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# Proposed Perchlorate Reference Dose (RfD)

*Prepared for:*

The Perchlorate Study Group (PSG)

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Toxicology Excellence for Risk Assessment

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# Reference Dose (RfD) for Perchlorate

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# Executive Summary

A reference dose (RfD) of 0.01 mg/kg/day is proposed for perchlorate. This RfD is based on a critical study in sensitive humans which identifies a lowest observed adverse effect level (LOAEL) of 1.4 mg/kg/day based on early manifestations of thyroid-pituitary disturbance. No human studies have identified a definitive no observed adverse effect level (NOAEL); the RfD is based on the lowest human LOAEL identified. This study is supported by short-term animal studies which find NOAELs in the range of 0.1-1.5 mg/kg/day. Recommended uncertainty factors include 3 to protect sensitive subpopulations, 3 to account for extrapolation from short-term studies, 3 to account for database deficiencies and 3 for the use of a minimal LOAEL. Confidence in the RfD is medium-to-low because of the general lack of information about the effects of chronic exposure at low doses.

Until the mid 1960's perchlorate was used to treat hyperthyroidism caused by Graves' disease. Therefore, perchlorate has been extensively studied in Graves' disease patients and to a lesser extent in normal humans. In addition, both short- and long-term studies in various rodent species have been conducted. The data in both humans and animals indicate that perchlorate exerts its effects by competitively inhibiting uptake of iodide into the thyroid thereby inhibiting the production of iodide-containing thyroid hormones. The short-term consequence of this action is a response by the pituitary gland to produce TSH which in turn stimulates diffuse cell division and growth of the thyroid gland. Effects related to disturbance of the thyroid-pituitary axis have been seen in studies in humans, both Graves' patients and normal humans, and in both short-term and long-term studies in animals. Thus, disturbance of the function of the thyroid-pituitary axis appears to be the critical effect from exposure to perchlorate.

The human studies demonstrate that Graves' disease patients treated with doses in the range of 6 to 14 mg/kg/day occasionally developed fatal aplastic anemia. However, this response is likely to be the result of patients with an improperly functioning immune system suffering an immune mediated hypersensitivity reaction to perchlorate. In a single Graves' disease patient, a dose level of 3 mg/kg/day controlled hyperthyroidism with no side effects after 22 years of treatment. Thus, this dose, while having a beneficial effect in Graves' patients, might be a LOAEL in normal humans with a lifetime of perchlorate exposure. When the underlying mechanism of toxicity is examined (i.e., prevention of iodide uptake by the thyroid), a dose of 1.4 mg/kg/day (LOAEL) in Graves patients caused complete release of iodide by the thyroid while lower doses caused only a partial release. No human studies which identify a dose that has no effect at all on thyroid function were found.

release. No human studies which identify a dose that has no effect at all on thyroid function were found.

Most animal studies were conducted at doses that were too high to identify the threshold for perchlorate's effect on the thyroid-pituitary axis. However, two animal studies identified NOAELs. A four day study identified a NOAEL of 1.5 mg/kg/day and a 14-day study identified a NOAEL of 0.12 mg/kg/day (this dose was a LOAEL in females) based on decreased thyroid hormone levels and increased TSH levels. A third study suggested a NOAEL of 0.25 for increased secretion of iodide from the thyroid. However, this study was poorly reported and inadequate for risk assessment purposes. A defect of the animal studies is that few of the studies examined any organs or tissues other than the thyroid.

# 1. Introduction

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. Because perchlorate use was required in the performance of Department of Defense and National Aeronautic and Space Administration contract, government and contractor facilities are potential locations requiring extensive perchlorate remediation. These compounds have been found as contaminants in soils and groundwater. In addition, until recently, perchlorate salts, particularly potassium perchlorate, have been used therapeutically to treat hyperthyroidism resulting from Graves' disease. Perchlorate, $ClO_4^-$, is an anion which forms salts with most cations. These salts dissociate completely when dissolved in water or aqueous tissues.

This paper will discuss the human and animal toxicity data for perchlorates and calculate an oral reference dose (RfD) for the non-cancer health endpoints following the U.S. Environmental Protection Agency (U.S. EPA) methods. Several important issues related to perchlorates' potential for causing adverse health effects in humans will be discussed to better characterize the health risk

## 1.1 Existing Provisional Reference Dose (RfD)

RfDs for perchlorate-containing compounds, including potassium perchlorate (CAS# 7778-74-7), ammonium perchlorate (CAS# 7790-98-9), lithium perchlorate (CAS# 7791-03-9), sodium perchlorate (CAS# 7601-89-0) or perchloric acid (CAS# 7601-90-3) are not available on U.S. EPA's Integrated Risk Information System (IRIS) or Health Effects Assessment Summary Tables (HEAST). In late 1992, U.S. EPA's Superfund Health Risk Technical Support Center in the National Center for Environmental Assessment (NCEA) assessed the toxicity of potassium perchlorate and developed a provisional RfD for the perchlorate compounds. This provisional value has been used as the basis for developing clean-up levels by U.S. EPA Regional Superfund personnel. In addition, U.S. EPA Region III has placed this provisional value on its Risk-Based Concentration Tables, which are a widely-used risk assessment reference for many state agencies.

The provisional RfD is based on an acute study by Stanbury and Wyngaarden (1952) in which single doses of potassium perchlorate caused the release of iodide from the thyroids of patients with Graves' disease. The NOAEL was determined to be 0.14 mg/kg/day because iodide release was incomplete at

this dose. The 1000-fold uncertainty factor included a factor of 10 for the use of a less-than-chronic study, 10 to protect sensitive subpopulations, and 10 to account for database deficiencies. The resulting provisional RfD was 0.0001 mg/kg/day.

## 1.2 Purpose of this Document

In 1995, the Perchlorate Study Group (a consortium of companies that use and/or manufacture perchlorates) submitted a revised assessment of the perchlorate RfD to U.S. EPA-NCEA for review. At that time, several issues regarding the association of perchlorate treatment with fatal hematological disorders and the deficiencies in the overall database were identified and remained unresolved. The purpose of this document is to develop an RfD for perchlorate based on a comprehensive discussion of its likely critical effect and uncertainty factors that incorporates the latest information on interhuman variability, interspecies extrapolation, extrapolation across durations, and strengths and limitations of the overall database. These issues are discussed below.

## 1.3 The Method Used

The RfD method of U.S. EPA was used to evaluate and quantitate the non-cancer toxicity of perchlorate. The determination of RfDs lies squarely in the area of hazard identification and dose response assessment as defined by the National Academy of Sciences (NAS, 1983) report on risk assessment in the federal government. U.S. EPA defines the reference dose as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. (Barnes and Dourson, 1988; Dourson, 1994).

For health effects that are not cancer, the U.S. EPA and others first identify the critical effect(s), which is the first adverse effect(s) or its known precursor that occurs in the dose scale. Human toxicity data adequate for use in the estimation of RfDs are seldom available, but if so, they are preferred in the selection of this critical effect. The use of human data has the advantage of avoiding the uncertainties inherent in interspecies extrapolation.

After the critical effect(s) has been identified, U.S. EPA generally selects an experimental dose rate from a study that represents the highest level tested at which the critical effect was not demonstrated. This level (i.e., the NOAEL) is

the key datum gleaned from the toxicologist's review of the chemical's entire database and is the first component in the estimation of an RfD. If a NOAEL is not available, the use of a LOAEL is recommended. Alternatively, a benchmark dose (BMD) may be used in this part of the assessment. A BMD is a statistical lower confidence limit on the dose that produces a predetermined level of change in adverse response compared with the response in untreated animals (called the benchmark response or BMR). Advantages and disadvantages of NOAELs and BMDs are described elsewhere (U.S. EPA, 1995).

Presented with data from several animal studies, U.S. EPA first seeks to identify the animal model that is most relevant to humans based on the most defensible biological rationale, for example using comparative pharmacokinetic data. In the absence of a clearly most relevant species, however, U.S. EPA generally chooses the critical study and species that shows an adverse effect at the lowest administered dose. This is based on the assumption that, in the absence of data to the contrary, humans may be as sensitive as the most sensitive experimental animal species.

In the absence of adequate human data U.S. EPA generally considers a "complete" database, that is, complete for the purpose of calculating a RfD for noncancer health effects, to be composed of:

- two adequate mammalian chronic toxicity studies by the appropriate route in different species;
- one adequate mammalian multi-generation reproductive toxicity study by an appropriate route; and
- two adequate mammalian developmental toxicity studies by an appropriate route in different species.

An adequate study is one which tests a sufficient number of animals of both sexes at two or more nonzero dose levels and identifies a NOAEL and LOAEL. The determination of study adequacy rests on professional judgment. A detailed discussion of the factors to be considered when evaluating the adequacy of a database and a study can be found in U.S. EPA (1994).

Uncertainty factors (UFs) are reductions in the dose rate or concentration to account for areas of scientific uncertainty inherent in most toxicity databases. The choice of appropriate uncertainty and modifying factors reflects a case-by-case judgment by experts and should account for each of the applicable areas of uncertainty and any nuances in the available data that might change the magnitude of any factor.

Typically, U.S. EPA uses uncertainty factors to account for five areas of uncertainty. The UF for human variability (designated as H) is intended to account for the variation in sensitivity among the members of the human population. The UF for experimental animal-to-human extrapolation (designated as A) is intended to account for the extrapolation from animal data to the case of humans and is considered to have components of both toxicokinetics and toxicodynamics. The subchronic-to-chronic UF (designated as S) is intended to account for extrapolating from less than chronic levels to chronic levels. The UF for LOAEL-to-NOAEL extrapolation (designated as L) is applied when an appropriate NOAEL is not available to serve as the basis for a risk estimate, and extrapolation from an experimental LOAEL is necessary. Database completeness (designated as D) is intended to account for the inability of any single study to adequately address all possible adverse outcomes. U.S. EPA currently uses an additional factor, referred to as a modifying factor (MF), as an occasional adjustment in the estimation of an RfD to account for areas of uncertainty not explicitly addressed by the usual factors.

The traditional default value of 10 has been generally used for each of these UFs; U.S. EPA, however, through experience of calculating thousands of RfDs has developed criteria for reducing UFs (generally to a half-log value of 3, or 1), when data warrant. U.S. EPA also recognizes the potential overlap between UFs and attempts to accommodate this. A recent publication discusses the use of factors other than default based on these criteria (Dourson et al., 1996).

The equation that U.S. EPA uses to determine the value of the RfD is:

$$RfD = NOAEL \text{ or } LOAEL(mg/kg/day) \div (UF \times MF)$$

where:

NOAEL = No Observed Adverse Effect Level
LOAEL = Lowest Observed Adverse Effect Level
UF = Uncertainty Factor
MF = Modifying Factor.

Finally, U.S. EPA provides a statement of confidence in their noncancer risk estimates (Barnes and Dourson, 1988; Dourson, 1994). High confidence indicates a judgment that additional toxicity data are not likely to change the RfD. Low confidence indicates that at least a single, well-conducted, subchronic mammalian bioassay by the appropriate route is available. For such a minimum database, the likelihood that additional toxicity data may change the RfD is greater. Medium confidence indicates a judgment somewhere between high and

low. Example of confidence statements for RfDs can be found on U.S. EPA's IRIS (U.S. EPA, 1996).

# 2. Hazard Identification

## 2.1 Review of Relevant Data

Perchlorate was used until the mid-1960's in the treatment of people who are hyperthyroid because of Graves' disease. 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 do not look at long-term exposure to perchlorate. Long-term studies in animals, clearly show thyroid toxicity at high doses; although, generally, these studies did not examine targets other than the thyroid. 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. This had lead to investigation of the effects that lower doses of perchlorate have on the pituitary-thyroid axis.

### 2.1.1 Toxicity Data in Humans

The thyroid gland appears to be the principal target organ for perchlorate toxicity in humans. In humans, the only other effects seen are hematological effects in Graves' patients at doses 100-fold higher than those needed to affect iodide concentration in the thyroid. However, experts in the field have suggested that these hematological effects are a hypersensitivity reaction and unrelated to the effects that perchlorate have on iodine balance in the thyroid.

In normal humans, the synthesis and secretion of thyroid hormones are controlled by a feedback mechanism involving the production of thyroid stimulating hormone (TSH) by the anterior pituitary. Iodide levels in the thyroid also play a role in the control of thyroid hormone levels. TSH causes the thyroid to initiate new hormone synthesis. Its production in the pituitary gland responds to blood levels of T3 and T4. When circulating levels of T3 and T4 decrease, the production of TSH in the pituitary increases. Increased levels of circulating T3 and T4 lead to decreased pituitary production of TSH. In vitro studies of iodide transport in sheep thyroid tissue slices (Wolff and Maurey, 1962) and phospholipid vesicles (Saito et al., 1983) have confirmed that perchlorate competitively inhibits iodide transport into the thyroid. A summary of the human studies of perchlorate is presented in Table 1.

### 2.1.1.1 Studies in Patients with Graves' Disease

Potassium perchlorate has been used to treat Graves' disease in humans and most of the data on perchlorates effects on humans are in patients with this disease. Graves' disease is an autoimmune disorder in which patients carry immunoglobulins in their blood which bind to the TSH receptors on thyroid cells and act like TSH to stimulate DNA synthesis and cell divisions leading to a hyperthyroid state. Symptoms of the disease include increased synthesis and secretion of iodide containing hormones into the blood by the thyroid gland, thyroid gland enlargement, increased basal metabolism and loss of weight. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones by inhibiting the accumulation of iodide in the thyroid.

Stanbury and Wyngaarden (1952) evaluated perchlorate in patients with Graves disease and found that perchlorate caused the discharge of iodine accumulated in the thyroid and blocked the uptake of iodine into the thyroid. Within 30 minutes of administration, a single dose of 100 mg potassium perchlorate caused the nearly complete release (~80%) of $I^{131}$ from the thyroids of 8 Graves' disease patients previously treated with tracer amounts of $I^{131}$ and 1-methyl-2-mercaptoimidazole (MMIA)[a]. A single dose of 10 mg perchlorate appeared to cause about a 50% release of accumulated iodine and the authors reported that perchlorate doses as low as 3 mg caused detectable, but incomplete, release of iodide from the thyroid (data for doses less than 10 mg were not presented). In addition, Stanbury and Wyngaarden (1952) reported that the uptake of tracer levels of $I^{131}$ into the thyroid glands of patients with Graves' disease was markedly inhibited for as long as 6 hours when 100 mg of potassium perchlorate was given orally 1 hour prior to administration of the tracer.

[a] 1-methyl-2-mercaptoimidazole (MMIA) is an antithyroid agent that inhibits incorporation of iodide into thyroid hormone molecules. Pretreatment with MMIA ensured that any $I^{131}$ accumulated in the thyroid was not used to produce thyroid hormone.

**Table 1. Human Studies of Perchlorate**

| Study | Duration/ Number of Subjects | Doses mg/kg-day | Effects | Notes | Uncertainty Factors | RfD mg/kg-day |
|---|---|---|---|---|---|---|
| Stanbury and Wyngaarden (1952) | Single dose<br><br>3 subjects | 0<br>0.04<br>0.14<br>1.4 LOAEL | Release of iodine from thyroid. Inhibition of iodine uptake by thyroid. | Graves' disease patients. | 3L<br>3S<br>3H<br>3D<br><br>100 | 1E-2 |
| Connell (1981) | 22 years<br>1 subject | 3 LOAEL | No adverse effects with clinical control of hyperthyroidism | Graves' disease patients | 3L<br>3H<br>3D<br><br>30 | 1E-1 |
| Godley and Stanbury (1954) | 28 weeks<br>24 subjects | 0<br>8.6 LOAEL | Gastrointestinal irritation in 2/24 patients. Decrease of iodine uptake by thyroid | Graves' disease patients | | [a]NA |
| Crooks and Wayne (1960) | unknown duration<br>35 subjects<br>165 subjects<br>10 subjects<br>40 subjects | <br>8.6 LOAEL<br>14<br>21<br>28 | Skin rash, nausea at 8.6-14. Also agranulocytosis at 21 | Graves' disease patients | | [a]NA |
| Morgan and Trotter (1960) | 2-3 weeks<br>180 subjects<br>67 subjects | <br>6-14 LOAEL<br>17-28 | Skin rash, sore throat, GI irritation, lymphadenopathy (3% in low dose, 18% in high dose) | Graves' disease patients | | [a]NA |
| Hobson (1961) | 33 weeks<br>1 subject | 9-11 FEL | Fatal aplastic anemia | Graves' disease patients | | [a]NA |
| Johnson and Moore (1961) | 3 months<br>1 month<br>1 subject | 14 FEL<br>9 FEL | Fatal aplastic anemia | Graves' disease patients | | [a]NA |

| Study | Duration/ Number of Subjects | Doses mg/kg-day | Effects | Notes | Uncertainty Factors | RfD mg/kg-day |
|---|---|---|---|---|---|---|
| Fawcett and Clark (1961) | 6 months<br>2 months<br>1 subject | 9 FEL<br>6 FEL | Fatal aplastic anemia | Graves' disease patients | | [a]NA |
| Krevans et al. (1962) | 2 weeks<br>10 weeks<br>4 months<br>1 subject | 11 FEL<br>9 FEL<br>6 FEL | Fatal aplastic anemia | Graves' disease patients | | [a]NA |
| Gjemdal (1963) | 4 months<br>1 subject | 6-9 FEL | Fatal aplastic anemia | Graves' disease patients | | [a]NA |
| Barzilai and Sheinfeld (1966) | 2 months<br>2 subjects | 14 FEL | Fatal aplastic anemia and fatal agranulocytosis | Graves' disease patients | | [a]NA |
| Burgi et al. (1974) | 8 days<br>5 subjects | 0<br>9.7 LOAEL | Release of iodine from thyroid | Healthy volunteers | 3L<br>3S<br>10H<br>3D<br>300 | 3E-2 |
| Brabant et al. (1992) | 4 weeks<br>5 subjects | 12 LOAEL | Decrease in thyroid iodine concentration, free T4, thyroglobulin, and TSH (Follow up study shows increase thyroid volume at same dose) | Healthy volunteers pretreated with iodine for 4 weeks before perchlorate exposure | | |

* Table is ordered in roughly increasing dose.

Note a: This may not be an appropriate study on which to base an RfD because the doses are within the range of those that cause frank effects in Graves' patients. However, this frank toxicity may be caused by susceptibility unrelated to perchlorate exposure. See text for discussion.

Inhibition of iodine occurred both in two patients treated with MMIA and three patients without MMIA treatment.. The authors state that no toxic effects were encountered in any of these patients who were given no more than three doses for a total of not more than 600 mg potassium perchlorate. . This study identifies a definitive LOAEL of 1.4 mg/kg/day[b] for complete release of iodine from the thyroid. Since it is not clear what degree of iodine release constitutes an adverse effect, we have not designated a NOAEL for this study.

Godley and Stanbury (1954) report using potassium perchlorate to treat 24 patients with Graves' disease. Patients were treated with 600-1200 mg/day for at least 11 weeks and as long as 45-52 weeks. Two patients developed gastrointestinal problems that were assumed to be due to perchlorate treatment. In one patient, these effects occurred at 600 mg/day, but the dose which the other patient received is not specified. Other side effects of antithyroid agents, such as hematological changes, liver damage, or skin rash, were not observed. This study suggests a LOAEL of 9 mg/kg/day.

Crooks and Wayne (1960) observed one case of skin rash and three cases of nausea (2%) among 35 patients treated with 600 mg/day (9 mg/kg/day) and 165 patients given 1,000 mg/day (14 mg/kg/day). In another group of 10 patients given 1500 mg/day (21 mg/kg/day) and 40 patients given 2000 mg/day (29 mg/kg/day), five cases of skin rash, two cases of nausea and one case of agranulocytosis occurred (16%). Leukocyte counts returned to normal in the patient with the agranulocytosis when perchlorate treatment was stopped. The length of treatment in unclear but appears to have been up to 22 weeks. The authors report the "time to cure" for perchlorate of approximately 9 weeks. The authors also report 1 of 12 infants born of mothers given 600 to 1000 mg/day, was born with a very slightly enlarged thyroid which returned to normal size in six weeks; no other abnormalities were noted. This study defines a LOAEL between 9 and 14 mg/kg/day.

Morgans and Trotter (1960) reported that 3% of 180 patients treated with 400 to 1,000 mg/day (6 to 14 mg/kg/day) potassium perchlorate and 18% of 67 patients treated with 1,200 to 2,000 mg/day (17 to 29 mg/kg/day) displayed a variety of adverse reactions including skin rash, sore throat, gastrointestinal irritation and lymphadenopathy. Reactions occurred within 2-3 weeks of drug administration. This study defines a LOAEL between 6 and 14 mg/kg/day.

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[b] Unless otherwise indicated, for human studies in which the actual body weight of the subjects was not reported, the dose in mg/kg/day was calculated assuming a body weight of 70 kg. Thus a dose of 100 mg/day ÷ 70 kg is 1.4 mg/kg/day.

Connell (1981) reported a case study of a single Graves' disease patient who was treated with potassium perchlorate at 200 mg/day (3 mg/kg/day) for 22 years without any indication of adverse side effects. This dose level provided sufficient clinical control of the hyperthyroidism.

Between 1961 and 1966, the occurrence of severe hematological side effects in patients receiving long-term potassium perchlorate treatment for Graves' disease led to a decreased use of potassium perchlorate as a therapeutic agent. Several authors (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clark, 1961; Krevans et al., 1962; and Gjemdal, 1963) report case studies where a single patient suffered fatal aplastic anemia after treatment with doses ranging from 6 to 14 mg/kg/day. The duration of treatment ranged from 3 months (Johnson and Moore, 1961) to 8 months (Hobson, 1961). In all cases, patients were started out at the high end of the treatment range for a period of time and then were reduced to the lower end of the treatment range after the appearance of side effects. In two cases (Hobson, 1961 and Gjemdal, 1963) patients had co-exposures to other drugs. Other case reports are available which report nonfatal agranulocytosis in patients treated with 14 mg/kg/day for 12 days (Southwell and Randall, 1960) or 3 months (Sunar, 1963). Barzilai and Sheinfeld (1966) report that 11% of 76 patients developed leukopenia or other unspecified side effects after treatment with 1,000 mg/day (14 mg/kg/day) for as little as 2 months. Within this group, there was one case of fatal aplastic anemia and one case of fatal agranulocytosis. These studies indicate that doses in the range of 6 to 14 mg/kg/day represent a frank effect level (FEL) inpatients with Graves' disease. There is no information to suggest that humans without Graves' disease would have a similar reaction to perchlorate (See Section 2.1.1.3).

### 2.1.1.2 Studies in Normal Humans

Far fewer data are available to demonstrate the effects of perchlorate in normal, healthy individuals. In the available studies, exposure to perchlorate was short - just a few days to 4 weeks. Burgi et al. (1974) examined the effects of perchlorate on the secretion of endogenous iodine by the normal human thyroid gland. Five healthy volunteers received tracers of $I^{125}$-iodide and $I^{131}$-thyroxine for 17 days followed by 600 mg/day perchlorate (9.7 mg/kg/day, based on actual reported average body weight of 61.8 kg) perchlorate for 8 days. Urine and serum were analyzed for $I^{125}$ and $I^{131}$ to determine if perchlorate can cause the discharge of endogenous as well as exogenous iodide from the thyroid. Results show that this dose of perchlorate was sufficient to completely block iodide uptake by the thyroid. In addition, perchlorate caused a 65% increase in excretion of non-thyroxine iodine over background. The authors attributed this increase to additional secretion of endogenous iodide from the thyroid.

Treatment with carbimazole plus perchlorate caused a further increase in the secretion of non-thyroxine iodine, suggesting that perchlorate causes only a partial, not complete, release of endogenous iodide. This study defines a minimal LOAEL of 9.7 mg/kg/day.

Brabant (1992) administered potassium perchlorate to healthy volunteers as a means to study changes in TSH concentration and release in response to a decrease in iodine supply to the thyroid. During the first 4 weeks of the study, the volunteers were given 200 ug/day iodine. After iodine supplementation was discontinued, the volunteers were orally administered 900 mg/day of potassium perchlorate for four weeks to induce a state of iodine depletion. At the end of the 4-week perchlorate treatment, levels of thyroid hormones were measured. Although perchlorate treatment had no effect on thyroid volume or levels of T3 and T4, intrathyroidal iodine concentration was significantly decreased, serum levels of TSH were significantly decreased, and serum levels of thyroglobulin were almost doubled. The authors speculate that the decrease of TSH, which is opposite of the expected response, may be an early adaptive mechanism to the iodine deficiency induced by perchlorate. They suggest that early in iodine deficiency, the thyroid becomes more sensitive to TSH, creating a feedback mechanism that decreases TSH levels. Only as iodine deficiency becomes more prolonged do TSH levels increase. This study defines a LOAEL of 13 mg/kg/day for thyroid effects.

In a follow up study, Brabant (1994) repeated his studies with perchlorate treatment longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all subjects, although TSH levels did not increase.

#### 2.1.1.3 Role of Perchlorate in Autoimmunity and Hematological Effects

Treatment of Graves' disease patients with perchlorates has resulted in serious hematological effects in a small number of people. These effects include fatal aplastic anemia and agranulocytosis as well as less serious effects including reversible agranulocytosis, lympadenopathy, and leukopenia. Skin rash has also been frequently reported as a side effect of perchlorate treatment and may be related to the effects of perchlorate on the hematological system. For risk assessment purposes, several questions regarding the relationship between Graves' disease patients and normal humans must be answered.

- Will perchlorate have the same hematological effects in normal humans after prolonged exposure?

- Are Graves' disease patients uniquely sensitive to the hematological effects of perchlorate?

- By what mechanism does perchlorate cause hematological effects and are these effects related to perchlorate's effect on the thyroid?

The development of aplastic anemia is highly variable in the population and related to individual susceptibility. The data suggest that the altered immune function of Graves' disease patients renders them uniquely susceptible to these types of hypersensitivity reactions.

As described above, Graves' disease is an autoimmune disease in which patients carry autoantibodies to thyroid tissue which mimic TSH stimulation. Although cells from Graves' patients have an increased prevalence to express certain HLA (major histocompatibility complex) antigens (Robbins, 1979; Holland et al., 1991), Graves' disease is thought to be mediated by altered function of activated T lymphocytes (Holland et al., 1991; Panayi, 1995). Most Graves' patients have a lymphocytic infiltrate of the thyroid (Robbins, 1979). Holland et al. (1991) report the development of Graves' disease in a male patient eight years following a bone marrow transplant from his sister who had Graves' disease. The clinical findings support a role for circulating lymphocytes in the initiation of the disease.

While Graves' disease is the product of disrupted immune function, there is also evidence that hyperthyroidism itself alters immune function. In animals, hyperthyroidism results in diminished suppressor T cell function (Wenzel and Lente, 1984). In addition, Graves' disease patients in whom hyperthyroidism was not in control had decreased T cell counts but Graves' patients in whom hyperthyroidism was under control had normal T cell counts (Wenzel and Lente, 1984). Thus, it seems probable that thyroid hormone levels alter lymphocyte populations and properties. Also, patients with Graves' disease are likely to be more susceptible to idiosyncratic reactions to compounds which act on the immune system.

Antithyroid drugs appear to exert their effects on the hematopoietic system through an immune mechanism. Wing and Fantus (1987) reviewed the adverse effects of two antithyroid drugs, propylthiouracil and methimazole, and concluded that most reactions were related to immunologic effects of these drugs. They noted that skin rash and granulocytopenia were among the most commonly reported adverse effects of these drugs. Less commonly reported effects include aplastic anemia, leukopenia, and antibodies to insulin and glucagon. In fact, Wing and Fantus (1987) recommend that patients be instructed to report skin rash immediately as this may be an early sign of adverse immune reaction caused by

the antithyroid drugs. Although these authors did not include perchlorate in their investigation, the similarity of the effects seen after perchlorate treatment, including rash, leukopenia, agranulocytosis, and aplastic anemia, suggest that perchlorate may also act to induce an immune effect in a similar fashion.

There is a tight functional connectivity between the immune and endocrine systems, which is mediated, at least partly, by shared receptors and mediators among the systems (Kammuller, 1995). Thus, although the mechanism of perchlorate action on the hematopoietic system is not known, it is likely to be an immune reaction. Although it is possible that perchlorate may cause the hematological effects in normal humans, it appears that Graves' patients are likely to be more sensitive to this type of immune-induced adverse effect than normal humans. The underlying abnormal immunologic function in Graves' disease makes these patients more sensitive to immunologic challenges. Immunoreactivity to antithyroid drugs is another expression of the abnormal immune system in these patients (Wall et al., 1984; Wing and Fantus, 1987). Thus they are expected to have drug allergies with increased frequency (Wall et al., 1984).

### 2.1.2 Toxicity Data in Animals

Both short-term and long-term studies in animals have evaluated the effects of perchlorate on the thyroid. These studies established LOAELs at high doses, and they generally did not examine tissues and systems other than the thyroid. The long-term studies demonstrate that continual disruption of the thyroid-pituitary axis by perchlorate will result in the development of thyroid tumors.[c] A summary of the animal studies of perchlorate is presented in Table 2.

#### 2.1.2.1 Short-term and Subchronic Studies

Mannisto et al. (1979) measured serum levels of TSH, T3 and T4 by radioimmunassays in groups of 5-6 male Sprague-Dawley rats weighing 180-220 grams who were exposed to potassium perchlorate in their drinking water at concentrations of 0, 10, 50, 100, or 500 mg/L for four days. Potassium perchlorate doses of 0, 1.5, 7.6, 15.3, and 76.3 were calculated assuming a body weight of 0.2 kg and a water consumption rate of 0.0305 L/day (U.S. EPA, 1987). Perchlorate produced statistically significant increases in serum TSH levels and decreases in serum T3 and T4 levels. Significant changes in all three parameters were measured in the 100 and 500 mg/L (15.3 and 76.3 mg/kg/day) dose group. In the 50 mg/L (7.6 mg/kg/day) dose group levels of T3 and T4 were significantly decreased; TSH levels were slightly increased, but the increase was not significant. At the low dose, T3, T4, and TSH levels were unchanged from controls. This study identifies a NOAEL of 1.5 mg/kg/day and a LOAEL of 7.6 mg/kg/day.

Caldwell et al.(1996) administered ammonium perchlorate in drinking water at concentrations of 0, 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L to Sprague-Dawley rats (6/sex/group) for 14 days. The authors calculated the corresponding doses to be 0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, and 22.16 mg/kg/day for males and 0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, and 24.86 mg/kg/day for females. Thyroid weights were measured and thyroid hormone levels were measured using a radioimmune assay technique. Relative thyroid weights were statistically significantly increased in the two highest dose groups compared with controls. Thyroglobulin levels and TSH increased in both male and female rats in a dose-dependent manner. The TSH increase was statistically

[c] Because this assessment concerns the non-cancer effects of perchlorate, the findings of tumors in the long-term animal studies are not reported or evaluated. According to U.S. EPA's thyroid cancer policy, the development of thyroid cancer after continual disruption of the thyroid-pituitary axis is considered to be by a threshold mechanism. The implications of this policy for quantitative risk assessment of perchlorate are discussed in Section 2.2.2.

**Table 2. Animal Studies of Perchlorate***

| Study | Species (n) | Duration | Doses mg/kg-day | Effects | Notes |
|---|---|---|---|---|---|
| Kessler and Kruskemper (1966) | Male Wistar rat (6-8/group) | 2 years | 0 (water)<br>1339 LOAEL | Increased absolute and relative thyroid weight. Follicular cell hyperplasia | No tissues other than thyroid examined |
| Pajer and Kalisnik (1991) | Female Balb/c mice (36/group) | 46 weeks | 0 (water)<br>2147 LOAEL | Increased thyroid volume, pituitary TSH, histopathologic changes in thyroid. Follicular cell carcinoma | No tissues or organ systems other than thyroid examined |
| Shigan (1963) | Rabbits, Rats (# and sex not specified) | 9 months | 0 (water?)<br>0.25 NOAEL<br>2 LOAEL<br>40 | Increased secretion of iodine from thyroid; no effects on other organs | Other effects examined were cardiac electrical activity, liver function, and conditioned reflexes. |
| | Rabbits, Rats (# and sex not specified) | 3 months | 0 (water)<br>190 LOAEL | Changes in cardiac electrical activity and liver function | Study not well enough reported or translated to be useful for risk assessment |
| Gauss (1972) | Female NMRI mice | 160 days | 0 (diet)<br>2011 LOAEL | Increased thyroid volume and histological changes to thyroid | |
| Hiasa et al. (1987) | Male Wistar rats (20/group) | 20 weeks | 0 (diet)<br>81 LOAEL | Increased absolute and relative thyroid weight, serum TSH. Decreased serum $T_4$ | No effect on body or liver weight. No other parameters examined. No histopathology. |
| Caldwell et al. (1996) | Male Sprague-Dawley rats (6/group) | 14 days | 0 (water)<br>0.11, NOAEL<br>0.44 LOAEL<br>1.11<br>2.26<br>4.32<br>11.44<br>22.16 | Increased relative thyroid weight, increased TSH and thyroglobulin, decreased $T_3/T_4$ | No other tissues or organs examined |

| Study | Species (n) | Duration | Doses mg/kg-day | Effects | Notes |
|---|---|---|---|---|---|
| | Female Sprague-Dawley rats (6/group) | 14 days | 0 (water)<br>0.12 LOAEL<br>0.47<br>1.23<br>3.06<br>4.91<br>11.47<br>24.86 | Same effects as in males. Females are more sensitive. | No other tissues or organs examined |
| Mannisto et al. (1978) | Male Sprague-Dawley rats (5-6/group) | 4 days | 0 (water)<br>1.5 NOAEL<br>7.6 LOAEL<br>15.3<br>76.3 | Increased TSH and decreased $T_3/T_4$ | No other endpoints examined; no histopathology. |
| Brown-Grant (1966) | Female Wistar rats (11/group) | gestation days 2-8 | 0 (water)<br>63<br>246 | None | Developmental effects and maternal toxicity not evaluated. Only endpoint examined was the number of live litters. |
| Brown-Grant and Sherwood (1971) | Female Wistar rats (10/group) | gestation day 0 to 12/13 | 1% in water LOAEL | Decreased number of dams with implantation sites, increased maternal and pup thyroid weight | No untreated controls |
| Postel (1957) | Female guinea pigs | gestation day 21-48 | 0 (water)<br>740 LOAEL | Increased fetal thyroid weight | Fetuses were not examined for other developmental effects |

* Table is ordered in roughly decreasing exposure duration.

significant at the 0. 47 mg/kg/day dose for females and at the 1.11 mg/kg/day dose for males. Both T3 and T4 showed statistically significant decreases; however, the T4 effect did not show a dose relationship. For T3, the decrease was statistically significant at the lowest dose, 0.12 mg/kg/day, in females and at the 0.44 mg/kg/day dose level in males. This study suggests that female rats are more sensitive than male rats to the effects of perchlorate. This study defines a LOAEL in females of 0.12 mg/kg/day. The same dose in males is a NOAEL.

Shigan (1963) administered 190 mg/kg/day in water to rabbits and white rats (number, sex and strain not identified) for 3 months. The author does not indicate if the compound was administered in drinking water or gavage with water. The animals were examined for cardiac function, liver function based on changes in serum proteins, immune function based on leukocyte phagocytosis, and adrenal function. Perchlorate at the dose tested caused a change in the EKG and a decrease in serum proteins indicating a disruption of the glycogen -forming function of the liver. The authors do not indicate if these changes were observed in both rabbits and rats. Perchlorate had no effect in the remaining tests. This study suggests a LOAEL of 190 mg/kg/day; although the study is incompletely reported and/or translated, limiting its usefulness for risk assessment.

In a second set of experiments, Shigan (1963) also treated rabbits and white rats (number, sex, and strain not identified) with 0, 0.25, 2, and 40 mg/kg/day of potassium perchlorate for 9 months. The medium for dosing was not reported. The animals were examined for cardiac function, liver function, and conditioned reflexes. In addition, uptake and discharge of iodine by the thyroid was examined. In the two highest dose groups, there was a statistically significant increase in the amount of iodine excreted from the thyroid; this increase was not observed in the 0.25 mg/kg/day dose group. The study does not indicate if the effect was seen in one or both species tested. This study suggests a NOAEL of 0.25 mg/kg/day and a LOAEL of 2 mg/kg/day for thyroid effects.

Hiasa et al. (1987) measured serum levels of T3, T4, and TSH by radioimmunassay in groups of 20 male Wistar rats administered 0 or 1,000 ppm potassium perchlorate in the diet for 20 weeks. Assuming a body weight of 0.34 kg (the average final body weight of rats treated with perchlorate alone) and a food consumption rate of 27.4 g/day (U.S. EPA 1987), an estimated dose of 80.7 mg/kg/day can be calculated. Absolute and relative thyroid weights were significantly increased compared with controls in perchlorate treated rats. No effects were seen on liver weights. T4 levels decreased slightly, but the decrease was not statistically significant. T3 levels were unchanged compared with controls. TSH levels were statistically significantly increased compared with controls. Histological exam of the thyroid revealed diffused small follicles in

perchlorate treated rats and 1 case of follicular hyperplasia. The 80.7 mg/kg/day dose is a LOAEL.

Gauss (1972) fed female NMRI mice a diet containing 0 or 1% potassium perchlorate for up to 160 days. Mice were between 50 and 60 days old at the beginning of treatment and weighed between 19 and 28 grams (average of 23.23 g). During the first two months of treatment, body weights increased about 12%; body weight data for longer treatment periods were not reported. Assuming a body weight of 23 g and a food consumption value of 4.625 g/day (U.S. EPA 1987), a dose of 2,011 mg/kg/day can be calculated. Thyroid glands were examined histologically at 10-20 day intervals through the 160 days. Thyroid volume, nuclei volume and height of epithelial follicles were increased in treated mice throughout the treatment period compared with controls. The English translation summary of the histological examinations described a progressive change in the histological appearance of the thyroid of treated mice, beginning with colloid loss, nuclei volume expansion and rising epithelium height, followed by the appearance of hyperplasia and hypertrophy of the thyroid parenchyma. At later stages of the treatment period, hyperplastic follicles, areas of adenomatic tissue, adenoma complexes and secreting cystadenomas were observed. However, no progression to malignancy was apparent. The 2,011 mg/kg/day dose is a LOAEL.

2.1.2.2 Long-term Studies

Kessler and Kruskemper (1966) provided potassium perchlorate in drinking water at a concentration of 0 or 1% to male Wistar rats for 2 years. Body weights and thyroid weights were reported for groups of 6-8 rats sacrificed after 0, 40, 120, 220, and 730 days of treatment. Thyroid glands from the animals were examined histologically. Using body weight data provided in the report to calculate a time-weighted average body weight of 0.336 and using an estimated water consumption of 0.045 L/day [calculated with the allometric equation recommended by U.S. EPA (1987)], a dose of 1339 mg/kg/day is derived. Body weights of control and treated animals were comparable throughout the experiment. In contrast, thyroid weights (both relative and absolute)were markedly increased in treated rats compared with controls at each examination interval. Histological examination of thyroids from treated rats at 40 days revealed follicular cell hyperplasia.[d] The authors characterized these changes as typical for a thyroid gland stimulated by TSH for a relatively short period of time. After 200 days of perchlorate treatment, diffusely degenerative

[d] Follicular cell hyperplasia is defined by small follicle with high epithelia and large nuclei, numerous mitoses, colloid resorption and low-grade mesenchymal reaction.

changes with fibrosis and increased colloid were observed. The authors commented that the course of the histological changes in the thyroid was similar to that produced by long-term administration of thiouracil, another antithyroid agent. The authors further reported that four of eleven rats treated with potassium perchlorate for 2 years displayed benign tumors of the thyroid gland and that 20 untreated Wistar control rats displayed no thyroid gland tumors. The 1,339 mg/kg/day dose is a LOAEL.

Pajer and Kalisnik (1991) administered 0 or 1.2% sodium perchlorate in drinking water to groups of 36 female BALB/c mice (12/group) for up to 46 weeks. Eight or 12 weeks after the beginning of the experiment, one group of treated and control mice were totally irradiated with 0.8 Gy on 5 consecutive days, at a dose rate of 1.45 Gy/minute, so that each mouse received a total of 4 Gy. Assuming a body weight of 0.0353 kg and a water consumption rate of 0.0063 L/day (U.S. EPA 1987), a dose of 2147 mg/kg/day can be calculated. Thirty animals died during the experimental period although details about the cause of death were not provided. Forty-two animals were sacrificed at 46 weeks for histological examination of the thyroid and pituitary. No other tissues were examined. Obvious treatment related histological changes were observed in the thyroid and pituitary, including thyroid follicular cell carcinoma. Immunoperoxidase staining of pituitary thyrotropic cells with antihuman TSH serum provided qualitative evidence of increased TSH production in the pituitary. Perchlorate treatment was associated with increased total volume of the thyroid gland and the distal parts of the anterior pituitary (adenohypophysis). In addition, increased average volume and increased numbers of epithelial, thyrotropic and parafollicular cells was observed. Irradiation appeared to enhance the effects of perchlorate treatment. This study identifies a LOAEL of 2147 mg/kg/day for thyroid effects.

### 2.1.2.3 Developmental/Reproductive Toxicity Studies

Brown-Grant (1966) examined perchlorate for its effects on pregnancy in rats. Potassium perchlorate at a 1% solution in drinking water was administered to pregnant Wistar rats from day 2 to day 8 of gestation. Average doses were reported to be 237 mg/rat/day which is equivalent to 741 mg/kg/day assuming a body weight of 0.32 kg (U.S. EPA, 1987). Birth of a live litter occurred in 8/11 treated dams compared with 7/11 of potassium chloride treated control dams. Examination of fetuses for developmental defects was not conducted. Neither the perchlorate treated dams nor the KCl controls which did not give birth displayed any visible sign of implantation in their uteri. The author concluded that 1% potassium perchlorate in the drinking water had no effect on the course of pregnancy in rats. This study identifies a free standing NOAEL of 741

mg/kg/day; although, in the absence of examination of fetuses this judgment is tentative.

Brown-Grant and Sherwood (1971) administered 1% potassium perchlorate or 0.1% potassium iodide in drinking water to pregnant Wistar rats that were also lactating. Administration began on day 0 of pregnancy and continued until day 12 or 13. Non-lactating pregnant rats were provided with 0.1% KCl or KI by similar protocol. Untreated controls were not included in the experiment. The suckling litters were removed on days 9 or 10 and all dams were killed on day 12 or 13 and examined for the number of implantation sites. There was 100% incidence of dams with implantation sites for all groups but the perchlorate treated group in which 70% of the dams had implantation sites. The number of implementation sites per dam was comparable for all groups. Thyroid weights in the perchlorate treated dams appeared to be increased compared with the chloride or iodide treated dams. Also, thyroid weights of the offspring of perchlorate treated dams was increased compared with offspring from iodide treated dams. The authors concluded that treatment with potassium perchlorate had no significant effect on blastocyst survival or the ability to implant under conditions delaying implantation (i.e., concurrent lactation). This study defines a LOAEL of ≈ 740 mg/kg/day (assuming body weights and water intakes were similar to those in Brown-Grant, 1966) for both maternal and fetal thyroid effects.

Postel (1957) reported that administration of 1% potassium perchlorate in drinking water to pregnant guinea pigs during gestation days 21 through 48 produced enlarged thyroids in the fetuses compared with thyroids of control fetuses. In contrast, perchlorate treatment did not have any effect on the thyroids in dams. Enlarged fetal thyroids also occurred when perchlorate treatment was accompanied by daily subcutaneous treatment with T3 doses as high as 32 ug/kg/day. From water intake and body weight data, the authors calculated an average daily dose to the dams of 740 mg/kg/day. The fetuses were not examined for other developmental effects. In a separate experiment, 0 or 1% potassium perchlorate was administered to nonpregnant female guinea pigs for 30, 60, or 90 days. Thyroid enlargement and hyperplasia were apparent in treated animals after 60 or 90 days of treatment. This study identifies a LOAEL of 740 mg/kg/day for fetal thyroid enlargement.

## 2.2 Characterization of Hazard of Perchlorate

In both humans and animals, perchlorate acts to competitively inhibit iodide accumulation in the thyroid thereby inhibiting the production of iodide-containing thyroid hormones. The short-term consequence of this action is a response by the pituitary gland to produce TSH which in turn stimulates diffuse

cell division and growth of the thyroid gland. Effects related to disturbance of the thyroid-pituitary axis have been seen in studies in humans, both Graves' patients and normal humans, and in both short-term and long-term studies in animals. Thus, disturbance of the function of the thyroid-pituitary axis appears to be the critical effect from exposure to perchlorate salts. Disturbance of the thyroid-pituitary axis leads to both noncancer and cancer effects in both humans and experimental animals.

### 2.2.1 Non Cancer

Experience with treatment of Graves' patients shows that repeated oral administration of 200 mg doses taken 1 to 5 times per day (3 to 14 mg/kg/day) were effective in inhibiting the excessive production of thyroid hormones and controlling other aspects of hyperthyroidism (Connell, 1981; Godley and Stanbury, 1954; Crooks and Wayne, 1960, Morgans and Trotter, 1960). However, perchlorate within a dose range of 6-14 mg/kg/day also resulted in a small number of fatal hematological effects in Graves' patients. In each of these cases, patients were treated with perchlorate at the high end of the dose range (i.e., 9 to 14 mg/kg/day) until hematological symptoms appeared; then patient's dosage was reduced to the low end of the range (i.e., 6 mg/kg/day). Thus, the threshold for these effects appears to be between 6 and 9 mg/kg/day. Because of the serious nature of these effects, the low end of the dose range (6 mg/kg/day) should be considered a Frank Effect Level (FEL).

The hematological effects of perchlorate appear to be a hypersensitivity reaction unrelated to perchlorate's effects on iodine uptake and secretion by the thyroid. The hematological effects of perchlorate may be mediated through an immune reaction such as a drug-induced autoimmune response. Since Graves' patients already have an unbalanced immune system, they are more susceptible to the hematological effects of perchlorate (Wall et al., 1987; Wing and Fantus, 1987) and thus should be considered a sensitive subpopulation for these effects.

In addition to being a sensitive population for the hematological effects of perchlorate, it has also been suggested that Graves' disease patients are the sensitive population for perchlorate's effects on iodine balance in the thyroid. Because the thyroids of Graves' disease patients are continually stimulated by antibodies which stimulate the TSH receptor, they have essentially unregulated iodine uptake. Endocrine experts have suggested that it is plausible to assume that Graves' patients will be more sensitive to the iodine blocking effects of perchlorate than normal humans (Capen, 1996; Fagin, 1996). No data are available to identify a mechanism for this phenomenon. The same phenomenon is likely to be observed in people who are iodine deficient; because their thyroids

are also under constant stimulation, they are expected to be as sensitive to perchlorate as Graves' disease patients (Capen, 1996; Fagin, 1996). Conversely, people who are hypothyroid are likely to be less sensitive to perchlorate.

Two short-term studies in normal humans support the conclusion that the target organ for perchlorate is the thyroid. Healthy volunteers dosed with 9 mg/kg/day for 8 days (Burgi, 1974) or 12 mg/kg/day for 4 weeks (Brabant 1992, 1994) both experienced a decrease in the intra-thyroidal iodide concentration of 65% and 25% respectively. A short-coming of the Brabant studies is that the volunteers were pretreated with iodine for 4 weeks prior to perchlorate treatment. It is likely that this iodine loading affected the response to perchlorate.[e] There are no studies in normal humans which demonstrate the effects of perchlorate after long-term treatment.

Animal data support the conclusion that perchlorate affects the thyroid-pituitary axis. Both short- and long-term studies found effects such as decreased T3 and T4 levels, increased TSH levels, secretion of iodine from the thyroid, and increased thyroid weights (both relative and absolute). A short-coming of the animal database is that there are few studies adequate for risk assessment which examined any tissues or systems other than the thyroid. One study, Shigan (1963) appeared to examine cardiac and liver function in addition to thyroid function. However, either because of inadequate reporting or translation from Russian, very little information is available about the methods used or the results obtained., making this study unsuitable for risk assessment purposes. Therefore, the database does not completely rule out the possibility that perchlorate has effects on systems other than the thyroid after long-term treatment.

### 2.2.2 Cancer

A convincing body of evidence suggests that long-term interference with the thyroid-pituitary axis can lead to thyroid follicular cell neoplasia. This phenomenon has been the subject of extensive review (Hill et al., 1989) and is summarized below. As described in Section 2.1, TSH stimulates the thyroid follicular cells to synthesize T3 and T4, which in turn inhibit the synthesis of additional TSH. Thus, high plasma levels of T3 and T4 reduce the amount of TSH produced and low levels increase the amount of TSH produced. If thyroid hormones are not produced in response to TSH, plasma levels of TSH remain

[e] According to Godley and Stanbury (1954), "No patient failed to respond [to perchlorate therapy] in time, but several responded slowly. One of these was receiving large doses of iodine just before perchlorate was begun. Presumably the thyroid of this patient was filled with iodine, which had to be exhausted before a therapeutic effect could be achieved."

high, resulting in an ongoing stimulation of the thyroid gland. This occurs following every condition which interferes with these feedback mechanisms, including iodine deficiency, thyroidectomy, or chemical disturbance.

A series of progressive morphological changes occurs in the thyroid in response to prolonged elevated levels of TSH, regardless of the nature of the stimulus causing TSH elevation (Hill et al., 1989). Initially thyroid weight remains constant although there are significant changes in thyroid morphology including resorption of colloid from the follicular cell lumen and increases in cell volume and vascularity. With continued TSH stimulation, there is a rapid increase in thyroid weight and size associated with follicular cell hyperplasia. Ultimately with continued TSH stimulation, the diffuse hyperplasia progresses to nodular proliferation of the follicular cells and eventually to benign and malignant tumors. This progression is similar regardless of the cause of thyroid insufficiency. Hill et al (1989) lists several conditions which can lead to this progression of pathology including dietary iodine deficiency, blockage of iodine into the thyroid, interference with thyroid hormone synthesis, suppression of thyroid activity by high concentrations of iodine, enhanced metabolism of thyroid hormones, and damage to the thyroid gland. It has been suggested that rats are more sensitive to these effects of increased TSH. Rats cannot withstand a sustained increase of TSH without developing tumors; just an initial rise in TSH levels will have promoting effects (Capen, 1996).

From the foregoing discussion, it is apparent that thyroid cancer induced by interference with thyroid -pituitary homeostasis is a threshold phenomenon. In fact, U.S. EPA (1996) has adopted the policy that an assumption of a threshold is appropriate for the dose-response assessment of chemicals which cause a disruption of thyroid-pituitary homeostasis and do not have genotoxic activity relevant to carcinogenicity. Perchlorate ion was cited repeatedly by U.S. EPA (1996) as an example of a chemical known to disrupt thyroid-pituitary homeostasis by acting directly on the thyroid.

The two long-term studies of perchlorate in animals (Kessler and Kruskemper, 1966; Pajer and Kalisnik, 1991) demonstrated that perchlorate induces follicular cell carcinogenesis. The shorter term studies (Gauss, 1972; Hiasa et al., 1987) indicate that carcinogenesis is preceded by the morphological changes typical of the progression induced by TSH stimulation described by Hill et al. (1989). No genotoxicity studies were located in the literature, so it is not possible to state with certainty that perchlorate does not have any genotoxic activity relevant to carcinogenicity. However, perchlorate is so clearly acting to disrupt thyroid-pituitary homeostasis that the assumption of a threshold for dose-response assessment is appropriate. Therefore, the RfD developed in the

following section is likely to be protective for both noncancer and cancer effects of perchlorate.

# 3. Dose Response Assessment

## 3.1 Choice of Critical Study

Because it is inappropriate to derive an RfD from a FEL, the clinical data which define a FEL in the range of 6-14 mg/kg/day do not provide a suitable basis for RfD derivation. For this same reason, the studies in normal humans, which were also conducted at doses which are within the range of the FEL, are also not suitable for RfD derivation. Only one long-term study in humans reported patient response to a dose less than the range of the FEL. Connell (1981) reported that a 22 year treatment with 3 mg/kg/day perchlorate effectively controlled symptoms of hyperthyroidism without any adverse side effects. However, this study is limited because only one case was reported.

The early experiments by Stanbury and Wyngaarden (1952), however, examined the influence of acute doses of potassium perchlorate at lower dosage levels. In these experiments, single 100 mg doses of potassium perchlorate (1.4 mg/kg/day) caused a complete release of iodide from the thyroid and prevented the accumulation of subsequently administered iodide for about 6 hours. Single doses as low as 3 mg potassium perchlorate (0.04 mg/kg/day) also produced a detectable, but incomplete, release of iodide. Thus, 1.4 mg/kg/day appears to be a definitive LOAEL for acute disturbance of iodide accumulation in the thyroid. It is assumed for the purposes of RfD derivation that repeated exposure to 1.4 mg/kg/day would lead to a functional disturbance of the thyroid-pituitary axis, including decreased synthesis of thyroid hormones and increased production of TSH. Exposure to lower doses would insufficiently impair iodine accumulation and would not disturb the function of the thyroid-pituitary axis.

This LOAEL is supported by the findings of the animal studies. In Caldwell et al. (1996), T3 levels in female rats were significantly decreased by a 14-day exposure to 0.12 mg/kg/day. At a dose of 0.47 mg/kg/day an effect on both T3 and TSH was observed, with T3 levels significantly decreased and TSH levels significantly increased. In addition, Mannisto et al. (1979) reported a NOAEL of 1.5 mg/kg/day and a LOAEL of 7.6 mg/kg/day based on decreased T3/T4 and increased TSH after 4 days exposure to perchlorate.

## 3.2 Choice of Uncertainty and Modifying Factors

The choice of uncertainty factors to be used with the appropriate critical effect of perchlorate depends on the areas of uncertainty that exist given the quality of the database.

### 3.2.1 Human Variability (H)

Do existing data account for sensitive individuals?

If yes, this suggests an uncertainty factor other than a default value of 10---as low as a value of 1 in some instances [see for example, the description of the uncertainty factor for nitrates on U.S. EPA's IRIS (1995) where a NOAEL of a sensitive population was used as the basis of the RfD]. Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences among humans. Some recent work has attempted to quantify these distinctions (Renwick, 1993).

Perchlorate's critical effect in both humans and animals is disruption of the thyroid-pituitary axis. Subpopulations who may be sensitive to this effect are Graves' patients and people who are deficient in iodine intake (Capen, 1996; Fagin, 1996). About an order of magnitude difference exists between normal human LOAELs (9.7 and 13 mg/kg/day) compared with Graves' patients LOAELs (1.4 and 3.0 mg/kg/day). These differences also suggest that the Graves' patients in the Stanbury and Wyngaarden (1952) study represent a sensitive population. Thus, a reduced factor appears warranted. Since the critical study was conducted in sensitive individuals, we recommend a $UF_H$ of 3.

### 3.2.2 Inter-Species Variability (A)

Do existing data allow for a quantifiable extrapolation of animal dose to the expected human equivalent dose for effects of similar magnitude? Or as is more likely the case, for NOAELs?

If yes, this suggests an uncertainty factor other than a default value of 10---with RfDs for example, a value of 3 is often used when dosimetric adjustments are used in the determination of HEC [see U.S. EPA's IRIS (1995) for numerous examples]. Scientists familiar with this area have also considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences between experimental animals and humans, but also recognize that some overlap with the uncertainty factor for intra-species variability exists. Some recent work has also attempted to quantify these distinctions in general (Renwick, 1993).

Since human data are used as the basis of this RfD, this factor is not needed (i.e., $UF_A = 1$). However, data suggest that rats are more sensitive to the toxicity of perchlorate than humans. Thus, if the rat LOAEL from Caldwell (0.12 mg/kg/day) or the rat NOAEL from Mannisto (1.5 mg/kg/day) are used as the basis of an RfD, a reduced $UF_A$ might be warranted.

### 3.2.3 Subchronic-to-Chronic Extrapolation (S)

Do existing data allow for a quantifiable extrapolation of the critical effect after subchronic exposure to that after chronic exposure? Will NOAELs of different critical effects after subchronic and chronic exposure, differ quantitatively?

U.S. EPA has occasionally used values less than 10 (nearly always 3-fold) with less than chronic exposures when data were available to support such a reduction [for example, see the RfD for arsine on U.S. EPA's IRIS (1995)]. Scientists familiar with this area also recognize that some overlap with this factor occurs with the database uncertainty factor (see following discussion).

For perchlorate, the initial step in the progression to overt signs of toxicity is the inhibition of iodine uptake by the thyroid and the discharge of unbound iodine from the thyroid. Without this preliminary action of perchlorate, the cascade of events that lead to altered thyroid function and morphology will not occur. Therefore, short term assays that measure the initial step in the cascade of effects are likely to be as accurate predictors of toxicity as chronic assays. Some reduction in the default $UF_S$ of 10 seems reasonable, but a reduction to 1-fold is not. Thus, a 3-fold factor was applied. This partial reduction is supported by the Connell (1981) study which showed that a 22 year exposure to perchlorate in a sensitive individual (a Graves' patient) did not result in any adverse effects.

### 3.2.4 Insufficient Database (D)

Do existing data allow for a reasoned judgment of likely critical effect, given that any one toxicity study is unable to adequately address all possible outcomes?

If data exist from at least five studies (two chronic standard toxicity bioassays in different species, one two-generation reproductive bioassay and two developmental toxicity studies in different species), an uncertainty factor of 1 is applied. U.S. EPA has occasionally used values less than 10 (nearly always 3-

fold) when data were available on several, but not all 5 studies [for example, see the RfD for acetaldehyde on U.S. EPA's IRIS (1995)], and factors of 10 (generally) when data were only available from a single study. Scientists familiar with this area also recognize that some overlap occurs with the subchronic to chronic uncertainty factor (discussed previously). The general solution to this problem when subchronic studies are available in two species, is to assign the uncertainty to the subchronic to chronic factor, and not to the database factor.

For perchlorate, the database consists of a wealth of knowledge about the long-term administration of perchlorate to patients with Graves' disease, chronic studies in rats (Kessler and Kruskemper, 1966) and mice (Pajer and Kalisnick, 1991); short-term studies in normal humans (Burgi, 1974 and Brabant, 1992), rats (Hiasa et al., 1987), and mice (Gauss, 1972); and acute studies in sensitive humans (Stanbury and Wyngaarden, 1952) and rats (Mannisto et al., 1979 and Caldwell et al., 1996). However, the chronic and subchronic studies are limited by the fact that they generally only examined thyroid effects. There are also three studies that examined the effects of perchlorate on dams during gestation, on pregnancy outcome, and on fetal thyroid (Brown-Grant, 1966; Brown-Grant and Sherwood, 1971; and Postel, 1957). These studies are limited because they did not examine developmental effects other than thyroid effects. No multigenerational studies are available. Thus, although there are a variety of studies, the database is missing some elements, such that some factor is stiill warranted. A $UF_D$ of 3 is reasonable.

### 3.2.5 LOAEL to NOAEL (L) Extrapolation

Do existing data allow for the use of a NOAEL, rather than a LOAEL for the estimation of an RfD?

If a well-defined NOAEL does not exist, a factor of 10 is often used. However, U.S. EPA has often used values less than 10 (nearly always 3-fold) when data suggest that the LOAEL is for a minimally adverse effect, since the hypothesized NOAEL would likely be closer to this LOAEL then to a LOAEL with greater severity. For example, compare the RfDs for acrylonitrile and 1,2 epoxybutane on U.S. EPA's IRIS (U.S. EPA, 1996). The former RfD uses a 3-fold factor with degeneration and inflammation of nasal respiratory epithelium; the later RfD uses a 10-fold factor with more severe degenerative lesions of the epithelium.

The principal study identifies a LOAEL (1.4 mg/kg/day) for a biochemical endpoint that serves as a precursor to a presumed clinical disease (i.e., hypothyroidism) in normal individuals. At this dose, however, there are no overt

signs of toxicity. Signs of toxicity in humans, including skin rash, gastrointestinal problems, and increased thyroid volume, occur at dose levels of about 6 mg/kg/day or higher. Therefore, the LOAEL of 1.4 mg/kg/day appears to be a minimal LOAEL which suggests that a full 10-fold UF for this area of uncertaintyis not needed. A $UF_L$ of 3 is appropriate here.

### 3.2.6 Modifying Factor

A modifying factor is not considered necessary with this database. This is because the outstanding uncertainties can be adequately addressed with the standard factors. U.S. EPA only occasionally uses a modifying factor; for example, see the RfD for methyl ethyl ketone on U.S. EPA's IRIS (1995). The default value of 1 is appropriate for perchlorate.

### 3.2.7 Composite Uncertainty and Modifying Factors

The composite uncertainty factor to use with a given database for developing RfDs is a case-by-case judgment by experts, and should be flexible enough to account for each of the applicable five areas of uncertainty and any nuances in the available data that might change the magnitude of any factor. U.S. EPA describes its choice of composite UF and subcomponents for individual assessments on its IRIS database (U.S. EPA, 1996). For perchlorate, the recommended overall uncertainty factor is 100.

## 3.3 Determination of the RfD

Our proposed RfD is 1E-2 mg/kg/day. This value is determined from the following equation using parameters previously described.

$$\begin{aligned} RfD &= (LOAEL) \div (3_H \bullet 1_A \bullet 3_S \bullet 3_D \bullet 3_L \bullet 1_{MF}) \\ &= 1.4 \text{ mg/kg/day} \div 100 \\ &= 0.014 \text{ mg/kg/day, rounded to 1E-2 mg/kg/day.} \end{aligned}$$

where:

$3_H$ is a 10-fold factor, applied to account for the uncertainty inherent in the differences in human sensitivity

$1_A$, an interspecies UF was not needed.

$3_S$ is a 3-fold factor to account for the uncertainty in extrapolating from short-term results to chronic exposure.

$3_D$ is a 3-fold factor to account for the uncertainty due to an incomplete database.

$3_L$ is the factor to account for extrapolation from a LOAEL to a NOAEL.

$1_{MF}$, a modifying factor different than 1-fold was not needed.

## 3.4 Confidence in the RfD

Confidence in the critical studies is medium-to-low because these studies examined only short-term manifestations of the perchlorate critical effect. However, while Stanbury and Wyngaarden only examined the initial step in disruption of the thyroid-pituitary axis (inhibition of iodide uptake by thyroid), Caldwell et al. (1996) reported a further step in the critical effect, that is decrease of thyroid hormone levels with accompanying increase in TSH. Confidence in the database is medium-to-low. The effect of chronic exposure to low doses of perchlorate has not been adequately examined in either normal humans or animals. In addition, the database lacks adequate developmental and multigenerational studies. Reflecting confidence in the principal studies and the database, confidence in the RfD for potassium perchlorate is medium-to-low.

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