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January 29, 1999

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Susan Goldhaber / Ella Darden
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RE: Technical Workshop on Perchlorate Risk Issues: Comments for the peer reviewers

Dear Ms. Darden:

Attached please find a copy of my report "Benchmark Doses for Perchlorate Obtained from Lamm *et al.* (1999) Study of Thyroid Function in Perchlorate Workers", which was developed for Kerr McGee, that I would like provided to participants at the technical workshop on perchlorate risk issues and included in the workshop record.

Thank you very much.

Sincerely,

Kenny S. Crump, Ph. D.

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Benchmark Doses for Perchlorate Obtained from Lamm *et al.* (1999) Study of Thyroid Function in Perchlorate Workers

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January 28, 1999

Introduction

As an aid to determining an appropriate reference dose (RfD) for perchlorate, a benchmark analysis was applied to thyroid function data from a study by Lamm *et al.* (1999) conducted at a perchlorate manufacturing plant. This work was requested and paid for by Kerr McGee Corporation.

Background

Lamm *et al.* (1999) studied thyroid function in 39 subjects who worked in direct perchlorate production at a perchlorate production plant near Cedar City, Utah. Also studied were 21 workers in the same facility who were assigned to azide production. Pre- and post-shift urine samples and post-shift blood samples were collected. Levels of TSH [thyroid stimulating hormone], T_3 [triiodotyronine], T_4 [thyroxine], and THBR [thyroid hormone binding ratio] were obtained from the post-shift serum measurements. FTI [free thyroxine index] was calculated as the product of the T_4 concentration and the THBR. Differences between pre-shift and post-shift urinary perchlorate measurements were used to estimate the amount of perchlorate absorbed during the shift (mg/shift). Thyroid function was not found to be related to the absorbed dose of perchlorate.

Benchmark Dose (BMD) Methodology

In the past, USEPA has calculated an RfD by applying uncertainty factors to a NOAEL (no-observed-adverse-effect-level). Whenever a NOAEL was not defined by the available studies, USEPA would use a LOAEL (lowest-observed-adverse-effect level) in place of a NOAEL and apply an additional uncertainty factor of 10. This approach was developed primarily for experimental data in which animals were placed in discrete dose groups, and is less appropriate for epidemiological data. When an effect was found in an epidemiological study, the mean exposure in the study was often assumed to be a LOAEL. However, all that may have been actually known was that an effect occurred somewhere within the range of exposures in the epidemiological study – a range that might span several orders of magnitude. More recently USEPA has begun using the benchmark as a replacement for the NOAEL in determining RfCs

and RfDs (USEPA, 1997). This approach has now been applied to several chemicals by the Agency, and results are provided in the IRIS data base.

A benchmark dose (BMD) is a dose that corresponds to a specified level of additional response called the benchmark response (BMR). A BMD is calculated by fitting a mathematical dose-response model to dose-response data. The BMDL, which is a lower statistical confidence bound on the BMD, replaces the NOAEL in the calculation of an RfC or RfD.

The BMD method has several advantages over the NOAEL, including making better use of dose-response information and reflecting sample size more appropriately (Crump 1984, Barnes et al. 1995). When evaluating an epidemiological study, use of the BMD method allows one to consider the dose-response over the entire exposure range rather than making the crude assumption often made in connection with the NOAEL that any effect seen in a population would occur at the mean exposure level.

A BMDL can be calculated from a negative study like that of Lamm *et al.*, as well as from a positive one. In a negative study, the upper bound on the BMD corresponding to any level of increased risk, no matter how small, is undefined (i.e., infinite). Moreover, in a negative study, the slope of the mean response curve could be negative, in which case the estimate of the BMD would also be undefined. Nevertheless, even in a negative study, the statistical lower bound on the BMD (i.e., the BMDL) can always be calculated as a finite number (Crump 1995). It is possible that a BMDL calculated from a negative study reflects only the statistical constraints imposed by the experimental design. Even so, since it is a lower statistical confidence bound, it represents a conservative (in the sense of being health protective) value, since even though a study was negative, exposure could have resulted in a small undetected increase in health effects, and the BMDL represents a statistical lower bound for the dose that could have produced an undetected increase.

Calculation of a BMD involves choosing the dose-response model, the definition of additional response (BMR) used to define the BMD, and the size of the statistical confidence interval represented by the BMDL. Somewhat different approaches must be used to calculate a BMD based on whether the underlying data are quantal or continuous. Quantal data are data that indicate simply whether or not a subject had a particular response. Continuous data, on the other hand, indicate the magnitude of response and theoretically can assume any value in a range. The thyroid function data being modeled in this report (TSH and FTI) represent continuous endpoints.

The k-power dose-response model (Crump 1995), which was one of the models used to make BMD calculations reported herein, has the form

$$\mu(d) = \mu_0 \pm \beta d^k, \quad (1)$$

where d is dose of perchlorate absorbed over a shift, $\mu(d)$ is the expected thyroid test result in a worker with an absorbed perchlorate dose of d, μ_0, b and $k \geq 1$ are parameters estimated from the data. The parameter k was included to allow the dose response for perchlorate to be nonlinear. It was further assumed that test scores were

normally distributed (and consequently represented continuous data) with a variance, σ, that was independent of the amount of perchlorate absorbed.

There are two equivalent ways to define the BMD when using this model (Crump 1995). Both will be described, since each has certain conceptual advantages. One way is to define the BMD as the exposure that corresponds to a given change, BMRC, in the mean response normalized by the standard deviation; i.e., the BMD is defined as the exposure that satisfies $[\mu(BMD) - \mu(0)]/\sigma = BMRC$. Although this formulation is straightforward, there is no clear-cut, non-arbitrary way for deciding upon an appropriate value for BMRC.

The equivalent alternative approach involves first defining a proportion p_0 of an unexposed population that would be considered to have an abnormal response. The value p_0=0.05 was used in analyses reported herein, which is consistent with the convention that the "normal range" of a clinical test is assumed to encompass test results of 95% of a normal population. With p_0 fixed, the probability, P(d), that a subject exposed to d has an abnormal response can be calculated from p_0, σ, and the mean response, $\mu(d)$ (Crump, 1995). This formulation therefore provides a link between quantal and continuous data, since quantal data are generally used to model the probability of an adverse response. In benchmark analyses based upon quantal data, the BMD is often defined as the exposure that causes a predetermined increase, BMRQ, in the probability of an adverse response, e.g., as the value that satisfies

$$P(BMD) - P(0) = BMRQ. \quad (2)$$

Since we now can calculate P(e) using continuous data, we can use the same expression to define the BMD for continuous data. To complete the definition of the BMD we must choose a value for BMRQ. USEPA has published several risk assessments in its IRIS data based that employ the BMD, and in each case USEPA defined BMRQ = 0.1. Therefore we defined the benchmark using expression (2) with BMRQ = 0.1. I.e., we defined the benchmark as the exposure that causes the probability of an abnormal response to increase by 0.1, where an abnormal response is defined so that responses of 5% of unexposed subjects are expected to be abnormal. Allen et al. (1994a, 1994b) determined from analyses of a large number of quantal animal studies that choosing BMRQ = 0.1 produced BMDLs that, on average, were smaller (more conservative) than, the corresponding NOAELs.

Crump (1995) showed that the two approaches described above for defining a BMD from continuous data using the k-power model are completely equivalent, i.e., that given a value for BMRQ, a corresponding value for BMRC can be determined so that BMDs and BMDLs obtained using the two approaches are exactly the same. Crump (1995) also provides a formula for converting from a value for BMRQ to the equivalent BMRC and vice-versa. With BMRQ = 0.1 and p_0 = 0.05, the equivalent BMRC is 0.61. Therefore, our definition of the BMD is equivalent to defining the BMD as the exposure for which the mean response above background is equal to 0.61σ.

It was noted above that if the underlying data are normally distributed with a common standard deviation, specification of a dose response model, $\mu(d)$, for the mean response, and specification of a proportion, p_0 of unexposed subjects whose responses are considered abnormal, determines the probability, $P(d)$ of an adverse response as a function of exposure. Alternatively, with normally distributed data and a common standard deviation, one can specify p_0 and a functional form for the probability of an adverse response, and that determines the mean response, $\mu(d)$. This allows one to define a model for continuous data that corresponds to a predetermined functional form for the probability of an adverse response, and consequently permits the same dose-response model to be applied to quantal and continuous data (Crump 1995). As an alternative to the k-power model applied to continuous data, the Weibull dose-response model,

$$P(d) = p_0 + (1 - p_0)\{1 - \exp[-(Ad)^k]\}, \quad (3)$$

was also applied to the continuous responses of TSH and FTI.

In addition to determining the BMDL from continuous responses, the data for TSH and FTI were discretized by defining a subject to be "affected" if his or her value was outside the normal range. The Weibull dose response model was applied to these quantal data, and the BMD was defined as the exposure corresponding to a 10% increase in probability that the response was outside the normal range.

The conventional 95% level of statistical confidence was used in all cases to define the BMDL. (I.e., the BMDL was defined as the 95% lower confidence bound on the BMD.)

Age and sex were incorporated as covariates in each BMD calculation -- age as a continuous variable and sex via an indicator variable. The method for incorporating these covariates into the models for continuous data are described in Crump and Van Landingham (1996). Covariates were incorporated into the Weibull model calculations for discrete data by making p_0 in equation (3) a function of the covariates.

Since the BMD analyses of the continuous endpoints assume that the responses are normally distributed, the Shipiro-Wilks statistical test for normality (SAS, 1990) was applied to the residuals after including age, sex and perchlorate dose in a linear regression. The FTI responses were normally distributed ($p > 0.05$). Although the TSH levels were not normally distributed ($p < 0.01$), the log-transformed TSH levels were normal ($p > 0.05$). Consequently the log-transform of the TSH levels were used in the modeling.

Results

Table 1 shows the BMDLs (in units of mg perchlorate/shift) obtained from the Lamm *et al.* (1999) study. One worker, who had medium perchlorate exposure was found to have a previously undiagnosed autoimmune condition of the thyroid, euthyroid Hashimoto's thyroiditis. This worker was omitted from all analyses. In addition, one azide production worker had been previously diagnosed with Grave's disease. This

worker had a TSH level of 38, which was more than four times the next highest TSH value. However, this worker's FTI level was not remarkable. BMD calculations for TSH were performed both including and not including this worker and results were similar. The results reported in Table 1 are from analyses that included this worker. Thus, all analyses reported in Table 1 include data on 57 workers.

Figures 1 and 2 give a visual explanation of the BMD calculations reported in Table 1 for TSH and FTI, respectively, based on the k-power model. These graphs contain the partial residuals of the Log TSH and (untransformed) FTI values plotted against the estimated perchlorate intake (mg/shift). The partial residuals are the observed values adjusted for the predicted effects of age and sex. The solid line represents the best estimate of the mean response, and the dotted curve represents a 95% statistical upper confidence bound on the mean response used to calculate the BMDL (95% lower bound on BMD). Graphically the BMDL is determined by projecting a horizontal line from the value on the y-axis corresponding to the mean response plus 0.61s (where s is the estimated standard deviation of the responses), to the point of intersection with the BMDL (dotted) curve. The x-coordinate of the point of intersection is the BMDL. As explained earlier, defining the BMD by a change in the mean response of 0.61σ is equivalent to assuming that 5% of responses are abnormal and defining the BMD by an increase of 0.1 in the probability of an abnormal response. The estimated BMDs are determined graphically in the same way as the BMDLs, except that the best estimate of the mean response (solid curve) instead of the 95% confidence bound (dotted) curve; however, in these examples the estimated BMDs are very large values that cannot be shown on these graphs.

The BMDLs for perchlorate in Table 1 range from 18 mg/shift to 58 mg/shift. The BMDLs obtained from the quantal data (18 mg/shift for FTI and 28 mg/shift for TSH) are smaller than those obtained using continuous data. This difference may be a result of the loss of information incurred from converting from continuous to discrete data. If so, the BMDLs obtained from the continuous data would be more reliable.

Considering only BMDLs based on continuous data, the BMDLs for TSH were 57 mg/shift (k-power model) and 58 mg/shift (Weibull model). The corresponding BMDLs for FTI were 44 and 46 mg/shift. Consequently, the BMDLs were not sensitive to the form of continuous model employed.

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Table 1

BMDLs for Perchlorate Calculated for TSH and FTI

Data Type	Thyroid Function		Model	BMDL mg/shift
TSH	log Thyroid stimulating hormone	Continuous	KPower	58
			Weibull	57
		Quantal	Weibull	28
FTI	Free T4 index	Continuous	Kpower	46
			Weibull	44
		Quantal	Weibull	18

Figure 1: Residuals for TSH Fit by k-Power Model

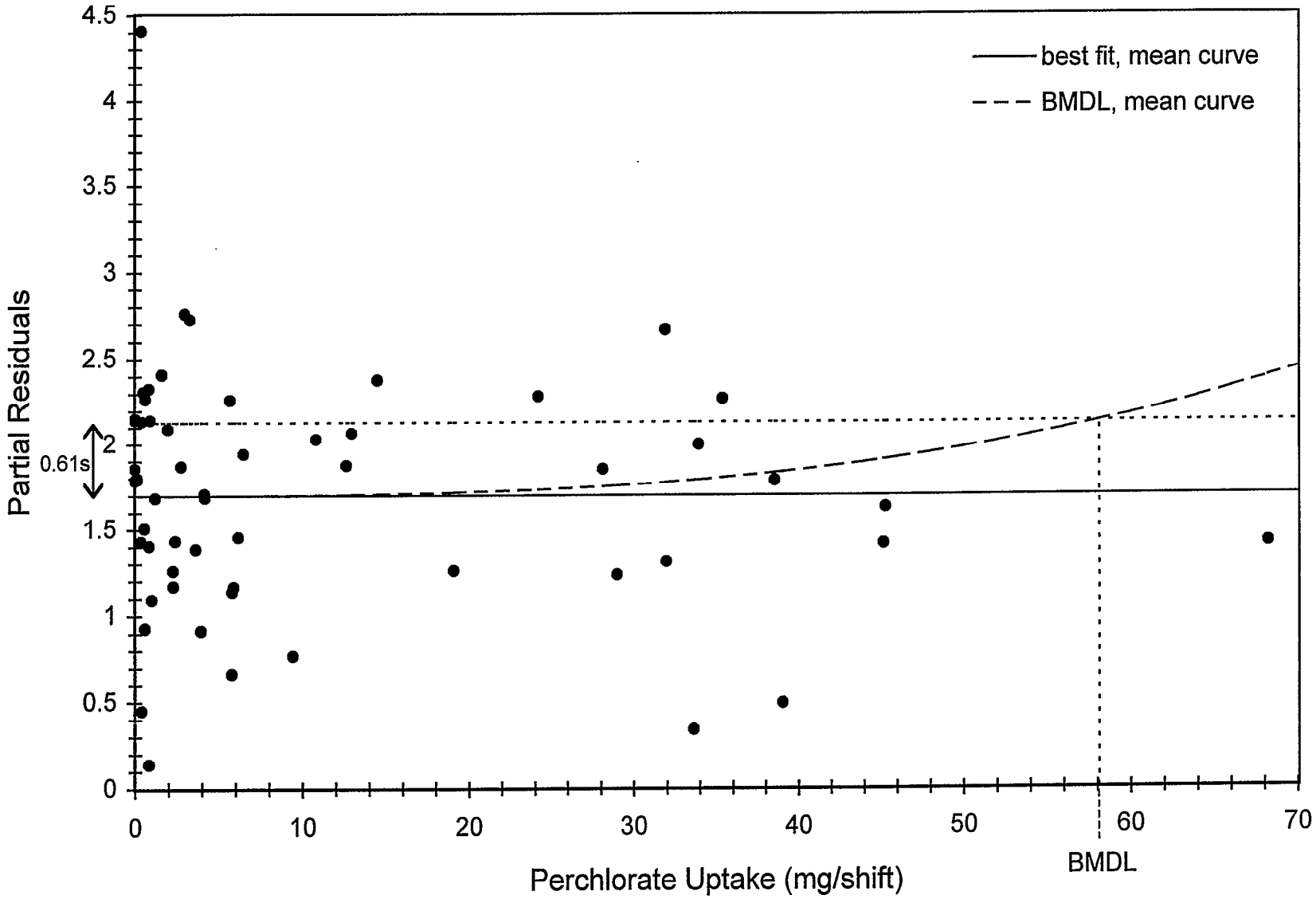
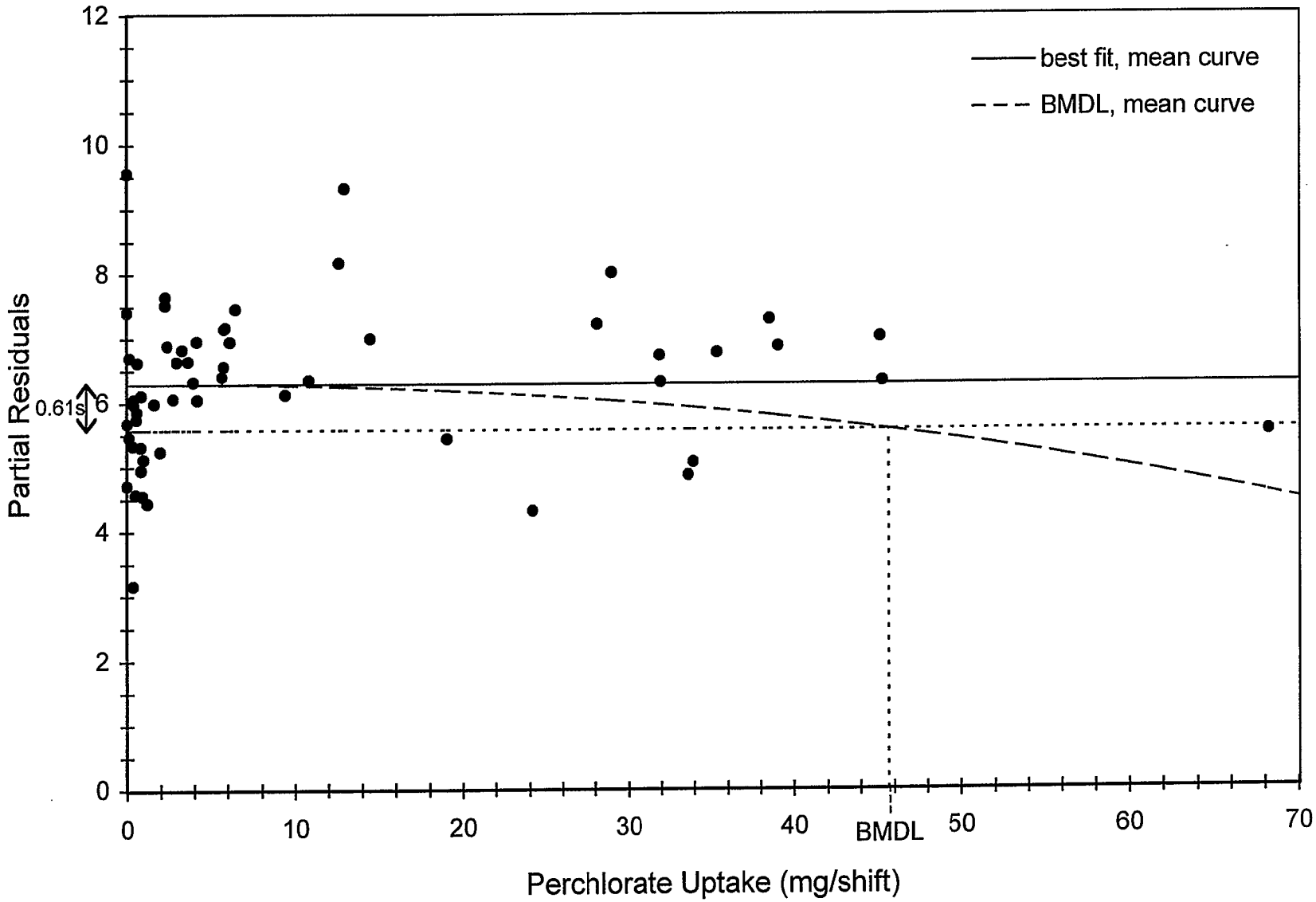


Figure 2: Residuals for FTI Fit by k-Power Model



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January 29, 1999

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RE: Technical Workshop on Perchlorate Risk Issues: Comments for the peer reviewers

Dear Ms. Darden:

Over the past 30 days, new information has been made available that is significant in evaluating the human toxicological risk of perchlorate environmental contamination. An important occupational health study that has just been accepted for publication demonstrates that humans do not experience thyroid-pituitary perturbations with daily perchlorate doses of 40-60 mg/day.

In addition to this latest study, it is equally important that all scientific data related to human health effects be evaluated and considered in developing a reference dose for perchlorate. On behalf of Kerr-McGee Chemical LLC, I offer the following comments regarding the new information, choice of critical effect and uncertainty factors:

I New information strongly suggests reconsideration of recent occupational studies

In establishing an RfD, EPA is attempting to estimate a daily dose in humans that is likely to be without appreciable health effects during a lifetime. The most relevant information possible on which to base this estimate is human health data following many years of low dose exposure to perchlorate. Only in the absence of these type of data, extrapolation from short term high dose studies or animal studies is appropriate.

As with most other chemicals of environmental concern, occupational exposures during the production or use of ammonium perchlorate are much higher than potential environmental exposures. At the beginning of 1998 there were two production facilities in the U.S. and by June, there was only one remaining (Kerr-McGee sold its ammonium perchlorate business to American Pacific).

Both of these worker populations were studied and reported during 1997-1998 and submitted to EPA by September, 1998. Exposure to perchlorate in these two plants was predominantly through inhalation of ammonium perchlorate dust. EPA expressed concerns regarding the "route to route" comparability of dose that occurs through the respiratory route to that that occurs through the oral route. They also expressed concern that no "dose" would result from this type of exposure. Their reasoning is based primarily upon arguments relative to insoluble particulates.

In direct response to those concerns, we offer the following for consideration. Due to the hygroscopicity and high aqueous solubility of ammonium perchlorate (about the same as sodium chloride at body temperature), concerns regarding deposition site are relatively unimportant. Total deposition of particles measured in the two production facilities is expected to be in the 40-90% range[6,7,8,9,11]. Particles that lodge in the anterior nasal airways will undoubtedly clear by extrinsic processes such as nose blowing[3,7,11]. The highly soluble particles that deposit in the posterior nasal passages, pharynx, mouth, larynx, bronchial or bronchiolar regions will either absorb directly through the mucous membranes or clear through the gastrointestinal tract.[3,7,11] Although the exact location of absorption will depend upon solubility rate and particle size[11], once absorbed into the bloodstream, it is physiologically irrelevant what the route of absorption was. Attached are table 10-8 and figures 10-13, 10-14 and 10-34 from reference 11 supporting these arguments.

EPA did not consider the study submitted by Lamm et al[4] because it had not yet been accepted for publication in a journal (although it had been externally peer reviewed). The study was peer reviewed and accepted by mid-January, 1999 for publication in JOEM. It is unclear whether or not EPA will provide copies of that study to the external peer review committee.

EPA comments that "the lack of particle size diameter and distribution data is perhaps one of the most significant limitations of the [Gibbs et al[1]] study". They assert that particle size of the dust was more likely 200 microns based on a personal correspondence. (One of the standard ammonium perchlorate grades is screened as a 200 micron product, of

which less than 0.1% is smaller than 40 microns and 90% larger than 100 microns.) As a result, EPA "was reluctant to assign a NOAEL or LOAEL estimate from this study because of the considerable uncertainties in the exposure estimates".

In direct consideration to that comment, we offer the following. Particle size distribution data for workplace dust that EPA considered key for the Gibbs et al study was presented for a nearly identical production process [as part of the Lamm et al study[4]]. (The Kerr-McGee production facility was closed at the time EPA suggested collecting particle size data.) Additional recent particle size distribution data from the Utah facility are presented in figure 1 showing that 70-90% of the workplace dust is less than 21 microns in size. Additionally, EPA should consider that the standard dust collecting methods used in both studies have well characterized efficiencies and would exclude 200 micron particles. In fact, the upper cutpoint of the 'total dust' (NIOSH method 0500 which very closely approximates "thoracic dust" or PM10 dust) measurements in both studies is 30-40 microns.

Additionally, the Lamm et al[4] study documented significant systemic absorption from workplace dust exposures (calculated from preshift and post shift urinary perchlorate and creatinine levels). Lamm et al[4] found a strong correlation between doses calculated from exposure estimates and doses calculated from urinary perchlorate excretion, thus validating the assumptions relating exposure with dose in the Gibbs et al[1] study.

EPA also commented that failure to control for circadian rhythms in TSH was a limitation to the study. All blood samples were collected during day shifts however, some shifts were 8 hour and some were 12 hour shifts. Statistical analysis was used to control for this effect and a circadian effect was seen.

Combined, the two occupational studies include 86 exposed workers (75 male, 11 female) ranging in age from 25 to 60. Average tenure on the job was about 7 years. Daily workplace doses ranged from 0.1 mg/day to 70 mg/day with the highest exposure group (perchlorate C in Lamm et al[4]) averaging 34 mg/shift. These two related occupational studies demonstrate:

- *Perchlorate dust exposure in the workplace far exceeds most potential environmental exposures (by 1-4 orders of magnitude)*

- *Perchlorate dust in the workplace is readily absorbed systemically through inhalation and is excreted in the urine*

- *No effect in thyroid hormone levels with acute (single shift) exposure to perchlorate dust*

- *No effect on thyroid hormone levels after years of exposure to perchlorate dust*

- *No clinically apparent thyroid abnormalities, even in the highest exposure group*

- *No increase in the percentage of abnormal thyroid tests with perchlorate exposure (no apparent unusually sensitive subpopulation of workers)*

- *No effect on renal, hepatic or hematopoetic systems after chronic exposure*

Although both of these studies were negative with respect to finding adverse thyroid health effects, the data are sufficiently robust to detect anticipated:

- *circadian changes in TSH (thyroid stimulating hormone)*

- *gender effects on hemoglobin, hematocrit, SGPT, GGTP and creatinine*

- *race effects on hemoglobin, SGPT and creatinine*

Kerr-McGee requested Dr. Kenny Crump to apply a benchmark modeling approach to the Lamm et al[4] data set. His analysis indicates a BMDL in the range of 0.3 - 0.8 mg/kg-day with the most reliable estimate in the 0.6 - 0.8 mg/km-day range. Documentation of that analysis is being sent separately to EPA by Dr. Crump.

II Choice of critical effect

There is no question that disturbance of thyroid homeostasis is the critical effect of perchlorate. However, since hormone changes are expected to occur before thyroid histopathology, then hormone changes should be the critical effect. EPA states that it is "fairly well established that rats are more sensitive to thyroid-pituitary perturbations [than humans]". Since the RfD is designed to *protect humans from chronic low level exposures*, the most appropriate critical effect should be derived from humans with chronic low level exposures with due consideration that potential sensitive subgroups such as children are protected. The BMDL derived from the

Lamm et al[4] study represents the most appropriate critical effect level upon which to base the RfD.

III Uncertainty factors

EPA applied an uncertainty factor of 100 to the RfD, explained as:

- *3 for minimal LOAEL for histopathology findings (which some internal reviewers thought should be a NOAEL).*
- *3 for database uncertainty based on two out-standing studies*
- *3 for pharmacokinetic portion (based on rat-human extrapolation)*
- *3 for intrahuman variability*

The use of the BMDL based on Lamm et al[4] obviates the need for the two of these uncertainty factors (LOAEL to NOAEL and rat to human). After the completion of the immunotoxicity study (mid June, 1999) and the two-generation reproductive study (end April, 1999), the need for a database uncertainty factor will be obviated.

A factor of 10 for intrahuman variability (instead of 3) is appropriate considering that there is still concern regarding fetal effects. Towards this end, a new study[5] has been accepted for publication and is included as new information not yet considered by EPA. This study, based on neonatal thyroid tests in southern California and southern Nevada, demonstrates no excess congenital hypothyroidism in areas where water is contaminated with perchlorate.

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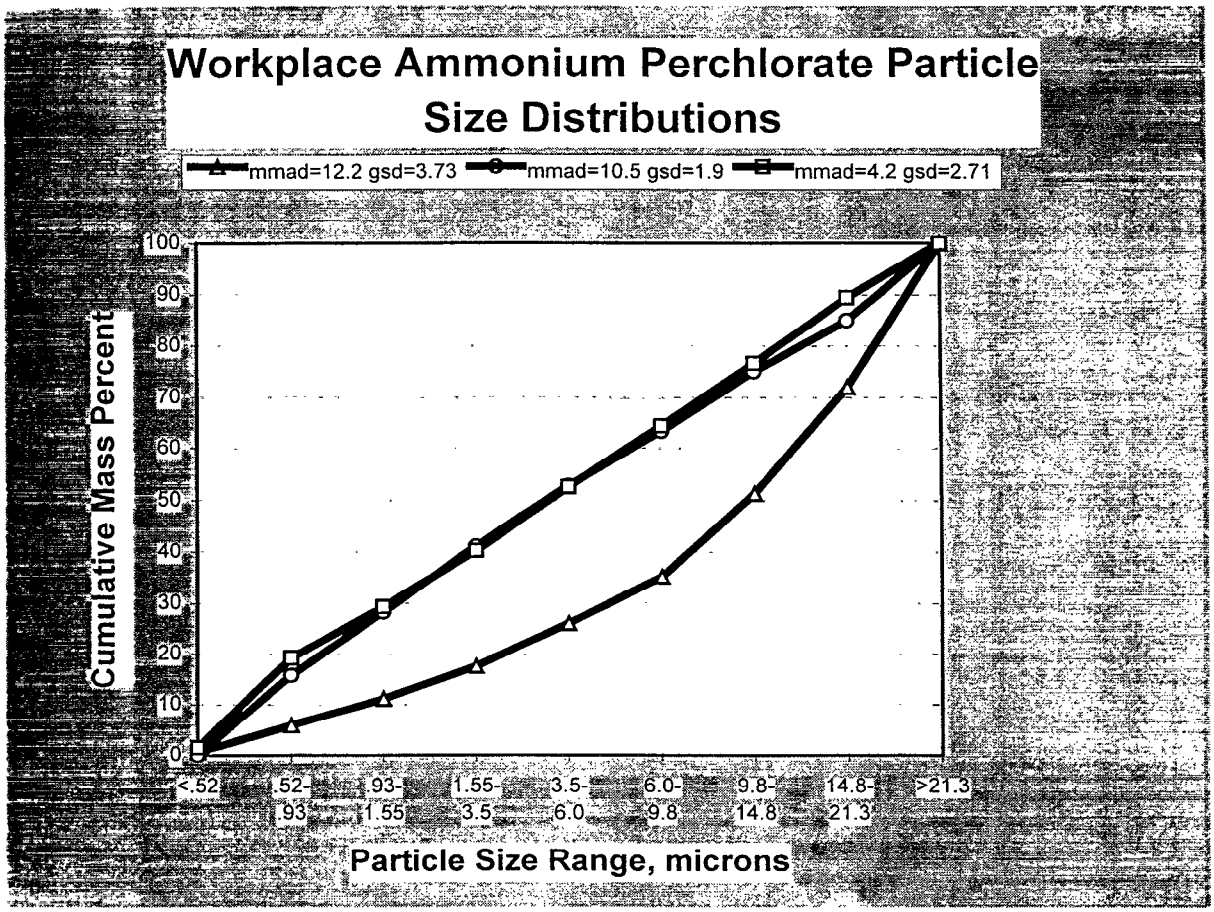

FIGURE 1 Particle size distributions of workplace dust representative of Lamm et al[4] (Cedar City Utah facility). Data collected by David Houck, CIH using a Graseby-Anderson Model 298 eight-stage Marple Cascade Impactor at 2 liters per minute flow rate for full shift - Jan 26-28, 1999.

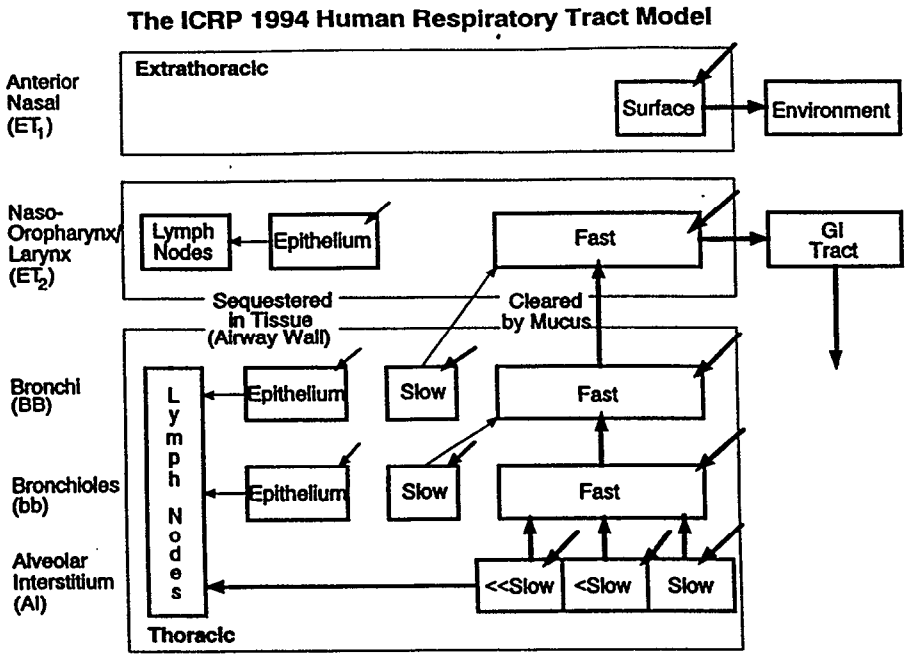

Figure 10-34. Schematic of the International Commission on Radiological Protection (ICRP66, 1994) model. Respiratory tract compartments in which inhaled particles may be deposited are illustrated. An explanation of clearance pathways, clearance rates, and subfractions of activity committed to different pathways is provided in the text.

Source: International Commission on Radiological Protection (ICRP66, 1994).

TABLE 10-8. OVERVIEW OF RESPIRATORY TRACT PARTICLE CLEARANCE AND TRANSLOCATION MECHANISMS

Extrathoracic region
Mucociliary transport
Sneezing
Nose wiping and blowing
Dissolution (for "soluble" particles) and absorption into blood
Tracheobronchial region
Mucociliary transport
Endocytosis by macrophages/epithelial cells
Coughing
Dissolution (for "soluble" particles) and absorption into blood
Alveolar region
Macrophages, epithelial cells
Interstitial
Dissolution for "soluble" and "insoluble" particles (intra-and extracellular)

Source: Schlesinger (1995).

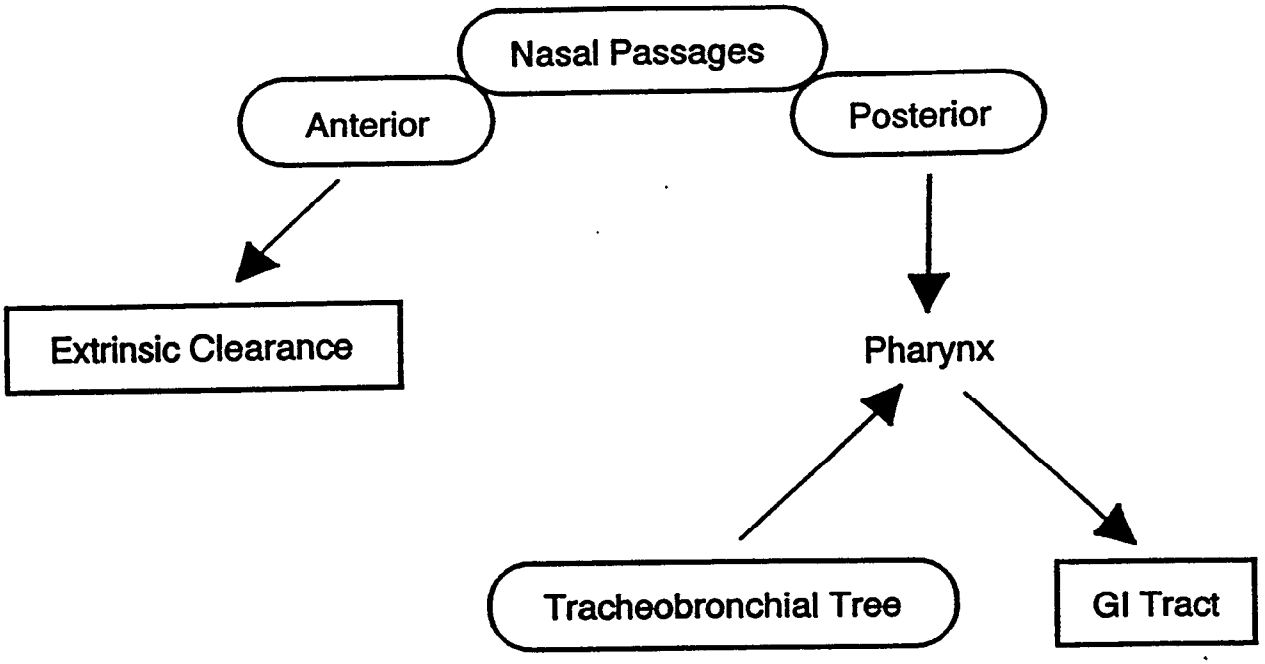

Figure 10-12. Major physical clearance pathways from the extrathoracic region and tracheobronchial tree.

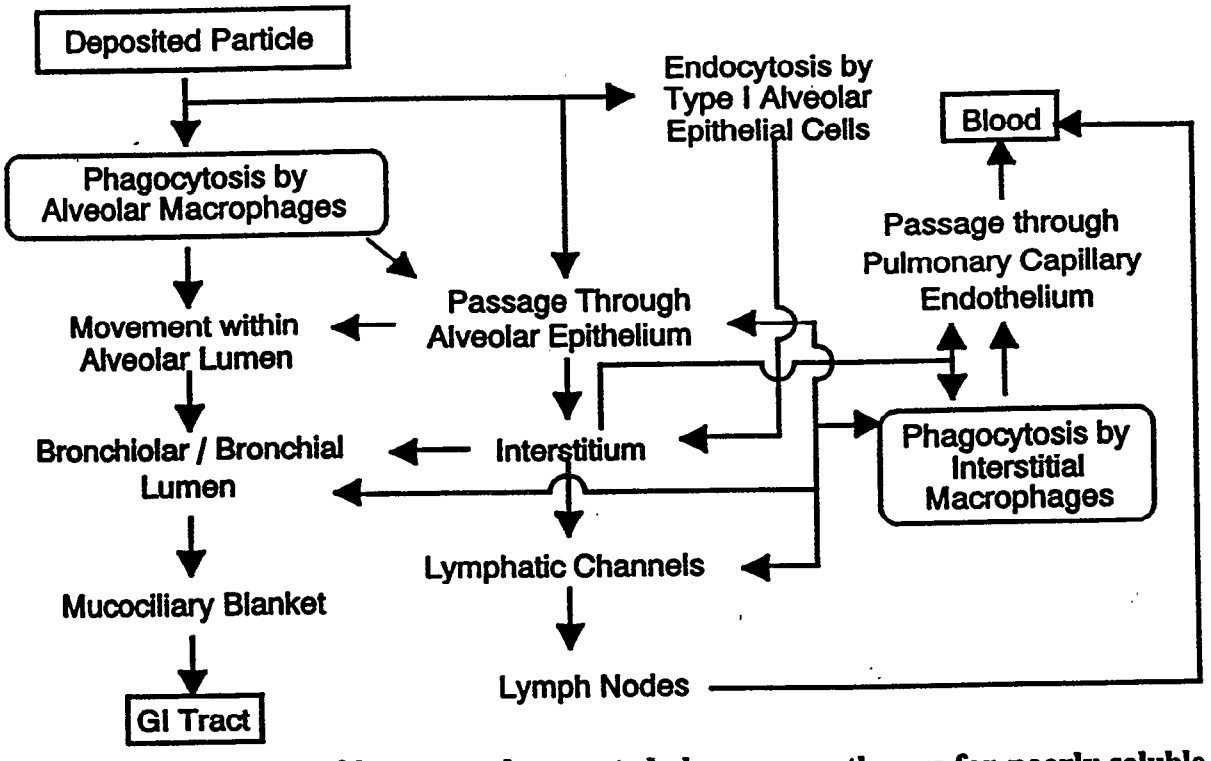

Figure 10-13. Diagram of known and suspected clearance pathways for poorly soluble particles depositing in the alveolar region.

Source: Modified from Schlesinger (1995).

MEMORANDUM

To: Susan Goldfarb

From: William M. Stiteler, PhD

Subject: Statistical Review of Perchlorate Report

Date: February 1, 1999

As requested by TERA I have reviewed the report *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information.* My comments and questions refer to Sections 5 and 6 of that report. In some cases details were not sufficient for me to see clearly what was done. I was able to fill in some of the information gaps on the TERA website, but the short time frame did not permit me to become completely familiar with all of the details. As a result, the questions and comments that follow may in some cases reflect an erroneous conclusion on my part about what was presented.

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1. On lines 6 and 14 of page 5-9 reference is made to "step down ANOVA tests". I am not familiar with the term "step down" used in the context of analysis of variance. (It is a term commonly associated with multiple regression, however.) Further, a review of several reference texts on ANOVA did not reveal the use of that term. I think it would be helpful to provide a brief description of what a step down ANOVA test is or perhaps provide a reference.

2. On lines 7-10 of page 5-9 the procedure for correcting for multiple comparisons (five individual ANOVA tests, one for each response) is said to involve dividing the acceptable alpha by the square root of n. This appears to be related to the Bonferroni procedure which involves dividing the acceptable alpha by n. The Bonferroni procedure is widely used and described (or derived) in many textbooks (Snedecor and Cochran, 1980 for example). I would like to see a reference for the use of the square root. Alternatively the analysis could be done simultaneously for all of the responses using multivariate analysis of variance (MANOVA) for an "exact" solution.

3. Dose is a quantitative factor but the ANOVAS of Section 5 treat it as a qualitative factor. What this means in effect is that the natural order of the dose groups is lost - the result would be the same if dose 0.0 is called A, 0.1 is called B, etc. In fact the five doses could be assigned names A through E in random order without having any effect on the ANOVA. The ANOVA simply evaluates whether the means of the five levels of dose are equal and the order in which they are presented is of no consequence.

   On pages 5-33 through 5-38 the author(s) express frustration with the failure to detect statistical significance where the data suggest something biologically significant. But if Figure 5-14 is redrawn with the levels of the treatment (dose) in random order the biological significance would likely evaporate as well. It is not the fault of the tool that it is being used for the wrong job or that another tool would do the job better. This analysis could (perhaps should) be performed with the general linear model, treating dose as a quantitative variable. Then the dose-response relationship would be considered. It is interesting that the discussion of this topic is concluded on page 5-38 with a suggestion that standard parametric statistical methods are inadequate and that the benchmark dose method may be a better alternative. I would argue that the benchmark

dose method is a standard parametric statistical method; but it is one that treats dose as a quantitative variable. Ironically, however, the benchmark dose method is designed for a quantal response and to use it for a continuous response like hormone level it is necessary to make some sort of conversion to quantal form.

4. I disagree with the comment on page 5-37 lines 23-25 that "The large variability coupled with the complicated design (treatment, age, gender, and block) will tend to wash out anything other than extremely large effects". As a rule a more complicated design allows us to account for more sources of variation. In a simple design, that variance would not be accounted for and would show up in the error mean square. For example, if age, gender and block (and associated interactions) are eliminated to give a simple one-way design any variability contributed by those factors would become part of the error term, making it <u>more</u> difficult to discern differences in the treatment levels (doses in this case). This relates also to the discussion of benchmark dose on page 5-38 where it says that this ".. approach may be useful because of the inverse relationship between data variability and the benchmark dose." (Lines 21-22). We should attempt to identify and account for as many sources of extraneous variability as possible so that the benchmark dose is not driven by factors unrelated to exposure to the chemical.

5. Are intra-litter correlations being taken into account?

6. The legends for the majority of the figures in Section 5 are confusing because they give a symbol for both males and females while the caption indicates that only one sex is plotted or that the sexes are combined. Figure 5-14 has two legends and they are contradictory.

7. It appears that the decision on combining data across sexes was based on presence or absence of a gender*treatment interaction. If there was no interaction they were combined. I do not understand this. If we plot response versus dose for the males and females separately, a lack of gender*treatment interaction means that each of the line segments connecting doses for the males is parallel to the corresponding line segment for the females. This is illustrated in Figure 1. If one or more of

the corresponding segments are not parallel then an interaction is indicated. Figure 2 shows a gender*treatment interaction at higher doses. I agree that it would not be appropriate to combine across sexes in the presence of an interaction even if the main effect for gender was not significant. The absence of an interaction, however, is a necessary but not sufficient condition for combining. It would also be a requirement that there be no difference between the sexes. For example, assuming a significant main effect for gender in Figure 1 it would not be appropriate to combine.

8. Section 6.1.1.1 discusses the results of correlation analyses of hormone and thyroid histopathology. I have no problem with the methods used here but the discussion is very misleading. With a few exceptions the correlations are mediocre at best and yet they are being touted as highly significant. In fact, the discussion does not even focus on the actual magnitude of the correlations, presenting instead what is "highly significant", "correlated highly", "highly associated", etc. I believe this results from a misunderstanding about what the p value associated with the correlation represents. That p value results from a test of the hypothesis that the underlying population correlation is 0.0. If the population correlation is in fact only slightly different from zero, say equal to 0.00000002, then the null hypothesis is not true and a sufficiently large sample would detect that fact; the null hypothesis would be rejected (with a significant p value). In fact we can say that any sample correlation, no matter how small, is highly significant if the sample is large enough. Therefore claiming a correlation is highly significant conveys absolutely no information about whether the correlation is strong; only the magnitude of the correlation itself can do that. I recommend that results of this section be reevaluated using the p value as an initial screen. If the p value is not small then the correlation is not important no matter how large it is. If the p value is small, then the correlation may or may not be important depending on its magnitude.

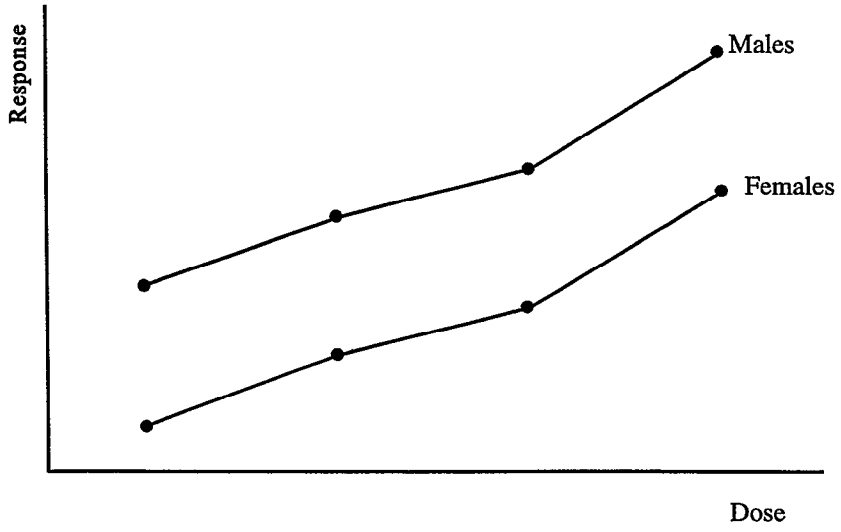

Figure 1 No Gender*Treatment Interaction

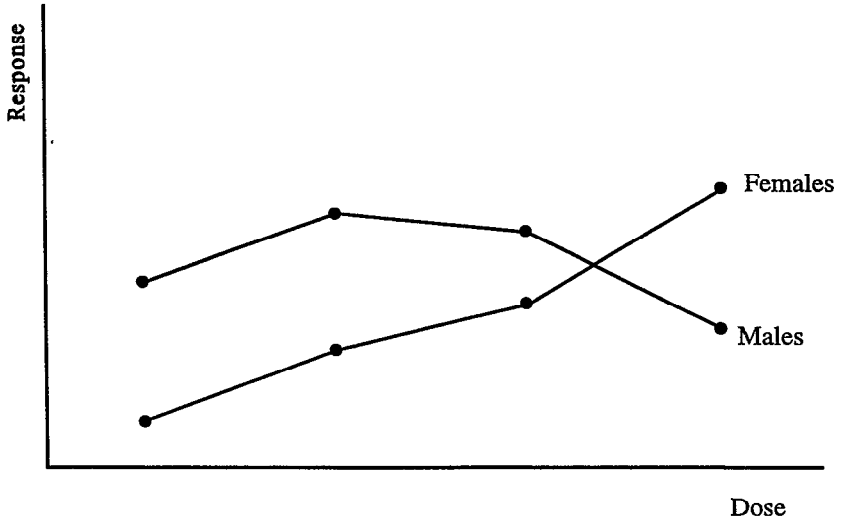

Figure 2 Gender*Treatment Interaction at Higher Doses

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January 29, 1999

Susan Goldhaber/Ella Darden
Research Triangle Institute
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

Dear Ms. Goldhaber and Ms. Darden,

Enclosed please find a copy of "Perchlorate and Human Health: Literature Summary" which was submitted to the U.S. Environmental Protection Agency on September 1, 1998. Please forward the document to the perchlorate external review panel for their consideration.

I would like to request five minutes at the meeting to present my summary of the literature.

Sincerely,

Arnold Engel

Arnold Engel, MD

Perchlorate and Human Health: Literature Summary

January 29, 1999

Arnold Engel, MD and Steven H. Lamm, MD

For the External Peer Review Workshop on Perchlorate[1]
San Bernardino, California
February 10-11, 1999

Prepared for American Pacific Corporation by
Consultants in Epidemiology & Occupational Health, Inc.
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Perchlorate and the Human Adult:

The thyroid gland makes thyroid hormone that circulates in the blood and regulates tissue metabolism, particularly protein synthesis. The thyroid gland concentrates iodine from the blood, iodinates tyrosine, and subsequently generates the thyroid hormones (T_4 and T_3) within the large glycoprotein thyroglobulin. T_4 and T_3 are then hydrolyzed and secreted into the blood. A large store of preformed hormone is present in the thyroid. The thyroid gland is regulated by thyroid stimulating hormone (TSH), which is secreted by the anterior pituitary gland.

Anti-thyroid drugs act by either blocking the uptake of iodine or by blocking the synthesis of T_4 and T_3. Perchlorate's effect on the thyroid has been well studied (Wolff, 1998). It is a competitive inhibitor for iodine uptake by the thyroid and does not affect the synthesis of thyroid hormone. Perchlorate is the most effective drug at blocking the uptake of iodine. Perchlorate passes rapidly through the body. The half-life ($T_{1/2}$) for perchlorate in humans is about 6 hours (Eichler, 1929). Ninety-five percent of the ingested dose is excreted as perchlorate into the urine. The thionamides (such as carbimazole, methimazole [MMI, Tapazol], or propylthiouracil [PTU]) are effective at blocking hormone synthesis. In some cases of thyrotoxicosis, both types of drugs are given simultaneously.

Perchlorate was used for treating thyrotoxicosis (hyperthyroidism) in the 1950s. Its use diminished in the 1960s after a few cases of fatal aplastic anemia or agranulocytosis developed in patients treated with very large doses of perchlorate (600-1600 mg/day) for long duration (months). No case of fatal aplastic anemia or fatal agranulocytosis has been reported in a patient receiving less than 600 mg/day perchlorate (Rokke and Vogt, 1968). There is a case report of a lady who was successfully treated with 200 mg/day perchlorate for 22 years without complications (Connell, 1981). Wenzel and Lente (1984) reviewed the literature on adverse reactions to perchlorate and concluded that "severe adverse reactions, such as granulocytosis, were likely to occur only when large doses of more than 1000 mg perchlorate were administered, while doses lower than 1000 mg perchlorate had fewer side effects than thionamide drugs." They reported treating Graves' disease patients with perchlorate for twelve or more months with doses beginning at 900 mg/day and diminishing to 40-120 mg/day.

Currently, perchlorate is used for patients who are sensitive to other drugs and, particularly, for patients who suffer from iodine-induced thyrotoxicosis. The drug amiodarone is used to treat heart patients with ischemic heart disease or, more commonly, those with rapid heart rate (tachycardia). Amiodarone may be a life-saving drug; however, because it contains nearly 40 percent iodine, it can be toxic to the thyroid gland. Case series of patients with amiodarone associated thyrotoxicosis (AAT) have been published for patients treated for nearly a month or more with 1,000 mg/day perchlorate with no adverse effect on their blood cells (Martino et al, 1986a, 1986b; Richert et al, 1989; Trip et al., 1994). There is a report of one patient who developed a mild neutropenia after forty days of treatment at 1,000 mg/day perchlorate (Martino et al., 1986b). Occupational health studies of workers with many years of employment in perchlorate-manufacture showed no effect on either thyroid hormones or blood cell studies (Gibbs et al., 1998; Lamm et al., 1999).

A small block in iodine uptake (such as by perchlorate) is likely to be compensated by the thyroid. For example, an Austrian study showed that nearly 40 percent of euthyroid people had normal thyroid hormone levels in spite of having low iodine intake (< 100 ug I per gm creatinine in the urine) (Buchinger et al., 1997). Similarly, a Swedish study showed that women who drank milk which contained thiocyanate (8 mg per day for 12 weeks) showed no change in their thyroid hormone status (serum T_3, T_4, and TSH) (Dahlberg et al, 1994). Thiocyanate is both an antithyroid drug and a bacteriostatic agent. The thyroid regulation system is usually able to maintain a normal thyroid hormone level unless the thyroid is completely blocked from the uptake of iodine for a prolonged period of time.

Perchlorate and the Human Fetus:

In the past, perchlorate has specifically been used for the treatment of thyrotoxicosis in pregnancy. Thus, the evidence of the effect of perchlorate on the human fetus comes from these patients. Crooks and Wayne (1960) reported in Lancet that

> Opinion is almost unanimous that the most appropriate form of therapy for women who develop thyrotoxicosis during pregnancy is the administration of an antithyroid drug. Goitre and hypothyroidism have been reported in the infants of mothers who have received these substances but it is rare, and when it occurs the dosage has almost always been unduly high (Macgregor and Goodwin, 1953). We have treated 12 pregnant thyrotoxic patients with potassium perchlorate (600 mg/day or 1000 mg/day) and in each have achieved satisfactory control of the disease. One of the infants had a very slight enlargement of the thyroid which disappeared within 6 weeks. The remainder showed no abnormality of any kind. We report these results to show that the different mode of action of potassium perchlorate and its special physical properties do not render it more liable to affect the fetal thyroid than the antithyroid substances in more common use.

The concern about perchlorate and the fetus is that it might present the possibility of the development of congenital hypothyroidism with mental retardation (cretinism). This is a severe form of mental retardation that is found in areas with endemic iodine deficiency. In these cases, the fetus has exceptionally low iodine exposure both in-utero and after birth. That is not the situation anywhere in the United States. The United States diet is not iodine-deficient, and all states have neonatal screening programs that detect early cases of congenital hypothyroidism.

Congenital hypothyroidism is found in approximately 1 in 3500 live births, but is easily detected and readily treated among children born in hospitals throughout the United States. In counties in California that reported cases of congenital hypothyroidism in 1996, the rates were no higher in counties reporting perchlorate in drinking water or wells as compared to counties that did not (Lamm, 1998). The 1996-7 data demonstrated that no more than the expected number of cases of congenital hypothyroidism occurred in the counties of Southern California and Nevada that had perchlorate reported in their drinking water supply (Doemland and Lamm, 1999). The California Department of Health Services

examined the incidence of congenital hypothyroidism in the zip code areas of Sacramento with perchlorate contamination and found no more cases than expected, based on statewide rates (CDHS, 1997). The risk of congenital hypothyroidism appears to be rather stable. Delange et al. (1986) showed that the incidence of congenital hypothyroidism was no greater in European cities with moderate iodine deficiency (50-100% of the newborns had urinary iodine levels below 5 ug/dl) than in European cities without iodine deficiency (about 5 % with urinary iodine below 5 ug/dl).

Newborn infants with congenital hypothyroidism who are identified by screening programs and treated promptly usually have IQs in the normal range at five to seven years of age, as well as normal growth and development. Even children born without a thyroid have normal intellect if thyroid treatment starts early (Burrows et al, 1994). The thyroid system of the fetus develops independently of the mother's, although it is dependent on the maternal-placental system for adequate iodine supply (Fisher DA, 1997). Even in the absence of a functioning fetal thyroid, the maternal thyroxine that crosses the placenta is usually able to sustain a fetal blood level of 40-60 nmol/liter (Vulsma et al., 1989; Larsen PR, 1989). The maternal thyroxine is generally sufficient to supply enough thyroid hormone to the fetus to take care of the fetal brain, although the fetal thyroid hormone levels are lower than normal. Children's IQs are generally normal if the newborn's thyroxine level is 43 nmol/liter or higher (Tillotson et al., 1994) and if the post-natal treatment is sufficient to restore the serum TSH to normal levels (about 6-11 ug L-thyroxine/kg/day; Illicki and Larsson, 1991). Fetuses with a normal fetal thyroid whose mothers are taking appropriate doses of anti-thyroid medications have normal levels of thyroid hormones (Momotani et al, 1997). Studies of children born to mothers who were treated with anti-thyroid drugs during pregnancy have normal IQs (not different from comparison children) (Burrow et al., 1978; McCarroll et al., 1976; and Messer et al., 1990).

The primary medical use of perchlorate has been to block the uptake of iodine by the thyroid. Hormonal changes do not appear to occur unless the blockage is largely complete, which in therapeutic situations occurs with one hundred or a few hundred milligrams per day. Under most therapeutic conditions, the fetus generally appears not to have been adversely effected. The large body of medical literature published over the past forty years suggests that low levels of perchlorate exposure will not affect thyroid health in the adult or cause congenital hypothyroidism in the fetus.

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January 29, 1999

Susan Goldhaber/Ella Darden
Research Triangle Institute
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

Dear Ms. Goldhaber and Ms. Darden:

Enclosed for submission to the perchlorate external peer review panel is the following article: "Thyroid Health Status of Ammonium Perchlorate Workers: A Cross- Sectional Occupational Health Study" which has been peer reviewed and accepted by the Journal of Occupational and Environmental Medicine and is currently in press. The paper's abstract that summarizes the findings is below:

Title: Thyroid Health Status of Ammonium Perchlorate Workers: A Cross- Sectional Occupational Health Study

Authors: Steven H. Lamm, MD[1], Lewis E. Braverman, MD[2], Feng Xiao Li, MD, DrPH[1,3], Kent Richman, PhD[4], Sam Pino, BSc[2], Gregory Howearth BSc[4]

Institutions: [1]Consultants in Epidemiology and Occupational Health Inc. (CEOH, Inc.), Washington, DC
[2]Brigham & Women's Hospital, Boston, MA
[3]University of Calgary, Calgary, ALB
[4]American Pacific Corporation, Cedar City, UT

Abstract:

Since pharmaceutical exposures to perchlorate are known to suppress thyroid function in patients with hyperthyroidism, a study of employees at a perchlorate manufacturing plant has been conducted to assess whether occupational exposure to perchlorate suppresses thyroid function. Exposure to perchlorate was assessed by measurement of ambient air concentrations of total and respirable perchlorate particles, and systemic absorption was assessed by measurement of urinary perchlorate excretion. Airborne exposures ranged from 0.004 to 167 mg/day total particulate perchlorate. Urinary perchlorate measurements demonstrated that exposure to the airborne particulate perchlorate resulted in systemic absorption. Workers were in four exposure groups with mean perchlorate absorbed dosages of 1, 4, 11 and 34 mg perchlorate per day. Thyroid function was assessed both by TSH, FTI, T_4, T_3, THBR, or TPO antibodies and by clinical examination. No differences in thyroid function parameters were found between the four groups of workers across about three orders of magnitude of exposure and of dose. Thus, the human thyroid function was not affected by these levels of absorbed perchlorate. In addition, no clinical evidence of thyroid abnormalities was found in any exposure group. The blood cell counts were normal in all groups indicating no evidence of hematotoxicity in this exposure range. The absence of evidence of an effect on thyroid function or blood cells from occupational airborne perchlorate exposure at a mean absorption of 34 mg/day demonstrates a human no observed adverse effect level that can assist in the evaluation of human health risks from environmental perchlorate contamination.

I would appreciate the opportunity to present this paper to the panel and to answer their questions.

Cordially,

Steven H. Lamm, MD

Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational Health Study, Lamm SH, LE Braverman, FX Li, K Richman, S Pino, and G Howearth

Introduction

This occupational health study was conducted in July of 1998 at the only US industrial site currently manufacturing ammonium perchlorate (the Cedar City, Utah site of American Pacific Corporation). The purpose was to assess the health status, specifically thyroid function, of workers with long-term (months to years) exposure to perchlorate. This study included measures of exposure to perchlorate particulates, measures of urinary perchlorate to determine the magnitude of systemic absorption from perchlorate particulates, and measures of thyroid function to determine whether (and if so, to what degree) the thyroid function was affected by the perchlorate exposure. While this study was primarily designed to develop a health assessment of occupationally exposed perchlorate workers, the information gathered may also be useful in assessing health risks to persons environmentally exposed to perchlorate.

Perchlorates have been used industrially for over 50 years in propellants and explosives as oxidizers because of the strong oxidizing potential of their salts. Ammonium perchlorate is used as the oxidizer in solid propellant for rockets and missiles, such as the boosters for the space shuttle and the Titan rocket, as well as for fireworks. Other perchlorate salts include sodium perchlorate, which is used as an oxidizer in slurry explosives manufacturing, and potassium perchlorate, which is used in road flares and in air bag inflation systems. Perchlorate salts are soluble in water. Perchlorate ions have been detected in ground and surface waters near sites where perchlorates are used or manufactured. The perchlorate salts fully ionize, and the perchlorate ion (ClO_4^-) persists for several decades in surface and ground waters. Recent improvements in the laboratory method for detecting perchlorate in drinking-water supplies have lowered the limit of detection from 400 parts per billion (ppb) to 4 ppb. Subsequent surveys of water supplies in California have detected perchlorate in a number of wells, the sources of which have been traced back to various sites of industrial perchlorate use, and in a major water supply (the Colorado river, downstream from Lake Mead), the source of which has been traced back to an area of perchlorate manufacture in Nevada. Some of the well measurements in California have exceeded 18 ppb, and the water measurements from the Colorado River have ranged from 5-8 ppb. The potential health risks from short-term and long-term consumption of such levels of perchlorate in drinking water supplies are currently under assessment by the US Environmental Protection Agency.

Although thyroid function is dependent upon an adequate dietary intake of iodine (100-200 μg/day) as substrate for hormone synthesis, the thyroid readily compensates for a modest decrease in iodine intake by enlarging and actively transporting a larger fraction of the circulating iodine. An Austrian study showed that euthyroid subjects (n=2,308) had normal levels of the thyroid hormone thyroxine (T_4) in spite of having mildly low iodine intakes (as indicated by urinary excretion of < 100 μg I per gram creatinine).[1]

Studies in humans and rodents demonstrate that the primary effect of perchlorate is to block the uptake of iodine by the thyroid gland, thus potentially decreasing the production of the thyroid hormones T_4 and triiodothryronine (T_3). Perchlorate (as potassium perchlorate) has been used medically since the late 1950s to treat hyperthyroidism, and its effects on the thyroid have been well studied.[2] It is a competitive inhibitor of the sodium/iodide symporter of the thyroid follicular cell, which actively transports iodine from the blood into the thyroid. It does not appear to have an important effect on thyroid hormone synthesis. Perchlorate is the most effective drug for blocking the thyroidal uptake of iodine. It is excreted unmetabolized, with approximately 95% recovery in urine over 72 hours.[3] Eichler reported that within 6-8 hours the urine contained 50% of a 1 or 2 gram oral dose given to an adult male.[3] Similarly, Durand reported that within 5-9 hours the urine contained 50% of a 0.8 gram oral dose given to an adult male.[4]

In 1952, Stanbury and Wyngaarden demonstrated the effectiveness of perchlorate in treating hyperthyroidism due to Graves' Disease at dose levels of 200 mg potassium perchlorate three times daily.[5] Subsequently, treatment doses were increased to 1,200 mg per day to accelerate the induction of normal thyroid function. Following case reports of fatal aplastic anemia or agranulocytosis in patients treated with perchlorate at doses of 600-1,600 mg/day for extended periods, the use of perchlorate for treating Graves' disease markedly decreased. More recently, Wenzel and Lente treated hyperthyroid patients with Graves' disease with perchlorate at doses of 900 mg and less daily for 1 year with excellent results and no mention of blood dyscrasias.[6] Perchlorate is now used to treat patients who have iodine-induced thyrotoxicosis (e.g., amiodarone-associated thyrotoxicosis [AAT]). Amiodarone, a potent drug used to treat cardiac tachyarrythmias, contains nearly 40 percent iodine. AAT patients treated for one month or longer with perchlorate at doses up to 1,000 mg/day perchlorate had no evidence of agranulocytosis or aplastic anemia.[7,8,9,10]

Few studies have been conducted on the effect of perchlorate on healthy human subjects. In one study, Burgi et al. showed in five healthy volunteers that 600 mg/day was sufficient to completely block iodide uptake by the thyroid.[11] In another study, Brabant et al. were unable in five healthy male volunteers to induce a state of iodine depletion by administering orally 900 mg/day of potassium perchlorate for four weeks.[12] Brabant is reported to have observed mild goiters without an increase in TSH levels in a five week long repeat of that study. These are the only studies that indicate toxicity levels of perchlorate exposure in healthy humans.

The present occupational health study focuses on thyroid health status and was conducted in an ammonium perchlorate manufacturing plant. The manufacturing process at this plant begins with the electrolysis of brine (sodium chloride in water) to first form sodium chlorate ($NaClO_3$) and then sodium perchlorate ($NaClO_4$). Ammonium chloride (NH_4Cl) is formed from ammonia (NH_3) and hydrochloric acid (HCl). The sodium perchlorate is reacted with the ammonium chloride to form ammonium perchlorate (NH_4ClO_4) and salt (NaCl). The solution is cooled, and the ammonium perchlorate crystals are dried and blended to specifications.

Study Design

This was a cross-sectional study of two similar worker populations from the same industrial complex - ammonium perchlorate production workers and sodium azide production workers; the latter served as a control group. The purpose was to assess perchlorate exposure and thyroid function in both groups. The two production plants are in close proximity, and the workers share locker facilities, storage areas, training and administrative areas, etc., but not production areas.

Perchlorate exposure was measured using full-shift breathing zone air sampling for both total perchlorate particles and respirable perchlorate particles. Urinary perchlorate concentration was assessed at both the beginning and end of the twelve-hour shift in which the particulate exposure was measured. Particle-size-selective sampling was conducted to obtain the mass mean aerodynamic diameter of the particles.

Thyroid function was assessed by measuring serum thyroid stimulating hormone (TSH), T_4, and T_3 concentrations, the thyroid hormone binding ratio (THBR), and the free T_4 index (FTI). Thyroid peroxidase (TPO) antibody concentrations were measured to identify workers with underlying Hashimoto's thyroiditis. If the occupational exposure to perchlorate were suppressing the thyroid by blocking iodine uptake, the expected observation would be that the TSH levels would increase.

Urinary iodine concentrations were obtained to determine if workers had adequate iodine intake. Blood samples for complete blood counts (CBC) and serum samples for a chemistry panel were also obtained. Physical exams and medical histories with careful thyroid evaluation were performed on all subjects.

Materials and Methods

A. Study Population

The perchlorate production plant is located in the industrial facility of the American Pacific Corporation in Iron County, Utah, west of Cedar City. The facility, which began production in 1989, employs approximately 190 workers in four major divisions. The thirty-nine employees assigned to direct perchlorate production and the twenty-one employees assigned to direct azide production were eligible to be study participants. Employees assigned to administrative, engineering, maintenance, and supervisory positions were not eligible to be study participants. Fifty-eight of the sixty employees eligible for participation did participate. Two of the eligible perchlorate workers were not at the plant at the time of the study because of vacation or military duty and did not participate. The production employees work 12-hour shifts (on three days; off three days), with rotation from days to night approximately monthly. The employee population from both plants are similar in that they are drawn from the same population base, have the same management procedures and policies, work similar rotating shifts, and have participated in prior medical monitoring programs at the facility.

All participants were instructed as to the nature of the study and informed consent was obtained. The study protocol and consent forms were approved by the Georgetown University School of Medicine institutional review board. Pre-shift and post-shift urine samples were obtained from all participants, and post-shift blood samples were obtained from all but one participant who declined to give a blood sample. Air sampling equipment was used throughout the shift for determining both total and respirable perchlorate particle exposure. Participants completed a medical questionnaire and underwent a physical examination conducted by a local physician's assistant and a thyroid examination conducted by a thyroid specialist (LEB). Examiners were not aware of a participant's study group. Laboratory samples were prepared with

participant code numbers to keep the laboratory personnel blinded as to a participant's study group. The personnel and director of each laboratory maintained quality control and assurance procedures.

B. Perchlorate exposure groups

The job assignments of the perchlorate production workers were classified into three categories of presumptive exposure (low, medium, and high) based on the visible dust generated. The categories of low, medium and high were used as follows to classify workers:

- A- Low: employees handling only solutions or slurries of perchlorates. This includes electrolysis through crystallization processes.
- B- Medium: employees handling limited quantities of dry perchlorates, resulting in only minor visible-dust exposure. This includes the initial drying process.
- C- High: employees handling large quantities of dry perchlorates, resulting in significant visible-dust exposure. This includes the blending and packaging operations.

C. Urine samples

Pre- and post-shift urine samples were collected from all participants to measure urinary perchlorate, iodine, and creatinine levels. Urine samples were frozen after collection and thawed prior to analysis. Urinary creatinine and iodine measurements were performed in the Iodine Research Laboratory at the Brigham & Women's Hospital (Boston, MA), using the Jaffee alkaline picrate method for creatinine and the Sandell-Koltoff reaction for iodine.[13] Urinary perchlorate measurements were performed by Dr. Kent Richman at the American Pacific Corporation laboratory, using a US Air Force developed modification of a Dionex conductivity detection method. The method (available on request) is capable of measuring urinary perchlorate concentrations of 0.5 parts per million (ppm) or greater.[14]

D. Blood samples

Post-shift blood samples were collected. Complete blood counts were performed at the clinical laboratories of Valley View Medical Center, Cedar City, Utah. The complete blood count included absolute counts of red blood cells, white blood cells, and platelets; absolute and relative counts of lymphocytes, neutrophiles, monocytes, eosinophiles, and basophiles; and hemoglobin, hematocrit, and red cell parameters (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration).

E. Serum samples for thyroid function studies

Thyroid function studies on post-shift serum samples were performed in the Endocrine-Hypertension Research Laboratory of Brigham & Women's Hospital. Thyroid function studies were carried out in duplicate, in the same assay, and in random order. Tests and their methods follow with the normal values for the laboratory indicated in parentheses. TSH [thyroid stimulating hormone] (0.45-4.5 μU/ml) was measured by chemiluminescence, (Beckman Access, Chaska, MN), T_4 [thyroxine] (5-11 μg/dl) and THBR [thyroid hormone binding ratio] (0.85-1.10) by radioimmunoassay (Diagnostic Products Corp, Los Angeles, CA), T_3 [triiodotyronine] (87-178 ng/dl) by radioimmunoassay (Beckman Access, Chaska, MN), and TPO [thyroid peroxidase] (<20 IU/ml) by ELISA (American Laboratory Products Co, LTD). The FTI [free thyroxine index] is the product of the T_4 concentration and the THBR. The results reported for each subject are the mean of the duplicate values for each test.

F. Serum sample for blood chemistry panel.

Post-shift serum samples obtained from the participants were collected for analysis of a chemistry panel by Quest Diagnostics of Cambridge, MA . The chemistry panel included serum levels of calcium, phosphate, glucose, blood urea nitrogen, creatinine, uric acid, cholesterol, total protein, albumin, alkaline phosphatase, lactic dehydrogenase, SGOT, and total bilirubin.

G. Air sampling

Full-shift air sampling for total (< 40 μm) and for respirable (< 10 μm) breathing zone particles was carried out under the direction of David Houck, CIH. For total particulate, 5 μm PVC filters in 37-mm three-piece, closed-face cassettes were used, with a sampling rate of about two liters per minute and a sampling duration of ten to eleven hours. For respirable particulates, SKC aluminum cyclones were used with 5 μm PVC filters in 37-mm cassettes, at a flow rate of 1.9 liters per minute.

Montgomery-Watson Laboratories analyzed the cassettes for perchlorate. Cassette samples were dissolved in a 10-ml aliquot of 30 mM sodium hydroxide, and perchlorate concentration was measured

using the California Department of Health Services analytic method for perchlorate in drinking water samples.

Size-selective sampling of airborne dust was performed during a production period with a Marple 8-stage Cascade Impactor in the blender building at a height of five feet above the floor in the area where the perchlorate C group worked. Particle size distributions were determined at the following eight cutpoints: 21.3 μm, 14.8 μm, 9.8 μm, 6.0 μm, 3.5 μm, 1.55 μm, 0.93 μm, and 0.52 μm. Dust particles in the range of 0.1 to 10 μm are generally considered to be "respirable", as they may enter and be retained by the deep regions of the lung. Total particle mass was also determined since the highly soluble perchlorate particle may be readily absorbed after deposition into the nasal passages or upper respiratory tract. Inhalable particles which may also precipitate in the upper areas were not separately measured. The mass mean aerodynamic diameter of the particles was calculated.

H. Urinary perchlorate excretion

To determine the time course of urinary perchlorate excretion, two workers were monitored for six days, with urine samples submitted every twelve hours. These employees worked in the high exposure area during the first twelve hours of each of the first three days; in the next three days they were assigned to the administrative building rather than the production area. Thus, the observation period includes three exposure periods followed by 3 ½ days of observation with no known exposure. The urinary perchlorate levels during the three unexposed days provide an indication of perchlorate elimination rates under the conditions of exposure experienced by these two workers.

I. Methods of Statistical Analysis

Statistical analysis of data was conducted using the Stata package for the personal computer. A t-test was applied to mean differences in all continuous exposure, outcome and demographic variables. Descriptive statistics for both exposure and outcome variables were calculated and presented as arithmetic and/or geometric means and standard deviations, medians, ranges (minimum and maximum), and interquartile ranges (25th percentile and 75th percentile). For categorical variables, a chi-square statistic was used. Two-tailed p values were calculated for each comparison. The absence of a statistically significant difference was inferred if the two-tailed p value was not less than 0.05. For outcome data, pair-wise t-tests were performed between the comparison group and each of the exposed groups. A non-parametric z-test for trend across ordered groups was conducted.[15]

Results

A. Population Description

A total of 58 employees participated in this occupational health study - 37 from the ammonium perchlorate production plant (35 male and 2 female) and 21 from the sodium azide production plant (19 male and 2 female), ranging in age from 20 to 56 years. The mean age of the ammonium perchlorate workers was 30 years compared to 35 years in the azide workers. Forty percent of the ammonium perchlorate production workers and fifty percent of azide production workers had been employed for more than five years.

B. Medical examination and questionnaire findings

No differences were found between the azide workers and the perchlorate workers or among the three perchlorate worker groups in the findings from their medical examinations or their responses on the medical questionnaire. Mean heights and weights were similar. The groups did not differ in their clinical findings (blood pressure, pulse, examination of body systems) from the medical examination. According to their answers on the medical questionnaire, the groups did not differ in alcohol or tobacco use, in medication use, in frequencies of family history of major systemic diseases (diabetes, hypertension, rheumatoid arthritis, thyroid disease, or cancer), or in reported medical problems.

Thyroid disease was identified in two workers. One worker in the low perchlorate exposure group (A) had been previously diagnosed with Graves' disease. His disease was diagnosed nine years prior to this employment and had been treated with radioactive iodine. He was now found to be hypothyroid and undermedicated. Previously undiagnosed thyroid disease was found in only one worker, a worker in the medium perchlorate exposure group (B) who was found in this examination to have an autoimmune condition of the thyroid, euthyroid Hashimoto's thyroiditis. Other than the first worker, no worker reported a history of either thyroid disease or thyroid medication.

C. Airborne Exposure

The thirty-seven perchlorate participants included fourteen employees in the nominally low perchlorate exposure jobs, 8 employees in the nominally medium perchlorate exposure jobs, and 15 employees in the nominally high perchlorate exposure jobs. All thirty-seven perchlorate participants wore air samplers. Thirty-two wore respirable particle samplers, and twenty-one wore total particle samplers. Seven of the twenty-one azide participants wore air samplers of whom two wore only a respirable particle sampler, one wore only a total particle sampler, and four wore both a respirable and total particle sampler. Table 1 presents the respirable and total airborne perchlorate exposures (mg/day) of the workers, stratified by exposure group. This was calculated from the laboratory report of the amount of perchlorate in the sampling cassettes, the air sampling rate (about 2 liters/min) and duration (about 10 hours), and the assumption of an inhalation rate of 1.2 cubic meters per hour. Data are presented as the arithmetic and geometric means and standard deviations, along with the range, median, and distribution by quartiles. This table demonstrates that airborne perchlorate particle levels are greater in the dusty parts of the perchlorate plant than in the azide plant and that both respirable particle and total particle perchlorate inhalation progressively increase with visible-dust level in the perchlorate plant exposure groups. The exposures of the high exposure perchlorate group are clearly discernible from the other worker groups, being three orders of magnitude greater than those of the azide workers. The minimal exposures of the azide workers may come from contamination from the shared non-production facilities or contamination of their work site. This table also demonstrates that patterns of distribution for respirable and total particles across the exposure groups are similar. Individual measures of respirable and total particle perchlorate were highly correlated with a statistically significant correlation co-efficient ($r = 0.82$; $p << 0.01$), based on 18 paired samples. Respirable particle inhalation accounted for 14% of the total particle inhalation rate (Figure 1).

Particle size-selective sampling conducted in the blender operation (perchlorate exposure group C area) yielded a mass median aerodynamic diameter of the particles of 7.4 μm with a geometric standard deviation of 3.8.

D. Urinary perchlorate levels

Table 2 presents mean urine perchlorate levels (mg/gm creatinine) for the azide plant workers and for each perchlorate exposure group. The data are presented for the pre-shift urine sample, for the post-shift urine sample, and for a post-shift (adjusted) measure. The data are presented as the arithmetic mean and its standard deviation, the range, the median, and the quartiles.

The pre-shift urine was collected prior to the beginning of the work shift, and the post-shift urine was collected at the end of the work shift. The post-shift urine perchlorate measurement reflects two components – (a) the excretion due to the residual in the body of the perchlorate that was present in the body at the time of the pre-shift urine perchlorate measure and (b) the incremental increase in urinary perchlorate due to the excretion of the perchlorate that was inhaled (or ingested) during the work shift.

The post-shift (adjusted) measure is defined to represent the post-shift urinary perchlorate component that reflects perchlorate inhaled during the shift. The body burden represented by the pre-shift urinary perchlorate measure is estimated by pharmacokinetic modeling (assuming first order kinetics and an 8 hour excretory half life) to be reduced by 65% after 12 hours. The post-shift (adjusted) urinary perchlorate estimate was obtained by subtracting 35% of the pre-shift urinary perchlorate measure from the post-shift urinary perchlorate measure as an exposure estimate adjustment. This first-order model follows the equation: $E_i - E_o e^{-kt} = D (1 - e^{-kt})$ where E is the excretion rate of perchlorate at time (o) and time (i) and D is the dose rate from exposure. For a half-life of 8 hours and at time 12 hours, e^{-kt} equals 0.354. The post-shift (adjusted) measure is $E_i - 0.354\ E_o$.

Table 2 demonstrates that workers in the three perchlorate exposure groups have progressively increasing levels of urinary perchlorate. Symmetry about the means and the similarities between the mean and the median in each strata suggest that log transformation of the data is unnecessary.

E. Absorbed dose

The absorbed dose can be calculated for each shift directly from the data presented above in Table 2. From the model equation above, with 12 hour work shifts and an 8 hour half life, the excreted dose (D) follows the equation: $D = k\ [E_i - 0.354\ E_o]/0.646$. The term $[E_i - 0.354\ E_o]$ is the post-shift (adjusted) level in mg perchlorate per gram creatinine. The value of 0.646 at the end of a 12 hour work shift is robust, as a value of 0.64 to 0.65 is applicable over a half-life range of 4 to 24 hours. The human adult creatinine excretion rate of 1 mg/min links perchlorate excretion rates in terms of creatinine to rates in terms of time. The percent absorbed that is excreted into the urine is assumed to be 95% as shown by Eichler (1929). The

exposure rate is assumed to be relatively constant throughout the work shift and was measured as a time-weighted average exposure. The excreted dose is then 12 hours x 60 min/hr x 0.001 gm/mg x 1 mg creatinine/min x [post-shift adj]/0.646 and the absorbed dose is the excreted dose/0.95 which is expressed in mg/shift. This represents a reasonable estimate of perchlorate absorption over a 12 hour shift (mg/shift) and is calculated independently of the exposure estimates.

F. Thyroid function status

Table 3 presents the mean thyroid function tests for the azide production workers and for each of the perchlorate exposure groups, along with the standard deviations, ranges, medians, and distribution by quartiles. There were no differences in thyroid function tests between workers in the azide and perchlorate plants or between the azide workers and any of the three perchlorate exposure groups. Extreme outlier values due to specific thyroid diseases were excluded from the analysis as indicated in Table 3. Pair-wise t-tests were performed between the azide group and each of the three perchlorate exposure groups. As shown in Table 3, none of the comparisons were statistically significant at $\alpha < 0.05$ level. A non-parametric z-test for trend across the ordered groups for each of the six thyroid function tests, using the method developed by Cuzick (1985), found no statistically significant trend (although the trend for TFI was of borderline significance but in the opposite direction than pharmacologically predicted).

Categorical data analyses also were conducted with normal values for T_4, T_3, FTI, THBR, TSH, and anti-TPO being defined as values within the normal ranges for the laboratory. There were no significant differences across the exposure groups or between the two plants. In no case did the proportion of abnormal values for the perchlorate workers exceed that for the azide workers. There were no suggestive trends, either statistically or clinically, for any thyroid function test. Further, no differences in the thyroid function tests were observed in the perchlorate plant workers due to their inhaled and excreted perchlorate levels. These findings show that thyroid function is not altered in workers exposed at the perchlorate levels found in this plant.

G. Thyroid examinations

Clinical examination of the thyroid of all participants revealed no significant thyroid abnormalities in any group. The thyroid glands did not differ in size, texture, or shape between the two groups or across the perchlorate exposure groups. No goiters were detected in any of the workers. A small nodule was detected in a worker in a low perchlorate exposure job. Secondary signs of hypothyroidism and of hyperthyroidism were sought by the thyroid examiner (LEB) and were not observed. There was no evidence of bradycardia, tachycardia, or tremor. Examination of the skin, eyes, and extremities did not reveal signs of thyroid disease.

H. Urinary iodine excretion

The pre-shift urinary iodine values of the azide and perchlorate workers met international standards with 95% or more having a urinary iodine level of 50 ug/l or greater. More than 90% of each group had pre-shift urinary iodine levels greater than 100 ug/l. These data indicate the absence of iodine deficiency in these groups of workers.

The pre-shift urinary iodine values did not differ between the perchlorate workers (mean = 318 μg/l; standard deviation = 164 μg/l) and the azide workers (mean = 344 μg/l; standard deviation = 180 μg/l). Analysis was limited to the forty-nine specimens with pre-shift urinary iodine values less than 800 μg/l, as greater values suggest contamination from extraneous sources. Additionally, the urinary iodine values adjusted for creatinine excretion did not differ between the perchlorate workers (mean = 192 μg/gm; standard deviation = 132 μg/gm) and the azide workers (mean = 211 μg/gm; standard deviation = 120 μg/gm).

Pre-shift and post-shift urinary iodine levels were compared. There was no evidence of increased iodine excretion post-shift compared to pre-shift for either the azide or perchlorate workers, based on those forty-four paired urine samples with values not suggesting contamination (i.e., < 800 ug/l). The pre-shift and post-shift urine iodine levels did not differ for the perchlorate workers [Pre-shift: mean = 294 ug/l, stn dev = 150; Post-shift: mean = 297 ug/l, stn dev = 175] or for the azide workers [Pre-shift: mean = 350 ug/l, stn dev = 189; Post-shift: mean = 325 ug/l, stn dev = 202].

I. Complete Blood Counts

The blood counts showed no difference between the perchlorate workers and the azide workers, either directly or when the perchlorate workers were stratified by exposure groups. The mean of the red cell

counts, white blood cell counts (including lymphocytes, neutrophils, monocytes, etc.) and platelet counts revealed no significant differences across the exposure groups (Table 4). The proportion of workers with red cell count, white blood cell counts, or platelet counts below the laboratory normal ranges were similar in all groups. No significant trends were observed in the blood cell data whether examined as continuous variables or as categorical variables. Neither the mean nor the proportion abnormal were found to be significantly different for the azide group or any of the perchloride groups when each of the eighteen cellular parameters were examined. There was no evidence for aplastic anemia, agranulocytosis, or neutropenia. No suggestion of hematotoxicity was seen among the perchlorate-exposed workers or the azide-exposed workers.

J. Other clinical parameters

Serum chemical profiles were conducted on workers. The clinical chemistry results showed no evidence of either renal or hepatic toxicity. The frequency of elevated serum cholesterol levels was greater than 5 % among both the azide and the perchlorate workers. The serum phosphate level was the only chemical variable that showed significantly higher values among the perchlorate workers than among the azide workers. No explanation for this finding is apparent. There were no differences between the groups in the distributions of any of the other twelve chemical profile parameters.

K. Perchlorate excretion rate

Urinary perchlorate levels for two workers in the high-exposure perchlorate group were monitored during three days with measured occupational perchlorate exposure and during the subsequent three days without known perchlorate exposure (Figure 2). The data indicate that the perchlorate body burden, as indicated by urinary perchlorate concentration, increases over the three days of work exposure with generally a decrease between the 12-hour work shifts. Figure 2 graphically illustrates that exposure is the reason for absorption. Figure 3 presents the same data, but as the logarithm of the urinary perchlorate concentration. The elimination of perchlorate after the last definite exposure period appears to follow a 1st order kinetics pattern, which is particularly noted when the urinary perchlorate level is between 0.1 and 10 mg/l (Figure 3). The average perchlorate elimination half-life post-exposure for Employee A was 7.9 hours (excluding the period in day 6 of apparent minimal exposure), and the average perchlorate elimination half-life post-exposure for Employee B was 8.2 hours. The graph of Figure 3 suggests that the excretion half-life may be longer when the urine concentration is greater than 10 mg/l than at lower levels, possibly indicating a re-distribution into an alternative component site, such as the digestive tract.

Discussion

This occupational health study was conducted to determine whether occupational exposure to airborne perchlorate particles and the resultant systemic absorption of perchlorate during the manufacturing process has adversely affected the thyroid status of perchlorate workers. The study revealed no differences in thyroid function tests between perchlorate workers and a comparison group (azide workers). No differences in thyroid function test results were found among the workers across the four exposure groups.

The perchlorate characteristics of these four groups can be described both in terms of airborne perchlorate exposure and of perchlorate absorption as determined from urinary perchlorate excretion. Based on industrial hygiene airborne measurements, these groups are found to have had exposures with group arithmetic mean exposures ranging from 0.01 to 60 mg perchlorate per day, group median exposures ranging from 0.01 to 45 mg/day, and group geometric mean exposures ranging from 0.01 to 30 mg perchlorate per day. Different analysts may consider different exposure metrics to best summarize the airborne exposure. Based on urinary perchlorate excretion data, these groups are found to have had absorbed dosages with means ranging from 0.9 to 34 mg/day and medians ranging from 0.6 to 33 mg/day. The dosage data distribute symetrically about the means and the means are almost identical to the medians. Therefore, geometric means would provide no additional information. These data demonstrate no adverse effect on thyroid function at perchlorate absorptions of 0.01 to 34 mg/day.

No occupational thyroid disease was found among these workers. The perchlorate exposures in this plant were not found to be associated with thyroid abnormalities or with an excess of abnormal thyroid function tests. Further, there was no evidence of differences between the groups with respect to renal, hepatic, or hematological parameters. Thus, there is no evidence that perchlorate adversely affects the thyroid or those other three systems at these absorption levels, a daily absorption of 34 mg/day.

This study is the first to measure and assess urinary perchlorate concentrations in workers exposed to perchlorate particles. This study includes two measures of perchlorate exposure (total and respirable

particles) and one measure of perchlorate absorption (urinary perchlorate). The two measures of perchlorate exposure have been found to correlate very strongly (Figure 1). Data analysis has been conducted to examine the relationship between airborne particle perchlorate (both respirable and total) and perchlorate absorption.

Figure 4 demonstrates the statistically significant association (correlation) between airborne respirable particle perchlorate exposure and perchlorate absorption. Figure 4 also demonstrates that direct inhalation of respirable particles is not the only source for absorbed perchlorate. The slope of the association ($b = 2.06$) is greater than one, indicating that although the rise in respirable particle exposure significantly tracts the rise in absorption, the increase in absorption is twice as great as the increase in this exposure metric. Thus, the respirable particle perchlorate exposure is insufficient to account by itself for the rise in the perchlorate absorption.

Figure 5 demonstrates the statistically significant association (correlation) between airborne total particle perchlorate exposure and perchlorate absorption. In this case, the slope of the association ($b = 0.31$) is less than one, indicating that the increase in total particle perchlorate exposure is sufficient to account for the increase in the perchlorate absorption. This analysis suggests that an absorption co-efficient of 31% could describe the association between total particle perchlorate exposure and perchlorate absorption. Inspection of Figure 5 suggests that at lower total particle perchlorate exposures (i.e., 0 to 50 mg/day) other factors, such as hand-to-mouth ingestion, may make a major contribution to the total perchlorate exposure and absorption.

The airborne total particle perchlorate, with its high aqueous solubility, appears to be readily absorbed and appears generally to be the source of the excreted perchlorate. The design of pre- and post-shift urine collections and perchlorate assessments and measurements of both respirable particle and total particle inhalation exposure to perchlorate throughout the shift has revealed that total particle (as well as respirable particle) perchlorate inhalation exposure leads to systemic absorption of perchlorate and to its urinary excretion. The lower respiratory tract is the primary site for respirable particle perchlorate to be absorbed. Whether the total particle perchlorate is also absorbed in the upper respiratory tract and is carried by mucous into the gastrointestinal tract, or it enters the gastrointestinal tract by direct contact, is not important to the post-absorption pharmacology. The perchlorate absorbed into the blood stream (whether from the respiratory or gastrointestinal tract) are equivalent since perchlorate excretion in the urine and pharmacological effects on the thyroid are both dependent upon absorption into the blood stream.

The data from the workers in this study contributes to the literature on perchlorate excretion rates in humans. The excretion half-lives of 7.9 hours and 8.2 hours in the workers observed for three days post-exposure is quite consistent with the 6-8 hours reported by Eichler in 1929 and the 5-9 hours reported by Durand in 1938. There is a quiet pleasure in observing that one's work replicates data published sixty to seventy years earlier, though the total number of subjects published is now only four.

The Eichler (1929) exposure was to a single oral dose of 1 or 2 grams. The Durand (1938) exposure was to a single oral dose of 0.8 grams. The two workers in this study had been working in perchlorate production area C during the prior work period and can be assumed to have had the equivalent of a 34 mg oral dosage over a twelve hour period. Since these workers are regularly in this employment, this exposure can be described as chronic or sub-chronic exposure at a moderate dosage (greater than environmental and less than pharmaceutical). Although there is a suggestion within our data that some other physiological processes may be occurring at higher exposure levels, these data do indicate that 8-hours is a reasonable estimate of the perchlorate excretion rate in humans. This 8-hr half-life has been consistent down to the limit of detection in the urine.

This study has provided an insight into the absorption and excretion of perchlorate among workers exposed to airborne perchlorate particles. It has also shown that these workers do not demonstrate any adverse effect on their thyroids at these occupational exposure levels at this perchlorate-manufacturing plant. This study also confirms the findings of Gibbs et al. that demonstrated the absence of an adverse effect on thyroid function among perchlorate-manufacturing employees at a different plant.[16] Gibbs demonstrated the absence of an effect on thyroid function both in examining across the work shift acutely and across the working life cumulative exposure chronically.[16] Gibbs et al. also demonstrated the absence of an effect on kidney, liver, or bone marrow function across the working life.[16] The present study has added observations of individual respiratory perchlorate particle exposures and subsequent urinary perchlorate measurements to the exposure measurements of Gibbs et al.[16] It has also added serum T_3 and TPO antibodies, and a clinical thyroid examination to their assessment of thyroid function. This study has also reported the absence of an effect on the liver, kidney, or blood cells at a range of perchlorate exposure and absorption rates. Both studies have demonstrated that occupational exposures to perchlorate have not

been hazardous to the thyroid health status of the workers studied at these plants or to the other examined organ systems.

Occupational health studies serve to advise workers, physicians, and managers on the safe limits of exposure. That is their primary function and their purpose of design. Such studies may also be helpful to toxicologists and other health scientists who desire clarification of mechanisms and parameters concerning perchlorate exposure, absorption, toxicity, and excretion. Additionally, such studies are useful to environmental health specialists who must consider the risks associated with low-level exposures to perchlorate, either through inhalation or ingestion. The US Environmental Protection Agency and a number of state health departments are currently attempting to assess the potential magnitude of risk associated with various levels of perchlorate contamination of drinking water. Studies such as this provide useful information for the assessment of such risks.

Current levels of perchlorate detected in drinking waters of Southern California and Southern Nevada are in the range of 5-8 ppb (μg/l) and up to 15 ppb, respectively. The assumed ingestion of two liters per day would yield an ingestion exposure and absorption of up to 30 μg perchlorate per day for an adult. This rate is about one to two orders of magnitude lower than that of the azide worker control group in this study. It is about three orders of magnitude lower than that of the perchlorate group C workers who showed no adverse effect on their thyroid health status with recurrent occupational absorbed exposures of about 34 mg perchlorate per day.

Two issues of perchlorate toxicity have arisen, hematotoxicity in adults and congenital hypothyroidism in the newborn. Cases of hematotoxicity associated with perchlorate exposure have generally been reported for Graves' disease patients being treated therapeutically with doses of 600 to 1,000 or more mg per day, and only once in a patient treated with 450 mg per day who had previously had a toxic reaction at 800 mg per day.[2, 17] No hematotoxicity was seen among the workers in this study and, in particular, not at 34 mg per day. The case report literature suggests that reported hematotoxicity findings in Graves' disease patients may occur, but at exposures at least one to two orders of magnitude greater than the occupational exposures and at least four to six orders of magnitude greater than the environmental exposures from the Southern California and Nevada waters. The dosages at which had been reported is vastly greater than that observed even in the occupational exposure ranges.

Prior to 1960, perchlorate was commonly used to treat women with hyperthyroidism during pregnancy. Crooks and Wayne reported in Lancet that they had "treated 12 pregnant thyrotoxic patients with potassium perchlorate (600 mg/day or 1000 mg/day) and in each have achieved satisfactory control of the disease.[18] One of the infants had a very slight enlargement of the thyroid that disappeared within 6 weeks. The remainder showed no abnormality of any kind." This is the only published report of perchlorate and the neonatal thyroid. Additionally, the California Department of Health Services (1997) has a preliminary health review for a Superfund site in Sacremento, California where perchlorate is a contaminant of concern.[19] They found no increase in congenital hypothyroidism in the zip codes of interest. Similarly, Doemland and Lamm reported that the counties in Southern California and Nevada with perchlorate-contaminated drinking water had no more cases of congenital hypothyroidism than would be expected, based on state rates for 1996 and 1997.[20] Thus, only one case of transient goiter in a newborn has been reported for those whose mother had therapeutic exposures and two research groups looking at different geographic areas found no evidence of an increased risk of congenital hypothyroidism with environmental exposures.

In conclusion, this study has (1) found no evidence of an adverse effect of perchlorate exposure on thyroid function among perchlorate workers, (2) demonstrated that airborne perchlorate is absorbed and excreted by perchlorate workers, (3) indicated that the exposure to perchlorate particles larger than respirable size is likely to account for the magnitude of perchlorate excretion, (4) provided an estimate for the urinary excretion half-life of perchlorate in perchlorate workers, and (5) developed information that may be useful in the assessment of human health risk from environmental exposure to perchlorate. The results of the present study do not support the hypothesis that chronic exposure to perchlorate at the levels encountered in this study has an adverse effect on thyroid function. There also is no evidence to support the hypothesis that perchlorate has an effect on the hematopoeitic system even at these occupational doses. The findings in this study demonstrate a "no adverse effect level" on thyroid function and hematotoxicity in a worker population of 34 mg perchlorate per day for humans.

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January 29, 1999

Susan Goldhaber/Ella Darden
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3040 Cornwallis Rd.
Research Triangle Park, NC 27709

Dear Ms. Goldhaber & Ms. Darden:

Please find enclosed the five tables and five figures for the article "Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational Health Study" which has been peer reviewed and accepted by the Journal of Occupational and Environmental Medicine and is currently in press.

I would appreciate the opportunity to present the data and graphs to the panel and answer any questions they may have.

Sincerely,

Feng Li

Feng Li, MD, DrPH

Table 2. Descriptive statistics of creatinine-adjusted urine perchlorate levels (mg/gm) by plant and exposure and adsorbed dose (mg/shift)

Groups	N	Mean	Std. Dev.	Min	P25	Median	P75	Max
				Pre-shift				
Azide	21	1.31	1.55	0.23	0.39	0.84	1.36	6.37
Perchlorate A	14	2.05	2.42	0.42	0.59	1.09	1.50	8.02
Perchlorate B	8	5.98	5.70	0.64	1.12	4.87	9.91	15.41
Perchlorate C	14	11.30	9.93	0.53	1.09	14.89	17.61	30.22
				Post-shift				
Azide	21	1.19	1.16	0.16	0.52	0.81	1.52	4.97
Perchlorate A	14	4.07	2.15	0.46	2.38	3.53	5.71	8.24
Perchlorate B	8	11.27	7.59	3.63	4.93	9.79	16.45	24.22
Perchlorate C	15	32.22	13.14	11.16	27.81	33.09	38.89	64.38
				Post-Shift (adjusted)				
Azide	21	0.75	1.00	-0.85	0.14	0.53	0.78	3.62
Perchlorate A	14	3.39	2.29	0.32	1.69	2.87	5.02	8.04
Perchlorate B	8	9.28	7.41	2.67	3.13	7.16	13.32	23.98
Perchlorate C	14	28.66	12.38	10.80	21.04	28.43	33.70	58.51
				Absorbed Dosage (mg/shift)				
Azide	21	0.88	1.17	-1.00	0.16	0.62	0.92	4.25
Perchlorate A	14	3.98	2.69	0.38	1.98	3.37	5.89	9.43
Perchlorate B	8	10.89	8.69	3.13	3.67	8.40	15.63	28.13
Perchlorate C	14	33.62	14.52	12.67	24.68	33.35	39.54	68.65

Table 3. Descriptive statistics of thyroid function parameters by plant and exposure groups

Groups	N	Mean	Std. Dev.	Min	P25	Median	P75	Max	P-value*
					T4 (5 – 11 µg/dl)**				
Azide	21	6.73	1.479	4.60	5.40	6.80	7.40	9.90	--
Perchlorate A	13	7.13	1.583	4.00	6.40	6.90	7.80	10.60	0.46
Perchlorate B	8	7.34	1.115	5.40	6.70	7.50	8.00	8.90	0.31
Perchlorate C	15	7.03	1.301	4.40	6.00	7.30	8.10	8.60	0.54
					T3 (87 – 178 ng/dl)				
Azide	21	142.52	17.543	113.00	129.00	143.00	156.00	169.00	--
Perchlorate A	13	148.38	25.178	96.00	145.00	159.00	166.00	174.00	0.43
Perchlorate B	8	152.13	23.234	120.00	134.00	148.00	176.00	181.00	0.24
Perchlorate C	15	152.13	20.368	108.00	141.00	150.00	165.00	192.00	0.14
					TSH (0.45 - 4.5 µU/ml)				
Azide	21	3.14	1.870	0.67	2.00	2.80	4.10	8.40	--
Perchlorate A	12	2.68	1.143	1.20	1.70	2.50	3.75	4.50+	0.45
Perchlorate B	8	2.41	1.271	0.75	1.45	2.15	3.50	4.30	0.32
Perchlorate C	15	3.33	2.338	0.65	1.50	2.80	4.20	8.20	0.80
					FTI (5.0 - 11.0)				
Azide	21	6.05	1.248	4.40	5.30	6.00	6.70	9.60	--
Perchlorate A	13	6.33	1.435	3.20	5.80	6.40	6.90	9.40	0.55
Perchlorate B	8	6.56	0.847	5.10	6.25	6.50	6.90	8.10	0.29
Perchlorate C	15	6.56	1.022	4.40	5.60	6.90	7.20	8.20	0.20
					THBR (0.85 – 1.10)				
Azide	21	0.90	0.071	0.80	0.84	0.91	0.95	1.08	--
Perchlorate A	13	0.89	0.064	0.79	0.83	0.89	0.93	0.99	0.47
Perchlorate B	8	0.90	0.069	0.79	0.85	0.90	0.96	0.99	0.81
Perchlorate C	15	0.94	0.094	0.78	0.87	0.95	1.01	1.09	0.19

	Anti-TPO (< 20 IU/ml)								
Azide	21	9.28	11.274	5.00	5.00	5.00	6.30	53.20	--
Perchlorate A	13	9.26	10.611	5.00	5.00	5.00	5.00	42.70	0.98
Perchlorate B	8	11.38	9.524	5.00	5.00	5.00	18.20	29.60++	0.65
Perchlorate C	14	8.63	6.797	5.00	5.00	5.00	9.50	29.60	0.85

* All t-tests were performed assuming equal variances based on Bartlett's test for equal variances (at α level of 0.05).

** Values in parentheses represent the laboratory's normal range for the assay.

\+ One extreme outlier (TSH = 38 µU/ml) was excluded. This worker was diagnosed with Graves' Disease nine years before employment and is insufficiently treated for his hypothyroidism that developed following ^{131}I therapy.

++ One extreme outlier (Anti-TPO = 709 IU/ml) was excluded. This worker has euthyroid Hashimoto's thyroiditis.

Table 4. Descriptive statistics of blood cell counts by plants and exposure groups

Groups	N	Mean	Std. Dev.	Min	P25	Med	P75	Max	P-value
				Red Blood Cells (4.40 – 5.80 x 10^3/nl)**					
Azide	21	5.16	0.333	4.30	5.00	5.20	5.40	5.73	--
Perchlorate A	14	5.06	0.500	4.10	4.90	5.22	5.30	5.70	0.48
Perchlorate B	8	5.11	0.233	4.74	4.95	5.14	5.28	5.40	0.66
Perchlorate C	15	5.37	0.367	4.40	5.00	5.50	5.60	5.76	0.10
				White Blood Cells (3.6 – 10.6 cells/nl)					
Azide	21	8.62	5.608	4.90	6.81	7.60	8.30	32.60	--
Perchlorate A	14	7.67	1.380	5.50	7.20	7.30	7.90	11.00	0.47*
Perchlorate B	8	7.83	2.631	4.40	5.45	7.85	9.90	11.80	0.61*
Perchlorate C	15	7.99	1.554	5.90	6.50	7.80	8.70	11.80	0.63*
				Neutrophiles (1.8 –8.0 cells/nl)					
Azide	21	4.96	5.222	1.80	3.50	3.60	4.40	27.50	--
Perchlorate A	14	4.41	0.796	2.90	4.10	4.65	4.80	5.50	0.64*
Perchlorate B	8	4.20	1.546	2.30	3.00	4.40	4.65	7.20	0.55*
Perchlorate C	15	4.30	1.214	2.20	3.40	4.30	4.70	7.70	0.58*
				Lymphocytes (1.2 –3.4 cells/nl)					
Azide	21	2.66	0.691	1.30	2.30	2.60	2.80	4.10	--
Perchlorate A	14	2.39	0.659	1.60	1.90	2.20	2.60	4.30	0.27
Perchlorate B	8	2.83	1.516	1.50	1.75	2.30	3.55	5.90	0.77*
Perchlorate C	15	2.81	0.822	1.10	2.40	2.80	3.10	4.40	0.56
				Platelets (140-440 platelets/nl)					
Azide	21	233.00	40.107	149.00	206.00	230.00	268.00	304.00	--
Perchlorate A	14	235.07	47.798	159.00	204.00	245.00	270.00	317.00	0.89
Perchlorate B	8	221.88	61.989	144.00	182.50	213.00	246.00	348.00	0.57
Perchlorate C	15	230.53	57.679	127.00	193.00	222.00	258.00	343.00	0.88

* t-tests were performed assuming unequal variances based on Bartlett's test for equal variances (at α level of 0.05).

** Values in parentheses represent the laboratory's normal range.

Figure 1

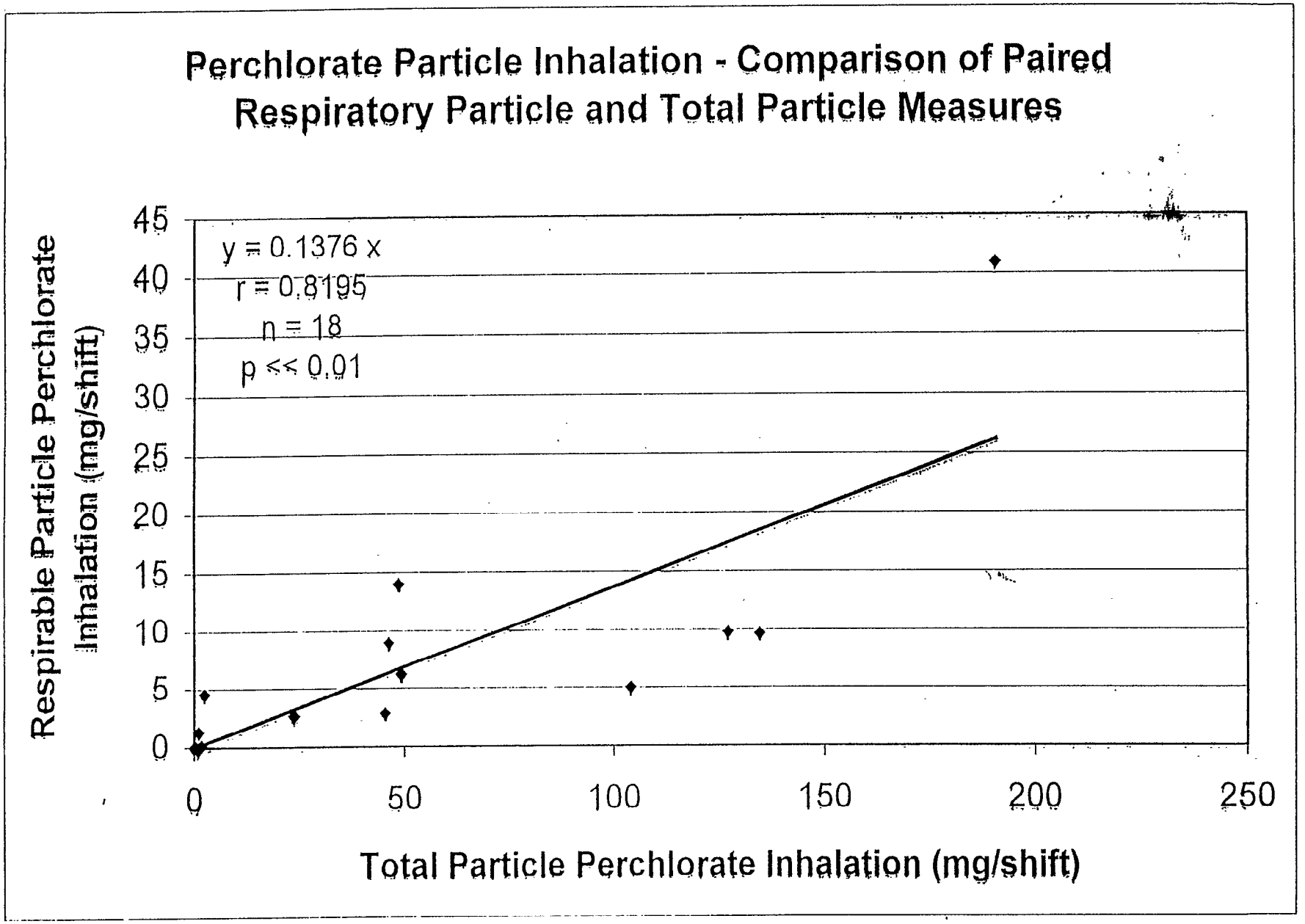
Perchlorate Particle Inhalation - Comparison of Paired Respiratory Particle and Total Particle Measures
y = 0.1376 x
r = 0.8195
n = 18
p << 0.01
Respirable Particle Perchlorate Inhalation (mg/shift)
Total Particle Perchlorate Inhalation (mg/shift)
0
5
10
15
20
25
30
35
40
45
0
50
100
150
200
250

Figure 2

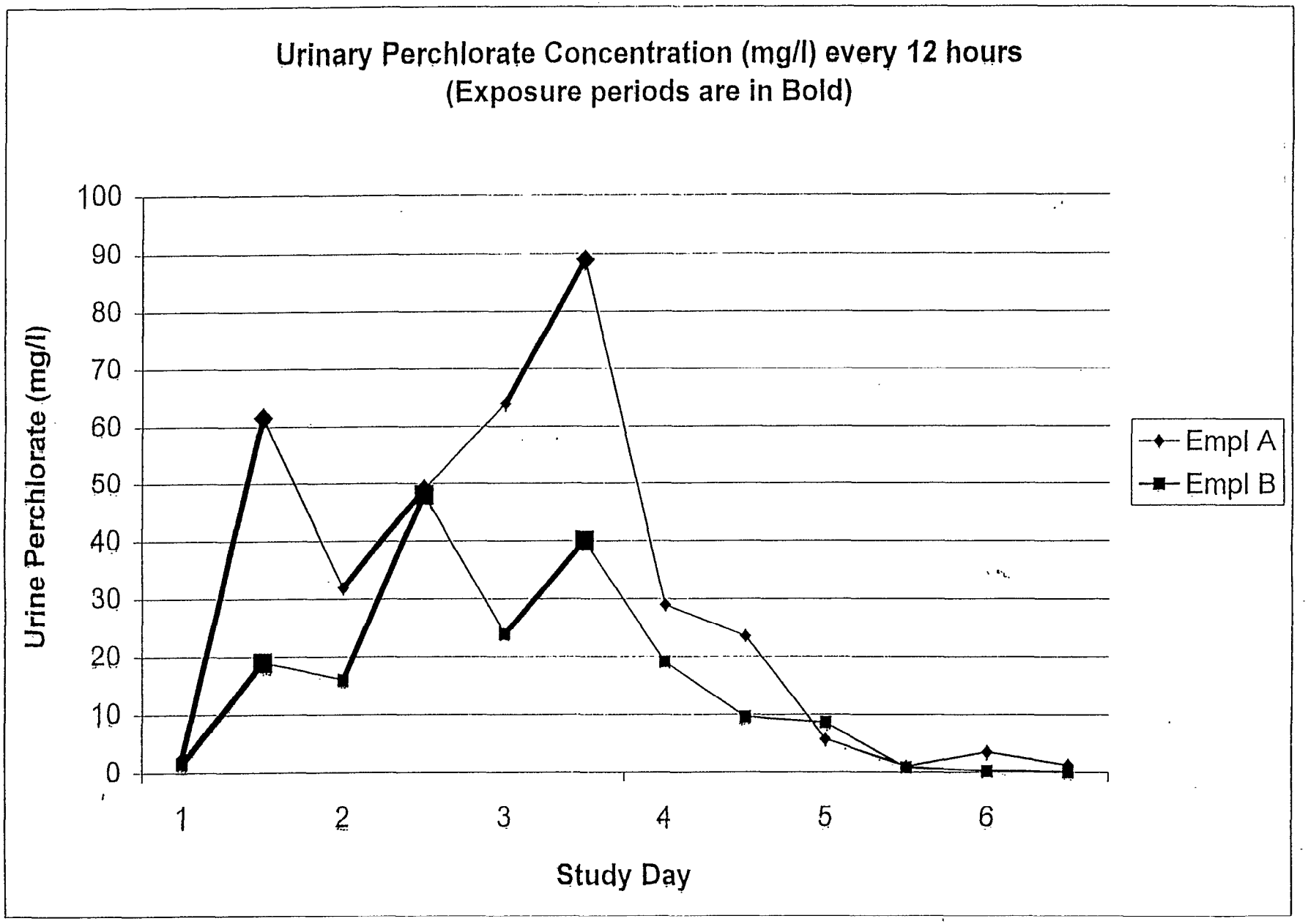
Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in Bold)
Urine Perchlorate (mg/l)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Study Day
Empl A
Empl B

Figure 3

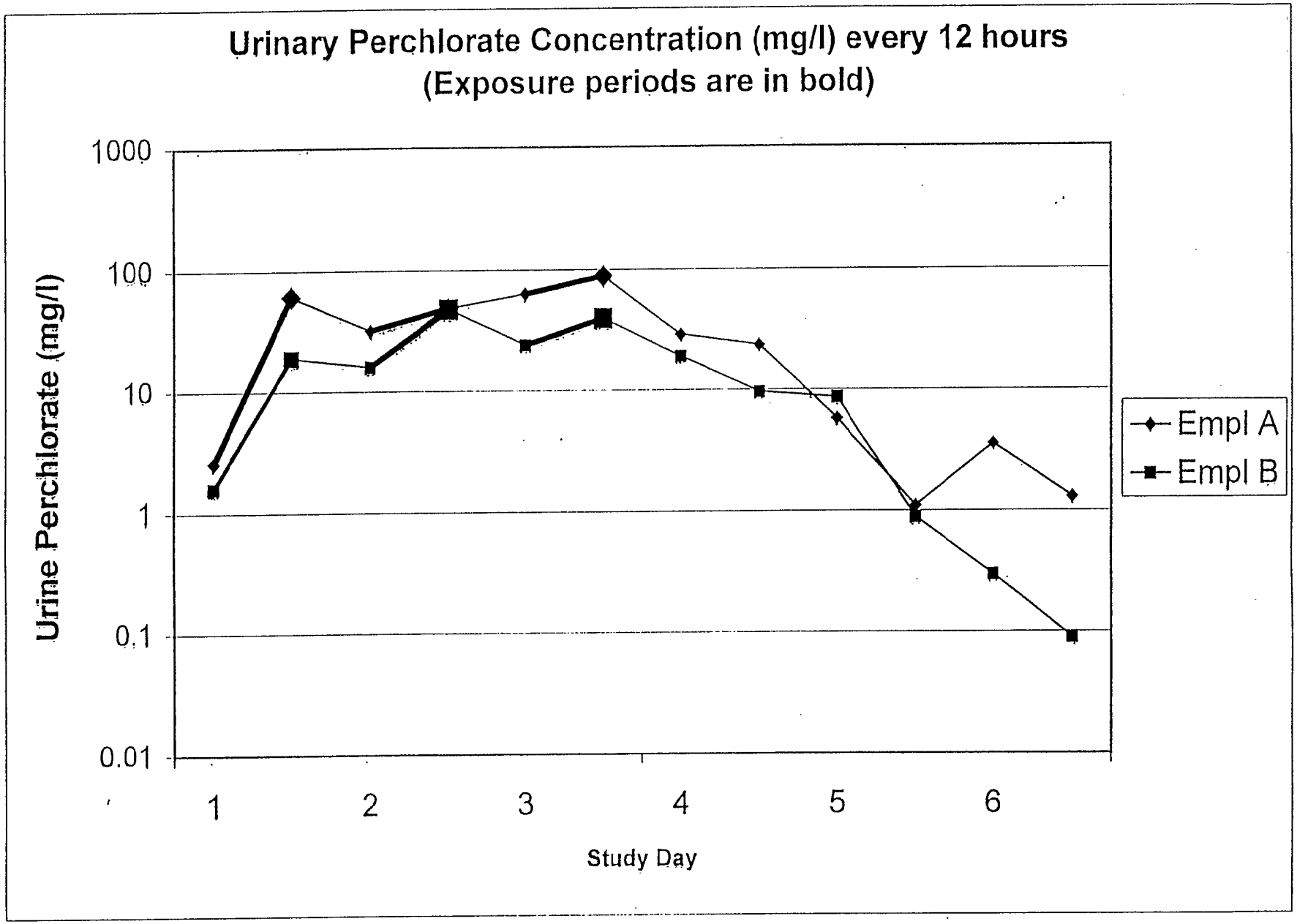
Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in bold)
Urine Perchlorate (mg/l)
1000
100
10
1
0.1
0.01
1
2
3
4
5
6
Study Day
Empl A
Empl B

Figure 4

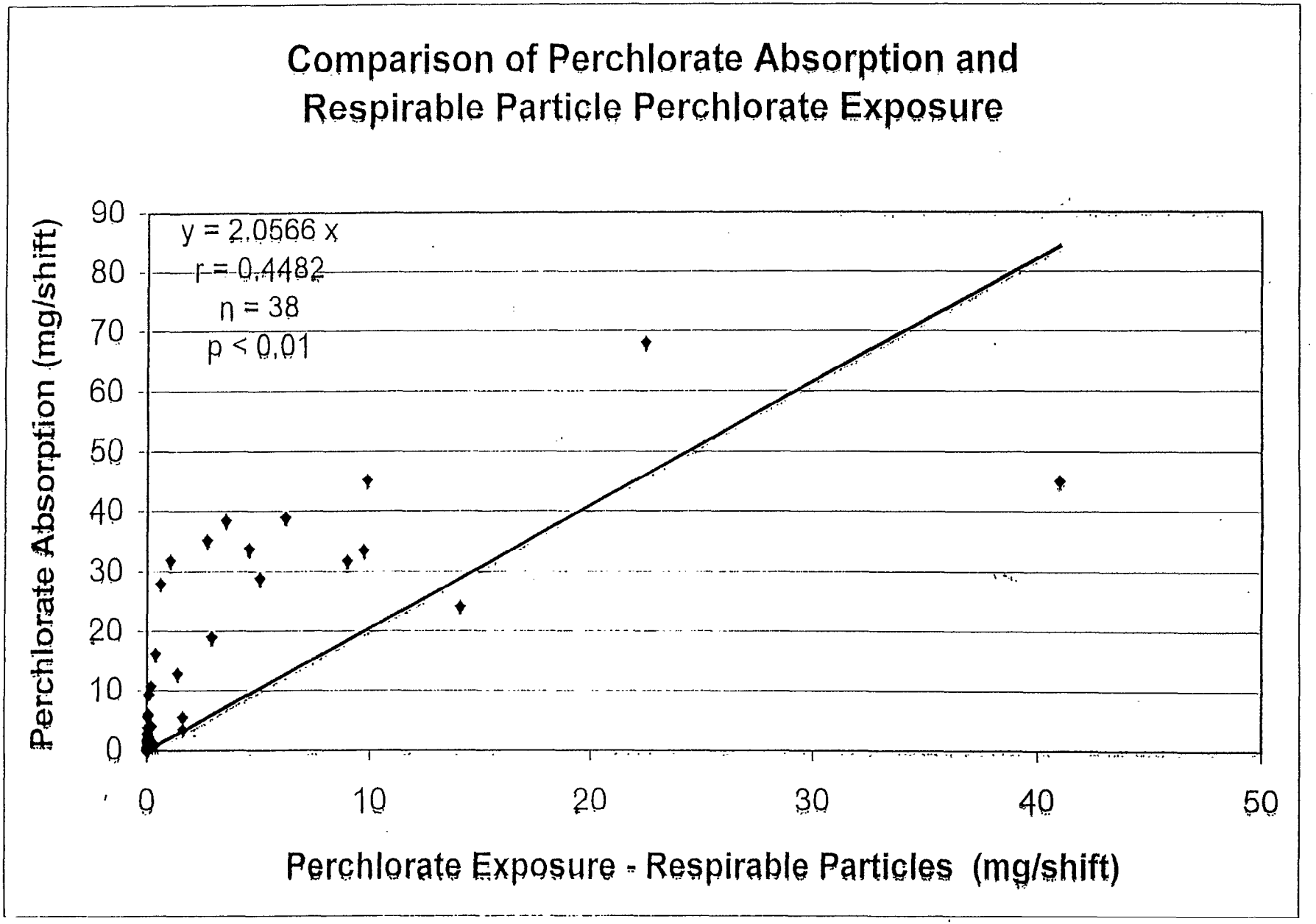
Comparison of Perchlorate Absorption and
Respirable Particle Perchlorate Exposure
y = 2.0566 x
r = 0.4482
n = 38
p < 0.01
Perchlorate Absorption (mg/shift)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Perchlorate Exposure - Respirable Particles (mg/shift)

Figure 5

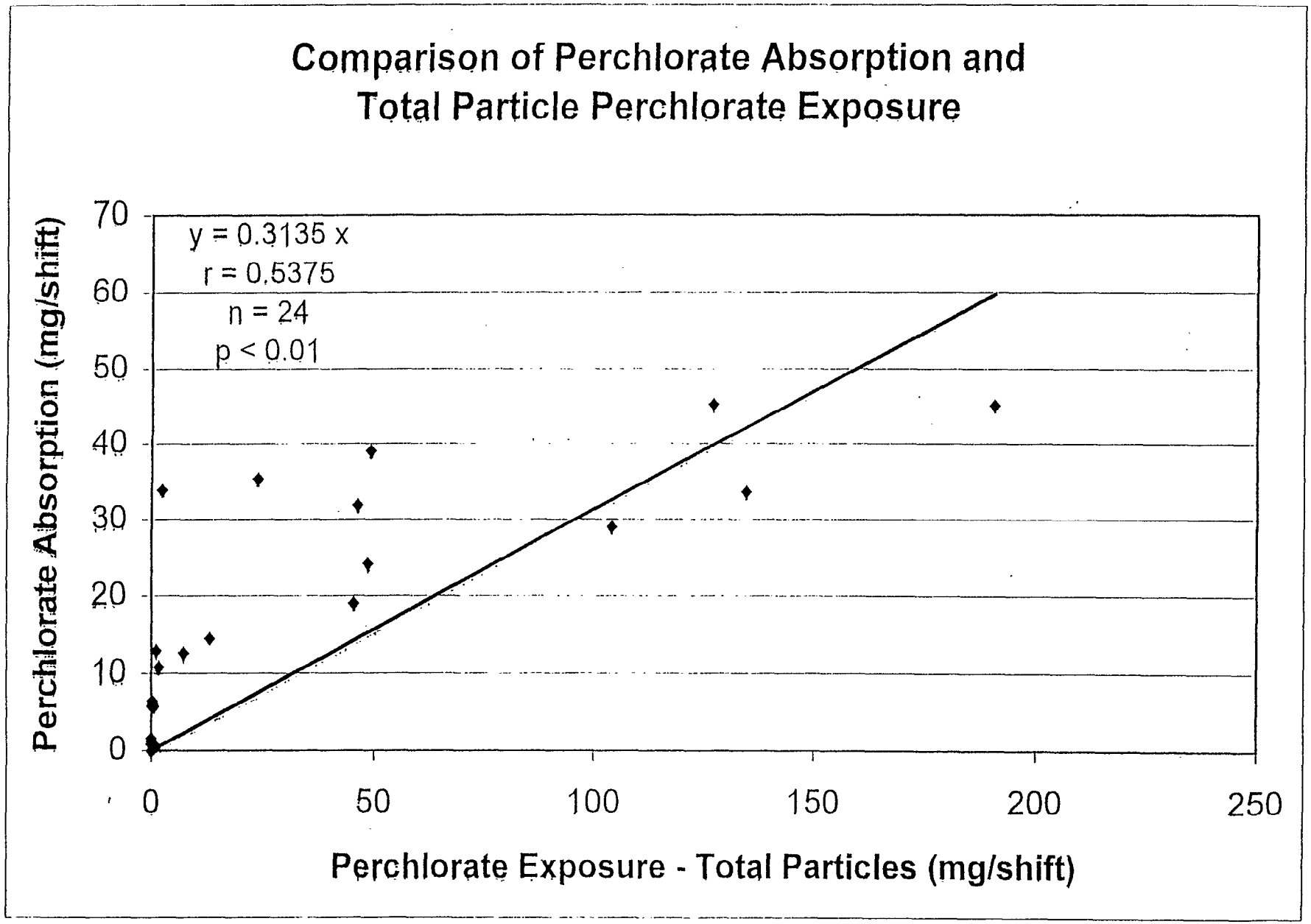
Comparison of Perchlorate Absorption and
Total Particle Perchlorate Exposure
y = 0.3135 x
r = 0.5375
n = 24
p < 0.01
Perchlorate Absorption (mg/shift)
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Perchlorate Exposure - Total Particles (mg/shift)

Table 1. Descriptive statistics of respirable and total airborne perchlorate levels (mg/day) by plant and exposure groups

Groups	N	Ar-Mean	Ar-STD	Geo-Mean	Geo-STD	Min	P25	Median	P75	Max
					Respirable (mg/day)					
Azide	6	0.021	0.014	0.017	1.925	0.009	0.010	0.014	0.038	0.039
Perchlorate A	11	0.091	0.095	0.057	3.019	0.006	0.040	0.067	0.083	0.331
Perchlorate B	7	0.601	0.671	0.255	5.196	0.031	0.031	0.374	1.522	1.575
Perchlorate C	14	8.591	9.386	5.414	2.740	0.957	2.643	5.040	10.160	35.852
					Total (mg/day)					
Azide	4	0.014	0.012	0.011	2.482	0.004	0.006	0.012	0.023	0.030
Perchlorate A	6	0.337	0.187	0.288	1.921	0.107	0.168	0.330	0.487	0.602
Perchlorate B	2	6.567	7.139	4.200	---	1.519	1.519	6.567	11.615	11.615
Perchlorate C	12	59.378	53.605	28.674	5.101	1.036	11.772	44.890	103.749	166.996

Legend:

N = number; Ar-mean = arithmetic mean (average); Ar-STD = standard deviation of the arithmetic mean; Geo-mean = geometric mean (logarithmic mean); Geo-STD = standard deviation of the geometric mean; Min = minimum value; P25 = 25th percentile value; median = 50th percentile value; P75 = 75th percentile value; and Max = maximum value.

Consultants in Epidemiology and Occupational Health, Inc.

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January 29, 1999

Susan Goldhaber
Research Triangle Institue
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

Dear Ms. Goldhaber,

Enclosed please find a copy of the final accepted version of "Has Perchlorate in Drinking Water Increased the Rate of Congenital Hypothyroidism?" for publication in the Journal of Occupational and Environmental Medicine. I would appreciate you forwarding the paper to the perchlorate external peer review committee for their review.

Also I would like to formally request five minutes at the meeting in San Bernadino to present the key points of the paper.

Thank you for your time and consideration.

Sincerely,

Martha Doemland

Martha Doemland, PhD

Has Perchlorate in Drinking Water Increased the Rate of Congenital Hypothyroidism?

Running Title: Perchlorate and Congenital Hypothyroidism

Word Count: 777

Steven H. Lamm, MD & Martha Doemland, PhD

From Consultants in Epidemiology and Occupational Health, Inc. (CEOH, Inc.), Washington, DC (Drs. Lamm and Doemland)

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Journal of Occupational and Environmental Medicine
In press (January 22, 1999)

Abstract

Perchlorate, known to inhibit the human thyroid at doses above 200 mg/day, was detected in the drinking water supplies of seven counties in California and Nevada at levels of 4 to 16 ug/l in 1997. The data from the neonatal screening programs of the state health departments were analyzed for any increased incidence of congenital hypothyroidism in those counties. County-specific, ethnicity-specific data for Nevada and California were obtained for 1996-1997. Within these seven counties, nearly 700,000 newborns had been screened. 249 cases were identified, where 243 were expected, for an over all risk ratio of 1.0 (95% confidence interval, 0.9-1.2). The risk ratios for the individual counties ranged between 0.6 and 1.1. These data in this ecological analysis do not indicate any increase in the incidence of congenital hypothyroidism with the reported perchlorate levels.

Keywords: Perchlorate, thyroid, congenital hypothyroidism

Introduction

Congenital hypothyroidism is a preventable cause of mental retardation and is detected at birth through neonatal screening programs. Perchlorate, now a known environmental contaminant or drinking and surface waters, is known to block thyroid hormone formation by competitively inhibiting the uptake of iodine by the thyroid gland. An analysis has been conducted to determine whether the counties with perchlorate-containing water have an increased rate of congenital hypothyroidism.

Methods

The source of perchlorate contamination in California and Nevada originated from industrial sites manufacturing or using perchlorate for missiles, rockets, or fireworks. An industrial site in Nevada led to contamination of Lake Mead which contaminated the Colorado River water supply for Southern California and the water supply for Las Vegas (Clark County) Nevada at levels of 5-8 ppb and up to 16 ppb, respectively. The US Environmental Protection Agency (EPA) Region 9 has identified six counties in California and one in Nevada with perchlorate in the drinking water supply. (Figure I)

The health departments of both Nevada and California have conducted neonatal screening programs for congenital hypothyroidism for over ten years. A heel-stick blood sample of all newborns is used to assess the presence of a variety of congenital metabolic diseases. Participation is mandatory and covers all hospitals with birthing units. Follow-up after diagnosis and referral for treatment is supervised by the state health departments.

The county-specific congenital hypothyroidism case counts and live birth counts for 1996 and 1997 have been obtained for both California and Nevada. Theses data were supplied by the respective state Health Departments (George Cunningham, MD, MPH, Chief of the Genetics Disease Branch, Primary Care & Family Health Division, California Department of Health Services; Gloria Dayhli, Bureau of Family Health Services, Nevada State Health Division). The California data were stratified by ethnicity since fifty percent of California births are Hispanic and Hispanic ethnicity has been shown to be a risk factor for congenital hypothyroidism.

Results

California and Nevada comprise a population of about 35 million people with a birth rate of about 16%. The neonatal screening programs cover essentially one hundred percent of the live births in each state, including the 700,000 newborns who were screened during 1996-97 in the seven counties with perchlorate-contaminated drinking water.

Based on state incidence rates of congenital hypothyroidism, 243 cases would have been expected in the seven county area during 1996-1997 and 249 cases were observed [Table 1]. This risk ratio is 1.02 (95% confidence limits, 0.9-1.2). The risk ratios (congenital hypothyroidism standardized birth prevalence ratio) for each county was calculated for the individual counties ranged between 0.6 and 1.1. Thus, in Nevada and California, the counties with detectable levels of perchlorate in the drinking water have prevalence rates for congenital hypothyroidism than do not differ from the expected based on state rates.

Nearly the entire water supply for Clark County, Nevada comes from the primary source of perchlorate contamination (Lake Mead). The California counties have more spotty and intermittent exposure. Nonetheless, this ecological examination of the congenital hypothyroidism data (1996-97) shows no increase in the prevalence of congenital hypothyroidism in counties with detected perchlorate levels in the drinking water.

Discussion

Perchlorate was detected in the range of 4-16 ppb (μg/l) in drinking water supplies for California and Nevada. Assuming water intake of two liters per day, this might provide a daily dosage of perchlorate of approximately 20 μg per day. A daily intake rate of perchlorate at 20 μg per day can be compared with the minimum effective dose of 200 mg/day (200,000 μg/day) that has been used medically to suppress the thyroid in treatment of hyperthyroidosis.

Congenital hypothyroidism occurs when both the maternal thyroid and the fetal thyroid are unable to supply adequate thyroid hormone to the fetus. This occurs endemically only in the presence of severe iodine deficiency, a condition rarely known in the United States, and sporadically with structural or metabolic defects in the thyroid. Children born without a thyroid have normal intellect if thyroid treatment starts early [3] because the maternal thyroxine that crosses the placenta is usually sufficient to sustain the fetus. [4, 5] Even moderate iodine deficiency in a population yielded only transient changes in thyroid hormone levels (T_4, TSH) and no increase in congenital hypothyroidism.[6]

Comparison of the county-specific rates of congenital hypothyroidism (based on prevalence rates derived from mandatory reporting programs) in California and Nevada reveal that counties with detected levels of perchlorate in the drinking water do not have higher rates of congenital hypothyroidism. These data, at an ecological level of analysis, seem to indicate that no increased rate of congenital hypothyroidism is associated with the levels of perchlorate found in the drinking waters of California and Nevada.

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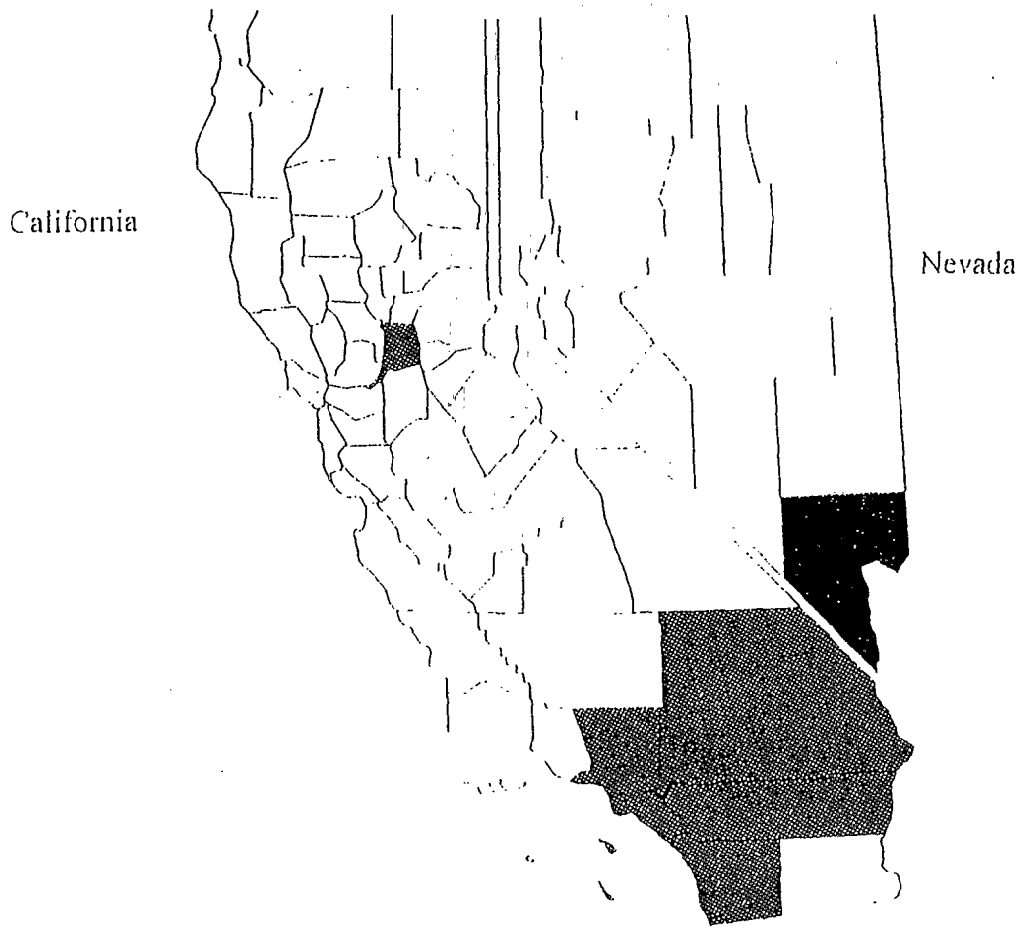
Table 1

Congenital Hypothyroidism Cases (Observed and Expected*) for 1996 and 1997 in Nevada and California Counties with Perchlorate Reported in Water Supply

State	County	Newborns Number Screened	Congenital Hypothyroidism Cases Observed	Expected	Observed/Expected	95% Conf. Limits
Nevada	Clark	36,016	7	8.3	0.84	(0.34-1.74)
California	Los Angeles	338,934	136	123.5	1.10	(0.92-1.30)
	Orange	101,227	40	35.9	1.12	(0.80-1.52)
	Riverside	43,577	11	15.6	0.71	(0.35-1.26)
	Sacramento	39,235	8	12.9	0.62	(0.27-1.22)
	San Bernardino	51,637	17	18.4	0.92	(0.54-1.48)
	San Diego	80,582	30	28.2	1.06	(0.72-1.52)
	Total	655,192	242	234.6	1.03	(0.90-1.16)
All seven counties		691,208	249	42.9	1.03	(0.90-1.16)

* Expected numbers have been adjusted for Hispanic ethnicity.

Figure 1. Counties in California and Nevada with Perchlorate Detected in Drinking Water



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Comments on EPA's "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information"

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1.0 INTRODUCTION

The Perchlorate Study Group asked the Jellinek, Schwartz and Connolly Corporation and the Gradient Corporation (both subsidiaries of the International Technology Group [IT Group] to review the draft EPA Reference Dose (RfD) Document for perchlorate and provide comments to EPA's Peer Review Panel regarding the scientific basis for the proposed Reference Dose (RfD) for perchlorate. A team of seven toxicologists from the IT Group has completed its initial review of this draft document. The team concludes that, given the severe time constraints under which the document was prepared, EPA has done a good job in addressing the issue of perchlorate toxicity. There are, however, several issues that the Peer Review Panel should consider as they review the draft RfD document. Specific issues include:

- Biological Relevance of the Critical Effect Chosen by EPA
- Questions Regarding the Interpretation (especially the Histopathology) of the Key Studies
- Application of Appropriate Uncertainty Factors in Setting the Reference Dose
- Significance of Study Data Not Yet Received
- Ecological Effects Data

These issues are discussed sequentially below.

2.0 BIOLOGICAL RELEVANCE OF THE ENDPOINTS DESCRIBED BY EPA

2.1 EPA's decision not to use changes in T3/T4 levels as the basis for deriving an RfD for perchlorate is scientifically correct.

EPA correctly determined that changes in circulating levels of T3 and T4 are not appropriate for use as the critical endpoint for derivation of a perchlorate RfD. This conclusion is based on the understanding that decreases in T3 and T4 levels represent a normal response to a reduction in iodine and are within the limits of homeostatic control. The levels of thyroid hormones fluctuate in response to a wide variety of changing environmental conditions and changes in these hormone levels should be considered normal. For example, EPA states that the hormonal changes observed in the Caldwell study (1995) represent a response in hormone homeostasis (USEPA, 1998, pp.6 –31). Similarly, changes in TSH also constitute an important part of the normal feed-back mechanism for regulation of thyroid hormone levels and reflect an adaptive response to decreases in T3 and T4.

2.2 EPA's use of follicular cell hypertrophy as a critical adverse effect needs to be examined. Hypertrophy is part of a normal homeostatic thyroid response. It is not in itself an adverse effect and is not causally related to an adverse effect.

A LOAEL of 0.1 mg/kg/day for follicular cell hypertrophy in 5 day post-natal rat pups in the neurodevelopmental study was selected by EPA as a suitable endpoint from which to derive the perchlorate RfD. The LOAEL is, by definition, the lowest measured dose of a chemical associated with an <u>adverse</u> effect. In the risk characterization for perchlorate, EPA recommends expanding the concept

of the LOAEL and identifies a "critical effect" as "the effect that is adverse that first appears in the dose scale as it is increased or is a known precursor to the first adverse effect" (EPA, 1998).

However, we are concerned about the application of this definition to the data available on perchlorate. The toxicological literature provides no support for the assumption that thyroid follicular cell hypertrophy is either adverse or a known precursor of an adverse effect (McClain, 1995; Capen and Martin, 1989; Hill, et al., 1998). Hypertrophy is indicative of a slightly increased functional activity of the thyroid gland and should be regarded as an adaptive functional change. Hypertrophy often "precedes" hyperplasia both in time and at lower dose levels, but is not thought to progress biologically to hyperplasia and is not, itself, a biological indicator or predictor of hyperplasia or other adverse effects. There is a general consensus among toxicologists that some degree of thyroid inhibition with resulting hypertrophy can be accommodated within the bounds of the normal feedback mechanism without the induction of either hyperplasia or neoplasia (Paynter *et al.*, 1986). In this context we conclude that follicular cell hypertrophy is an adaptive response to the need for increased thyroid hormone production, and is not an adverse effect, nor a known precursor to an adverse effect, nor a reliable biological indicator of an adverse effect. We believe that it is more appropriate to use the diagnosis of hyperplasia as a point of departure for setting the RfD (see Section 2.4). Although hyperplasia is also an adaptive change, it can be considered a response to altered thyroid gland function that is potentially adverse. That is, since under some circumstances, mild hyperplasia can progress to severe hyperplasia and potentially to neoplasia. Thus even mild hyperplasia can be considered a possible precursor to an adverse effect.

2.3 Inconsistencies in recording and interpreting the histopathological data limit the reliability of the endpoint selection process. Additional histopathological analyses are warranted.

There is considerable inconsistency in the evaluation and reporting of histopathological effects in several of the rat studies on which the EPA relies in selecting an endpoint for establishing a RfD. Specifically, the very different endpoints, hypertrophy and hyperplasia (see Section 2.2 above), appear not to have been adequately defined or accurately distinguished and may have been inconsistently or incorrectly read and reported by the investigators. For example, pathology reviews of two of the studies (Caldwell et al., 1995; Argus Research Laboratories, 1998) used in the derivation of the RfD, report hypertrophy at relatively low doses. In contrast, the 90-day study (Springborn, 1998) makes no mention of thyroid follicular cell hypertrophy but reports only hyperplasia at the highest dose employed (10 mg/kg/day). In this study, the hyperplasia was described as "minimal in most cases with only an occasional thyroid with a severity grade of mild." Even at the high dose, the effect in the 90 day study was apparently "difficult to differentiate from increased activity or an 'active' thyroid." Despite the clearly different reports of hypertrophy and hyperplasia, Section 6 of the EPA document refers to all these effects as hyperplasia (see Table 6-1D). We recommend that the critical histopathological-based NOAELs and LOAELs in rows 1 and 3 of Table 6-1D be reconsidered pending resolution of the difference between hypertrophy and hyperplasia. As discussed later, it is also recommended that EPA consider reevaluating all of the rat studies by a pathology working group or a single pathologist with specific expertise in thyroid histopathology. Several discrepancies between EPA's report of these studies and the studies themselves are further described in Section 3 below.

2.4 Hyperplasia is a more biologically relevant adverse effect than the non-adverse effect of hypertrophy for the critical endpoint with which to determine the reference dose for perchlorate.

As discussed above, while it is appropriate for EPA to base the perchlorate RfD on a histopathological endpoint, the Agency may wish to consider using hyperplasia instead of hypertrophy as a critical endpoint. Thyroid hyperplasia was observed at 10 mg/kg/day at 14 days in the 90-day rat study. Hyperplasia is a reasonable basis for a NOAEL and LOAEL in that persistent, highly elevated hyperplasia in the rat can lead to thyroid follicular cell neoplasia, adenoma, or other adverse effects. It should be emphasized that the endpoint of hyperplasia in the rat model is still very conservative with respect to humans. This is due to the exquisite sensitivity of the rat to minor fluctuations in thyroid hormone and TSH levels compared to humans, and the much greater incidence of spontaneous thyroid tumor development in the rat (McClain, 1995). However, as described in Section 2.2 of this document, not all forms of hyperplasia (e.g., mild hyperplasia) are adverse. Determination of the severity of the hyperplasia should be based on the professional judgment of a pathologist who has had considerable experience with thyroid gland pathology.

3.0 ANALYSIS OF THE KEY STUDIES

Three major rat studies were available for consideration by EPA in its determination of appropriate endpoints for setting a reference dose for perchlorate:

A. Caldwell *et al.* (1995) 14-day study (and its EPA reanalysis)
B. Springborn Laboratories (1998) 90-day study
C. Argus Research Laboratories (1998) developmental neurobehavioral study

A discussion of these studies in terms of their relevance to the RfD determination is provided in this section.

3.1 The Caldwell *et al.* (1995) study

The Caldwell study was a 14-day study in which groups of rats (6/sex/treatment group) were administered ammonium perchlorate (AP) in drinking water at doses of 0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, or 24.86 mg/kg-day for females and 0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, or 22.16 mg/kg-day for males. After 14 days, rats were sacrificed and thyroid weights and thyroid hormone levels were measured. Although not published, thyroid histopathology was also evaluated.

The histopathology data from the Caldwell *et al.* (1995) study were used by EPA to support the critical effect driving the RfD used in the risk assessment. There are several problems associated with the Caldwell *et al.* (1995) study that make it of questionable value for supporting the selection of follicular cell hypertrophy as a critical effect. These problems include:

- The study's histopathology report (Eggers, 1995) provides no methodology or diagnostic criteria and contains errors. For example, the dose levels discussed throughout as mg/kg are, in fact, concentrations of AP in the water (mg/L x 0.1) administered to the rats.

- The Air Force memorandum to EPA (Channel, 1998) to which Eggers' narrative pathology report was attached also contains errors and inconsistencies.

In addition to the problems with the original study reports, EPA's interpretation of the Caldwell *et al.* (1995) study and associated histopathological evaluation (Channel, 1998; Eggers, 1995) provided on page 5-8 of the perchlorate document contains several errors that are repeated in EPA's risk assessment in Chapter 6.

- EPA states that the mean severity scores for decreased lumen size alone were statistically significantly different from controls above the 0.44/0.47 mg/kg-day group. This conclusion is at odds with the Channel (1998) memo which clearly states that the change in lumen area occurs at and above 2.3/3.1 mg/kg/day (males/females) which would result in a NOAEL for this parameter of 1.1/1.2 mg/kg/day. The EPA document states that the histopathology data identify a LOAEL at 0.11/0.12 mg/kg-day based on follicular epithelial cell hypertrophy. However, EPA provides no discussion or justification for this conclusion.
- Both Eggers (1995) and Channel (1998) report thyroid follicular epithelial cell hypertrophy. The criteria for making a finding of hypertrophy are not actually described, although in the data spreadsheets they are referred to as hypertrophy/hyperplasia. In the EPA perchlorate risk assessment document, the endpoint finding is correctly reported as hypertrophy on page 5-8. However, in Chapter 6, (see 6-2, lines 23-24) and in Table 6-1D (see footnote definition of SH-FCH), EPA refers to the histopathology findings as hyperplasia.

EPA's decision to use the results of the Caldwell *et al.* (1995) study, particularly the histopathological analysis, to support the critical effect used to derive the RfD used in the risk assessment is questionable. Additionally, we recommend that EPA revise the perchlorate review document to ensure that the study results are accurately reported in the review document.

3.2 Springborn Laboratory (1998), 90-Day study

The Springborn study was a 90-day study in which groups of rats (20 or 30/sex/treatment group) were administered ammonium perchlorate (AP) in drinking water at measured doses of 0, 0.01, 0.05, 0.2, 1.0 and 10.0 mg/kg-day. The study was well conducted.

Except for observations relating to thyroid function and structure, the findings were unremarkable with respect to treatment-related effects. Statistically significant changes in T3, T4, and TSH were observed at 14, 90 and 120 days. In general, these changes consisted of dose-related decreases in T4 and T3 and an increase in TSH although the actual values tended to be quite variable between sexes and at different times even after the 30-day recovery period. The reasons for this are not clear and may relate to the times during which they were measured in the diurnal cycle. Thyroid hormone levels vary considerably at different times of the day, and it is important to restrict comparisons to carefully synchronized control and treated samples.

Significant treatment-related increases in thyroid weight and histopathological changes (described as mild hyperplasia) were observed in both sexes, but only at the highest dose tested (10 mg/kg-day). The term follicular cell hyperplasia was employed "because of the increased number of small follicles in the central portion of the thyroid" (Springborn Laboratories, 1998). Even at the high dose, the effect was "difficult to differentiate from increased activity or an 'active' thyroid." Although significant weight and histopathological changes were observed at both 14 and 90 days, they returned to control levels at 120 days following the 30 day recovery period. Severity of effect appeared to be greater at the 14-day interval, implying a potential adaptation to continued exposure to the test material. Based on the histopathological effects observed, the Study Director assigned a NOAEL of 1.0 mg/kg-day for the oral administration of AP in drinking water. There is no mention of an increased incidence of thyroid follicular cell hypertrophy. However, it should be noted that the study pathologists' reporting criteria for hyperplasia includes some elements of both hypertrophy and hyperplasia.

In its reanalysis of the Springborn data, EPA concluded that the NOEL for decreased T3 and T4 was 0.01 mg/kg-day and that the NOEL for TSH was 0.05 mg/kg-day. Because these small changes in hormone levels were not considered adverse, these values were not used in the derivation of the RfD. The Agency appropriately concluded that the basis for a NOAEL should be the histopathological effect of hyperplasia. Because of the good quality of this study and the biological relevance of mild hyperplasia as a potential precursor to an adverse effect, EPA should consider using the 90-day study, rather than the neurodevelopmental study, as the basis for RfD development.

3.3 Argus Research Laboratories, Inc., 1998

The neurodevelopmental toxicity study of perchlorate did not show any unequivocal treatment-related evidence of developmental neurotoxicity. The study did not show evidence of increased susceptibility to pups for effects on the thyroid, based on the histopathological findings in the pups. These findings were remarkably consistent with the observations in young adult rats in the Caldwell 14-day study at similar dose levels (even though two different pathologists read these tissues, both were within the same institution potentially leading to more consistent reporting criteria). Hypertrophy was seen in both studies, but there was no evidence of hyperplasia. Comparison of pup and maternal thyroid hormone levels cannot be made in this study, because of a non-random maternal sampling design, and because sampling was done from too few dams per group. Since maternal thyroids in the developmental neurotoxicity study were evaluated by yet a third pathologist, comparison of effects in dams and pups should await histopathological re-evaluation of the thyroids using consistent evaluation criteria. There is also a need to resolve the significant difference in the results obtained by standard histology and morphometric techniques.

In its review of the developmental neurotoxicity study data, EPA reached two conclusions that do not appear to be supported by the underlying study data, and that are in conflict with the conclusions of the experienced investigators who performed this study. These include EPA's evaluation of the treatment-relationship of changes in the high dose groups in motor activity in the PND 14 pups and of

morphometric findings in a single brain region (corpus callosum in high dose female offspring). Further comments on these parameters will be submitted subsequently.

3.4 Conclusions regarding critical studies used.

There appear to be several inconsistencies in the histopathological examinations and reports in all of the relevant studies.

- There is apparent confusion between use of the terms hypertrophy and hyperplasia in both the study reports and in the EPA RfD document. This results, in part, because the studies do not provide diagnostic criteria for thyroid follicular cell hypertrophy and thyroid follicular cell hyperplasia.
- Multiple pathologists are involved and it is not clear that all used consistent criteria for the diagnosis. This is very important because this is the basis for the point of departure for establishing the RfD. The histopathology of the thyroid gland should be reevaluated by one pathologist with experience in rat thyroid gland histology using consistent criteria. The revaluation should be conducted under blind and randomized conditions.
- In the Argus and Caldwell studies there are apparent discrepancies between the results of the histological and morphometric analyses.

4.0 APPLICATION OF UNCERTAINTY FACTORS IN SETTING THE REFERENCE DOSE

4.1 The Subchronic-to-Chronic uncertainty factor of 1 is appropriate.

The UF of 1 is appropriate for the following reasons:

- The thyroid lesions and hormone changes in the rat pups in the neurodevelopmental study were shown to be reversible.
- Perchlorate has a relatively short half-life (USEPA, 1998).
- The thyroid histopathology and hormone perturbations observed in the Springborn study at 90 days were generally less severe and less frequent than those observed at 14 days, suggesting that the effects do not progress with continued exposure and may even diminish (Springborn, 1998). Additionally, reversibility was seen after 30 days without exposure.

4.2 If EPA continues to rely on hypertrophy as a critical effect, the uncertainty factor of 3 for extrapolating from a minimal LOAEL of 0.1 mg/kg-d to a NOAEL should be reduced to 1, since hypertrophy does not represent an adverse effect.

The selected critical effect is based on thyroid follicular cell hypertrophy in PND5 pups at 0.11 mg/kg/day. Since, as discussed, hypertrophy should not be considered an adverse effect, it is a highly conservative point of departure for the RfD and the UF should be reduced to 1. The basis for this conclusion is described in Section 2.4. If, on the other hand, hyperplasia is selected as the critical

endpoint on which to base the RfD, the comment would be moot because results of the 90-day study would supply a NOAEL and not a LOAEL to use in developing an RfD.

4.3. The uncertainty factor of 3 for interspecies uncertainty is conservative and may need further evaluation.

EPA retained the pharmacokinetic interspecies uncertainty factor of 3 due to the lack of pharmacokinetic parameters such as half life of perchlorate in rats *vs.* humans; dose of perchlorate at the symporter following administration of perchlorate; and ability of perchlorate to inhibit iodine uptake in the rat *vs.* the human. This factor warrants additional evaluation after pharmacokinetic data are available.

With respect to pharmacodynamics for interspecies uncertainty, EPA appropriately decided not to apply a UF because:

- There is a robust database indicating that that rats are considerably more sensitive to thyroid hormone imbalance than humans (Capen, 1997).
- Human blood contains thyroid binding proteins that have a much higher affinity for thyroid hormones than those in the rat blood. The reduced binding affinity in rat blood contributes to a significantly reduced half-life for circulating thyroid hormones in the rat (Capen, 1997).
- An elimination pathway for T4 involving conjugation with glucuronide is much more prominent in rats than it is in humans (Curran and DeGroot, 1991).
- The rat's physiology results in a significantly reduced buffering capacity as compared to humans, that accelerates and enhances the rat's response to minor fluctuations in thyroid hormones and subsequent increase in thyroid activity.
- There are also differences between rats and humans with respect to the neoplastic response to prolonged elevations of TSH. Prolonged exposures to elevated levels of TSH in rats are associated with neoplasia. However, epidemiological investigations of individuals with elevated TSH provide no evidence of neoplasia occurring by a similar mechanism in humans (Curran and DeGroot, 1991) although such elevations are associated with thyroid enlargement (DHHS, 1998).

The application of an UF of 3 to address differences in pharmacokinetics is justified at this time; however, since the pharmacodynamic differences between rats and humans would support an uncertainty factor of less than 1, the value of 3 for an overall interspecies UF is conservative and needs to be modified.

4.4 EPA should retain an uncertainty factor of 3 for database uncertainty until the remaining studies (two-generation reproductive study and immunotoxicology study) are completed. However, on receipt of the new data (assuming adverse effects are not indicated) this factor should be reduced to 1.

We support EPA's decision to apply an UF of 3 for database gaps (UF_D) based on the fact that both a two-generation reproductive study and immunotoxicity study are underway and have not yet produced definitive results. However, it is reasonable to assume that upon receipt of the new data this factor will be removed to reflect the completeness of the database, assuming adverse effects are not indicated at lower doses than those in the completed studies. Preliminary reports on the unfinished studies do not support any expectations of cause for concern (see Section 5.0 below). While it is unlikely from our review of the preliminary data that either reproductive or immunological endpoints will generate a lower RfD, we support EPA's use of an UF_D of 3 until all the data are received.

5.0 SIGNIFICANCE OF INCOMING STUDY DATA

5.1 Immunotoxicity

We agree with EPA's conclusion that immunological effects are likely to occur only at higher concentrations than those at which thyroid hormone and histology effects have been observed. Keil *et al.* (1998) performed a series of 14- and 90-day immunotoxicity studies in female mice. Immunotoxicity results were highly variable. With respect to decreased macrophage phagocytosis of *Listeria monocytogenes* (the most significant effect noted), one experiment identified a LOAEL of 0.1 mg/kg-day, a second identified a NOAEL of 1 mg/kg-day, and a third identified a LOAEL of 3 mg/kg/day, while in other experiments, no alteration in macrophage phagocytic activity was observed (USEPA, 1998). As described by EPA, one would expect a decrease in macrophage phagocytosis to result in decreased resistance to *L. monocytogenes* infection. However, instead, a trend toward *increased* resistance to *L. monocytogenes* was suggested by the currently available data, drawing into question the significance of the reported effect on macrophage phagocytosis. Therefore, a critical endpoint based on immunological effects from perchlorate appears to be less sensitive than the thyroid histopathological endpoint chosen.

5.2 Two-Generation Reproductive Study

We disagree with EPA's conclusion that the reproductive toxicity results as summarized in the EPA draft raise any concerns regarding potential effects of perchlorate on estrous cyclicity, mating, or fertility of the P_1 adults. The mating, fertility and estrous cycle data show no relation to perchlorate treatment, and fall well within the limits of normal biological variation of these parameters.

As noted above, however, there are still key data from this study that are not available, and we concur with EPA's conclusion that an additional uncertainty factor of three for an incomplete data base be retained until all the results are received and an audited report is available.

6.0 ECOLOGICAL EFFECTS DATA

EPA's analysis of the potential ecological effects of perchlorate raises a number of issues that deserve additional examination before conclusions can be reached. These include:

- EPA does not provide actual data on the environmental fate properties of perchlorate to support its determination that perchlorate is both mobile and persistent in the environment. Some of the data on effects in plants (different toxicity values in different soils) suggest that in fact perchlorate may bind to some extent to certain types of soils. It would be appropriate to collect some basic laboratory environmental fate data before concluding about mobility and persistence.
- EPA applies very large safety factors to the available data and then concludes that perchlorate concentrations at specific locales may exceed the resulting levels of concern. The justification for such large safety factors is not clear and should be reviewed. It would also be appropriate to collect environmental samples before concluding that levels of concern are likely to be exceeded. The very small number of available environmental samples also brings into question EPA's conclusion that adverse effects are likely for both individuals and populations of terrestrial herbivores. This conclusion appears to be premature.

7.0 Conclusions

On the basis of our review of the EPA perchlorate RfD document and the key underlying studies, we have reached the following conclusions:

- The studies completed to date demonstrate that the thyroid gland is the primary target organ for perchlorate.
- EPA usually bases a RfD on an adverse effect. EPA appropriately decided to base the perchlorate RfD on a histological rather than a hormonal endpoint. However, EPA uses a critical point of departure for the RfD, thyroid follicular cell hypertrophy, that is neither an adverse effect nor a precursor to an adverse effect.
- Hypertrophy is indicative of a slightly increased functional activity of the thyroid gland and should be regarded as an adaptive functional response to a need for increased thyroid hormone production. Consequently, hypertrophy should not be considered an adverse effect or a precursor of an adverse effect.
- It is more appropriate to use the diagnosis of hyperplasia as a critical point of departure for setting the RfD. Although hyperplasia *per se* is also an adaptive response, it is usually indicative of a change in thyroid gland function that can, under some circumstances, be considered adverse.
- The critical distinction between hypertrophy and hyperplasia has been lost in the review because of the lack of clarity and consistency in the histopathological data from the key studies used by the EPA. This resulted from the use of multiple pathologists in these studies, the absence of consistent diagnostic criteria and use of different rating scales among pathologists.
- Because of the apparent confusion between the terms hypertrophy and hyperplasia, we recommend that the histopathology data from the three key studies be reevaluated by one pathologist with experience in rat thyroid gland histology using consistent criteria.

- If hyperplasia had been selected as the critical effect, the current data suggest a NOAEL of 1.0 mg/kg/day (from the 90-day study) rather than the "minimal LOAEL" of 0.1 mg/kg/day for thyroid hypertrophy from the neurodevelopmental study.
- There are marked differences in thyroid gland physiology between rats and humans that make quantitative extrapolation difficult. EPA's selection of uncertainty factors were generally conservative. In its review of the existing and incoming study data, EPA should consider reducing, the interspecies and database uncertainty factors.
- A final decision on appropriate endpoint selection and uncertainty factors to be applied should be withheld until complete immunotoxicological and two-generation reproductive toxicity study data are available. Similarly, the available ecotoxicological data for perchlorate suggests the need for further environmental testing prior to the assessment of a level of concern.

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February 1, 1999

Ms. Susan Goldfarb
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Dear Ms. Goldfarb:

On behalf of the Perchlorate Study Group, *TERA* has reviewed EPA's document titled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information*. This letter transmits to the Peer Review Panel our comments on the scientific basis of the proposed reference dose. Overall, we wish to commend EPA on the quality of its review. Several issues may elicit differing scientific judgment, however, which warrant the consideration of the Peer Review Panel as it reviews the document.

Thank you for the opportunity to highlight these issues for the Peer Review Panel. If you need additional information, please feel free to contact me by phone at (513) 542-7475, or by email at dourson@tera.org.

Sincerely,

Michael L. Dourson

Michael L. Dourson, Ph.D, DABT
Director
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Comments on EPA's Choice of Critical Study and Data Set[1]

EPA has identified disturbance in thyroid homeostasis as the critical effect for perchlorate, and selected incidence of thyroid hypertrophy in pups culled on postnatal day 5 (PND 5) from the neurobehavioral developmental study (Argus 1998) as the data set that best represents the critical effect. However, we ask several questions regarding the reliability, validity, and use of these data and feel that answers are needed before these data are used as the basis of a RfD.

1. Was this part of the study designed properly to detect effects caused by *in utero* exposure?
2. Was the correct data and statistical analysis selected for the evaluation of PND 5 pups?
3. Were appropriate data used in the benchmark modeling that was used to confirm the dose level for the critical effect?

As EPA notes on page 6-12 of its report, any effects observed in PND 5 pups are likely to have been the result of *in utero* exposure. Since the effect in this case actually occurs in the female exposed to the chemical, the appropriate measure of response is the litter, not the individual offspring. While the main study design for the neurobehavioral developmental study evaluated one pup/sex/litter to control for litter effects, the histopathological analysis of the culled pups did not follow this design. All thyroids were collected by Argus Laboratories at culling, fixed in 10% neutral buffered formalin, and shipped to the Sponsor (AFRL/HEST).

Moreover, six thyroids per sex/dose group were selected for examination; selection of thyroids was accomplished by randomizing the samples from each treatment group using a computer program for randomization. Separate randomization was done for selection of males and females. First, it should be noted that there were 25 dams/group in the study, but thyroid histopathology from only 6 pups/sex/group was evaluated. This means that, for a given sex, less than 25% of the litters were sampled. EPA pooled the male and female data, improving the sampling of litters. However, in each dose group, between 30 and 60% of the combined male and female pups were littermates (York, personal communication). EPA appears to have taken the littermates into account by analyzing the data on a "per litter" basis. This meant that when a given litter was represented by *both* a male and a female, the average severity score of the male and the female were used as the litter score (Geller, USEPA, personal communication). Given the small sample size from each litter (1-2 animals/litter), is this an appropriate analysis method? Furthermore, given the small number of litters represented, is this analysis appropriate? We suggest that the panel evaluate whether enough litters were sampled to detect an effect caused by *in utero* exposure. In addition, we suggest that the panel consider what would be an adequate study design to address these issues.

As to the second question, in Section 5.2.3.2, EPA evaluates the thyroid histopathology results from the neurobehavioral developmental study. EPA notes (page 5-27, line 29-30) that the measurement of follicular epithelium cell height is a more sensitive indicator of thyroid effects than follicle diameter. In Appendix O of the Argus study report, Dr. William Baker describes two histopathology analyses of changes in cell height. In the first analysis, Dr. Baker conducted a subjective analysis of changes in cell height, ranking changes on a scale of 0 to 4. This is described in Appendix O (Results I) as follicular cell hypertrophy. In the second analysis

[1] All references are as per EPA Report or otherwise footnoted.

(reported in Appendix O under Results II), Dr. Baker quantitatively measured the height of follicular epithelium cells. Five follicles from each of three sections were measured. Results of the first (subjective) analysis indicate that there was no statistically significant increased incidence or severity of increased cell height in females. In males, there was a statistically significant increase in severity only at 10 mg/kg/day. Statistically significant increases in the number of males with follicular cell hypertrophy (i.e., subjective scoring of increased epithelial cell height) were observed at all doses. As reported in a personal communication from Dr. Baker, the follicular cell hypertrophy was subjectively scored as 0 (no change), 1 (minimal changes in general height), or 2 (mild changes in general height). No animals received a score of 3 (moderate), which would have included any increase in cell number or cell division. The incidence of severity scores for increased epithelial cell height in males, as provided by Dr. Baker (personal communication) is shown in Table 1.

Table 1. Severity scores for increased epithelial cell height from PND5 pups

Severity Score	0 mg/kg-day	0.1 mg/kg-day	1.0 mg/kg-day	3.0 mg/kg-day	10.0 mg/kg-day
Males					
0	5/6	1/6	2/6	1/6	0/6
1	1/6	4/6	3/6	4/6	2/6
2	0/6	1/6	1/6	1/6	4/6
Females					
0	4/6	3/6	1/6	3/6	0/6
1	1/6	2/6	2/6	2/6	5/6
2	1/6	1/6	3/6	1/6	1/6

This table shows that the incidence of males with either no or minimal changes in cell height (severity scores 0 or 1) remains essentially the same until the 10 mg/kg-day dose group. The incidence of animals with mild increased cell height (severity 2) also does not change until the 10 mg/kg-day dose group. Consistent with the results of the subjective analysis that there is no clear effect until 10 mg/kg/day, statistically significant increases in the quantitative measurement of follicular cell height were observed only in the 10 mg/kg-day dose group. (This interpretation is in contrast to EPA's interpretation of a disparity between the subjective and quantitative evaluations.) As was observed in the subjective assessment, there was no statistically significant effect on measured cell height in females. Therefore, should the panel conclude that the PND 5 pup data is valid and reliable enough to be used in the risk assessment, we suggest that the panel evaluate whether the histopathology data from these animals has been appropriately evaluated and whether the quantitative measurement of follicular height should be considered a more accurate indicator of changes in thyroid histopathology.

As to the third question, EPA used a benchmark dose analysis of the data from the subjective assessment in order to confirm that a LOAEL of 0.1 mg/kg-day was appropriate EPA evaluated and modeled the frequency of occurrence of severity ratings of 0.5 and up. Given that severity 1 was classified as "minimal," should average severity ratings of 0.5 or 1 be considered an appropriate basis for risk assessment, or should only severities of 2 or greater be considered? The study pathologist noted in a personal communication that hyperplasia would have been graded severity 3 ("moderate") or higher, if it had been seen, while hypertrophy would have been graded 1 or 2.

Comments on EPA's Choice of Uncertainty and Modifying Factors for the Perchlorate RfD

Human Variability (H) [Default Value is 10-Fold; EPA's choice is 3-Fold]

Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences among humans.

EPA chose a value of 3 for this factor (obtained by reducing the toxicodynamic factor to 1), because the animal model used is for a fetal effect and the adult human hormone homeostasis is likely to be more stable. We agree with EPA, and add that the use of this factor will also likely protect sensitive adult subgroups such as Graves' patients and people who are deficient in iodine intake (*TERA*, 1997). EPA's description of the overlap between this factor with the factor used for LOAEL to NOAEL is conceptually correct, but difficult to communicate. We suggest that the distinction be maintained between these two factors. We encourage EPA to review data on the current pharmaceutical use of perchlorate, and, if appropriate reevaluate the dose-response relationship from existing human studies as described in EPA's report (Chapter 3). Such an analysis was requested at a previous peer review meeting (*TERA*, 1998a). Moreover, two occupation studies are now available (Gibbs et al., 1998; Lamm et al., 1999[2]) which should be gleaned for any relevant information in this reevaluation of dose-response relationship. Please see a separate section of this comment (on use of human data) for more details.

Inter-Species Variability (A) [Default Value is 10-Fold; EPA's choice is 3-Fold]

Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences between animals and humans.

EPA chose a value of 3 for this factor (obtained by reducing the toxicodynamic factor to 1), because rats are known to be more sensitive than humans to thyroid disturbance. We agree with EPA's initial choice, but encourage EPA to analyze existing comparative data on rats and humans to quantify the rat's greater sensitivity to the iodide blocking effects of perchlorate. Sufficient human and rat data now exist on which to make a tentative assessment of this sensitivity. For example, The data of Meyer (1998) found on page 5-62 of EPA's report, could be compared to the data of Burgi et al. (1974). Moreover, the report of Sterner and Mattie (1998)[3] gives information on the perchlorate discharge tests in humans and rats. These data should be compared as well. Such an analysis was requested at a previous peer review (*TERA*, 1998a).

Subchronic-to-Chronic Extrapolation (S) [Default Value is 10-Fold; EPA's choice is 1-Fold]

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[2] Lamm, S.H., L.E. Braverman, F. Xiao, K. Richman, S Pino and G. Howearth. 1999. Thyroid health status of ammonium perchlorate workers: A cross-sectional occupational health study. Accepted for publication in J Occup and Environ Medicine.

[3] Sterner, T.R. and D.R. Mattie. 1998. Perchlorate literature review and summary: Developmental effects, metabolism, receptor kinetics and pharmacological uses. AFRL-HE-WP-TR-1998-0106.

EPA chose a value of 1 for this factor, because the mode of action of perchlorate indicates that if the current critical effect is maintained then it will be protective of downstream events. EPA supported this choice of a 1-fold factor with the fact of the relatively short half-life of perchlorate in the body. We agree with EPA's choice of factor for the reasons stated.

Insufficient Database (D) [Default Value is 10-Fold; EPA's choice is 3-Fold]

EPA chose a value of 3 for this factor, because of the data outstanding from the 2-generation reproductive and immunotoxicity studies. We agree with EPA's choice, and also agree with EPA that appropriate data from these two studies, when finalized, will likely reduce the need for this factor in the future.

LOAEL to NOAEL (L) Extrapolation [Default Value is 10-Fold; EPA's choice is 3-Fold]

EPA chose a value of 3 for this factor for perchlorate, because the critical effect and its LOAEL represent minimal toxicity. In fact, as EPA suggests, an argument could be maintained that the critical effect represents a normal homeostatic response to the decrease in T3 & T4 and increase TSH, and should therefore be considered a NOAEL. As one way forward, we encourage EPA to ask experts in thyroid disturbance whether this effect should be consider adverse, or whether it represents part of the normal homeostatic response of the rat (see a later section of these comments for more details). If experts agree that EPA's suggested critical effect represents homeostasis, then a different effect could be selected, or this dose could be considered a NOAEL, which would obviate the need for this factor.

Modifying Factor (MF) [Default is 1-Fold; EPA's choice is 1-Fold]

EPA considers a default value of 1 as appropriate for a modifying factor for perchlorate. This is because the outstanding uncertainties for perchlorate can be adequately addressed with the standard factors. We agree with EPA on the use of 1-fold for this factor.

Composite Uncertainty and Modifying Factors [Each Data Base Has Its Own; EPA's Choice for Perchlorate is 100]

EPA recommends an overall uncertainty factor of 100 for perchlorate. We agree with EPA's initial recommendation, but anticipate that the inclusion of the complete 2 generation reproductive and immunotoxicity studies in the perchlorate database will likely reduce the composite factor of 100 to 30-fold. This is because the data base uncertainty factor of 3 will likely not be necessary after the new studies are finished. Moreover, if thyroid experts suggest a different critical effect as the basis of the RfD, or if an analysis of existing comparative data on rats and humans yield a firm quantitative value, the issue of uncertainty factors may need to be revisited.

Comment of EPA's Use of Human Data in Hazard Characterization

Although the EPA assessment presents information on some of the available human data, these data do not greatly contribute to EPA's hazard characterization of perchlorate. We suggest that the available human data should be reanalyzed. This would lead to a better understanding of the perchlorate dose-response curve in humans, and to a clearer description of the relative sensitivity of rats and humans to perchlorate. Such an analysis was suggested at a previous peer review meeting (*TERA*, 1998a).

Several types of human data are available for consideration of the perchlorate RfD; some of these data are new. These studies include a small epidemiology study (Lamm Doemland; 1999[4]---new), occupational exposure of perchlorate workers (Gibb et al, 1998; Lamm et al., 1999---new)) experimental dosing of normal human volunteers Burgi et al, 1974; Brabant et al., 1992), and treatment of Graves' disease patients with perchlorate (section 3.1 of EPA report). Human studies are also available on use of perchlorate discharge tests in infants as a diagnostic tool (Sterner and Mattie, 1998---not cited in EPA report), which may prove to be very useful in the comparison with animal perchlorate discharge tests. Treatment of patients given the heart drug amioderone (Sterner and Mattie, 1998) are also available, but we feel that the use of these latter data to determine and RfD is problematic. We very briefly describe several of these studies below and in Table 2.

Lamm and Doemland (1999) conducted an analysis to determine whether the perchlorate-containing drinking water increases the risk of congenital hypothyroidism. Perchlorate was detected in the drinking water supplies of seven counties in California and Nevada at levels of 4-16 ug/L in 1997. The data from the neonatal screening programs of the state health departments were analyzed for incidence of congenital hypothyroidism in those counties in 1996-1997. Total 700,000 newborns were screened during this period. Two hundred and forty-nine cases were identified in these newborns while 243 were expected based on the state-wide incidence. The risk ratio is 1.02 with 95% confidence limits of 0.9-1.2. Studies such as this can assist the determination of whether an increase of perchlorate-induced thyroid problems is evident. They are much more limited in assisting in the determination of a RfD, however.

Gibbs et al. (1998) conducted a cross-section occupational epidemiology study to evaluate thyroid, liver, kidney, and bone marrow function of workers at an ammonium perchlorate production facility. The study examined the health effects due to either single-shift exposure or working lifetime exposure. A total of 101 workers participated in the single shift study. Among them, 18 workers were exposed to ammonium perchlorate at doses ranging from 0.0002 to 0.436 mg/kg-day with an average of 0.036 mg/kg-day, and 83 workers who were never exposed to perchlorate were used as controls. The thyroid function measured before and after the work shift by T4, T3 resin uptake, TSH and free T4 index was analyzed. The estimated exposure was not a significant predictor of the cross-shift change in any of the thyroid parameters. The only significant finding in this study was cross-shift TSH changes that were greater for those who worked 12-hour shifts than for those who worked 8-hour shifts.

[4] Lamm, S.H. and M. Doemland. 1999. Has perchlorate in drinking water increased the rate of congenital hypothyroidism? Submitted to J Occup and Environ Medicine.

In the working lifetime study (Gibbs et al. 1998), 66 workers in low dose group exposed to perchlorate at doses ranged from 0.5 to 7.0 mg/kg (mean = 3.5), and 108 workers in high dose group exposed to doses ranged from 8.0 to 88 mg/kg (mean = 38). One hundred and ninety-two workers who never exposed to perchlorate were used as controls. Duration of these workers' exposure ranged from 1 to 27 years with an average of 8.3 years. The thyroid functional parameters as mentioned above were analyzed. Additionally, standard clinical blood test parameters of liver, kidney, and bone marrow function were evaluated to determine effects of chronic perchlorate exposure on these organs. The study shows that chronic perchlorate exposure did not have effects on thyroid, liver, kidney, or bone marrow functions.

EPA quite correctly pointed out several difficulties in the use of the Gibbs et al. (1998) study for risk assessment. The recent study of Lamm et al. (1999), however, may help resolve some of these difficulties and we feel that EPA should reconsider the use of the Gibbs et al work.

Moreover, Lamm et al. (1999) conducted a cross-sectional study to investigate thyroid health status of ammonium perchlorate workers. Thirty-seven employees from an ammonium perchlorate production plant and 21 from a sodium azide production plant (served as a control) participated in this study. The exposure workers was divided into 3 groups with average exposure levels at 0.33, 6.5, and 59 mg/day perchlorate in total airborne particulates while control group exposed to only 0.01 mg/day. Urinary perchlorate measurements indicated that workers in the exposed groups absorbed 4, 11 and 34 mg perchlorate per day while control workers absorbed 1 mg perchlorate. Thyroid function was evaluated based on clinical examinations and measurements of serum TSH, FTI, T4, thyroid hormone binding ratio (THBR), or TPO antibodies. No difference in the thyroid function was observed within the four groups. In addition, there was no evidence of hematotoxicity measured as blood cell count in these groups. Thus, this study found no evidence of an adverse effect of perchlorate exposure on thyroid status among workers. We feel that EPA should consider this study in the determination of an RfD.

EPA describes the following three studies, but did not use them directly in the determination of an RfD. 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. 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. 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.

These 3 studies by themselves are not strong enough to form the basis of a RfD (*TERA*, 1998a). However, they may help contribute to an understanding of the potential dose response curve in humans in conjunction with the occupational studies shown above. Furthermore, we believe that

data from these three studies might be compared with data from Meyer (1998) or Alterwill et al. (1987) in rats to ascertain whether an uncertainty factor of 3 for toxicokinetics should be replaced with data. If the human and rat data are not strictly comparable, then a generalization might be possible to allow a different choice of uncertainty factor between rats and humans.

- Sterner and Mattie (1998) also describe a series of studies on perchlorate discharge tests in humans and rats. The information in humans encompasses a number of conditions and includes children. EPA may wish to compare some of these studies to the information found in Meyers (1998) and Atterwill et al. (1987). Such a comparison may allow the use of a different uncertainty factor for animal to human variability.

Comment on Evaluation of Thyroid Homeostasis and Choice of Critical Effect

EPA has proposed a model for perchlorate mode of action that shows a sequential progression of effects (Figure 6-1). However, maintenance of normal thyroid function is the result of a series of feedback mechanisms, and adverse effects develop after the normal homeostatic capability of the thyroid has been exceeded. An alternative model is proposed in Figure 1. No change in a single thyroid parameter is indicative of adverse thyroid effects. Rather the degree of change, and the interaction of all different thyroid effects, including hormone level alterations, thyroid histopathology, and thyroid weights, should be evaluated to identify the perchlorate dose which results in animals moving from normal homeostasis into an altered function. Therefore we suggest that the panel establish criteria for distinguishing thyroid homeostasis from thyroid adversity. Some suggested criteria include focusing on changes in T4 as reflective of thyroid activity more than T3 because many other factors affect T3, and focusing on thyroid histopathology only when it is accompanied by changes in TSH at the same dose levels (Capen, personal communication).

For example, in all of the studies evaluated, EPA has designated statistically significant changes in hormone levels as LOAELs. However, since thyroid hormone levels can be widely variable due to homeostasis, it is important to evaluate the biological significance of altered hormone levels in addition to statistical significance. A slight, but statistically significant, decrease in hormone levels could have no biological consequence at all. Therefore, it is important to compare the dose levels that result in slight hormone changes with those that demonstrate alterations to the thyroid gland. Looking at the database as a whole, a common pattern is observed (e.g., see Figure EPA 5-9). Statistically significant changes in hormone levels occur at the lower doses, but then hormone levels remain constant over a range of doses up to 100-fold higher. At doses higher than 1 mg/kg-day hormone levels change again and at this point, thyroid histopathology and increased thyroid weight is observed. This pattern suggests that between the doses of 0.01 and 1 mg/kg-day, perchlorate is having an effect, but within this dose range the thyroid is able to maintain normal homeostasis. Conversely, alterations to the thyroid gland in the absence of hormone changes are not likely to be due to perchlorate exposure. In the neurobehavioral developmental study, hypertrophy/hyperplasia is observed in the pups at postnatal day 5 at the lowest dose (0.1 mg/kg-day). However, T3 was not statistically decreased in these animals until 0.3 mg/kg-day, T4 was not statistically decreased until 3 mg/kg-day, and

TSH was not statistically increased until 10 mg/kg-day. Are the effects on thyroid histopathology likely to be the result of altered thyroid hormones?

Table 2. Selection of Available Human Studies Ordered (Roughly) by Increasing Dose.

Study	Subject	Dose (mg/kg-day)	Duration	Response	Comments
Lamm and Doemland 1999	Newborn babies (n=700,000)	0.004-0.016 mg/L in drinking water (0.00011-0.00046)[a]	Pregnancy	No increase in incidence of congenital hypothyroidism	Incidence analysis
Gibbs et al. 1998	Perchlorate workers *Single shift study* (n=83 in control group, n=18 in exposed group) *Lifetime study* (n=192 in control group, n=174 in exposed group)	0.036 Low cum. dose avg.=3.5 mg/kg, high cum. dose avg.=38 mg/kg	One work shift (8 or 12 hours) Avg. exposure =8.3 years	Greater cross-shift TSH changes in 12-hour shift workers than 8-hour shift workers. No change in other thyroid functions No significant effect on thyroid, bone marrow, liver, or kidney function	Cross-sectional study
Lamm et al. 1999	Perchlorate workers (n=21 in control group, n=37 in exposed group)	0.01-59 mg/day in airborne particulate (absorbed doses of 0.014-0.486)[b]	12 h/day, 3 days/week for >5 years	No changes in thyroid function and clinical examination	Cross-sectional study
Stanbury and Wynyaarden 1952	Graves' disease patients (n=8)	3 mg -500 mg $KClO_4$ (0.04 - 7.14)[b]	Once	*Thyroid I release:* 10 mg: partial (40-45%) decrease of thyroid ^{131}I count; 100 mg: ^{131}I count decreased to control level (measured at thigh); *Thyroid I uptake inhibition:* 100 mg: inhibited ^{131}I accumulation	Measure radioactivity (I^{131}) in thyroid
Burgi et al. 1974	Healthy humans (n=5)	3 x 200 mg perchlorate (9.7)[c]	8 days	Increase in non T_4 bound I secretion from 40 ug/24 h (control) to 66 ug/4 h (perchlorate)	Double labeling (^{125}I and ^{131}I) and measure radioactivity in blood.
Branbant et al. 1992	Healthy humans (n=5)	3 x 300 mg (12.9)[b]	4 weeks	Decrease thyroid I from 4 mmol/ml (before treatment) to 3.0 mmol/ml (after perchlorate); decrease in blood TSH and increase in blood thyroglobulin	Measure thyroid (radioactivity) Measure blood hormone

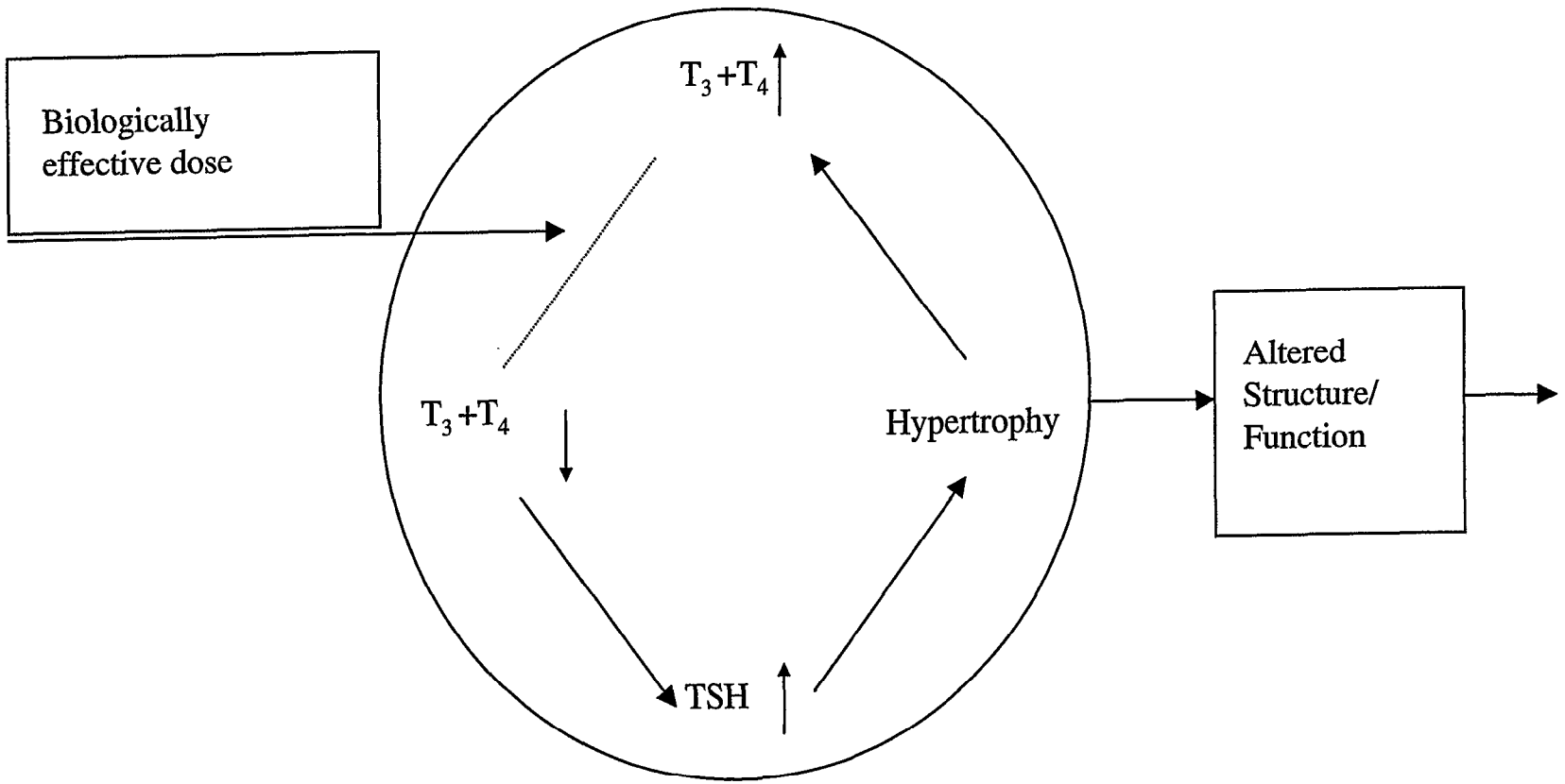
Estimated value(s) based on:

a. default 2 liter water consumption per day and adult body weight of 70 kg;

b. a default body weight of 70 kg for an adult;

c. the average body weight of 61.8kg reported by authors;

Early Biological Homeostasis



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Comments on the December 31, 1998, External Review Draft of the EPA/NCEA Document, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information"

Submitted to the External Peer-Reviewers

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February 1, 1999

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I. Introduction

The External Review Draft[1] describes the derivation of a revised RfD for perchlorate based on animal data. In Section II of this commentary, the derivation of this revised RfD is critiqued and suggestions are offered for a more physiologically integrated approach to using the animal data as the basis of the RfD.

The RfD derivation presented in the External Review Draft is essentially devoid of reference to available information on safe levels of human exposure to perchlorate. More to the point, additional human data are currently being gathered; these address the effects of short-term perchlorate exposures on thyroidal uptake of iodide, serum levels of thyroid hormones, and the kinetics of perchlorate elimination. Preliminary results are expected within the month, and final results a few months later. Section III briefly suggests how the human data could be utilized to ensure greater relevance to humans in the derivation of a revised RfD.

II. Animal Data As the Basis of the RfD

A. Choice of the Critical Study and Critical Endpoint

In the External Review Draft, EPA/NCEA evaluated the results of six new, animal-toxicity bioassays. Final study reports were available for four of these (Caldwell *et al.*'s 14-day study in rats, Springborn Laboratories' 14-day and 90-day study in rats, a neurodevelopmental study in rats, and a developmental toxicity study in rabbits), while only incomplete study results were available for the other two (a two-generation reproductive study in rats and an immunotoxicity study in mice). Based upon the results of the new studies, including some reanalyses of raw data by EPA/NCEA, critical effects were chosen and a principal study was identified. According to the External Review Draft (p. 6-2), "the overwhelming weight of the evidence from these studies support[s] the use of the hormone and thyroid histology evidence as the choice for critical effects." The critical study chosen was the neurodevelopmental study. The dose-response for follicular hypertrophy and decreased lumen size in postnatal-day-5 (PND5) pups (as revealed by standard histopathology techniques) formed the basis of the RfD derivation. To quantify the dose-response for these endpoints, EPA/NCEA performed contingency-table analyses that allowed severity and incidence to be considered together. For both sexes combined, EPA/NCEA found that the lowest dose tested, 0.1 mg/kg-day, produced a statistically significant increase in the incidence and severity of follicular-cell hypertrophy (*i.e.*, increased cellular height and/or diameter) and decreased size of the follicular-cell lumen. Based on these results, the lowest-observed-adverse-effect level (LOAEL) for the critical effect in the principal study was determined to be 0.1 mg/kg-day.

EPA/NCEA has made a good case for focusing on the rat neurodevelopment study, particularly the PND5 data, above all other animal data considered. The new animal bioassays support the premise that the thyroid is the most sensitive target organ. Furthermore, rats exposed to perchlorate *in utero* are, as expected, more susceptible to changes in follicular-cell morphology than are weanling rats exposed for 14 or 90 days. The question remains, however, whether such changes are adverse in and of themselves. Are these changes merely histologically identifiable or are they indicative of a pathological process?

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[1] "External Review Draft" is used herein as an abbreviation for the EPA/NCEA/ORD document, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information."

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B. Physiological Significance of Follicular Hypertrophy and Decreased Lumen Size

EPA/NCEA has argued, persuasively, that an exposure which produces transient effects of no toxicological significance to the adult animal might, on the other hand, produce permanent damage to the fetus or neonate. If exposure to a substance at a given dose produces irreversible histopathological changes, then it would be reasonable to conclude that a toxic threshold has been exceeded. However, no evidence for a permanent lesion has been found in rat pups exposed *in utero* and *via* breast milk to a maternal perchlorate dose as high as 10 mg/kg-day. The increased incidence of follicular-cell hypertrophy observed on PND5 in the 0.1 and 1.0 mg/kg-day dose groups was no longer evident in pups sacrificed on PND10. In pups with dosing discontinued on PND10, levels measured on PND22 were elevated relative to control levels only in the 1.0 mg/kg-day group; however, the statistical significance was not given and the absence of an effect in the 3.0 and 10 mg/kg-day groups argues against the relevance of this elevation. Table 1 shows the incidence data as given in Tables 5-3 (p. 5-29) and 5-4 (p. 5-30) of the External Review Draft.

EPA/NCEA has classified increased follicular-cell size (hypertrophy) and decreased follicular-cell lumen size as adverse effects. Indeed, there might be circumstances when the histological findings in question are corollary to an underlying pathology. But EPA/NCEA has not provided convincing evidence that such cellular changes, in and of themselves, fall outside the realm of physiologically normal thyroid morphology.

Table 1. Incidence Ratio of Any Evidence of Follicular-Epithelial-Cell Hypertrophy Among Rat Pups in the 1998 Neurodevelopmental Study of Perchlorate by Argus Laboratories, as Determined by Standard Histology

		Perchlorate Dose to the Dams (mg/kg-day)			
Time of Sacrifice	**Control**	**0.10**	**1.0**	**3.0**	**10**
PND5	0.25 (3/12)	0.67 (8/12)	0.75 (9/12)	0.67 (8/12)	1.00 (12/12)
PND10	0.40[a]	0.40[a]	0.40[a]	1.00[a]	1.00[a]
PND22[b]	0.52[a]	0.48[a]	0.68[a]	0.52[a]	0.48[a]

[a] The External Review Draft did not provide the absolute number of animals examined.
[b] Perchlorate exposure discontinued on PND10.

C. Correlation Analyses (Figures 6-3 to 6-16 of the External Review Draft)

In the External Review Draft, EPA develops a toxicologic mode-of-action model "proposed to map the relationships between external dose, internal dose, the biologically effective dose, and altered structural and functional parameters of established relevance to risk assessment" (p. 6-10.) Asserting that "the earliest biological effect, changes in thyroid and pituitary hormones" is the precursor for potential adverse outcomes, EPA notes, "The difficulty in designating an effect level for these perturbations, however, was in the degree of change to designate as adverse." Thyroid hormone/thyroid histology correlation analyses were used to "further support the mode-of-action mapping" model (p. 6-12). The analyses consist of the paired comparisons outlined in Table 1. EPA's hypothesis is that positive correlations for T3 *vs.* T4 and negative correlations for T3 (or T4)

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vs. TSH "are expected if these perturbations are affecting thyroid economy." The hypothesis is extended further: "Positive correlations between TSH and thyroid histopathology are expected, whereas T3 or T4 would be correlated negatively (inversely) with thyroid histopathology." Note that the use of the word "histopathology" in this context suggests the unwarranted assumption that the observed histological changes are indeed pathological in nature.

There is no doubt that, in most cases, the analyses revealed statistically robust correlations of the expected sign. The question is whether the results were skewed in favor of the relationships corresponding to higher perchlorate doses. In other words, would the same correlations be found if only the parameter values corresponding to perchlorate doses of, say, 1 mg/kg-day and below were included in the analyses? In the absence of access to the original data, much can be gleaned by visual inspection of figures 6-3 to 6-16. For T3 *vs.* T4, a positive correlation seems to be present even at the lower perchlorate doses. For T4 *vs.* TSH, there is evidence for and against a negative correlation. For the rank order of T4 or TSH *vs.* histopathological severity, it is doubtful that any statistically meaningful correlation would be found at lower doses.

- In Caldwell *et al.*'s 14-day rat study, even if doses above 1.1 mg/kg-day are ignored, an inverse relationship between T4 and TSH is evident upon visual inspection of Fig. 6-3 (p. 6-15). However, this finding contradicts the results of Springborn Laboratories for rats exposed likewise for 14 days (Fig. 6-8, p. 6-20) or of Argus Laboratories for PND5 rats following exposure *in utero* and *via* breast milk (Fig. 6-12, p. 6-24). In the latter two studies, removal of the 10 mg/kg-day data (leaving 1.0 mg/kg-day as the highest dose) would yield a *positive* relationship between T4 and TSH, *i.e.*, opposite to what the EPA/NCEA model predicts. For the remaining analyses of T4 *vs.* TSH (Figs. 6-6, 6-10, and 6-15, pp. 6-18, 6-22, and 6-27), visual inspection of the figures is insufficient to allow firm prediction of the consequences of removal of the data corresponding to perchlorate doses above 1 mg/kg-day; for these analyses, direct analysis of the raw data would be required.
- With respect to the analyses of T4 or TSH rank-order *vs.* severity classification for hypertrophy or decreased lumen size (as determined by standard histopathology), visual inspection of Caldwell *et al.*'s 14-day rat data (Figs. 6-4 and 6-5, pp. 6-16 and 6-17) does not allow prediction of the consequences of removal of the data for higher perchlorate doses. However, visual inspection of Springborn Laboratories' 14-day/90-day combined (Fig. 6-7, p. 6-19) or 14-day (Fig. 6-9, p. 6-21) data in rats indicates that, in both cases, the correlations are clearly dependent on the high-dose group (100 or 10 mg/kg-day). Thus, over perchlorate dose ranges of 0 to 30 mg/kg-day for the 14-day/90-day combined data and 0 to 1mg/kg-day for the 14-day data there appears to be no correlation of the severity of hypertrophy or decreased lumen size with the rank order of either T4 or TSH. To definitely confirm (or disprove) this assertion, one would have to perform sequential statistical analyses: removing the top dose, then the next highest dose, and so on, to see what remains of the relationship between T4 or TSH rank order and follicular-cell morphology after each round of data removal. Performance of this task would require access to the raw data.

If there is no correlation between the thyroid hormones T4 or TSH and thyroid histopathology (follicular hypertrophy or decreased lumen size) at perchlorate doses of 1.0 mg/kg-day and below, it is not useful to argue that such correlations support a model in which thyroid hormone changes *of any magnitude* are considered to be potentially causal to histological changes. It is far more likely that, even in an animal as sensitive as the rat, some fluctuations in T3, T4, and TSH are tolerated without consequent alteration of follicular-cell morphology.

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D. Historical Control Data on Serum T3 and T4 in Rats

In the External Review Draft, EPA/NCEA examines the relationship between perchlorate dose and thyroid hormone levels. Although statistical analysis of the dose-response is straightforward, lingering questions remain concerning the physiological significance of small, statistically significant changes. One means of addressing such concerns is to examine the historical data base for thyroid hormones in the control groups of animal bioassays. If a given dose of perchlorate produces a change that falls within the normal range of thyroid-hormone levels seen in animal-bioassay control groups, it would seem unreasonable to argue that such a response is part of a continuum leading to adverse effects.

Using data from bioassays sponsored by the National Toxicology Program (NTP), Dr. Greg Travlos of the National Institutes of Environmental Health Sciences (NIEHS) has assembled a data base of blood-chemistry parameter values found in control animals at the 13-week sacrifice. After excluding data considered by Dr. Travlos to be invalid or unreliable, there were data from thirteen bioassays for T3 and sixteen for T4. At this time, the entire data base on TSH is considered to be unreliable, either because of standardization issues, measurement errors, reporting errors, or some combination thereof (Dr. Greg Travlos, personal communication).

Figures 1 and 2 present mean values of serum total T3 and T4 in control-group rats at the 13-week termination point in NTP-sponsored studies. Also shown in Figures 1 and 2 are serum total T3 and T4 levels measured in the 90-day, drinking-water exposure study of perchlorate in rats. Note that mean serum T3 levels were higher in both male and female perchlorate controls than in any of the NTP control groups. Mean serum T4 levels in the perchlorate controls of both sexes were in the middle of the range defined by the NTP control groups. Up to the highest perchlorate dose tested, 10 mg/kg-day, serum T3 and T4 levels were well within the ranges defined by the means of the NTP control groups.

Possible inter-strain differences in T3 levels cannot be excluded, although any such difference are unlikely to be of consequence. With respect to T4 levels, the available data do not support the hypothesis that inter-strain differences are significant. Sprague-Dawley rats were used in the 90-day, drinking-water exposure study of perchlorate, while Fisher-344 (F344) rats were used in all NTP studies reporting T3 levels and in fourteen of the studies reporting T4 levels; Sprague-Dawley rats were used in the other two. The latter two studies reported mean serum T4 levels of 3.7 and 3.9 μg/dl in males and 4.7 and 5.0 μg/dl in females. T4 levels in the NTP Sprague-Dawley males fell at the low end of the NTP range, in between the levels observed for the 1 and 10 mg/kg-day perchlorate groups. By contrast, T4 levels in the NTP Sprague-Dawley females were near the middle of the NTP range, above the level observed in the zero-dose perchlorate group. These observations on the two NTP Sprague-Dawley control groups suggest that this rat strain is likely to demonstrate patterns of T3 and T4 variability similar to those observed for the larger, F344 rat-dominated NTP control data base.

In summary, the historical data base provides evidence *against* the assumption that in rats treated for 90 days with drinking water containing perchlorate at levels as high as 10 mg/kg-day, the resultant depression in T3 and T4 should be considered as physiologically abnormal or adverse.

E. Motor Activity Measurement in the Neurodevelopmental Study

It should be kept in mind that, as indicated on p. 5-37 of the External Review Draft, the non-significant increase in motor activity on PND14 in the pups of dams receiving the lowest-dose, 0.1 mg/kg-day, occurred "in only one gender on only 1 day out of 4 test days" and that "the effect seen

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in the males on PND14 may indeed be a Type I error and would not be found again if this experiment was repeated." It is reasonable for EPA/NCEA to request that the trial be performed again, but the lack of dose-response observed among the 0, 0.1 and 1.0 mg/kg-day groups indicates that the most likely outcome is no effect of treatment at doses below 3 mg/kg-day.

F. Recommendations for a Critical NOAEL Based on the Animal Data

1. Increased Size of the Corpus Callosum on PND12

Although the thyroid is certainly the principal target organ for the actions of perchlorate, it is perhaps a mistake to conclude that the critical effect can be found by looking at the thyroid or thyroid hormones directly. The thyroid apparently functions quite well (*i.e.*, in an adaptive fashion) in weanling rats given perchlorate for 90 days at doses up to 10 mg/kg-day, the highest dose tested; this is true also for the PND5 pups of rat dams given perchlorate at 10 mg/kg-day and below. In the latter (neurodevelopmental) study, recovery from increased thyroid follicular-cell hypertrophy in the 10 mg/kg-day group occurred by PND22 when perchlorate treatment was stopped on PND10 (see Table 1, above). From Figs. 5-11 and 5-12 of the External Review Draft (pp. 5-33 and 5-34) one can see that in PND5 rats, T3 and T4 were decreased approximately 60% and 30%, respectively, at a perchlorate dose of 3 mg/kg-day, while TSH was increased approximately 20% at a perchlorate dose of 10 mg/kg-day (Fig. 5-13, p. 5-35). It is conceivable that some developing organs might be affected by thyroid hormone changes of this magnitude during development. For example, it is possible that the 27% increase in the size of the corpus callosum in males and females combined (observed on PND12 in the pups of rats given perchlorate at 10 mg/kg-day) is secondary to altered thyroid hormone levels. Although the significance of this finding is unclear, it seems reasonable that "EPA considers a 27% increase in the size of any brain region to be a potentially adverse effect [ref.]" (p. 5-26). The LOAEL and NOAEL derived by EPA/NCEA from this study, 10 mg/kg-day and 3 mg/kg-day, respectively, constitute an appropriate departure for deriving an RfD.

Note, however, that Argus Laboratories' neurodevelopmental study is a poor model for the effects of chronic perchlorate administration on the developing fetus. Perchlorate exposure began on GD0, thus ensuring that the rat dams (and thus their embryonic fetuses) experienced the shock of shifting serum levels of thyroidal hormones at a very critical time. If the dams had begun perchlorate treatment several weeks prior to mating, it seems likely that the changes in thyroid hormone levels, thyroid histology, and corpus-callosum size observed on PND5 would have been significantly muted, perhaps to the point of insignificance. In this context, it is interesting that one study has found that the fetal rat brain is able to maintain T3 homeostasis to a greater extent than other fetal tissues under the stress of variations in the maternal supply of iodine, T3, or T4 (Morreale de Escobar *et al.*, 1992).

2. Immunotoxicity Results in Mice

The results of the ongoing immunotoxicity study should be evaluated carefully before proceeding with the derivation of a new RfD. The results on macrophage phagocytosis are too inconsistent and too transitory to form the basis of an RfD. According to the External Review Draft, results from a test of humoral immunity (antibody response to SRBC antigen) are anticipated by the date of the peer-review workshop, while repeats of several tests of host resistance (to *L. monocytogenes* and B6F10 tumor cells) are not expected to be complete before June 1999.

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III. Utilizing Human Data and Rat/Human Comparisons in the RfD Derivation

A. Ongoing Exposure Studies

One flaw of the External Review Draft is its failure to allow for existing health effects data gathered in occupational and clinical studies of perchlorate exposures. A more important flaw is that the revised RfD was derived during the time that two pertinent exposure studies were in the planning stages (or had been initiated). The Air Force Research Laboratory (AFRL) is currently conducting single-dose and 14-day exposure studies of the kinetics of perchlorate inhibition of thyroidal iodide uptake in rats. Dr. Lewis Braverman of Brigham and Women's Hospital, a well-known expert on the human thyroid, is in the process of conducting an exposure study in human volunteers. The Braverman study is examining thyroidal iodide uptake, serum levels of thyroid hormones, and the elimination kinetics of perchlorate at doses considerably lower than those hitherto tested in humans. Because perchlorate appears to exhibit nonlinear elimination kinetics, it is important to obtain animal and human toxicity health-effects data at doses as close as possible to the environmentally relevant range of exposures (Goodman, 1998). It is also important to be able to compare the inhibition of iodide uptake and the kinetics of perchlorate elimination in rats with those in humans. The data gathered by AFRL and Dr. Braverman should facilitate this comparison. The AFRL studies in rats and the Braverman study in humans are expected to conclude their data-gathering phases in February. The scientific basis of the revised RfD for perchlorate would be greatly strengthened by delaying the risk assessment process until the data from these studies can be taken into consideration.

B. Thyroid Homeostasis

It is well known that humans are able to maintain normal thyroid function over a wide range of iodine intake. This manifests, for example, in a narrower distribution of T4 values in normal humans than in control rats. In a study of 115 nongoitrous, Greek subjects exhibiting a broad spectrum of iodine intakes (*i.e.*, with urinary I/creatinine ratios varying from <50 to >250 μg/g), nearly all serum T4 values were in the 110 to 130 nM range (Moulopoulos *et al.*, 1988). This yields an estimated human T4 variation of approximately 15%, which is to be compared with the two- to three-fold variation in T4 for control rats in 13-week NTP studies (Table 1). Simply put, the rat is expected to be more sensitive than the human to fluctuations in dietary iodine and to agents which affect thyroid hormone levels. EPA/NCEA would do well to explore these established differences before deciding on the appropriate uncertainty factors to use in deriving a revised RfD from a rat LOAEL or NOAEL.

IV. References Not Cited in the External Review Draft

Goodman, G. 1998. "Perchlorate Pharmacokinetics: Critical Review and Evaluation of Relevance to Low-Level Exposures in Humans." September 1, 1998. Submitted to EPA/NCEA.

Morreale de Escobar, G., Calvo, R., Obregon, MJ, Escobar del Rey, F. 1992. Homeostasis of brain T3 in rat fetuses and their mothers: Effects of thyroid status and iodine deficiency. *Acta Med. Austriaca* Suppl. 1: 110-116.

Moulopoulos, D.S., Koutras, D.A., Mantzos, J., *et al.* 1988. *J. Clin. Invest.* 11: 437-439.

Table 2. Paired Comparisons of Thyroid Hormone and Thyroid Histopathology Data as Reported in Figures 6-3 to 6-16 of the External Review Draft

Figure	Comparison	Data Description/Citation (as given in the External Review Draft)	Perchlorate Doses (mg/kg-day)
6-3	T3 *vs.* T4; T4 *vs.* TSH	14-day rat Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-4	T4 rank order *vs.* severity rating for follicular hypertrophy; T4 rank order *vs.* severity rating for decreased follicular lumen size	14-day rat study Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-5	TSH rank order *vs.* severity rating for follicular hypertrophy; TSH rank order *vs.* severity rating for decreased follicular lumen size	14-day rat Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-6	T3 *vs.* T4; T4 *vs.* TSH	14-day and 90-day rat, combined data Springborn Laboratories, Inc. (1998)	0, 0.01, 1.0, 10, 30, 100
6-7	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	14-day and 90-day rat, combined data Springborn Laboratories, Inc. (1998)	0, 0.01, 1.0, 10, 30, 100
6-8	T3 *vs.* T4; T4 *vs.* TSH	14-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-9	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	14-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-10	T3 *vs.* T4; T4 *vs.* TSH	90-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-11	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	90-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-12	T3 *vs.* T4; T4 *vs.* TSH	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998a); York (1998c); Channel (1998b); Crofton (1998f)	0, 0.1, 1.0, 3.0, 10
6-13	T4 rank order *vs.* severity rating for follicular hypertrophy; T4 rank order *vs.* severity rating for decreased follicular lumen size	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998b); York (1998c); Channel (1998b); Crofton (1998e,f)	0, 0.1, 1.0, 3.0, 10
6-14	TSH rank order *vs.* severity rating for follicular hypertrophy; TSH rank order *vs.* severity rating for decreased follicular lumen size	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998b); York (1998c); Channel (1998b); Crofton (1998e,f)	0, 0.1, 1.0, 3.0, 10
6-15	T3 *vs.* T4; T4 *vs.* TSH	F0 rabbits on GD29 (developmental)	0, 0.01, 1.0, 10, 30, 100
6-16	T4, TSH rank order *vs.* severity rating for follicular hypertrophy	F0 rabbits on GD29 (developmental)	0, 0.01, 1.0, 10, 30, 100

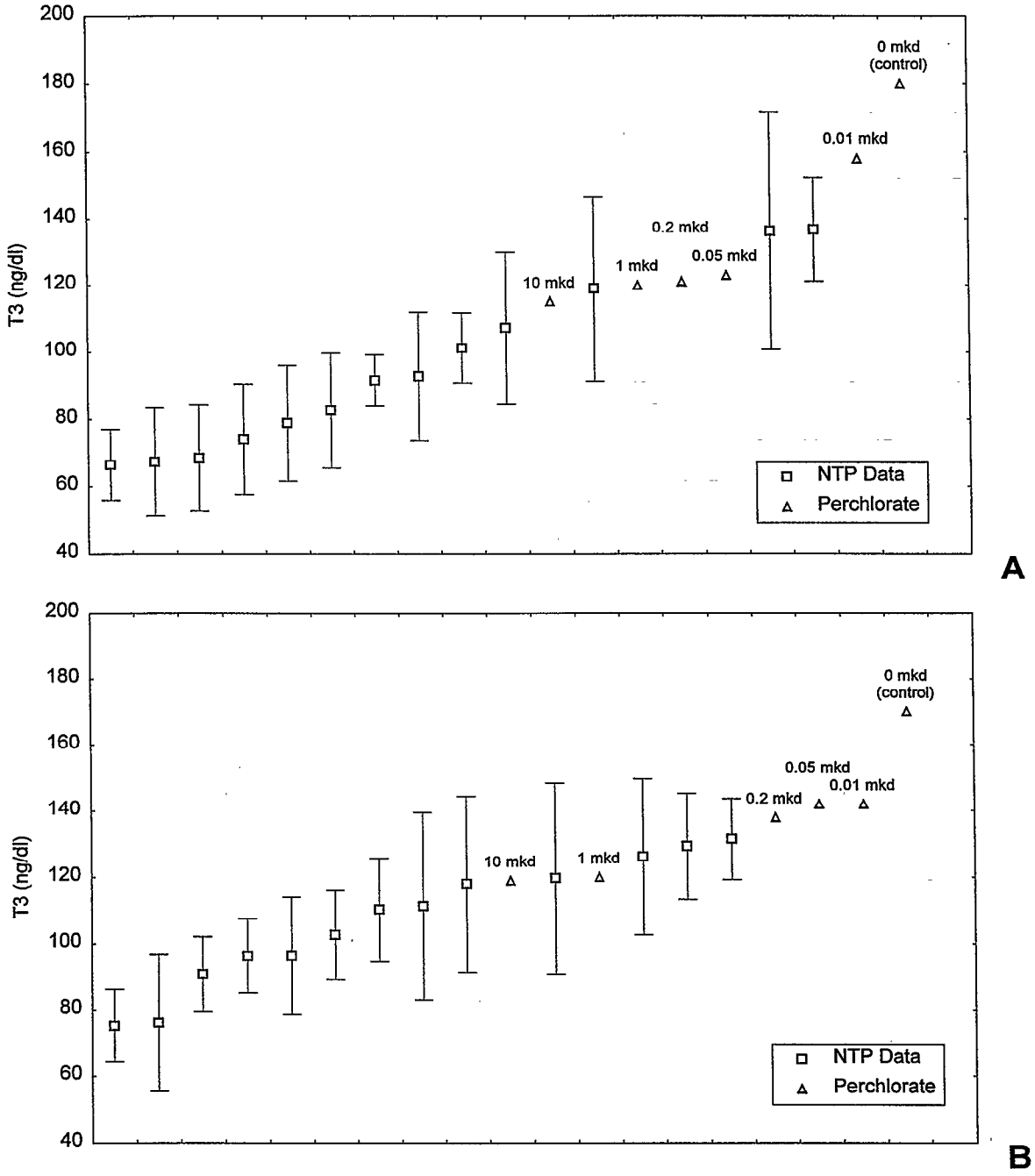

Figure 1. Serum total T3 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard deviations for the control groups in all thirteen bioassays for which reliable serum T3 measurements are available; courtesy of Dr. Greg Travlos, NIEHS. *Perchlorate data:* mean values; from Springborn Laboratories, Inc., as reported in Fig. 5-6 of the External Review Draft. *Abbreviation:* mkd, mg/kg-day.

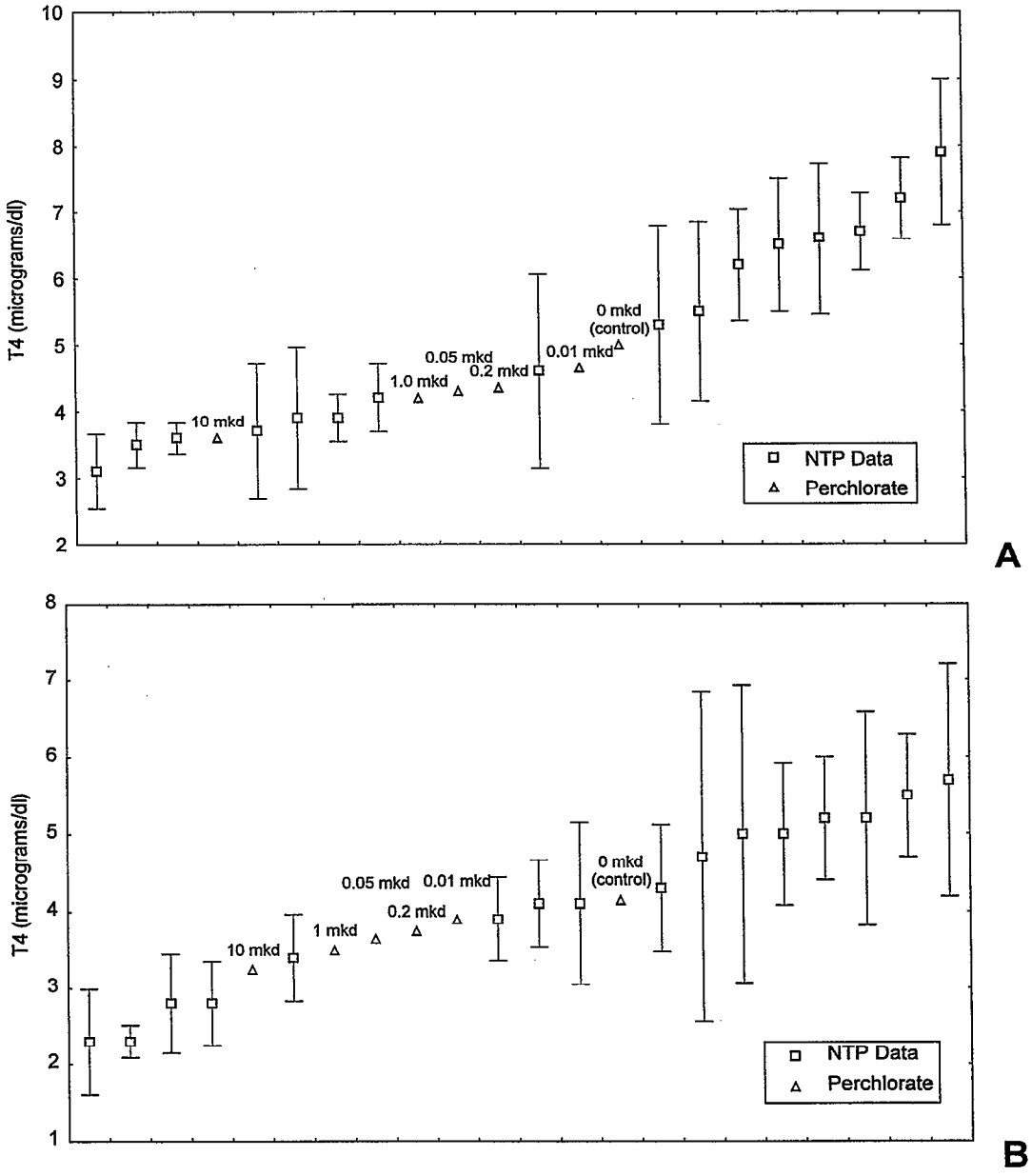

Figure 2. Serum total T4 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard de*via*tions for the control groups in all sixteen bioassays for which reliable serum T4 measurements are available; courtesy of Dr. Greg Travlos, NIEHS. *Perchlorate data:* mean values; from Springborn Laboratories, Inc., as reported in Fig. 5-8 of the External Review Draft. *Abbreviation:* mkd, mg/kg-day.

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1 February 1999

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RE: Comments on EPA Draft Document: Perchlorate Environmental Contamination: "Toxicological Review and Risk Characterization Based Upon Emerging Information" and Recent Mode-of-Action Studies with Ammonium Perchlorate

Dear Ms. Dardin:

The results of recent studies with ammonium perchlorate support earlier reports in the literature that the most critical effects of the compound are observed on the thyroid gland and interferes with the gland's ability to secrete thyroid hormones. This is consistent with the known mode-of-action of perchlorate as a potent inhibitor of the sodium-iodide symporter in the baso-lateral membrane of follicular cells, thereby, interfering with the ability of the thyroid gland to concentrate adequate amounts of intracellular iodide necessary for thyroid hormone synthesis to proceed at a normal rate.

I offer the following general comments for consideration by the agency as they finalize this important document.

1) It is important when interpreting thyroid hormone data and histopathologic findings of the thyroid gland, especially in the rat, to distinguish between adaptive (physiologic) responses from adverse (harmful) effects. This is particularly difficult in an endocrine organ such as the thyroid gland since it's physiologic function is to respond to changes in the internal environment of an animal or person and restore normal homeostasis. Therefore, mild (minimal) hypertrophy of follicular cells (as described in several of the recent studies) accompanied by modest elevations in circulating levels of thyroid stimulating hormone (TSH), in my judgement, should be considered an adaptive response by the thyroid gland to a mild interference in the ability to concentrate iodide ion. Similar adaptive changes are observed during pregnancy and periods of rapid growth, dietary iodine deficiency, and in response to stressful situations (e.g. low ambient temperature, severe infection), among others.

   I would suggest an adverse effect (low dose) of ammonium perchlorate to be one where there is definite evidence of follicular cell hyperplasia accompanied by a significant

increase in thyroid weights with significant reduction in both thyroxine (T4) and triiodothyronine (T3) as well as the expected compensatory increase in serum TSH. Based upon the known mode of action of perchlorate, the synthesis of both hormones (T4 and T3) should be equally interfered with when there is inadequate intracellular iodine. It is important to emphasize that all of the alterations in the thyroid follicular cells (hypertrophy or hyperplasia and increased thyroid weight) and changes in circulating levels of thyroid and pituitary hormones (decreased T4 and T3, increased TSH) following the administration of amonium perchlorate are fully reversible changes following cessation of compound administration.

2) The histologic changes described in the rat pups on post-natal day 5 should be interpreted with caution. In contrast to the pattern of change in fetal and neonatal thyroid function during pregnancy and extrauterine adaptation presented for humans in Figure 6-2, the rat thyroid may not be identical to humans. It has been my observation that the development of thyroid follicles and progressive accumulation of colloid continues into the early post-natal period in the rat making subjective histopathologic interpretation of the effects of a xenobiotic chemical on the thyroid difficult and subject to misinterpretation during this period of rapid growth. Therefore, I would not consider neonatal rats at post-natal day 5 to be an appropriate population to serve as a basis for setting the Rfd for perchlorate until this finding has been shown to be reproducible and separate from the normal developmental changes occurring at this time in the rat that result in the unique follicular structure of the thyroid gland (which is different from all other endocrine organs of the body).

   It is significant to note that although mild histologic changes were observed in developing follicular cells at post-natal day 5 at the lowest dose of perchlorate (0.1 mg/kg/day), changes in serum levels of thyroid hormones and TSH were detected only at higher doses of ammonium perchlorate (T3 significantly decreased at 0.3 mg/kg/day; T4 significantly decreased at 3 mg/kg/day; and TSH significantly decreased only at 10 mg/kg/day). This lack of correlation of histologic changes in thyroid follicular cells and circulating levels of thyroid and pituitary hormones suggests to me that the described histologic change at post-natal day 5 most likely is unrelated to the administration of ammonium perchlorate.

3) Review of Table 6-1A-D and 6-2 revealed there to be considerable variability in hormone levels and thyroid histologic changes between studies and at what dose level of ammonium perchlorate an effect was detected. Some of this variability may be related to the sensitivity in measuring circulating levels of a particular hormone in a species different from that of the primary antibody of the assay (e.g. measuring mouse TSH with a rat specified TSH assay). I would suggest that another factor accounting for the lack of correlation between circulating hormone levels in thyroid structure relates to determining an adaptive from an adverse effect in response to different doses of ammonium

perchlorate (refer to item 1 mentioned previously). In addition, histopathologic evaluations of thyroid glands from studies with known thyroid active compounds, such as perchlorate, should record follicular cell hypertrophy separate from follicular cell hyperplasia (not grouped together or only recording hyperplasia as in several of the recent studies).

4) The data package on the potential thyroid toxicity of ammonium perchlorate has been greatly enhanced by the recent subchronic (90 day) toxicity study, developmental neurotoxicity study, and the two-generation reproductive toxicity study in Sprague Dawley Rats; a developmental study in rabbits; and the immunotoxicology study in B6C3F1 mice. However, studies in a non-rodent species (e.g. non-human primate or dog) would be helpful in putting the thyroid data relating to perturbations in thyroid structure and function attributed to ammonium perchlorate in perspective due to the known marked physiologic differences between the human and rat thyroid (summarized in Table 4-1). Due to the wide potential human exposure to ammonium perchlorate, data from a primate species (whose thyroid gland is known to respond to potential thyrotoxic compounds in a matter more similar to the human than the rat), in my judgement would provide important information useful in quantifying the potential risk of this compound to humans.

Thank you for the opportunity to comment on the well prepared document that the USEPA has drafted on amonium perchlorate.

Sincerely,

Charles C. Capen

Charles C. Capen, DVM, PhD
Professor and Chairman