence of the intermediate lobe of the lungs, and absent gallbladder. Nevertheless, there was no significant difference in incidence of soft tissue alterations between the control group and treated groups.

A statistically significant difference in incidence of folded retina was observed; the fetal incidences in all the treated groups were significantly lower than the control group. Folded retina of the right and/or left eye occurred in 11, 1, 4, 5, 5, and 4 fetuses from 6, 1, 4, 4, 3, and 3 litters, respectively, in the control and five treated groups. However, this observation is considered unrelated to administration of ammonium perchlorate because this observation was made only in the heads examined with Wilson's technique and is considered an artifact of processing.

Although fetuses in some of the exposure groups showed significant increases in skeletal alterations, none of these changes are considered to be treatment related. Significant skeletal alterations include vertebral/rib malformations, incomplete ossification of the first sternal centra, and misaligned scapulae. The vertebral/rib malformations were observed in three fetuses from one litter in the 0.1 mg/kg-day group and in one fetus in the 10 mg/kg-day group. The incidence in the 0.1 mg/kg-day group was statistically significant, but it is not considered to be exposure-related because it was not dose-dependent, and the litter incidence, a more relevant parameter, was not significantly increased. The incompletely ossified first sternal centra was observed in two fetuses from two litters in the 0.1 mg/kg-day group and in five fetuses from three litters in the 30.0 mg/kg-day dose groups, whereas no case was seen in the control group. Although the incidences in the two dose groups were significantly higher than in the control group, they are also not considered to be treatment-related due to the same reason mentioned above for the vertebral/rib malformations. The misaligned scapulae occurred in 1, 0, 5, 0, 1, and 2 fetuses from 1, 0, 4, 0, 1, and 2 litters in the 0, 0.1, 1.0, 10.0, 30.0, and 100.0 mg/kg-day dosage groups, respectively. The increased incidence in the 1.0 mg/kg-day exposure group was significantly higher than in the control, but it is not considered to be treatment-related as well because of a non–dose-dependent response.

The incidence of other skeletal malformations or variations was comparable between the control group and treated groups. The average numbers of ossification sites in the hyoid, vertebrae (cervical, thoracic, lumbar, sacral, and caudal), ribs, sternum (manubrium, sternal centers, and xiphoid), forelimbs (carpals, metacarpals, and phalanges), and hindlimbs (tarsals, metatarsals, and phalanges) were also comparable between the control and the treated groups. In addition, all those values were within the ranges observed historically at the laboratory.

DISCUSSION

Perchlorate ion is not a developmental toxicant in pregnant rabbits at doses as high as 100 mg/kg-day ammonium perchlorate in the drinking water. No effects of perchlorate exposure were observed on any caesarean sectioning or litter parameters. No fetal alterations, either malformations or variations, were attributable to administration of ammonium perchlorate. Although the incidence of some alterations was statistically significant in at least one exposure group compared with the control group, all alterations were unrelated to ammonium perchlorate exposure. Folded retina, which was observed at lower incidence in all treated groups compared with the control group, was an artifact of processing. Incidence of vertebral/rib malformations, incompletely ossified first sternal centra, and misaligned scapulae were all significantly increased in one or more of the exposure groups, but was not treatment-related because the incidences were not dose-dependent and the litter incidence was not significant.

As is expected based on its mode of action, the thyroid is a target organ for the perchlorate ion in this study. Increased incidence of thyroid follicular hypertrophy was observed in does treated with $\geq$10 mg/kg-day ammonium perchlorate and significantly decreased T_4 was observed in does treated with $\geq$30 mg/kg-day.

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 developmental toxicity 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. Significantly increased incidence of all three parameters was observed in rabbit dams

exposed to ≥10 mg/kg-day; no effects on any of these parameters were observed in the 0.1 or 1 mg/kg-day dose groups.[2] Thus, the PWG analysis confirms that doses of ≥10 mg/kg-day result in adverse effects on the thyroids of pregnant rabbits.

No standard developmental toxicity study has been conducted on perchlorate to date. Perchlorate was tested by dosing 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 by rat dams during gestation resulted in increased thyroid weights in both dams and fetuses compared with controls. Thyroid enlargement was also observed in the fetuses of pregnant guinea pigs that received perchlorate in drinking water during gestation days 21 to 48 (Postel 1957).

Recently, developmental and reproductive studies have been conducted in deer mice (*Peromyscus maniculatus*) (Roots et al. 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. In the reproductive study, pup body weights were reduced on most observation days in the 1 mM 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.

Based on the data from this rabbit developmental toxicity study, the maternal no-observable-adverse-effect level (NOAEL) for ammonium perchlorate was 1.0 mg/kg-day. The 10.0, 30.0, and 100.0 mg/kg-day target doses caused hypertrophy of the follicular epithelium of the thyroid glands of the rabbits and there were decreases in serum T_4 levels at the doses ≥1.0 mg/kg-day. Although the T_4 decreases were only statistically significant at 30.0 and 100.0 mg/kg-day target dosages, a dose-dependent decrease at the lower doses correlated to the observations of thyroid hypertrophy in the exposed does. This correlation supports the identification of the thyroid hypertrophy as the adverse effect at doses ≥10.0 mg/kg-day. The developmental NOAEL was greater than 100.0 mg/kg-day. There were no adverse effects on embryo-fetal development as evaluated in the study, and based on these data, ammonium perchlorate should not be identified as a developmental toxicant in rabbits.

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

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