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Perchlorate Peer Review Workshop Report

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1.0 Summary of Workshop

EPA is in the process of conducting a toxicological review of perchlorate, including the development of a revised provisional Reference Dose (RfD), a cancer assessment, and an ecological assessment. As part of that process, EPA retained the Research Triangle Institute (RTI) to coordinate a scientific peer review of the Draft Toxicological Review Document on perchlorate, entitled "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information" and a set of supporting toxicological and ecological studies. The peer review was sponsored by EPA's Office of Solid Waste and Emergency Response (OSWER) and Office of Water (OW), while the Draft Toxicological Review Document was prepared by EPA's National Center for Environmental Assessment (NCEA).

In September, 1998, (63 FR 51918) RTI requested nominations from interested stakeholders of highly qualified scientists with expertise in general toxicology, thyroid function and toxicology, developmental toxicology, neurotoxicology, immunotoxicology, pharmacology, genetic toxicology, medical endocrinology with an emphasis on thyroid function, biostatistics, assessment of risks due to non-cancer and cancer health effects, and assessment of risks due to ecological effects.

From a field of outstanding nominees, RTI selected ten independent scientists to be peer reviewers and one of these scientists was selected to chair the workshop. The scientists were selected as peer reviewers based upon their demonstrated expertise in the subject areas outlined above and the need for balance in affiliation among the peer reviewers. At least one expert in each of the above disciplines was selected as a peer reviewer.

The scientists reviewed the following studies:

- A 90-day drinking water toxicity study in rats with ammonium perchlorate
- A neurobehavioral development study of ammonium perchlorate administered orally in drinking water to rats
- Oral (drinking water) dosage-range developmental toxicity study of ammonium perchlorate in rabbits
- Oral (drinking water) developmental toxicity study of ammonium perchlorate in rabbits
- Oral (drinking water) two-generation (one litter per generation) reproduction study of ammonium perchlorate in rats
- Effects of ammonium perchlorate on immunotoxicological, hematological, and thyroid parameters in B6C3F1 mice
- Genotoxicity assays for ammonium perchlorate

- Ecotoxicity assays for ammonium perchlorate

and the draft summary document:

- Perchlorate environmental contamination: Toxicological review and risk characterization based on emerging information.

In January 1999 (64 FR 2492), RTI presented additional information about the workshop. Stakeholders who had additional information on the perchlorate issue were invited to give a short presentation at the workshop.

The peer review workshop was held on February 10 and 11, 1999, in San Bernardino, California. The purpose of the workshop was to provide a forum for the peer reviewers to present and discuss their assessments of the individual studies and the Draft Toxicological Review Document on perchlorate. The public was invited to attend the workshop as observers, and stakeholders were invited to make presentations at the workshop.

Approximately 100 people attended the workshop as observers and 13 stakeholders made short presentations. The first day of the workshop consisted of an overview of the perchlorate issue by EPA, presentations by the stakeholders, and reviews of the individual studies and Draft Toxicological Review Document on perchlorate by the peer reviewers. The second day of the workshop consisted of summaries presented by the peer reviewers on the individual studies and a summary by the Chairman on the issues discussed at the workshop.

This report consists of:

- the Chairman's summary of the conclusions reached by the Peer Review panel at the workshop
- reviews of the studies and Draft Toxicological Review Document on perchlorate prepared by each peer reviewer after the conclusion of the workshop
- the following appendices:
  - Appendix A - the workshop agenda
  - Appendix B - a list of peer reviewers
  - Appendix C - short resumes of peer reviewers
  - Appendix D - a conflict-of-interest statement
  - Appendix E - the charge to the peer reviewers
  - Appendix F - a copy of written comments received by RTI from outside observers before the workshop

- Appendix G - a list of outside observers giving presentations
- Appendix H - a copy of material presented at the workshop by outside observers
- Appendix I - introductory presentations by EPA at the workshop
- Appendix J - a copy of additional analyses from EPA's NCEA.

Some of NCEA's analyses in Appendix J were not available to the peer reviewers prior to the workshop and were not subject to peer review.

2.0 Summary of Findings: Dr. Curtis Klaassen

Peer Review Summary of Perchlorate

HAZARD IDENTIFICATION

This document summarizes the published data, as well as the data that have just been accumulated on perchlorate. We were also informed about further data that are being collected and not yet thoroughly evaluated. In general, the presentation of these results by EPA staff was well done. However, the reviewers want to emphasize that the data that have recently been collected need to be thoroughly evaluated, and that all data should be published in peer-reviewed journals. There was consensus that the thyroid tissue slides from several studies need to be evaluated by a "pathology working group." There was also concern with quality control of TSH measurements across the studies.

There were a number of statistical issues that need to be addressed by the EPA. The major ones are: reexamining all the ANOVAs, making use of all available information in the statistical analyses, considering the elimination of the multiple comparisons correction factor, making a decision on how to consider litter effects, reconsidering the determination of certain no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs), deciding how the benchmark dose calculations are to be used, making certain that the statistical analyses described in the report are the ones actually used, carrying out heterogeneity tests prior to the ANOVAs, and, for reanalyses, comparing the raw data with those of the testing laboratories to make certain they are the same.

Despite these statistical issues it is clear that the numerous studies have delineated the hazards of perchlorate quite nicely. Perchlorate does not appear to produce toxic effects to most organs and is not mutagenic. Insufficient data has been collected to determine its effect on the immune system; however, these studies are in progress.

Toxic effects are produced by perchlorate on the thyroid, affecting T3, T4 and TSH levels and interfering with the developing central nervous system and the thyroid itself. These effects on hormone levels can lead to mental retardation and thyroid tumors. This is an extremely important conclusion, in that it indicates that the toxic effects of perchlorates appear to be limited to the consequence of its inhibition of iodide transport into the thyroid gland.

MODE OF ACTION

The physiology of thyroid hormones has been well studied. Thyroid hormones are important in many basic functions, including regulating basal metabolic rate and development of the central nervous system (as well as other basic functions such as reproduction). If thyroid hormones (T4 and T3) are low, the basal metabolic rate of humans decreases. If the thyroid hormones are low during development of the central nervous system, mental retardation can result, so-called "cretinism" in humans.

Thyroid hormone concentrations are highly regulated. Thus, when thyroid hormones are low, the hypothalamus secretes thyroid releasing hormone (TRH) and the TRH, together with the low levels of thyroid hormones in blood, result in the stimulation of the pituitary to increase its secretion of thyroid stimulating hormone (TSH). The TSH then is carried by the blood to the thyroid gland where it increases many metabolic processes so it can "normalize" the blood levels of thyroid hormones.

The data presented indicate that all of the adverse effects produced by perchlorate are associated with its initial inhibition of iodide uptake to the thyroid gland.

Thyroid Tumors: When perchlorate inhibits the uptake of iodide and production of thyroid hormones, there is a resultant increase in TSH. This adaptive response results in an increase in various enzymes and processes in the thyroid gland, including thyroid hypertrophy. However, if this stimulation of TSH to the thyroid gland is excessive and prolonged, thyroid tumors may result (this effect has been seen in rats). The thyroid tumors are not due to a genotoxic effect, because perchlorate is not mutagenic. These thyroid tumors are due to excessive stimulation of the thyroid gland, resulting in cell proliferation and subsequent neoplasia.

Decreased Mental Development: Thyroid hormones are required for normal mental development. Therefore, when perchlorate inhibits the uptake of iodide by the thyroid gland during the development of the nervous system during the pre- and early post-natal period, it results in a decrease in thyroid hormones in the blood necessary for normal mental development.

It is well accepted that an increase in serum TSH is a more sensitive index of an antithyroid effect and can be observed even if it is not possible to observe a clear decline in serum thyroid hormones (T4 and T3). There is little data to suggest that the increase in serum TSH produces an overcompensation in serum T4 and T3. Therefore the data sets relating to higher serum T3 and T4 levels in ammonium perchlorate-treated rats, such as in the Neurobehavioral Developmental Study, must be interpreted cautiously. In fact, the elevated T4/T3 levels may suggest a problem with the assays or experimental procedures, rather than reflect a real biological phenomenon. Given the current knowledge of the mechanisms of action of perchlorate on the thyroid it seems inappropriate to ascribe an antithyroid or toxic effect to perchlorate in sporadic instances where its treatment is associated with an increase above controls in serum T4 and/or T3.

DOSE RESPONSE

While the hazard identification of perchlorate appears to be in the final stages of being delineated, information on the dose-response is less robust. The panel concluded that thyroid cell hypertrophy (increase in cell size) was not a good biomarker for the adverse effect of perchlorate, but rather suggested the use of hyperplasia (increase in cell number). The present document and the study results often used the terms hyperplasia and hypertrophy interchangeably, which is potentially misleading. Hypertrophy is an adaptive, often reversible response, while hyperplasia is potentially adverse. The EPA also indicated that thyroid tissues from additional animals were available for histopathologic examination. Therefore, it was recommended that a "pathology-working group" be established to look at the existing thyroid slides from the subchronic study and the neurobehavioral study, as well as slides from additional pups in the neurobehavioral developmental toxicity study, to differentiate between hypertrophy and hyperplasia, and to establish dose-response curves for perchlorate-induced hyperplasia.

SPECIAL POPULATIONS

It is well known that thyroid hormones are essential for the normal development of the central nervous system. Therefore, it was suggested that the effects of perchlorate on mental development during fetal development, as well as the first few weeks of life be closely evaluated by histopathological, morphological, and behavioral assessments. Also infant exposure to perchlorate, reduced iodide concentrations, and/or alterations in maternal hormones in the mother's milk should be determined. In addition, it should be ascertained whether perchlorate is transferred in significant dosages from mother to pup via the milk.

RISK ASSESSMENT

***General Issues*:** The classic approach to risk assessment is to determine a NOAEL in laboratory animals, and to divide this by "safety factors" to assure protection of humans. Most of the descriptive studies needed to perform this type of risk assessment on perchlorate have been or are being performed.

A more scientific approach to risk assessment is possible when a greater scientific understanding of the mode and/or mechanism(s) of how the chemical produces the adverse effects has been achieved. With perchlorate, we know the mode of action probably as thoroughly as with any chemical. In addition, pharmacokinetic/toxicokinetic data of perchlorate in rats and humans will be available for building physiologically-based pharmacokinetic (PB-PK) models to decrease the uncertainty of extrapolating data from laboratory animals to humans. Thus, a predictive risk assessment for perchlorate is possible and should be pursued in the next iteration of this assessment.

Risk assessments that estimate human risk from data on human populations should be more accurate than ones that rely on animal data. This can be done with adequate occupational

exposure studies or administration to humans with thyroid disease. With a chemical like perchlorate, which has been used as a drug, a NOEL can be established in humans. The therapeutic dose given to humans is approximately 10 mg/kg. Thus, 10 mg/kg is too high for environmental perchlorate exposure. Further studies on healthy human volunteers are encouraged, such as those presently being conducted at Harvard and in Germany, to establish exposures that will protect humans from potential adverse effects of perchlorate exposure (using as a biomarker a dose that does not increase TSH).

***Mode of Action Approach*:** The document develops a rationale for a mode of action approach for assessing non-cancer and cancer risks of perchlorate. However, the mode of action data were not used in the RfD determinations. A clear statement of EPA's strategy for using mode of action and pharmacokinetic data would be very helpful.

A mode of action approach represents a move in the direction of harmonization of non-cancer and cancer risk assessment and permits a more rational use of available biological and toxicological data. EPA is to be commended for taking a move in the direction of such a harmonized approach.

ECOLOGICAL ASSESSMENT

The ecotoxicology studies were well done and support the screening ecological risk assessment. The major weaknesses of the screening ecological assessment were limited data on exposure and the potential for long-term chronic effects. These limited data resulted in an assessment that was quite conservative in terms of risk-based effect thresholds suggested, and in terms of the scope of additional studies recommended.

CONCLUSIONS

Before the RfD proposed by the EPA can be definitively evaluated, further work is needed. The EPA should convene a pathology working group, review the histopathology from the thyroid slides, and establish dose-response curves for hyperplasia and/or other adverse responses. The variable used by the EPA in the determination of the RfD (thyroid hypertrophy) was not considered by the peer review panel to represent an adverse effect, nor was it demonstrated to be correlated with an adverse response such as hyperplasia. It is not possible at this time to determine what uncertainty factor should applied in the RfD derivation. If the appropriate human data are accumulated, the uncertainty factor could be small. The magnitude of the uncertainty factor needs to wait until all the relevant studies are complete. Based on the lack of demonstrated adverse effects, the RfD proposed by the EPA (0.0009 mg/kg/day) is likely to be conservative.

3.0 Reviews of Toxicity Database, Toxicological Review Document, and Additional Testing Needs by Assigned Panel Members

3.1 General Statistical Issues: Dr. Joseph Haseman

General Comments

While there are a number of statistical issues that require the EPA's attention, all are correctable problems. Dealing with these issues may result in certain changes in the reported NOAELS and/or LOAELS, but it is unlikely that the final conclusions will be substantially altered. Except where noted, these recommendations are in response to Charges 1.1 (3), 1.1 (4), and 1.2.1 (3) from the list of Charges to the External Peer Review Panel. Ten specific and two general recommendations are given below.

Specific Recommendations

Recommendation 1: Re-examine all the ANOVA's, and in those cases where significant interactions and main effects are pooled with error, redo the ANOVA's and Tukey's test pairwise comparisons correctly. When an ANOVA is carried out, the primary objective is to identify important sources of variability and remove these corresponding sums of squares from the error term. These sources of variability may be main effects such as treatment, gender, or replicate, or they may be interactions among these factors.

Once the important sources of variability have been identified and removed from error, the treatment sum of squares is then compared with the error sum of squares to determine if it is significant. What is not appropriate is to identify an important source of variability and then in effect pretend it doesn't exist and add it back in with the error term. This artificially inflates the error and reduces study sensitivity when the treatment sum of squares is compared with this inflated error term. This problem occurs in a number of the EPA statistical analyses.

For example, consider the EPA Analysis of TSH in 90 Day Study at 120 days. From page 41 of Crofton (1998b), the following analysis of variance appears:

	df	SS	MS	F	p
Gender	1	883.7	883.7	310.1	<0.0001
Treatment	3	65.2	21.7	7.63	0.0002
Gender*Treatment	3	12.0	4.0	1.40	0.2497
Error	71	202.3	2.85		

This analysis is fine, and clearly identifies treatment as an important source of variability. However, from page 79 of Crofton (1998b), and Figure 5.9, Page 5-23 of EPA Draft Report, we have

	df	SS	MS	F	p
Treatment	3	65.7	21.9	1.50	0.2224
Error	75	1097.4	14.63		

By inappropriately pooling the gender (and gender*treatment) sum of squares with error, the statistical significance of the treatment effect has been lost. The EPA incorrectly concludes that there is no treatment effect.

For these data there are two possible fixes to this problem: (1) analyze each sex in a separate ANOVA, or (2) analyze the data as eight separate sex/species groups (my choice) . In either case, the Tukey's test comparisons should identify significant treatment effects, especially in females. In fact, these corrected analyses should identify all three doses of females as having shown significant effects. Figure 5.9 should then be re-plotted, showing separate male and female responses, and indicating the significant effects in females.

Similar comments apply to other EPA statistical analyses.

Recommendation 2: Make full use of all available information in the statistical analyses. The two variables that are most critical in the determination of the RfD are thyroid follicular cell hypertrophy and lumen size on PND5. For these variables, the EPA created four (0, 1, 2, 3) or five (0, 1, 2, 3, 4) ordered categories and assigned each animal to one of these categories depending upon the severity of the response.

However, when making pairwise comparisons of each individual dosed group to controls, these ordered responses were essentially ignored, and the data were collapsed into two categories, most often "no response" or "some response."

However, collapsing the data in this way sacrifices information and reduces study sensitivity. After I called this matter to the attention of the EPA, they revised their statistical analysis, and their new analyses seem appropriate and clearly demonstrate the gain in sensitivity by making full use of the study information. Because of the importance of thyroid histology in the RfD determination, these analyses should be brought forward and discussed in the EPA Report itself.

However, further work still needs to be done. For example, the benchmark dose calculations for hypertrophy ignore severity and are based on the collapsed data. The original benchmark dose calculations were also flawed in only making partial use of the data (i.e., using only those animals also having thyroid hormone data). Here again, I recommend making full use of all information available.

During the Peer Review we also learned that there were eight or nine hundred animals in the neurodevelopmental toxicity study that had not yet been examined for thyroid histology. Given the importance of this variable and the small sample sizes (six animals per sex per group) currently used, our panel strongly recommended that the EPA consider including additional animals, especially on PND5, to increase study sensitivity. This matter will be discussed in more detail later, but it is another example of making full use of the information available.

Recommendation 3: Consider eliminating the multiple comparisons correction factor. I understand the principle of why the EPA considered it necessary to use this "correction factor," but at least as currently used, I recommend against it for four reasons. (1) It is not a standard multiple comparisons adjustment. The EPA divides alpha by the square root of the number of variables examined. This is not the widely used Bonferonni adjustment, which divides alpha by the number of variables evaluated. At the very least, the EPA should reference their method of adjustment. (2) This adjustment is used in some studies but not in others. (3) It is reported as being applied to "interaction main effects," a term that is unfamiliar to me (I understand what an interaction is and what a main effect is, but not an interaction main effect. Moreover, this "correction" was reported as being applied to interaction main effects in a study with only one main effect and hence no interactions). (4) It is apparently not applied to the critical pairwise comparisons (Tukey's test), so its impact on study results is minimal in any case.

Recommendation 4: Make a decision about how to consider "litter effects" and be consistent throughout the report. In the contingency table analysis of thyroid follicular cell hypertrophy, the EPA ignores litter and simply reports the response rate among 12 males and females per group. In contrast, in the benchmark dose calculations, these responses are expressed on a per litter basis and an entirely different set of response rates are used for the same data. The EPA should be consistent in their approach to litter effects.

Although I am a strong advocate of the importance of litter effects in other contexts, in this instance I recommend that, for simplicity, the data be reported and evaluated on a per pup basis. There are apparently at most two (but mostly one) pup per litter, so there are insufficient

data to document litter effects if they are present. Moreover, since most litters have only a single pup, there would be little impact of litter effects even if they were present.

If the EPA accepts our panel's recommendation to obtain additional thyroid histology data, then the additional pups could be selected so that each litter had one male and one female. Thus, it would be possible to evaluate, and if necessary adjust for, the impact of litter effects on the response variables of interest.

Incidentally, I do not understand the EPA recommendation in the section on motor activity data to treat gender as a nested factor in an ANOVA. Gender is a crossed factor, not a nested factor. If additional (normally distributed) data are obtained so that there is one male and one female pup per litter, then the appropriate sources of variability for an ANOVA are

Gender
Treatment
Gender x Treatment
Litters (Treatment)
Error.

Litters is a nested factor; gender is not. This section of the draft report requires some work.

Recommendation 5: Reconsider the determination of certain NOAELs and LOAELs. Specific examples include, but may not be limited to:

(a) TSH in the 90 day study: Despite significant elevations in TSH in 0.05 mg/kg-day females on Day 15 (see Figure 5-9, page 5-23), the EPA nevertheless concludes (page 5-22) that "The NOAEL for TSH is 0.05 mg/kg-day." Furthermore, if the Day 120 data are analyzed appropriately, as discussed above, the elevated TSH response in 0.05 mg/kg-day females on Day 120 is also significant by Tukey's test. Thus, I recommend that the EPA lower the NOAEL to at least 0.01 mg/kg-day. Frankly, the data suggest a marginally significant effect at this dose as well, so 0.01 mg/kg-day may in fact be a LOAEL for TSH as well as for T3 and T4.

(b) rT3 in the 14 day study: In this case the EPA reported a NOAEL (0.17 mg/kg-day, see Table 5-2, page 5-14) that was between two doses actually used in the study (0.11-0.12 and 0.44-0.47 mg/kg-day). It is possible that this is just a typographical error, but if it is not, the EPA should explain in more detail how they were able to interpolate a NOAEL between two doses actually used in the study.

(c) Lumen size (morphometric analysis): In this analysis the EPA inappropriately pooled significant block effects with the error term, thereby reducing study sensitivity. I recommend simply comparing each individual dosed group with controls by ANOVA (males and females can be pooled, since there is no gender effect) using the same

basic approach that the EPA employed in the analysis of lumen size in the thyroid histology data. Such an analysis would reveal a significant reduction in lumen size at the 0.1 (but not the 1.0) mg/kg-day dose. Thus, I would conclude that 0.1 mg/kg-day is the LOAEL for lumen size, agreeing with the EPA interpretation for this variable based on the histology data.

However, it is very important to emphasize that even in the revised, more sensitive EPA contingency table analysis, the reduction in lumen size in the (combined male and female) 0.1 mg/kg-day group based on histology is not significant (p=0.0661; see Table 6A of revised Marcus analysis). The EPA was apparently confused by the results of their original statistical analysis and state (page 5-28) that "when data on both sexes were combined, the lowest dose, 0.1 mg/kg-day, was significant at 0.012 (df=8) for the follicular cell hypertrophy and for the lumen size at 0.008 (df=12)." However, these p values are for an overall Chi-square test, and one cannot infer significance at any specific dose based on significant overall differences among groups. These results suggest that the morphometric analyses may be more sensitive than the histology analyses for the detection of lumen size differences, at least at the 0.1 mg/kg-day dose. However, the morphology data had four extra animals per group that were not used in the thyroid histology data analyses.

(d) T4 in Neurodevelopmental Toxicity Study: In my opinion, the T3 and T4 responses track each other very closely in this study (compare Figures 5-11 and 5-12), and although I agree that the reduction in T4 at the 1.0 mg/kg-day dose is not quite statistically significant by Tukey's test, I feel it is biologically significant. Moreover, had a slightly less conservative multiple comparisons test been used (Fisher's protected LSD rather than Tukey's test) the reduction would in fact have been statistically significant. Thus, I see no reason not to consider this dose a LOAEL for T4, and thus 0.1 mg/kg-day a NOAEL for both T3 and T4.

(e) Motor activity data: The EPA seems quite convinced that the motor activity data in high dose males on PND14 show a biologically significant effect, despite (1) the lack of statistical significance at that dose; (2) a trend in the opposite direction in females at PND14; and (3) the lack of any supporting effect in either sex on PND18, PND22, or PND59. In an apparent effort to find statistical significance on PND14, the EPA asked Argus Laboratories to carry out an additional statistical analysis "using gender as a within subject variable, or alternatively, using a nested design with gender nested under litter" (page 5-33). The problems associated with trying to use gender as a nested factor have already been discussed in Recommendation 4 above, and Argus wisely did not attempt such an analysis.

Instead, they responded (York, 1998b) by carrying out an additional statistical analysis using gender as a between-subject variable. Since the females show the opposite effect of males, the overall dose effect is not significant, just as one would

expect (the male effect considered individually is also not significant). The EPA expressed disappointment, noting that the "secondary analysis submitted is still not what the EPA requested" (page 5-34). If the EPA had intended to ask Argus to adjust for litter effects, the analysis they should have requested is summarized in Recommendation 4 above. It is very unlikely that an adjustment for litter effects would have resulted in statistical significance. Indeed, adjusting for litter effects by a "per-litter" analysis generally tends to weaken, not strengthen, treatment effects that might otherwise be detected by a "per-pup" analysis.

The EPA also faults Argus Laboratories for failing to respond adequately to the request for an explanation of why the statistical analysis failed to detect significance in the PND14 motor activity for male rats (page 5-34). The primary reason for the lack of statistical significance is that these data show considerable animal-to- animal variability in motor activity. The EPA Draft report notes this large within group variability (page 5-37), but still concludes that the "increase in activity should be considered biologically significant until additional data can be marshaled to suggest or prove otherwise" (page 5-38). Ultimately, the decision of whether or not a non-significant increase is biologically important is a matter of scientific judgement, but I feel that the alternative explanation given on page 5-37 that this increase "may indeed be a Type I error and would not be found again if this experiment was repeated" is more nearly correct. However, referring to this result as a "Type I error" implies that a statistically significant effect was found, whereas this was not the case for these data.

Another factor that supports my point of view on this matter is that it is the male controls that appear to be unusually low, not the high dose group that is unusually high. For example, the mean motor activity response in the high dose male group (Number of movements = 404; Time spent in movement=364) is very similar to the mean control female response (Number of movements = 393; Time spent in movement = 430; see Argus, Appendix F, pages 509-512). There is nothing in these data that would suggest a gender difference in motor activity. In summary, in my opinion this non-significant increase in motor activity in one dosed group in one sex at one time period simply reflects random variability, nothing more. These comments also respond to Question III A-1 of the charge to the panel.

Recommendation 6: Make a decision about how the benchmark dose calculations are to be used, and make this clear in the report. The report carries out a series of benchmark dose calculations, but it is unclear how the EPA used this information in the RfD determinations. Since the ten page Executive Summary does not even mention the benchmark dose calculations, I can only assume that they played little or no role in the scientific decision making and were possibly carried out "after the fact" to see if such an approach would make a difference. My speculation may or may not be correct, but the EPA needs to address this matter in more detail

and at least mention the benchmark dose calculations in the Executive Summary if they are to remain in the report.

Recommendation 7: Make certain that the statistical analyses described in the report are the ones actually used. In several instances it is not clear what multiple comparison procedure is being used by the EPA. For example, for the morphometric data the actual statistical analyses provided to us (Crofton, 1998f) are Duncan's Multiple Range Test, but Crofton (1998f; page 2 of 29) states that the data were analyzed by "Turkey's Studentized Range Test." This should be "Tukey" rather than "Turkey," but the EPA Draft report also states (page 5-30) that Tukey's test was used. Either of these multiple comparison procedures (Duncan or Tukey) would be okay, but they are different tests. A similar problem occurs in the Segment II Developmental Toxicity studies (and possibly in others; the EPA should review all data analyses).

Similarly, in the original EPA contingency table analyses of the hypertrophy and lumen size data, the EPA Draft Report stated that the Likelihood ratio Chi-square test was being used, whereas in fact the p values reported in some cases were those of the "usual" Chi-square tests.

Recommendation 8: Correct the figures to eliminate the legend for males and females in those cases in which the graph is for one sex only (or for the pool of males and females). A number of figures incorrectly have this legend: Figures 5-2, 5-4, 5-6 (Day 120), 5-9 (Day 90 and Day 120), 5-10, 5-11, 5-12, 5-13, 5-14, 5-15, 5-16, and 5-17. Moreover, for some of these figures, the data should be plotted for males and females separately, since there is a significant gender effect that was ignored by the EPA. The EPA should redo all of these analyses, as indicated earlier, but it appears that at a minimum the following figures should have separate plots for males and females: Figures 5.2, 5-7, and 5.9 (Day 120). Figure 5-8 should also have separate plots for each time period.

Recommendation 9: Carry out heterogeneity tests prior to the ANOVAs and use a variance-stabilizing transformation on the data, if necessary. In the EPA Draft Report and supporting documentation, I could find no mention of the use of heterogeneity tests (e.g., Bartlett's test or Levine's test) prior to the ANOVAs. This is a concern, because an ANOVA assumes homogeneity, and for some variables (e.g., motor activity) it appeared that a data transformation should have been used to equalize the variances. This might also increase study sensitivity.

Recommendation 10: Before performing any reanalyses, compare the raw data with those of the testing laboratory to make certain that they are the same. There were several cases in which the summary statistics from the EPA reanalyses did not agree with the analysis of the same data carried out by the test laboratory.

This led to problems in some cases. For example, in Figure 5-19 for Day 14 (page 5-23), the plotted points for the 0.05 and 0.2 mg/kg-day males have been interchanged (see page 50 of 95 of Crofton, 1998b). This may have occurred in part because the mean responses reported by

the EPA (138.0 and 141.2) differ from the values reported by the test laboratory for these same two groups (140 and 139; see page 447 of SLI Study No. 3455.1).

General Recommendations

Response to Question 1.3.1 (3) charge to the panel. The following two recommendations are even more important than the ten statistical recommendations given above.

Recommendation A: Identify an adverse response upon which to base the RfD. After considerable discussion, it was the final consensus of our panel that thyroid hypertrophy and lumen size should not be considered "adverse responses" by the EPA. The transient or reversible nature of these responses (e.g., gone at the lower doses by PND10; gone from all doses by PND22) also supports this view. Our chairman also expressed the opinion (not challenged by the panel or by the EPA) that neither T3, T4, not TSH should be considered adverse responses from the standpoint of selecting an RfD. Apparently the EPA agrees, since there were statistically significant effects on T3 and T4 at doses as low as 0.01 mg/kg-day that were ignored by the EPA in their RfD calculations.

There apparently is a precedent for the EPA to base an RFD on a response that is not considered adverse, if the non-adverse response can be clearly shown to be predictive of an adverse response at higher doses. However, there is nothing in the EPA report that links the "non-adverse" thyroid hypertrophy response to any adverse response seen at higher doses. Attempts should be made to identify such an adverse response that might be used in the calculation of an RfD. The EPA Draft Report does note that T3, T4, and TSH are inter-correlated and that these variables may also be correlated with thyroid hypertrophy. However, correlating one non-adverse response with other non-adverse responses brings us no closer to identifying a correlated adverse response upon which to base an RfD.

Recommendation B: Carry out a Pathology Working Group (PWG) review of thyroid histopathology, including additional animals on PND5 and possibly other time points if these data are available. One possible adverse response that could be used to determine an RfD is thyroid follicular cell hyperplasia, and a PWG review would ensure that hypertrophy and hyperplasia are clearly defined, diagnosed, and differentiated. In my opinion, a variable this critical should not represent the subjective diagnoses of a single pathologist. I am somewhat surprised that a pathology review is not a standard protocol in EPA laboratory animal studies.

Adding to the possible confusion is the fact that the EPA Draft report and accompanying documentation use the terms hyperplasia and hypertrophy interchangeably (for example, see page 6-2 and also Figure 6-7, page 6-19), whereas they have quite different meanings. If a PWG identifies a dose-related response for thyroid hyperplasia, this variable could be the basis for an RfD rather than thyroid hypertrophy. Our Panel spent little time discussing the various uncertainty factors proposed by the EPA, since such discussions would have little value until an adverse effect can be identified upon which to base an RfD.

Finally, and perhaps most importantly, since no clear adverse effect could be demonstrated in the various studies in the EPA Draft Report (the NOAELs and LOAELs summarized in Tables 6-1 and 6-2 are all for thyroid hormones and thyroid hypertrophy/lumen size, all variables that are considered non-adverse), the RfD reported by the EPA (0.0009 mg/kg-day) is likely conservative.

Minor Comments

(1) In the first panel of Figure 5-9, Day 15 should probably be Day 14 (consistent with the figures for T3 and T4).

(2) There appears to be an error in the raw data for motor activity that requires correction. On page 611 of the Argus 1613-002 submission, the total number of movements for control female 1750 is 108, not 230, as reported in Table F8.

(3) According to page 16 of 19 in Crofton (1998e), the overall F for treatment effects for T3 is 216.89, not 214.89 as indicated in Figure 5-11.

3.2 Neurobehavioral Developmental Study: Dr. R. Thomas Zoeller

Review of Toxicological Review Document

1. In general, the review document appears to do a good job of capturing the key aspects of the protocols and conduct of the studies reviewed. The discussion appears to be balanced, providing a good sense of the strengths and weaknesses of the various studies.

2. In general, the review document appears to adequately evaluate the entire database. Specific issues of weaknesses both in the review document and in the design of studies are discussed below.

3. As written, the review document provides new information on analysis of data presented from the various studies. These new analyses appear reasonable and informative and help improve the relevance of the studies performed.

General Issues

1. Several aspects of the regulation of thyroid hormone "economy" are not fully presented throughout this document, and this weakness is reflected in the construction of experiments in the various studies. These are listed below:

   (a) Page E-4, line 13: "differences in plasma protein binding...." It is true that the half-life of thyroid hormones in circulation is different between rats and humans.

However, it is probably not true that this is simply due to differences in thyroid hormone binding proteins. First, rats do possess the same thyroid hormone binding proteins as expressed in humans (e.g., thyroid binding globulin, TBG, and transthyretin, TTR, [Tani, 1994 #1431]). These circulating proteins are regulated differently; however, pregnant and lactating rats, and neonatal pups, all express TBG in the circulation (Vranckx, 1994 #1432).

(b) Nearly 80% of circulating T_3 is derived from tissue metabolism of T_4. Moreover, tissue metabolism of thyroid hormones is dependent upon iodothyronine deiodinase activities, which are themselves regulated by circulating levels of thyroid hormones (Burmeister, 1997 #1332; Germain, 1997 #1351). Therefore, it is quite important to evaluate the potential effects of perchlorate on tissue metabolism of thyroid hormones, because these effects could be masking effects of perchlorate on the thyroid system.

2. The effect of perchlorate on the thyroid gland is the basis for focusing perchlorate studies on thyroid function and thyroid hormone action. This concept is well-developed in the review document and in the individual studies. However, there is no background information on the role of thyroid hormone in the development of endpoints used for any of the studies. This is a critical issue because thyroid hormone has very specific, and in some cases subtle, effects on the development of the nervous system and endpoints must be designed with this in mind to be valid. At the least, there is no discussion of endpoints that represent valid and sensitive measures of thyroid disruption during development. Moreover, most of the endpoints used do not appear to be sensitive to thyroid disruption and thus, would not be expected to be affected by perchlorate.

3. In discussions of the physiological effects of perchlorate, the document acknowledges that effects are likely to be selectively mediated by the sodium/iodide symporter. Specifically, perchlorate blocks iodide uptake by the symporter. Moreover, the document acknowledges that the symporter is expressed in several regions - not just the thyroid gland - including choriod plexus, gastric and intestinal mucosae, and mammary tissue. What does not appear to be recognized in the review report or in the design of the neurodevelopment studies is the possibility (likelihood), that perchlorate blocks iodide uptake into milk and into the gut and thyroid of the pup. Moreover, it does not appear to recognize the possibility that perchlorate may itself be selectively taken up into milk and transferred to the pup. This is most important in the design of the neurodevelopment studies. However, this issue should be addressed in the review report, especially with respect to the timing and duration of perchlorate exposure.

4. In discussions of perchlorate effects on thyroid hormone synthesis, there appears to be little recognition that the effect of perchlorate on thyroid hormone synthesis will take much longer to develop than the effect on iodide uptake. Because iodinated

thyroglobulin is stored in the colloid, a considerable amount of thyroid hormone precursor is stored. Thus, even if doses of perchlorate are used which completely inhibit iodide uptake, it will still take some time for thyroid hormone levels to respond. This is especially important in the design of the neurodevelopment studies and their interpretation where perchlorate is initiated on the day of conception. In fact, hypothyroxinemia would not be predicted to occur until much later, perhaps as late as parturition.

Specific Comments

1. P3-3, line 10: "This study suggested a LOAEL of 9 mg/kg-day in humans for short-term exposures." This dose is equivalent to 630 mg/day. This seems like a very large dose for a LOAEL when 9.7 mg/kg completely blocks iodide uptake.

2. P3-6, line 1: "There is no information to suggest that humans without Graves' disease would have a similar reaction to perchlorate." It is also true that there is no information to suggest that Graves' patients do not have a similar reaction.

3. Page 4-9, line 26: "...there is a high-affinity binding protein, thyroxine-binding globulin, which binds T_4 (and T_3 to a lesser degree); this protein is missing in rodents and lower vertebrates." <u>This statement is false</u> (Tani, 1994 #1431; Vranckx, 1994 #1432).

4. Is the review document, as currently written, useful for the purpose of characterizing the human health effects of ammonium perchlorate and the perchlorate ion? The database on perchlorate, as described in this review document, does not provide a great deal of confidence in establishing specific human health effects in a developmental context.

5. Development of Reference Dose (RfD)

   (a) The reported NOAELs/LOAELs accurately reflect the findings as reported within the various studies. However, because many of the endpoints were not the most sensitive to thyroid disruption, or analysis methods not the most sensitive, these values are not predicted to be the most accurate in fact. As an important example, the quantitative analysis of thyroid histology performed on pups in the neurodevelopment study were not taken in rigorous manner. Therefore, this LOAEL only reflects the fact that the lowest dose of perchlorate produced an observed adverse effect. If perchlorate treatment were initiated prior to mating, and if the quantitation of thyroid histopathology were performed more rigorously, the LOAEL would undoubtedly be lower than 0.1 mg/kg-day.

(b) The EPA's policy appears to be reasonable and it is appropriate to use the most sensitive endpoint on which to base the RfD. In this case, using the thyroid histopathology study for pups is reasonable because this is a critical and sensitive time for thyroid hormone action and disruption.

(c) The approach is reasonable, as is the identification of the neurodevelopment study. It is important to emphasize that thyroid hormone actions on brain development represent irreversible effects; thus, it is essential to protect the developing brain because there is no therapeutic remedy. However, for reasons discussed above and in the neurodevelopment review, the estimate of 0.1 mg/kg-day is probably high.

(d) Regarding the uncertainty factor of 3 for deriving the RfD: the greatest uncertainty that remains to be determined is the uptake of perchlorate in milk and the potential for concentration of perchlorate in the pup that occurs during lactation. If the assumption about the transfer of perchlorate to the pup is correct, and there is good evidence that it is not, then the uncertainty factor of 3 is reasonable. However, in the absence of these data, an UF of 10 seems justified.

(e) The UF of 3 for using the LOAEL should be increased to 10 because of the issues discussed above for the estimation of this particular LOAEL.

(f) The RfD calculated by the EPA and its justification are quite reasonable. However, the weaknesses in study design and data acquisition as described above introduce caution in this RfD.

Further Testing Needs for Perchlorate

1. It is essential to determine the concentration of perchlorate in milk of animals treated with different doses of perchlorate.

2. Endpoints need to be specifically designed to evaluate thyroid disruption. These are described in detail in the neurodevelopment review.

3. Although it is clear that perchlorate is a thyroid disruptor, the majority of endpoints were not designed to identify thyroid disruption. This is critical, especially considering the potential irreversible and specific effects of thyroid disruption during development. Thus, for an RfD to be developed with confidence, studies on perchlorate-induced disruption of thyroid hormone action are essential and these additional refinements would be important.

3.3 90-Day Subchronic Oral Bioassay Study: Dr. Charles Emerson

This was a study of the potential toxicity of ammonium perchlorate (AP), when ingested in drinking water, in male and female rats. AP was administered to groups of rats in doses ranging from 0 to 10 mg/kg/day for a period 90 days. Most endpoints were evaluated at 14 days and at 90 days as well as after a 30-day recovery period. The parameters evaluated were those relating to thyroid function as reflected by serum thyroid hormones, TSH and thyroid histopathology, indices of reproductive function, mutagenesis as assessed by bone marrow examination for micronuclei formation, routine blood chemistry profiles, and characteristics ascertained by complete gross necropsy and *in vivo* ocular examination. As the workshop progressed, the peer reviewers expressed and adhered to a consensus based on the empirical data and theoretical considerations presented. This consensus was that all of the foreseeable toxicity of perchlorate, and thus its potential toxicity, would be related to its ability to inhibit thyroid function. The potentially deleterious effects of perchlorate, therefore, would be due to the effects of hypothyroidism on tissues and organ systems. In this context the peer reviewers considered the general toxicity parameters evaluated in this study, many of which are not targets for hypothyroidism, to be broad, detailed, and performed in a thorough manner. One reviewer was concerned, however, about the high variability of this data in rats and the potential it held for Type II errors. The assessment of the effects of perchlorate on the thyroid was considered in two contexts. The first was an assessment of thyroid function itself, and the second was the effects of hypothyroidism on organ function and development. It was noted that, despite the major concern being the potential for perchlorate to cause hypothyroidism, the endpoints were not selected on the basis of their being sensitive to hypothyroidism or changes in thyroid status. An important corollary question, one that could not be resolved, is whether rats or humans were more or less sensitive to the same degrees of thyroid hormone deprivation. However, this discussion reinforced that notion that studies in primates might provide the most relevant data concerning the potential toxicity of perchlorate in humans. The most critical aspect of the studies, considering the potential mechanism for perchlorate toxicity, relates to the dose of perchlorate that caused an adverse effect on thyroid function. Studies in humans strongly suggest that a rise in serum TSH is one, if not the most, sensitive and specific index of primary hypothyroidism. A question was raised as to whether the TSH assay used in this study was as sensitive and specific for the rat as TSH assays that use materials (labeled rat TSH, primary antiserum developed against rat TSH, highly purified rat TSH standard) provided by the NIH NIADDK National Hormone and Pituitary Program (Baltimore, MD). It was recommended that serum samples from the 90-Day Subchronic Oral Bioassay Study be tested using the NIH NIADDK rat TSH assay since most research studies in the endocrine literature utilize this assay. As far as hormone assay results themselves were concerned, it was noted that the results for the 10 animals of each sex assumed major importance, as they provided the comparison group for all the treated groups. A larger number of control rats would have been preferable to provide more robust data. Although in some cases, such as was noted for T4 values for males treated for 90 days, relatively low doses of perchlorate were associated with a statistically significant decrease in T4, there was not a clear dose-response relationship that would be expected if the findings were biologically relevant. Only in the case of the 10 mg/kg/day dose at 14 days did the pattern of TSH, T4, and T3 suggest

a meaningful antithyroid effect. Notably, with the exception of the duration of treatment, this is consistent with the conclusion of the AMENDED FINAL REPORT (Joseph C. Siglin, Ph.D. DABT) in which 1.0 mg/kg/day was considered to be the NOAEL for oral administration of ammonium perchlorate to rats via drinking water for 90 days. This NOAEL was based on treatment-related thyroid pathology. In this context the peer reviewers noted that it was important to utilize uniform criteria for thyroid histopathological examination and blinded reading of slides provided the most optimal and scientifically correct way to evaluate samples.

In summary, this is a study of the potential toxicity of ammonium perchlorate when administered to rats for 90 days. The data available at the time of the workshop did not suggest a biologically meaningful adverse effect on a broad range of endpoints at the doses employed except for mild hypothyroidism at a dose of 10 mg/kg/day. It would be important to compare the TSH assay used in this study with that of the NIH NIADDK rat TSH assay. If large discrepancies are observed, strong consideration should be given to assaying all available samples using the NIH NIADDK rat TSH assay. The standards for thyroid histology examination in the 90-Day Subchronic Oral Bioassay Study should be similar to those for other studies and emphasize uniform grading criteria and blinded readings for definitive analysis. In the event of further studies, to confirm or expand the 90-Day Subchronic Oral Bioassay Study, it would be desirable to expand the number of rats in the groups. This would minimize the chances of Type II error and reduce the chance that the mean for the control group is, in fact, an outlier from the other groups.

3.4 Pilot Developmental Study/Segment II Developmental Study: Dr. Rochelle Tyl

I. REVIEW OF INDIVIDUAL STUDIES INITIATED SINCE MAY 1997

A. Oral (Drinking Water) Dosage-Range Developmental Toxicity Study of Ammonium Perchlorate in Rabbits. Final Pilot Report; Protocol No.: 1416-002P. R.G. York, Study Director, Argus Research Laboratories, Inc., Horsham, PA. Report Date: December 10, 1998.

1. Study Design

This is a relatively standard study design for a range-finding study to select target doses for a subsequent "definitive" developmental toxicity study. The design involved five naturally-mated does (presumed pregnant) per group (five groups) to achieve target intakes of ammonium perchlorate of 0, 0.1, 1.0, 10.0, and 20.0 mg/kg/day, in groups 1 through 5, respectively, based on body weights on gestational day (gd) 5 and estimated water consumption of 100 ml/kg/day, with subsequent adjustments based on actual body weights and water consumption values. Correcting the water concentrations, based on the previous week's body weight and actual water consumption values, will always be off

(too low) since the performing laboratory is basing the subsequent week's concentrations on the previous week's body weights, and the animals are gaining weight during the pregnancy. On gd 13, the target concentrations were increased in groups 2 (0.1 mg/kg/day) and 3 (1.0 mg/kg/day) to provide target intakes of 50 and 100 mg/kg/day, respectively, "in order to establish evidence of maternal toxicity." The protocol (Attachment 1) and the text of the report do not provide information on the statistical analyses used. The *ad libitum* exposure period to the does was for gd 6-28, with scheduled sacrifice on gd 29. At sacrifice, the does were examined for body weight, gravid uterine weight, fixed thyroid (and parathyroid) weight, and gross lesions of organs and body cavities. Maternal blood was taken at sacrifice (from the vena cava) for analyses of TSH, T3, and T4 (by AniLytics, Inc., Gaithersburg, MD), and the fixed thyroids were examined histologically by Research Pathology Services, Inc. (New Britain, PA). The number of ovarian corpora lutea and the number and status of uterine implantation sites (total, early, and late resorptions, live and dead fetuses) were recorded. Fetuses were counted, weighed, examined for gross (external) alterations, and sexed internally (one cannot sex rabbit fetuses externally). Fetal internal (visceral) examinations were not performed (except for sex determination), fetal thyroids were not weighed or examined, and fetal blood TSH, T3, or T4 levels were not evaluated. Fetal brains were also not evaluated histologically (a most likely target organ).

2. Conduct of Study

The study was performed under EPA (FIFRA/TSCA) GLPs with a QA statement provided (Attachment 4). The changes in target concentrations and intakes in groups 2 and 3 on gd 13 were done to provide higher intakes to ascertain maternal toxicity (obviously, to that point, the performing laboratory did not see any evidence of maternal toxicity through 20.0 mg/kg/day). This change was documented by Amendment No. 1, Attachment 1, dated December 30, 1997, but the amendment did not specify the calendar date(s) and gd that the change was initiated. (Hopefully, this information is in the study records.) This change makes interpretation of fetal findings at term very problematic, since embryos in groups 2 and 3 were potentially exposed to low doses early in major organogenesis (gd 6-13) and to very high doses (in a "hypothyroid" mother) for the rest of the embryonic (organogenesis) period and throughout the fetal period (see discussion in item no. 5 below). Fetal thyroids should have been examined. The performing laboratory removed one doe at 0 mg/kg/day from study since she had a one-horn pregnancy with only three live fetuses. This is reasonably appropriate (and should be specified in the laboratory's SOPs).

3. Statistical Methodology

Neither the protocol nor the report specifies which statistical tests were used. The protocol (p. 12) only states "Averages and percentages will be calculated. Litter values will be used where appropriate. Additional procedures and/or analyses may be performed

if deemed appropriate." This provides no useful information. I am assuming that nonparametric statistics were employed for continuous (body weights and changes, feed and water consumption, etc.) and discrete (malformation incidences, etc.) data because of the small group size (4-5 does per group), with a $p \leq 0.05$ as standard for statistical significance from the concurrent control group values. The summary data tables also do not specify which statistical tests were used for which parameters.

4. Presentation of Results

This was adequate, except there was no indication of which statistical tests were performed on which parameters. Three fetuses in three different litters (out of five, 60% litter incidence) at 20.0 mg/kg/day exhibited major external malformations. These fetuses were in the group which was exposed to the highest dose during the first half of major organogenesis (the 50 and 100 mg/kg/day groups were not switched to the higher doses until gd 13). Based on the performing laboratory's historical control data (Appendix J to the definitive study final report), meningocele and umbilical hernia (in this study in male fetus 6747-3) were present in low incidences (litters 0.85%, range 0-50%, fetuses 0.11%, range 0-12.0%, for umbilical hernia; and litter 0.24%, range 0-16.7%, fetuses 0.03%, range 0-2.0% for meningocele). Female fetus 6748-3 had cleft palate and a number of other major malformations (only cleft palate in historical control, litter 0.12%, range 0-5.3%; fetuses 0.01%, range 0-0.6%). Male fetus 6749-1 exhibited distended abdomen (ascites), not in historical controls. The performing laboratory acknowledges this could be due to early organogenesis exposure or chance, and correctly suggests that this dose be employed in the definitive study. My copy is missing page 2 and following of Attachment 2, "Thyroid Hormone Levels" from Anilytics, Inc., so I cannot examine individual data for groups 3 through 5.

5. Use in Hazard Characterization

This study was designed, performed, and interpreted to assign target doses (concentrations in the drinking water and intake values) for the subsequent definitive developmental toxicity study. It served this purpose. The changes in doses on gd 13 mean that the does (and the embryos via probable transplacental transport) were exposed to low intakes (0.1 and 1.0 mg/kg/day) for the first half of the period of major organogenesis (gd 6-13), and the does (and embryos/fetuses) were exposed to high intakes (50 and 100 mg/kg/day) for the second half of major organogenesis (gd 13-18/19) and throughout the fetal period (gd 18/19-28). Since the fetal thyroid, at least in humans, begins to concentrate iodide in the second and third trimesters (the fetal period in humans), the embryos/fetuses in these groups potentially were exposed during a critical period of differentiation and prenatal function to high levels of perchlorate. Examination of their thyroids (at least) would have been informative.

6. Incomplete Study

The study is complete, and the final report is available.

B. Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in Rabbits. Final Report; Protocol No. 1416-002. R. G. York, Study Director, Argus Research Laboratories, Inc., Horsham, PA. Report Date: September 1, 1998 (final report), September 10, 1998 (report amendment).

1. Study Design

This is a relatively standard study design for a Segment II developmental toxicity study in rabbits. The study design involved 25 naturally-mated does (presumed pregnant) per group (six groups) exposed to the test material *ad libitum* in the drinking water, from gd 6 through 28, to achieve target intakes of ammonium perchlorate of 0, 0.1, 1.0, 10.0, 30.0, and 100.0 mg/kg/day, with adjustments to the concentration of test material based on the previous week's body weights and water consumption. Correcting the water concentrations based on the previous week's body weights and actual water consumption values will always be off (too low), since the performing laboratory is basing the subsequent week's concentrations on the previous week's body weights, and the animals are gaining weight during the pregnancy. Two of the target intakes in this study (10.0 and 30.0 mg/kg/day) bracketed the dose in the range-finding study (20 mg/kg/day) at which fetal malformations were observed. Does were examined and weighed daily, and feed and water consumption was measured daily. Maternal rabbits were sacrificed on gd 29, with blood taken from the inferior vena cava for subsequent analysis for T3, T4, and TSH by AniLytics, Inc. (Gaithersburg, MD). Body weights, gravid uterine weights, and fixed thyroids (plus parathyroids) were weighed, and the thyroids were evaluated histologically by Research Pathology Services, Inc. (New Britain, PA). The number of ovarian corpora lutea and the number and status of uterine implantation sites (total, early and late resorptions, dead and live fetuses) were recorded. Each fetus was weighed, examined grossly, and sexed internally. Live fetuses were euthanized. For approximately 50% of the fetuses per litter, a single cross section was made between the parental and frontal bones of the skull, and the brain was examined *in situ*. The remaining fetuses per litter were decapitated and the heads fixed and decalcified in Bouin's solution for subsequent free-hand serial sections and examination. All fetuses were examined viscerally (abdominal and thoracic organs) and skeletally after staining with alizarin red S (for ossified bone). The report indicates that the skeletal preparations were examined for "skeletal and cartilaginous alterations." A deviation from the protocol and SOPs (Appendix D) indicates that although the protocol and SOPs specify staining with alizarin red S and Alcian blue (the latter specifically for cartilage), "The skeletal specimens were stained with only alizarin red S, not also with Alcian blue." The deviation continues "This deviation did not adversely affect the outcome or interpretation of the study, because although staining with Alcian blue makes it easier to see the cartilage, it is not

necessary to stain the cartilage to evaluate it. Bones and cartilage are evaluated adequately using the single stain, and double staining is not a guideline requirement." Based on many years of experience by this reviewer, it is extremely difficult, if not impossible, to "adequately" assess ossified and cartilaginous skeletal components without double staining (tricks to enhance visualization of cartilage in single-stained specimens include bottom lighting and use of ethanol to make the cartilage "cloudy," but these are not adequate). Fetal thyroids were not weighed or assessed histopathologically, fetal blood was not evaluated for TSH, T3, or T4, and fetal brains (a probable target organ due to maternal and likely fetal hypothyroidism) were not examined histologically.

2. Conduct of Study

This study was performed under EPA (TSCA/FIFRA) GLPs, and there is a QA statement (Appendix K). See comments in answer to question 1 on the major deviation for the protocol on staining fetal skeletal specimens. The lack of staining for fetal cartilaginous skeletal components impairs the laboratory's ability to adequately assess the fetal skeleton at term and identify any possible treatment-related changes.

3. Statistical Methodology

It is adequately described in the protocol (Appendix C, page 14) and in the final report (pages 28-29). However, I have two concerns:

a. The decision tree provided uses the Bartlett's test for homogeneity of variances to decide whether to use parametric or nonparametric statistics. If Bartlett's is significant for a given parameter at $p \leq 0.05$, nonparametric analysis is performed. This sets the "gate" for parametric versus nonparametric analyses very (too) low. Most laboratories, including this reviewer's, use a $p<0.001$ as the gate. I went back and looked at our rabbit data; if we had used the cutoff of $p<0.05$ for Bartlett's, almost all parameters would have been examined nonparametrically. Nonparametric statistics are less robust than parametric statistics, so fewer differences would be identified as statistically significantly different using nonparametric statistics. In my laboratory, we perform an arcsine-square root transformation on all litter-derived percentage data to allow use of parametric methods. For these litter-derived parametric data, we also weigh the ANOVA according to litter size (litters with a larger number of fetuses have lower weight fetuses, relative to litters with fewer numbers of fetuses).

b. Neither the text, summary tables, nor individual tables indicate which tests were used to evaluate statistical significance, so the reviewer has no way of knowing whether parametric or nonparametric tests were used.

4. Presentation of Results

Adequate, although the specific statistical test used is not provided in text or summary tables for a given parameter (see answer to #3).

5. Use in Hazard Characterization

This study is useful and appropriate to use in hazard characterization. The intake values must be corrected for presence of the ammonium ion. Fetal thyroids, brain, and hormone status (TSH, T3, T4) were not evaluated, yet this is most likely the most sensitive life stage, and the brain and thyroid are target organs. The maternal toxicity NOAEL was designated at 1.0 mg/kg/day, due to findings of hypertrophy of the thyroid follicular epithelial cells (and concomitant reduction in follicular lumen size) at 10.0, 30.0, and 100.0 mg/kg/day. The developmental toxicity NOAEL was "greater than 100.0 mg/kg/day," based on the endpoints that were evaluated.

Question: Was a rat developmental toxicity evaluation performed (usually two species, rat and rabbit, are used for developmental toxicity assessments)? At the workshop, we were told there has not been a rat developmental toxicity study. Since the rat is apparently as sensitive as or more sensitive than the human, this is a critical study and an important data gap.

6. Incomplete Study

The study is complete, and the final report is available.

3.5 2-Generation Reproductive Study: Dr. Rochelle Tyl

A. Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats. Final Interim Report, Protocol No. 416-001. R. G. York, Study Director. Argus Research Laboratories, Inc., Horsham, PA. Report Date: September 15, 1998.

1. Study Design

This is a two-generation study in CD® (Sprague-Dawley) rats, one litter per generation, performed according to U.S. EPA FIFRA "Pesticide Assessment Guidelines Subdivision F, 83-3" (this is incorrect; it should be 83-4), with additions from the OPPTS draft guidelines (1996), and thyroid (and parathyroid) weights and histopathology for parental and weanling animals. Blood was also taken from parental and weanling animals for "possible analysis of TSH, T3, and T4" (report, p. 17). The study design involved 30 rats/sex/group (four groups) exposed *ad libitum* (summary, p. 16, is incorrect; it states "once daily") to ammonium perchlorate in the

drinking water, to achieve target intakes of 0, 0.3, 3.0, and 30.0 mg/kg/day. Concentrations in the drinking water were adjusted based on the previous week's actual body weight and water consumption values. Correcting the water concentrations, based on the previous week's body weight and actual water consumption values, will always be off (too low), since the performing laboratory is basing the subsequent week's concentrations on the previous week's body weights and the animals are gaining weight rapidly during the F0 and F1 prebreed exposure period and during the F0 and F1 gestational periods. There was also no difference in the concentration provided to the males versus the females, but they differ in their body weights and water consumption and will, therefore, differ (with a fixed concentration the same for both sexes) in their intake in mg/kg/day. There was a ten-week prebreed exposure period (with estrous cyclicity evaluated for the last three weeks), a two-week mating period (based on examination of report Figure 1; not specified in report), three-week gestation for F0 and F1 parents, and three-week lactation period for parents and F1 and F2 offspring. F1 offspring will be evaluated for acquisition of preputial separation (males) beginning on postnatal day (pnd) 39 and of vaginal patency (females) beginning on pnd 28 (too late; CD® rats begin to acquire vaginal patency prior to pnd 28). The current report is an INTERIM report through the necropsy of F1 weanlings; there is no analytical report (Appendix G), no histology report (Appendix H), and no indication that parental and offspring blood will be analyzed for TSH, T3, or T4. At the workshop, additional data were provided on thyroid histopathology of F0 and F1 adult animals, and TSH, T3, and T4 levels for F0 adults, F1 weanlings, and F1 adults at necropsy. It is not clear whether histopathology on F1 weanling thyroids was (or will be) done; it is strongly recommended. It would have been very useful to have brain histology done on F1 and F2 weanlings and to have used the F1 and F2 pups, culled on pnd 4 to standardize litter sizes, for thyroid and brain histopathology (especially to corroborate histopathologic changes in the thyroid observed in pnd 5 pups in the developmental neurotoxicity study), and to evaluate their brains histologically to see if *in utero* exposure in a hypothyroid mother results in neonatal brain alterations. The pituitaries in the F0 and F1 animals also appeared enlarged in a dose-related incidence. This may be indirect evidence that increased TSH synthesis and release occurred in response to recognized reductions in circulating T3 and T4 levels.

2. Conduct of Study

Based on the protocol and data available, it is a well-conducted study, according to current guidelines, with some additional study-specific endpoints added.

3. Statistical Methodology

Statistical analysis is described in the report (pages 37-38) and in the protocol (Appendix D, page 23). I have five concerns with the methodology as presented:

a. It does not provide adequate detail.

b. The use of Bartlett's test for homogeneity of variances to decide whether to use parametric or nonparametric tests with the "gate" at $p<0.05$ (see comments on the rabbit developmental toxicity study) and the implications for robustness of analyses.

c. There is no mention of analysis of covariance (ANCOVA) for day (age) of acquisition of preputial separation (F1 males) or of vaginal patency (females) with body weight at acquisition as the covariate. A number of laboratories running multigeneration studies under the new OPPTS guidelines have data indicating that acquisition of these developmental landmarks is at least partially dependant on body weight, so that lighter pups (due for example to systemic toxicity) will acquire them later.

d. There is no mention of analysis of covariance (ANCOVA) by body weight for anogenital distance at birth of F2 pups if triggered by alterations in reproductive development in F1 animals. Again, anogenital distance is sensitive to body weight.

e. There is no indication of which tests were used to assess statistical significance for a given parameter in the summary tables.

4. Presentation of Results

Based on the interim nature of this report, presentation is adequate. There should be an indication of which statistical test was used for each parameter, parametric or nonparametric.

5. Use in Hazard Characterization

When all the data through weaning of the F2 offspring are in, the thyroid weights and histopathology in parents and offspring, and hopefully parental and offspring TSH, T3, and T4 values, will prove sensitive endpoints to confirm (or refute) the current most sensitive LOAEL of 0.1 mg/kg/day for the pnd 5 pups in the developmental neurotoxicity study. The low dose in this study is 0.3 mg/kg/day, higher than the most sensitive LOAEL, but it will provide confirmation and confidence in the assessment of effects at 0.1 mg/kg/day in the other study. Based on the information provided at the workshop, it is this reviewer's opinion that no NOAEL can be identified, based on thyroid follicular histopathology at 0.3 mg/kg/day (the low dose) in F0 males and females and in F1 males, as well as in both higher doses for both sexes and both generations.

There was much discussion at the Peer Review Workshop on the use of the terms "hypertrophy" (increase in cell size) versus "hyperplasia" (increase in cell number) by the pathologist reading the slides for all three studies; they appeared to be used interchangeably, which is not correct. This has implications for identifying a NOAEL and, therefore, risk assessment, since "hypertrophy" can be reviewed as a transient physiological response within homeostatic constraints (and therefore not necessarily adverse and not a necessary and sufficient biomarker of subsequent hyperplasia and possible neoplasia); i.e., after longer exposure or higher dose). "Hyperplasia" was unanimously viewed by the Panel as adverse and most likely necessary (but not necessarily sufficient) for subsequent neoplasia since cell divisions are involved.

A strong recommendation is to have the slides and diagnoses reviewed by a panel of pathologists to standardize the terminology and confirm the assessments.

6. Incomplete Study

This report is INTERIM and ends at the weaning of the F1 offspring. It does not include analytical results of the drinking water, histopathology of parental and offspring tissues, including thyroid/parathyroid, and it does not include the postnatal growth, development (including reproductive) and mating, and pregnancy and lactational events for F1 parents and F2 offspring. The F1 generation is really the most important, since it was at least potentially exposed during gestation and lactation, with exposures continuing through its mating, gestation and lactation, to produce F2 offspring. Since the fetal hypothalamic-pituitary-thyroid axis may be at especial risk and there are known effects of hypothyroidism on growth, development, and reproduction, this generation may show effects at the low dose of 0.3 mg/kg/day. Since this dose is higher than the low dose for the developmental neurotoxicity study, 0.1 mg/kg/day, at which effects were observed in offspring on pnd 5, it will not alter the most sensitive LOAEL, but it will provide confidence in the assessment.

Additional data made available at the workshop provided thyroid weights and histopathology for F0 and F1 parental animals, and TSH, T3, and T4 levels in F0 and F1 parental animals and F1 weanlings (see comments to no. 5).

B. Review of Toxicological Review Document

1. Effects of Concern to Human Health

Key aspects of study design (protocol), conduct, and conclusions of each toxicology study have been adequately described. Limitations have been appropriately discussed.

2. The strengths of the analyses have been well described.

a. The rat is a good surrogate for humans (rat is at least as sensitive as humans and probably more sensitive).
b. The concept of merging cancer and noncancer endpoints is appropriate, given the early and lower dose histopathologic changes to the thyroid and changes in thyroid hormone homeostasis (TSH, T3, T4) as health risks in and of themselves, and as a predictor ("harbinger") of possible carcinogenic risk. The concern over the use and interpretation of "hypertrophy" versus "hyperplasia" of the thyroid follicular epithelium should at least be addressed and hopefully resolved. Even in the absence of mutagenic potential of perchlorate, the pre- and perinatal animal (and human) should be considered at especial risk.
c. The discussion of the hypothalamic-pituitary-thyroid axis is appropriate.
d. The uncertainty factors are appropriately conservative and protective.

The biggest weakness is the lack of a final report for the two-generation study (only an interim report is available). The F1 generation is crucial and potentially the most sensitive. However, the low dose is 0.3 mg/kg/day, so a lower NOAEL/LOAEL will <u>not</u> be identified.

3. The additional statistical analyses were useful and appropriate. The designation of 0.1 mg/kg/day as a minimal LOAEL, based on the pnd 5 thyroid effects for the developmental neurotoxicology study, depends on whether the findings are "hypertrophy" (increase in cell size, presumably transient and within homeostatic constraints) or "hyperplasia" (increase in cell number, viewed as adverse and a predictor of possible carcinogenic risk since it involves cell divisions). This designation should be in keeping with biological plausibility, mode-of-action, weight of the evidence, and the "precautionary principle."

4. I don't know of any additional references.

5. NA (Ecotox)

6. Under exposure characterization (e.g., page 7-8, line 1 and page 8-6, line 7), the document indicates that "0.37%" is equal to "37×10^6 μg/L." But, 0.37% = 3700 ppm (0.0037 [conversion from percent] x 1,000,000 [1 liter = 1,000,000 μl] = 3700 ppm). Therefore, the correct value must be 3.7×10^6 μg/L (3700 mg/L), at least based on my calculations, which is possible, given the solubility of perchlorate in water of 24.992 w/w % for the ammonium salt (page 2-3, Table 2-2).

7. The document is useful.

C. HAZARD CHARACTERIZATION

Development of Reference Dose (RfD)

1. The individual NOAELS and LOAELS for each study, based on EPA reanalysis, are appropriate.

2. The EPA approach, using a merged cancer and noncancer endpoint evaluation, and viewing changes in histopathology of the thyroid (certainly for hyperplasia, less certain for hypertrophy) and in TSH, T3, and T4 as noncancer endpoints of concern and predictors ("harbingers") of possible cancer risk, is appropriate and justified.

3. The selection of 0.1 mg/kg/day as the "minimum" LOAEL, based on histopathological changes in the thyroid in pups on pnd 5 in the developmental neurotoxicology study, may be appropriate once terminology and diagnoses are standardized and confirmed.

4. Given the nonmutagenicity of perchlorate, the thyroid-pituitary disruptions in homeostasis (histopathology and circulating hormone levels), and the rat as an animal model at least as sensitive as humans (and apparently more sensitive), an uncertain factor of "3" is very conservative to go from rats to humans. A factor of "1" might be more realistic and still protective.

5. The additional uncertainty factor of "3" to go from a LOAEL to a NOAEL is appropriate, given that histopathologic changes are very sensitive and that there were such changes, albeit "minimal" at 0.1 mg/kg/day in the pnd 5 pups (again, depending on the concurrence of "adverse" for hyperplasia and the interpretation of hypertrophy).

6. The use of the RfD for a harmonized human health risk estimate should be protective for both noncancer health effects and cancer endpoints of perchlorate ion. The data support this position.

7. NA (Ecotox)

D. FURTHER TESTING NEEDS FOR PERCHLORATE

Toxicological Testing

1. The experimental designs of the toxicology tests undertaken since May 1997 are adequate, except for:

   a. The rat multigeneration study low dose is 0.3 mg/kg/day, while the low dose and the subsequently identified "minimal" LOAEL in the developmental neurotoxicology study is 0.1 mg/kg/day. "Hindsight is 20-20," but it would

have been very useful to run 0.1 mg/kg/day and a lower dose in the multi-generation study to confirm or refute the 0.1 mg/kg/day LOAEL and identify a true NOAEL in a study examining offspring at least potentially exposed during gestation and lactation, with exposure continuing through their reproductive phase (the F1 to make F2 litters).

b. I hope the blood samples retained from F0 and F1 parental animals and F1 and F2 weanlings for the multi-generation study will be analyzed for TSH, T3, and T4 to accompany the histopathological assessments on the thyroids from these animals. Additional data provided during the workshop included blood samples for F0 and F1 parents and F1 weanlings, and histopathology of F0 and F1 parental organs.

2. There has not been a Segment II developmental toxicity study of ammonium perchlorate in rats (the usual approach, mandated by EPA FIFRA and other regulatory testing requirements, is this test in two species a rodent, usually rat, and a nonrodent, usually a rabbit). It should be done, since the rat is as sensitive as/or more sensitive than the human. *In utero* exposure, up until term, will assess effects of maternal "hypothyroidism" and possible embryo/fetal "hypothyroidism" on *in utero* development, especially in the postembryonic fetal period, which corresponds in humans to when the fetus begins to concentrate iodide into the thyroid (does this indicate activation of the symporter?). The protocol should be a standard OPPTS (1998) developmental toxicity study with maternal blood and maternal (and fetal?) thyroids taken for serum TSH, T3, T4 (dams), and histopathology (dams and fetuses). Fetal brain histopathology should also be done.

3. Development of a PBPK model to address species differences in iodide uptake, perchlorate kinetics, and subsequent perturbations in the hypothalamic-pituitary-thyroid axis is an excellent idea, but:

   a. It will only be as good as the data available and will take considerable time to develop.
   b. It must model maternal, embryo/fetal and perinatal offspring parameters, with more than one timepoint during the pregnancy to detect anticipated changes in the parameters of concern (e.g., iodide uptake, as it changes in the maternal and embryo/fetal compartments during the pregnancy).

4. NA (Ecotox testing)

NOTE: There are a number of typographical errors and more substantive errors in the EPA Toxicological Review (review draft) which will need to be corrected prior to finalization. Examples (not complete) follow:

Review Page	Review Line	Error
E-6	12	Pimephales (fathead minnow) is an aquatic vertebrate (not invertebrate)
1-1	22-23	Thermal explosive decomposition occurs above (not below) 300°C.
3-2	23	"given no [not on] more than..."
3-6	17	"perchlorate" fix typo
3-6	19	"...adverse effect than healthy..."
3-7	17	"...radioimmunoassay..."
4-2	31	"from... to [not and] where..."
4-7	Table 4-1	Superscript "d" and "e" in table but not in footnotes; no superscripts "b" or "c" are present
4-8	8	"...quarternary...", fix typo
4-10	Figure 4-3	Item 9, "TSH-secretory..." [not THS]
4-11	Table 4-2	under "Indirect": (1) "- chemicals inhibiting TH [add "release"], and (2) "- chemical [add "s"] inhibiting [should be stimulating?] hepatic..."
4-18	6	"...developing conceptus..." not "fetus" (too narrow a term)
4-22	Table 4-6	middle column: (a) under rat dev. neurotox, maternal animals are "dams" not does; (b) designate initial parental generation "P0" (dev. neuro.) or "F0" (two-gen), but be consistent
5-43	13	Dev. Tox studies do not employ "brain histology," but serial freehand sections of fixed tissue (no embedment, microtome section or stain, etc.)
5-55	31	"affected" not "effected"
5-56	17	"perchlorate", fix typo

Review Page	Review Line	Error
5-57	30	The description of what the Ames test measures is incorrect. It measures "the reversion from a histidine- (histidine dependent) state to a histidine+ (histidine independent) state induced by chemicals..." (by growing the exposed organism on media without histidine, only his+, independent revertants will grow)
5-59	29	"...drinking water gavage" is incorrect, can't be both; should be "gavage" (I think)
6-10	5	"...diurnal...", fix typo
6-12	9	"...trichloroacetate...", fix typo
6-30	29	"...distributions...", fix typo
6-32	14	"...homeostasis...", fix typo
6-32	21	"...or those treated with anti-thyroid drugs..."; move "anti-thyroid"
6-41	8	"...exquisite...", fix typo
6-45	19	"...because it fell..." (not "if")
6-48	17	"...appropriate...", fix typo
6-52	7	"... because of the efflux..." (delete "to")
7-8, 8-6	1, 7	see comment on "0.37% (37 x 106 µg/L)"
7-14	2-3	If survival is reduced as indicated, it is not dose related; should it read "survival was reduced by 26%...etc."?
9-6	48	"...N-bis (2-hydroxypropyl) nitrosamine...", fix typo

3.6 Immunotoxicity Studies: Dr. Kimber White

Review of Immunotoxicological Studies

Information provided at the Perchlorate Workshop revealed that the laboratory conducting the immunotoxicological evaluation of perchlorate was just starting up and had limited experience in conducting many of the immunological assays. Furthermore, the personnel working on the project was limited to the Principle Investigator, a laboratory technician, and a graduate student. Due to the nature and high visibility of the perchlorate issue, it is unfortunate that a laboratory with more experience in conducting immunological studies was not involved in the project. In addition, based on the workscope undertaken, the personnel resources dedicated to the project do not appear to be sufficient. However, the Principle Investigator has accomplished a significant amount of work with very limited resources, and she should be complemented for her efforts.

The EPA has identified the evaluation of the effects of perchlorate on the immune system as a critical area in the characterization of perchlorate. The question being investigated in these studies (does perchlorate adversely affect the immune system?) is certainly germane to the evaluation of the overall health effects of perchlorate exposure. In general, the experimental design for the immunotoxicology studies appeared appropriate. One of the major strengths of the experimental design followed is the fact that the assays were conducted at multiple time points (14, 90, and 120 days) and, more importantly, each of the assays was conducted at least twice. This approach of repeating all functional assays at least twice is not routinely done in most immunotoxicological investigations, and the fact that the assays were repeated in the evaluation of perchlorate is considered to be a major strength of the study. A second significant strength is the inclusion of the 30-day recovery period (120 day results) in the experimental design. Most immunotoxicological evaluations do not include recovery information on the test compounds. The selection of the B6C3F1 mouse as the mouse strain for use in the immunological investigations is also considered a strength since significant data exist on the immunological responses of this test species. Furthermore, the use of sentinel mice during the study period also represents a strength since the immunological assays are very sensitive to viral infections.

The experimental design also had several weaknesses. First, the decision to use only 6 animals per group is considered to be a weakness. As reflected in the data, on several occasions multiple samples from the same group were lost as a result of technical error or for some other reason, resulting in small group sample size. A decrease in the number of samples in the control or treatment groups can have a significant impact on the statistical evaluation of the results. Another weakness in the design is the fact that a positive control was not included in each of the functional immunological assays. By having a positive control present in the assay, one insures that the assay was conducted correctly and that the assay was capable of detecting an effect, if one was present. The use of a positive control is also helpful as a reference point in evaluating the effect the test compound has on an immunological parameter being measured.

Only one sex, female animals, was evaluated for immunological effects. While this does represent a limitation of the studies, it is consistent with the usual approach used by other organizations, such as the National Toxicology Program, in evaluating the effects of test materials on the immune system. The decision to use female mice and not male mice is considered to be a strength in the design.

The major weakness in the experimental design was the selection of the immunological assays and host resistance assays used in the evaluation of perchlorate. It is unclear from the information provided what the rational was behind the selection of the assays. A tremendous amount of work has gone into developing, validating, and determining the sensitivity and predictability of various immunotoxicological assays (Luster et al., 1988, 1992, and 1993). This work appears not to have been considered in the assay selection for the evaluation of perchlorate. For example, the most predictive immunotoxicological assay, the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes (Plaque Assay), was not originally undertaken in the evaluation of perchlorate.

At the insistence of the EPA, the effect of perchlorate on humoral immunity was evaluated by the immunotoxicology laboratory and the preliminary results from these studies were provided at the workshop. Unfortunately, the assay conducted was not the Plaque Assay for which a significant data base exists, but an ELISA assay to sheep erythrocytes for which only minimum historical data exist. Also, a multilaboratory validation of the ELISA assay has not been conducted. Furthermore, due to the limited number of dilutions utilized in conducting the assays and the manner in which the data were evaluated, the results are essentially unusable for a proper evaluation of the effects of perchlorate on the humoral immune response.

Some of the assays conducted in the evaluation of perchlorate are no longer used by other organizations since they have been found not to be very predictive of immunotoxic effects. In addition, the procedures followed in conducting some of the assays differ significantly from those followed by Luster et al. For example, routinely a Delayed Type Hypersensitivity (DTH) assay, such as those conducted in the Luster et al. studies, consists of a holistic *in vivo* evaluation of the response to the test article. In the Luster et al. DTH studies, a mouse or rat is both sensitized and challenged *in vivo* and the endpoint monitored occurs *in vivo*, such as the swelling of a foot pad or recruitment of cells to the site of challenge. The "DTH" evaluated in these studies is more accurately described as an antigen specific proliferative response as opposed to a "classical" *in vivo* DTH response. As such, it loses the predictive value for identifying potentially immunosuppressive compounds.

It is also unclear what was the rational for selecting and conducting the host resistance assays prior to the completion of the functional assays. If humoral immunity, as evaluated in the plaque assay, is affected to a much greater extent than cell-mediated immune parameters, such as the cytotoxic T-cell response, a host resistance assay designed to evaluate humoral immunity may prove to be more appropriate in the overall immunotoxicological evaluation of perchlorate than the *Listeria monocytogenes* assay which was conducted. Since effects were observed on natural killer cell

activity, the selection of the B16F10 tumor was an appropriate model for evaluation of innate immunity host resistance.

The immunotoxicology studies conducted in the evaluation of perchlorate were reported to have been conducted under the guidelines of Good Laboratory Practices (GLPs). As such, the integrity of the data, from collection until inclusion of tables and graphs in the final report, would have been appropriately monitored by the Quality Assurance Manager and found free of error. Furthermore, the fact that the study was conducted under GLPs indicates that all Standard Operating Procedures (SOPs) for the various assays conducted were in place and were followed in carrying out the study.

A limitation which could decrease the relevance of the study findings is the fact that technical problems occurred with some of the assays. On several occasions the studies' results did not appear to repeat. This may just be due to the fact that the assays were conducted using living biological systems. However, technical problems can also contribute to the failure of an assay to produce reproducible results. The report authors themselves indicated that technical problems occurred in obtaining some of the data (i.e., spleen and thymus cellularity, nitrite production by peritoneal macrophages and *Listeria monocytogenes* study). The combination of the failure of assays to produce reproducible results and the concern raised by the technical problems associated with conducting the assays represents a limitation in the conduct of the study.

All of the immunotoxicological data presented was evaluated by using an analysis of variance and Tukey's multiple comparison to compare control and treatment groups. Results from immunotoxicological assays do not always follow a normal distribution and, at times, a more appropriate evaluation would be one using a non-parametric analysis. Accordingly, this fixed approach used by the authors in evaluating their data is considered to be a weakness. A better procedure would be to use a decision tree approach in which the data are first evaluated to determine if they are normally distributed and then evaluated for homogeneity of variances using the Bartlett's Chi Square Test or another statistical test. Homogeneous data would be evaluated by a parametric one-way analysis of variance. When significant differences occur, treatment groups would be compared to the control using the Dunnett's t Test. Non-homogeneous data would be evaluated using a non-parametric analysis of variance. When significant differences occur, treatment groups would be compared to the control using the Wilcoxon Rank Test.

In general, sufficient data were presented to confirm the findings in the report and usually the data could be tracked from the information provided in the tables to the final results in the figures. Areas of weakness include the lack of necessary information in several of the methods' write-ups. More information needs to be provided on the CD4/CD8 surface marker analysis, such as exactly which antibodies were used and an explanation why other cell types were not included in the evaluation. For example, why were B-cells and total T-cells not counted? Furthermore, the surface marker data need to be presented as absolute numbers as well as in percentage values.

More information needs to be provided on how the Lytic Unit was calculated for the natural killer cell assays. It appears that in some of the natural killer cell assays only three effector-to-target ratios

were used. With such a small number of points defining the curve, accurate determination of Lytic Units is questionable as some of the values have to be extrapolated when 10% lysis of the target cells is used to define the Lytic Unit. Finally, it would be extremely helpful to have all the graphs for a particular assay drawn to the same scale. This would make interpretation across repeat studies and at the different time periods much easier for the reader.

Based on the information provided at the Workshop by the Principle Investigator, including the preliminary ELISA data, as of this time the immunotoxicological evaluation of perchlorate still has significant data "gaps" which prevent us from knowing if perchlorate adversely effects the immune system. While some of the data generated is acceptable and can be used in the evaluation of the effects of perchlorate on components of the immune system, most of the critical information is still not available.

As indicated above, assays which have been shown to be predictive for identifying compounds which are immunotoxic still need to be conducted. As an minimum, the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes (Plaque Assay), needs to be carried out following both 14-and 90-day exposure to perchlorate. Serum from these same animals can also be correctly evaluated in the ELISA to sheep erythrocytes assay. Furthermore, because the *in vitro* phagocytosis assay showed an effect at all dose levels, the effect of perchlorate on macrophage phagocytosis needs to be further evaluated. Preferably, the study should be a holistic *in vivo* evaluation, such as the RES assay which measures the functional ability of the reticuloendothelial system; however, if this is not possible, a more conventional macrophage phagocytosis assay (i.e., using latex particles or radio-labeled sheep erythrocytes) should be conducted. While *Listeria monocytogenes* host resistance studies are currently ongoing, additional host resistance studies may be warranted if the results of the plaque assays identify the humoral immune response as a target for perchlorate exposure. Until these additional data are obtained, the potential for perchlorate exposure to adversely affect the immune system will remain unknown.

3.7 Genotoxicity Studies: Dr. David Brusick

Review and Summary of the Genotoxicity Testing of Perchlorate

Genetic Toxicology

This is a review of individual studies initiated since May 1997 and their impact on the Report.

Strengths and weaknesses of the experimental designs and validity of the interpretations contained in the report: Three studies were included in the series of assays for perchlorate (Salmonella reverse mutation, Mouse Lymphoma assay, and the Mouse Micronucleus). This is an acceptable battery for most chemical screening; however, this array of tests is not as comprehensive as one might want to have for this chemical, considering the extensive human exposure to perchlorate.

Salmonella reverse mutation assay: The assay reported was a very basic design. Most guidelines recommending this test include additional tester strains (e.g., TA104 and/or *E. coli* WP*2 uvr*A). These strains cover mutagenic mechanisms not detected by the initial set of strains and would have strengthened the assay and provided stronger support for assuming that perchlorate is nongenotoxic. Another method which is sometimes used to detect weak agents is the suspension assay which gives better contact between test material and the target organisms. The current assay meets minimum specifications.

Mouse Lymphoma: The design of the study and the conduct of the study were both inadequate. The design should have specific criteria for selecting the high dose on the basis of toxicity. Current guidelines (OECD) require that the high dose be selected at 80-90% toxicity. While it appears that the doses were selected to give the correct toxicity, the actual study data do not provide confirming information of concurrent toxicity. The retest of the -S9 trial should have been set at a 24-hour exposure period based on current protocol requirements. The results from this study are not acceptable for classifying the *in vitro* mutagenicity of perchlorate and should be repeated using current ICH or OECD guidelines.

The Mouse Micronucleus Test: The study design as described in the EPA review and in the contractor report, is incomplete. For example, the design description did not indicate if the mg/kg dose was delivered as a single dose or was given as two or more doses. If the dose was given as a single acute dose (which is what I conclude), there should have been two sample times; one at 24 hours and one at 48 hours. In this study, the sample time (only one) was not stated. I am also concerned about dose selection. The logic used to go from a dose of 2,000 mg/kg, which was moderately toxic, to the high dose in the test of 1,000 mg/kg was not well supported. There was no evidence for toxicity at 1,000 mg/kg, which is contrary to the recommendations from OECD. In order to provide the best possible support for negative genotoxicity, I would have selected a high dose of 2,000 mg/kg (or at least 1,500 mg/kg) and two lower dose levels at 50% and 25% of the high dose. The other potential issue resulting from the dose selection is that one has negative results with no evidence that the test material even reached the target cells (bone marrow). Consequently, the results from this assay are weak support for reaching a conclusion of nongenotoxicity and the study should be repeated with a better design and higher dose levels.

Hazard Characterization

Based on my concerns for at least two of the three tests conducted to define the genetic toxicity of perchlorate, it is my opinion that the classification of perchlorate as nongenotoxic is not well supported. This concern may have some influence on discussions of the type of risk analysis used in future evaluations.

Further Needs for Testing

While the general structure and toxicity of perchlorate do not fit with chemicals associated with DNA activity, I believe that additional testing for DNA damage should be performed including, the repeat of the Mouse Lymphoma assay and the mouse micronucleus assay. Based on completion of those repeats, additional studies may be appropriate.

Review of the Studies Repeated in 1998/1999

Following publication of the draft review report by EPA, all three of the genetic toxicology tests were repeated in different testing facilities.

Salmonella reverse mutation assay: The repeat study conducted by NIEHS contained all of the recommendations cited previously. Strains TA102 and TA104 were added. These strains would respond to active oxygen species or other DNA damaging radicals. In addition, the repeat tests were evaluated using the pre-incubation test methodology. The results of the repeat study were negative confirming the original Ames test.

Mouse Lymphoma: The study was repeated using the current ICH protocol. The toxicity profile seen in the first study was reproduced. The study design included a concurrent toxicity evaluation as part of the main mutation assay. The results of the study were negative and provided support for the negative results reported in the first study.

Mouse Micronucleus Assay: This study was repeated with an intraperitoneal injection route of administration. This route is more sensitive than oral administration (first study) and should have increased the opportunity to see even a weak effect. Dose selection in the repeat study was improved and resulted in treatment doses with evidence of toxicity to the test organism. The results were negative in the repeat study, confirming the results of the first study.

Hazard Characterization

The repeat studies were all well conducted with unequivocal negative results. Following the retests, the genotoxicity section of the EPA review was rewritten incorporating more of the study design information and integrating the two sets of data. The overall interpretation stated in the report was that "mutagenicity is not considered a possible mode of carcinogenic action for this chemical." This interpretation is consistent with the supporting data. The rewrite for this section is much improved over the original.

Summary of Genotoxicity Testing

The genotoxicity of perchlorate was assessed using a battery of three tests:

- The Ames test conducted by plate incorporation and pre-incubation methods.

- The Mouse Lymphoma assay for mutation at the thymidine kinase locus.
- The Mouse Micronucleus assay using both oral and i.p. injection routes of exposure.

All three tests were conducted twice yielding similar negative responses. The negative data from these studies provides strong evidence that perchlorate does not react with DNA to induce either point mutation or chromosome breakage. The EPA review of perchlorate concluded, reasonably, that "mutagenicity is not considered a possible mode of carcinogenic actions for this chemical."

3.8 Ecotoxicity Studies: Dr. Rick Cardwell

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information

Summary

EPA compiled data on the fate and effects of perchlorate on fish, wildlife, and plants, and from these prepared a screening ecological risk assessment (SERA). These data were sufficient to support preparation of the SERA, and the SERA was prepared in a competent and scientifically defensible manner. The authors demonstrated they were competent with respect to analysis of the ecotoxicological studies and in preparation of ecological risk assessments. The major weaknesses of the SERA reflected limited data concerning (1) exposure and (2) the potential for long-term chronic effects. The limited information resulted in a SERA that was quite conservative in terms of the risk-based effects thresholds suggested and in terms of the scope of additional studies recommended. Defining the concentrations of perchlorate that occur in surface waters, sediments, soils, and the tissues of plants and animals clearly is the most important data gap currently. The current information on exposure is so meager that it is impossible to judge the ecological problem posed by perchlorate, if one exists. The lack of this information makes it impossible to decide what types of fish, wildlife, and plants are at risk. The latter is important for making the risk assessments more specific and to guide testing needs. It is assumed that ponds, wetlands, and irrigation canals that are subject to evapoconcentration and occur downgradient from known perchlorate sources may be at greatest risk. If so, the fish, wildlife, and plants occurring in these habitats may be at greatest risk. Because of the possibly significant bioaccumulation of perchlorate that has been documented in leaves of some plants, effects to wildlife from consumption of perchlorate via dietary pathways may warrant special emphasis. However, bioaccumulation in another food base, invertebrates, should be examined, for both water-based and food-based exposures (e.g., insects consuming vascular plants or detritus). And finally, long-term chronic effects need examination in species and endpoints sensitive to the thyroid hormone. There have been no long-term studies of this type in species other than laboratory rodents (rat). The rat studies supporting human health risk evaluations should suffice for ecological risk evaluations made for mammals, but not for birds, fish, and invertebrates. The critical ecotoxicological studies may include the following. A long-term chronic exposure with fathead minnows encompassing gametogenesis and embryogenesis up

through to 28-day old fry is one such study. A study of the effects on tadpole development up through metamorphosis would be helpful in deciding risks to amphibians, as the frog embryo development study is inconclusive with respect to hormone-mediated effects. And finally, a chronic study using waterfowl should be considered if the foregoing rat, fathead minnow, and amphibian studies suggest effects at concentrations significantly lower than those currently predicted with the ecological screening risk analysis.

Specific Comments

***Ecotoxicological Effects of Concern* (Manuscript by Sprenger et al. (1998)**

Have the Key Aspects of Protocols, Methods and Ecotox Study Results Been Described Adequately?

EA Engineering, Science and Technology, Inc.: EA conducted the following tests: fathead minnow (*Pimephales*) and *Daphnia magna* acutes, fathead minnow 7-day chronic, *Ceriodaphnia* 7-day acute and chronic and 28-day lettuce chronic. The test protocols and methods used by EA Engineering, Science and Technology have been adequately described; more importantly, they conform to standard methods (e.g., ASTM and EPA). The test results meet the protocol requirements and are of good quality. The range-finding and definitive test results are in reasonable agreement, lending further confidence to the validity of the definitive test results.

Block Environmental Services, Inc.: BES conducted 6-7 day chronics with fathead minnow and *Ceriodaphnia* in essentially the same manner as EA. The protocol and test documentation were significantly less extensive than those of EA. No protocol was provided, although BEA said it was available and similar to methods described in a EPA chronic test method manual. Only data summaries were provided, and the results could not be independently verified. However, the *Ceriodaphnia* test results were very close to those reported by EA, and the *Pimephales* data were within a range (factor of 3-4) that is usual for inter-laboratory variability. This study is believed to be of acceptable quality.

Dumont and Bantle (1998): Drs. Dumont and Bantle studied mortality and malformations in the African toad *Xenopus* exposed to aqueous perchlorate. Although a formal test protocol did not accompany the report, it was performed according to ASTM and contained extensive test data. These data appear to be of acceptable quality.

Nzengung (1998): Dr. Nzengung studied the uptake and metabolism of perchlorate in three woody plants (willow – *Salix*; Eastern cottonwood – *Poplar*; and *Eucalyptus*), French Tarragon, spinach, the aquatic plant *Myriophyllum*, and microbial mats. There appeared to be no formal protocol, but the study was described adequately. These results should be treated as qualitative and indicative of trends, not as absolute values, because perchlorate concentrations in the medium were not constant. Rather, they declined with time as the initial addition of perchlorate

was not renewed during the course of the studies, and evidence was provided it was taken up by the plants as well as biodegraded.

Have Limitations in Studies Been Appropriately Discussed? There appeared to be two salient limitations in these tests, and they concern the chronic tests of *Ceriodaphnia* and *Pimephales* and Nzegung's (1998) phytoremediation study. I do not consider the 7-day chronics as definitive because their exposure durations were short, and for one species (*Pimephales*), they did not encompass the species' life cycle. With a chemical that affects hormonal function, I would have liked to have seen a multi-generation test with *Ceriodaphnia* and a full life cycle test with *Pimephales.* The question: were the 7-day test durations sufficient to check on hormonal effects with fish and invertebrates that had no prior exposure to the test substance? There is a lot of uncharted ground with endocrine disrupters, and we cannot yet answer this question. An analysis by Barnthouse, Suter and Bartell (1988) showed that full chronics, i.e., those encompassing the organism's life cycle, were more sensitive than early life stage tests. However, I would not extend the chronic testing unless documented surface water or sediment exposures exceed the 600 µg/L chronic threshold that the Toxicological Review calculated (i.e., Chapter 7, Screening Ecological Risk Assessment for Perchlorate).

The second caveat concerned Nzegung's phytoremediation study. The authors of the Toxicological Review noted the lack of toxicant renewal, and suggested that bioaccumulation probably was underestimated. The lack of perchlorate renewal seems to be a significant design flaw, and it may have underestimated significantly both degradation and bioaccumulation. Future tests should use a renewal or continuous flow technique to better simulate groundwater or irrigation water. The basis for biodegradation should be identified, as Nzegung proposed.

What are the Strengths and Weaknesses of Data Analyses? The data generally were analyzed with appropriate methodologies and to the extent necessary. One important toxicological index, the acute-chronic ratio, was not measured in one set of tests, because of their design. If the BEA tests had measured the 48-hr LC50 for *Ceriodaphnia* and the 96-hr LC50 for *Pimephales*, it would have been possible to calculate acute-chronic ratios for both species. These ratios are essential for estimating chronic toxicity from acute tests, and there are not enough of them available for perchlorate. Although they were calculated by EA for the same species, duplicate values would have afforded a check on precision.

Has the Document Adequately Evaluated the Results of all Relevant Studies and the Biological Significance of the Entire Database? In general, the existing toxicological data were comprehensively presented and interpreted by Sprenger et al. (1998). Including the test results for *Hydra*, *Bufo*, lamprey and Bringman and Kuhn's (1977) *Daphnia magna* test would strengthen the weight of evidence, unless these tests are seriously flawed. The Toxicological Review mentions these tests, but does not include them in the analyses or inferences. Reasons for their exclusion were not given. I believe these tests of other species, some very distant phylogenetically from fish and cladocerans, increase the weight of evidence suggesting that perchlorate probably is not chronically toxic to aquatic life below 600 ug/L.

Completeness of Technical Documentation? The technical documentation was excellent.

Are There Any Sections That Could Be Improved? Because of my concern about ephemeral pond-wetland exposure in arid regions, I think that more testing is needed concerning the following.

Highest Priority — Documentation of perchlorate concentrations in wetlands and small streams in arid regions near areas where significant quantities of perchlorate have been disposed. Small streams and ponds, perennial and ephemeral, in arid regions are very attractive to wildlife because these habitats and water are so limited. Such areas are subject to evaporative concentration. Thus, a combination of limited rainfall, evapo-concentration and bioaccumulation in wetland plants and invertebrates could create potentially higher exposure than assumed thus far.

Highest Priority — Perchlorate's chronic toxicity potential to nesting birds dependent on wetlands (e.g., waterfowl, shorebirds and blackbirds). Chronic tests with appropriate surrogate avian species may be necessary. Rationale: Various birds are very water-dependent for nesting; waterfowl, shorebirds, and blackbirds are often found using ponds and riparian habitat throughout the West. In some locations (San Joaquin, CA; Great Salt Lake, UT), they have sometimes been placed at risk from selenium that has bioaccumulated in their invertebrate food. Wildlife use of ephemeral ponds and perennial ponds subject to extensive evapoconcentration seems to increase their risk. Wildlife using evaporation ponds and wetlands draining areas with significant perchlorate in soils could be at risk, depending on water concentration, magnitude of bioaccumulation, and toxicity.

Highest Priority — Bioaccumulation potential in wetland plant species. Rationale: Bioaccumulation factors of up to 25-fold background were reported in the Toxicological Review, and Nzengung (1998) reported ClO_4 residues in leaves to be 3536 mg/kg in cottonwood, 813 mg/kg in willow, and 641 mg/kg in eucalyptus. Residues in leaves were the highest residues found in plants. These studies should examine bioaccumulation in leaves of woody riparian species, emergent macrophytes, submergent macrophytes, and algae. Species that are known to be important for food of wildlife should be emphasized.

Highest Priority — Perchlorate's chronic toxicity potential to a rodent. The mammalian tests of rodents conducted to support the human health risk assessment will

satisfy this data requirement. Rodents will also be attracted to the wetlands described above.

Highest Priority — Degradation potential in wetland plant species (include microbial role). This study can be dovetailed to the plant bioaccumulation study. It is designed to index persistence.

Medium Priority — Chronic toxicity to fish and amphibia, encompassing species and developmental endpoints that are sensitive to thyroid hormone effects. According to one of the peer reviewers, Dr. Rochelle Tyl, 14-20 days of perchlorate exposure appears to be sufficient to effect hormonal changes in mammals. Therefore, all developmental endpoints should encompass at least a 14-20 day pre-exposure. A long-term chronic exposure with fathead minnows encompassing gametogenesis and embryogenesis up through 28-day old fry is one such study. A study of the effects on tadpole development up through metamorphosis would be helpful in deciding risks to amphibians, as the frog embryo development study (FETAX) performed with perchlorate is inconclusive with respect to hormone-mediated effects.

Low Priority — Sediment toxicity to the freshwater amphipod, *Hyallela*. I accept the Toxicological Review's argument that water column toxicity should be indicative of sediment toxicity, so I believe sediment testing constitutes a low priority. Because of the concern about exposure in evaporative ponds and small wetlands, sediment exposure of invertebrates will occur.

No other recommendations appear to have sufficient priority to mention. I do not believe the evidence suggests risks are sufficient to justify the following studies that were recommended by the Toxicological Review:

Effects on aquatic plants: I saw no indication of phytotoxicity in the studies reviewed. Aquatic plants generally are protected by concentrations that protect aquatic life, because they are less sensitive (Kenaga and Moolenaar (1979).

Effects on nondaphnid invertebrates: Daphnids generally are the most sensitive group of aquatic invertebrates, and given perchlorate's low toxicity, the value of further testing appears questionable.

Effects on litter-feeding invertebrates: Rather than a toxicity study *per se*, examining bioaccumulation in species like corixids (backswimmers) and chironomids (midge flies) – abundant pond-dwelling species - may be more helpful. Some of the corixids and chironomids are tolerant species, and interest in them centers on what perchlorate residues they contain that would provide exposure to birds that eat them.

Testing in estuarine waters: The evidence does not suggest risks to large waterbodies. Groundwater concentrations are too low; groundwater flows are often relatively small relative to overall surface water flows, and dilution capacities are frequently large in large streams and estuaries.

Is the Document Useful for Characterizing Ecotoxicological Effects? If Not, Specify the Nature and Extent of Changes. Yes, the data presented are sufficient to estimate risks, and the Toxicological Review did a good job of estimating risks, from a screening perspective.

SCREENING ECOTOXICOLOGICAL RISK ASSESSMENT (Chapter 7 of Toxicological Review)

Have the Goals and Objectives of the Ecological Screening Analysis Been Adequately Described? Yes. This document was written by scientists well versed in ecorisk assessment and in the interpretation/use of ecotox effect and exposure data.

Does the Analysis Support the Summary and Conclusions Presented? Most of the conclusions are highly supportable.

I am reluctant to fully support the following conclusions/recommendations:

Risks to earthworms (assumed to be representative of soil invertebrates): I do not believe a interspecies safety factor of 242 is warranted, and believe it should be much lower. Strengthening the scientific basis for any safety factor would be helpful. This safety factor resulted in earthworms being categorized as one of the most sensitive species tested, which is inconsistent with the scientific literature on their relative sensitivity.

Risks to herbivorous mammals: The endpoint selected (effect on iodide uptake by the thyroid) has not, to my knowledge, been linked directly to population-level effects. If it is demonstrated to be directly translatable to a population-level effect, then it is acceptable. However, toxicological endpoints for aquatic life and wildlife must be population-level effects. For aquatic life and wildlife, sublethal physiologic or biochemical changes (biomarkers) are not accepted as appropriate surrogates by EPA (Stephan et al. 1985) or the scientific community (Gentile and Slimak1992). Effects on growth, survival and reproductive success are the commonly accepted population-level measurement endpoints.

Are Relevant and Important Aspects of Uncertainty Addressed Sufficiently? Yes. The uncertainty analysis was very thorough, though quite conservative.

Utility of Bioassays for Characterizing Hazard (Effects Potential): The bioassays were acceptable for characterizing hazard, subject to the caveats mentioned above concerning the potential for chronic toxicity over the life cycle of aquatic vertebrates (fish and amphibia). I was

disappointed that the screening ERA only used the *Ceriodaphnia* and fathead minnow acute and chronic data. It is my understanding this action was taken because of concern that the toxicological data for ammonium perchlorate may have reflected ammonia toxicity. Additional analysis might be desirable to see whether any of these data can be used, if it can be shown that ammonia toxicity was not a factor. Including more taxa in the Tier 2 chronic threshold analysis may make it less conservative and representative of more taxa.

Further Ecotoxicological Testing Needs For Perchlorate

Will the Additional Ecotoxicological Studies Currently Underway Be Sufficient to Characterize the Ecotoxicological Potential of Perchlorate? If not, what are data needs and why, and associated experimental designs? The following repeats recommendations made above: Because of my concern about ephemeral pond-wetland exposure in arid regions, I think that more testing is needed concerning the following. Details are provided in the review of Sprenger et al. (1998).

Highest Priority — Documentation of perchlorate concentrations in wetlands and small streams in arid regions near areas where significant quantities of perchlorate have been disposed. Small streams and ponds, perennial and ephemeral, in arid regions are very attractive to wildlife because these habitats and water are so limited. Such areas are subject to evaporative concentration. Thus, a combination of limited rainfall, evapo-concentration and bioaccumulation in wetland plants and invertebrates could create potentially higher exposure than assumed thus far.

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mg/kg in eucalyptus. Residues in leaves were the highest residues found in plants. These studies should examine bioaccumulation in leaves of woody riparian species, emergent macrophytes, submergent macrophytes, and algae. Species that are known to be important for food of wildlife should be emphasized.

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3.9 Risk Analysis: Dr. Melvin Andersen

Summary

This document develops a rationale for a mode of action based approach to assessing noncancer and cancer risks of perchlorate and also pursues a conventional assessment calculating an RfD from a specific critical study with application of a series of uncertainty factors. Unfortunately, the mode of action data do not appear to have much quantitative influence on the RfD determinations. Many of the studies upon which this hazard characterization is based are in the process of completion and several important recommendations for further analysis of the results were provided at the review. These new data should be forthcoming over the next several months and will almost assuredly influence the final RfD. It appears premature to offer a firm number in this current hazard characterization document. The chances are great that any provisional RfD will have to be changed within the next 6 to 9 months. While such a provisional standard could be set today, it would be better to postpone promulgation of a single number until the data set is completed and analyzed. An alternative would be to offer a range of RfD values depending on choices of LOAELs, LOELs, BMDs, and uncertainty factors.

A clearer articulation of the proposed strategy for using mode of action, pharmacokinetic, and iodide uptake inhibition data quantitatively should be added to the document either in the text or as an Appendix. This material would allow a reader to see clearly how the mode of action data are expected to influence the quantitative hazard characterization with perchlorate. The harmonization of cancer and non-cancer assessments proposed is appropriate, although not all the issues in the use of the data for conducting a cancer margin of exposure (MOE) are well-developed. Finally, some reorganization of the document would be helpful for clarity. Despite some limitations, this document is important in outlining the current status of the science for a mode of action assessment with perchlorate. It collates a large body of information, points the direction for a comprehensive assessment based on mode of action, and provides interim guidance while the data are collected, analyzed, and published. The US EPA staff and their collaborators are to be commended on the quality of this interim guidance for a perchlorate hazard characterization.

General

In contrast to other risk characterization documents from US EPA, this document is unusual. It actually represents a work in progress more than a completed characterization of health and ecological risks posed by perchlorate. Several studies are only partially reported (for instance, the immunotoxicity evaluations), and the development of a more integrated evaluation of the multiple toxic endpoints based on mode-of-action of perchlorate as an inhibitor of iodine uptake by the thyroid is outlined, but only partially implemented. The text in some places is uneven, for instance the extensive evaluation/BMD calculations conducted on the various data sets, given the final position that the thyroid changes in the post-natal day 5 (PND5) pups serves as the single response for estimating a provisional RfD. In addition, the placement in the text of the correlation testing with T4, T3, TSH and thyroid histology detracts from the flow of the document. These evaluations could be included as appendices with the main conclusions of the analyses captured in the body of the hazard characterization document.

The US EPA staff and collaborators have not simply provided a document based on conclusions drawn from other primary work and created a secondary document with interpretation. Instead, they have proactively participated in design of a research/testing program and the analysis and interpretation of the results. The statistics and correlations are a necessary part of the whole. With the new cancer guidelines, it will be important that the EPA conduct several mode of action based risk assessments in order to take an informed position on the manner in which these new guidelines should be implemented. This example with perchlorate is the first such effort by the US EPA staff. While there is room for improvement and clarification in the document, as noted below, US EPA staff deserves to be commended for taking this important first step under the new guidelines.

The mode of action based approach with perchlorate is important for assessing significance of hormonal changes and thyroid hypertrophy as a sentinel precursor for all relevant responses to perchlorate. The arguments for this approach are included in various portions of the text, although not clearly emphasized in the summary. This approach represents a move in the direction of harmonization of non-cancer and cancer risk assessment paradigms and permits better (i.e., more rationale) use of available biological and toxicological data. The presentation of the benchmark dose calculations points out the difficulties faced in fitting various models to the observed response data. After this fitting is completed, which model form should be applied and how confident are you that you have selected an appropriate mode to represent the data? The section on curve fitting and BMD calculations was the most difficult one to follow. Unfortunately, a more comprehensive biological model of impaired thyroid function for assessing curve shapes and assessing the significance of alterations in hormone concentrations has not yet been completely fleshed out, although the data for such an enterprise are apparently available or being collected. The presentation by Ms. Annie Jarabek (US EPA) included some detail about these pharmacokinetic and mechanistic studies. However, the manner in which the perchlorate pharmacokinetics and iodide inhibition data will be used in the final hazard characterization was never clearly articulated. The document could be improved by adding a

section that explained the quantitative impact of knowledge mode of action in the risk assessment.

Specific Comments

Most of the studies were adequately described and the interpretations were easily followed. However, the presentation and rational for the immunotoxicology studies were not well presented and the labeling of these studies by letter designation in the report was uninformative. It was also unclear whether any of these planned immunology studies will help at all in considering the importance of the human experience where altered hematology was observed in patients treated with perchlorate. The document states on page 3-6 that these hematological effects were believed to be due to an immunological response.

One of the strengths of the document was in conveying the results of the various thyroid hormone studies in tabular form. This presentation included the relationships for the thyroid hormone, TSH, and histology. These tables present a large amount of material in a readily comprehensible fashion. A tabulation of effect levels for specific toxicity in tissues other than the thyroid would also be useful. The presentation of the associations between T3, T4, TSH, and pathological changes was very important for confirming a link between the several steps: inhibition of I_2 uptake, impaired synthesis/release of T3 and T4, feedback increase of TSH, and overt histological alterations. This section should be improved by including an easily followed preamble letting the reader know exactly what is being done and why it is being done.

There are a number of sections that could be improved by some changes in organization and emphasis. In general, the text would be clearer if the specifics of the statistical analyses were provided in an appendix, with the results and implications emphasized in the text itself. The document has a conventional RfD assessment portion, i.e., critical study and application of uncertainty factors. It also contains a nascent structure in which mode of action is used to categorize effects and provide a larger structure for interpretation of studies. Some data are interpreted in terms of adequacy of a particular study to assess a NOAEL and others in terms of consistency, with the hypothesis that toxicity is secondary to precursor thyroid effects. The manner in which these different points are made and emphasized probably deserves more attention by the authors. This comment is not so much a criticism as point of emphasis. This presentation mode of action is relatively new for a non-cancer assessment. The authors need to think about how this emphasis might lead to reorganization of the presentation of materials. A possibility is to add text to the Executive Summary that lets the reader know what is coming in terms of the mode of action emphasis.

The document adequately captures the current state of knowledge for perchlorate toxicity. The new toxicity tests cover the concerns raised in earlier evaluations of the adequacy of the data base. One drawback is that many of the newer studies have not yet been adequately prepared, reviewed, and documented. All these results should be published and undergo peer-review separate from this evaluation. The argument supporting a precursor relationship for thyroid

dysfunction is well organized. However, this hypothesis is not implemented in a way that can have much quantitative impact on the assessment. If a quantitative implementation of the mode of action is planned, it would be useful to convene a panel either constituted internally at US EPA or jointly with external consultants to ask questions about what form the quantitative approach will take and whether the appropriate kinetic and pharmacodynamic data will be available to provide confidence in a more quantitative approach to guide the risk assessment.

Because of the mode of action emphasis, there is a tension between normal use of a single study and some consideration of the entire body of data in the determination of the RfD. There are two points of concern here. One is how the mode of action should influence the consideration of the entire data set; the second is the estimation of the toxic endpoints most sensitive to disruption of thyroid hormone homeostasis. It probably is appropriate and necessary to establish the most sensitive endpoint; however, depending on mode of action, the most sensitive endpoint in a rodent model may not turn out to be the most sensitive endpoint expected in humans (Barton and Andersen, 1998). The mode of action and evaluation of the totality of the data base might be more directly helpful in the selection of appropriate uncertainty factors after a critical study or several critical studies are identified. In addition, a quantitative model of disruption of thyroid hormone homeostasis (i.e., a BBDR model) might actually render some of the uncertainty factors unnecessary.

As noted earlier, it maybe necessary to select several critical studies. The critical study will depend on presumed mode of action and on characterization of animal-human differences in sensitivity of all organ systems. These targets include the target thyroid itself and the systemic tissues that respond to the thyroid hormone signals. The use of the PND5 results, with thyroid tissues as the critical study, seems sound given the present knowledge. However, it may not be chosen after all the new data are collected and ancillary studies of these tissues are completed, as suggested at the review. One concern is the consequences of the observed changes in the pup thyroid. Do they lead to adverse outcomes in these rats as they mature or are they simply a compensatory effect related to a temporary impairment in iodide incorporation that will be quickly rectified during nursing and weaning? These questions may require other assessments in the adult rats for altered behavior, etc. Also, are the same effects expected in humans? And, are there situations that make humans more sensitive or less sensitive at some point in life than the rat? These questions are all relevant to the selection and use of this observation as a minimal LOAEL. The Panel was not in agreement about the 'adversity' of the changes in thyroid in these rats and whether they represent a minimal LOAEL or simply a LOEL.

An uncertainty factor of 3.0 was used for animal-human differences because of pharmacodynamic differences in sensitivity across species. Given (1) the care taken to show the correlation of the various endpoints for thyroid effects, (2) the lack of finding suggesting other modes of action for perchlorate, and (3) the differences in human and rat plasma reserves of thyroid hormones, a factor of 3.0 seems appropriate at this time for this particular endpoint. It could be further adjusted by use of a PK/PD model for perchlorate kinetics/iodide uptake inhibition/ thyroid hormone alterations resulting from perchlorate. Further work on the kinetics

of perchlorate in different species and the kinetics of inhibition of the iodide transport mechanisms by perchlorate in rat and human thyroid tissues will be useful in confirming the assumptions above. One point that deserves emphasis throughout the text is the importance of mode of action in assessing animal-human differences with induced thyroid hormone suppression. It is clear that rats are more sensitive to promoting effects on the thyroid leading to carcinogenesis. However, the obverse side of this argument is the question of the relative sensitivity of rats and humans to toxic effects related to a hypothyroid condition arising from perchlorate exposure. This distinction of known species differences for cancer and the lack of knowledge of species-differences for non-cancer effects needs to be clearly articulated in this section dealing with species differences.

Three other uncertainty factors were applied, each with a value of 3.0, for using a minimal LOAEL versus LOAEL/intrahuman variability, data base deficiencies, and intrahuman variation (sensitive populations). All the corrections together provide a composite correction of 300. This is probably sufficient (and perhaps overly conservative) given the data because of the knowledge of the mode of action, the belief that rats are more sensitive than humans, and the evaluation of minimal effects in the postnatal rats. The number is large enough in composite; however, it is difficult to assert that the factors that constitute the 300 are correctly parsed between the individual factors. The data base and minimal LOAEL uncertainty factors are accessible to experiment, as is the term for interspecies extrapolation above. The Panel was reluctant to call the PND5 changes adverse and suggested further evaluations of these tissues before deciding whether the result should be called a minimal LOAEL or simply a NOEL. In general, I become concerned in reducing the sensitive subpopulation factor from 10, unless specific factors can be cited in support of the change for hypothyroid states. Nonetheless, the total value of 300 does appear health-protective at this time.

The development of a harmonized cancer/non-cancer assessment is a welcome direction within EPA. While not yet completed in the document, the steps taken in this direction are clear and appropriate for a harmonized approach. However, cancer and non-cancer assessments have some differences that still have not been reconciled. Unless they are discussed and reconciled, the authors should probably use different studies and different low dose extrapolation strategies for cancer and non-cancer effects. The present differences can be briefly outlined. First, mode of action has been more frequently (although still very infrequently) applied to cancer risk assessments. In the new US EPA carcinogen guidelines, there is provision to discuss mode of action and to evaluate the possibility that common modes of action could underlie both cancer and non-cancer endpoints. Nasal toxicity with vinyl acetate is an example. In the present case, the cancer assessment for compounds disrupting thyroid hormone homeostasis has to be extended to consider a much broader set of endpoints than simply carcinogenesis.

With the emphasis on MOE approaches for carcinogens with non-linear modes of carcinogenic action, it is likely that certain non-cancer endpoints will become the limiting or critical effects, not the cancer. This situation is likely to be true with perchlorate. For cancer, however, the use of the PND5 studies to assess lifetime cancer risk does not appear optimal.

Alterations in thyroid hormone function throughout life would probably be a better precursor step for a cancer risk assessment. (Emphasis in cancer pathogenesis on measures of disruption over a longer time argues for a cancer risk assessment based on thyroid hormone alterations occurring in adults rather than those occurring at a single time point in neonatal rats.) The authors should consider calculating a BMD for thyroid disruption in adult rats and follow a non-linear cancer risk assessment paradigm to arrive at a cancer-based RfD. This alternative exercise would provide a check on the consistency of conclusions reached for cancer and for the critical non-cancer effects. A problem with harmonizing cancer and non-cancer risk assessments at the present time is the US EPA requirement that non-cancer assessments decide on values of uncertainty factors, while the non-linear cancer assessment estimates a margin of exposure (MOE) by comparing a dose-adjusted BMD with presumed human exposure levels. The non-linear cancer assessments beg the question of how large does the calculated MOE have to be for the risk assessor to be comfortable. Somehow any harmonized assessment has to reconcile these two disparate endgames – uncertainty factors versus MOEs. Despite this unresolved issue of different quantitative strategies having to be applied to cancer and non-cancer assessments, this document has come closer to achieving a harmonized human health risk assessment than has been done for any other compound in the United States.

The utility of further studies depends partially on decisions regarding adequacy of the studies supported over the past few years. With the exception of the immunotoxicology, the test appeared to follow standard protocols accepted by the toxicology community and by EPA. The immunotoxicology requirements and the interpretation of these tests are both still evolving. Some standard immunotoxicity tests such as the sheep red blood cell antibody tests were not performed, and the studies did not appear to be tailored to create special test protocols based on thyroid alterations. In general, it was surprising that the design criteria used for most of the new studies didn't include a review of expected non-cancer endpoints for other iodide uptake inhibitors and considerations of modifying protocols based on that knowledge. (This may well have been a consideration; it's just not apparent from the materials reviewed.) Some other tests might still be considered, including evaluation of other developmental delays or of permanent impairment in adult animals following fetal/neonatal exposures. Have potential endpoints been adequately examined to rule out higher sensitivity of the fetus to transient periods of hypothyroidism?

There is a potential for considerable 'value added' in the assessment by development of a BBDR model for iodide uptake inhibition, thyroid hormone depletion, hypothyroidism, and compensation. Qualitatively, defining mode of action would assist in establishing non-linear approaches for cancer risk assessment and in supporting choices for uncertainty values. However, the analysis of dose response curves with perchlorate relied on external exposure concentrations and on curve fitting to forms that are not concordant with specific biological processes. The development of a PBPK model should permit a more quantitative approach to deriving uncertainty factors, to 'fitting' biological models with clearly defined parameters to data, and to inferring the impact of differences between humans and rodents for the risk assessments. What should this 'model' look like? It is important that the design of the

biologically based risk model begin as soon as a hypothesis is elaborated. In other words, it should be well along the development trail already.

One of the most important criterion in the modeling is a decision about which endpoint should be evaluated. This decision leads to the objective function against which model performance is to be judged. Thus it becomes important to define the measure of response or adversity that will be the basis for regulation - is it some proportionate change in circulating T4/T3, some alteration in TSH, or some characteristic alteration in the cellular structure of the thyroid tissue? The material presented in the document does not provide an adequate basis for commenting on any current model structure, although the methods for the PK, iodide uptake, and thyroid homeostasis models are fairly conventional and efforts in linking these individual elements have been previously reported.

There is presently inadequate documentation of the data that would be collected for the development of a quantitative model. However, the development of such a quantitative model represents the primary tool needed to move from qualitative application of the broader array of mode of action data to its quantitative impact on risk assessments. The approach needed is the organization of a biologically based dose response model as described in the cancer risk assessment guidelines. These models influence analysis in the range of observation and enlighten the extrapolation between species and from high to low doses. It may be too late in the effort to complete the perchlorate review to have a BBDR model that influences the risk assessment in any quantitative fashion. Nonetheless, pursuing such a model to completion now could give more confidence in the correctness of any decisions made in the short-term regarding perchlorate and, more importantly, guide future efforts with harmonized mode of action based assessments.

Some specific comments

The analogies to parking brakes and hillside parking were uninformative and confusing (see, page 2-8). Simply stated perchlorate is stable in biological matrices. That wording should be sufficient.

page 3-13 One of the potassium chlorates, third line up from the bottom, has the potassium as the labeled isotope vice the chlorine.

page 3-14 Perchlorate reduced the thyroid blood ratio of what? Iodide, I presume, although the sentence could be read to indicate perchlorate.

page 3-15 Is it true that the concentration of perchlorate in the thyroid was inversely related to dose, or is the concentration expressed as percent of administered dose related inversely to dose. The former seems hard to reconcile to the behavior of dose-dependent systems.

page 5-18 biological significance is .....suspect. Do you mean .....is uncertain?

page 5-41 The discussion of U-shaped dose response curves probably deserves mention later in the document when discussing limiting toxicity for different endpoints. Is it real? If it were used as an effect level, does it change the perception of which study gives the lowest adverse effect level? Should anything be done to pursue this observation?

page 5-54 Immunotoxicity – is there any data on immune function in hypothyroid individuals from which hypotheses could be generated about the endpoints that might be most worthwhile to pursue.

page 6-41 exquisite or equisite.

REFERENCES

Barton, H.A., Andersen, M.E., and Clewell, H.J., III (1998). Harmonization: Developing Consistent Guidelines for Applying Model of Action and Dosimetry Information to Cancer and Noncancer Risk Assessment. *Hum. Ecol. Risk Assess 4: 75-115.*

Appendix A

Workshop Agenda

Perchlorate Peer Review Workshop
Agenda
San Bernardino City Council Chambers
San Bernardino, California
February 10 - 11, 1999

Wednesday, February 10

8:30 - 8:35	Opening Remarks - Susan Goldhaber, Research Triangle Institute (RTI)
8:35 - 8:40	Opening Remarks - Dr. Curtis Klaassen, Workshop Chair
8:40 - 8:45	Background - Peter Grevatt, EPA Office of Solid Waste and Emergency Response (OSWER)
8:45 - 8:55	Background - Kevin Mayer, EPA Region IX
8:55 - 10:10	Presentation: Toxicological Review/Reference Dose/Cancer Assessment Policy- Dr. William Farland, Annie Jarabek, EPA Office of Research and Development (ORD)
10:10 - 10:25	BREAK
10:25 - 12:00	Presentations from Observers
12:00 - 1:00	LUNCH
1:00 - 3:30	Discussion of Toxicity Database and Draft Toxicological Review Document - Dr. David Brusick, Facilitator/Peer Reviewer General Statistical Issues - Dr. Haseman 90-Day Subchronic Oral Bioassay Study - Dr. Porterfield/Dr. Emerson Neurobehavioral Developmental Study - Dr. Zoeller/Dr. Emerson Pilot Developmental Study/Segment II Developmental Study - Dr. Tyl 2-Generation Reproductive Study - Dr. Tyl Immunotoxicity Studies - Dr. White/Dr. Zoeller Genotoxicity Studies - Dr. Brusick Ecotoxicity Studies - Dr. Cardwell
3:30 - 3:45	BREAK
3:45 - 6:00	Continued Discussion of Toxicity Database and Draft Toxicological Review Document

Perchlorate Peer Review Workshop
Agenda
San Bernardino City Council Chambers
San Bernardino, California
February 10 - 11, 1999

Thursday, February, 11

8:00 - 10:00	Continued Discussion of Draft Toxicological Review Document/ Hazard Characterization - Dr. David Brusick, Dr. Mel Andersen, Facilitators/Peer Reviewers
10:00 - 10:15	BREAK
10:15 - 12:00	Continued Discussion of Hazard Characterization/Additional Studies Required - Dr. Andersen, Facilitator/Peer Reviewer
12:00 - 12:30	Workshop Summary - Dr. Klaassen, Workshop Chair

Appendix B

List of Peer Reviewers

Peer Reviewers for Perchlorate Workshop

Chair

Dr. Curtis Klaassen
University of Kansas Medical Center
2018 Breidenthal Building
3901 Rainbow Boulevard
Kansas City, KS 66160
(913) 588-7714

Peer Reviewers

Dr. Melvin Andersen
Colorado State University
Center for Environmental Toxicology and Technology
Fort Collins, CO 80523-1680
(970) 491-8522

Dr. David Brusick
Covance Laboratories, Inc.
9200 Leesburg Pike
Vienna, VA 22182
(703) 893-5400

Dr. Rick Cardwell
Parametrix, Inc.
5808 Lake Washington Blvd. N.E. Suite 200
Kirkland, WA 98033-7350
(425) 822-8880

Dr. Charles Emerson
University of Massachusetts Medical Center
55 Lake Avenue North
Worcester, MA 01655
(508) 856-3166

Dr. Joseph Haseman
National Institute of Environmental Health Sciences
Biostatistics Branch
P.O. Box 12233
Research Triangle Park, NC 27709
(919) 541-4996

Dr. Susan Porterfield
Medical College of Georgia
CB-1104
Augusta, GA 30912-4765
(706) 721-3217

Dr. Rochelle Tyl
Research Triangle Institute
Center for Life Sciences and Toxicology
P.O.Box 12194
Research Triangle Park, NC 27709
(919) 541-5972

Dr. Kimber White
Medical College of Virginia
527 North 12th Street
Strauss Immunotoxicology Research Laboratory
Room 2011
Richmond, VA 23298
(804) 828-6789

Dr. R. Thomas Zoeller
University of Massachusetts
Department of Biology
Morrill Science Center
Amherst, MA 01003
(413) 545-2088

Appendix C

Short Resumes of Peer Reviewers

MELVIN ERNEST ANDERSEN, Ph.D., DABT, CIH

Professor, Environmental Health
Colorado State University
Fort Collins, CO 80523

Business Address
Colorado State University
Fort Collins, CO 80523
(970) 491-8522
(970) 491-8304 Fax
andersenme@aol.com

Home Address
8701 Drew Lane
Chapel Hill, NC 27516
Tel: (919) 942-4931

Dr. Andersen joined the faculty of Colorado State University in January 1999. He serves as Professor of Environmental Health and Director of the University's International Center for Risk Assessment, an organization that emphasizes novel approaches for applying emerging scientific knowledge to improve environmental and human health risk assessment and risk management. From 1994-1998, Dr. Andersen was Vice-President of the K.S. Crump Group of ICF Kaiser International Consulting. Between 1971 and 1994, he held positions in toxicology research and research management in the federal government (DoD and US EPA) and in private industry (Chemical Industry Institute of Toxicology). His career contributions are mainly in developing biologically realistic models of the uptake, distribution, metabolism, and biological effects of toxic chemicals and drugs and applying these models in safety assessments and quantitative health risk assessments. He is widely known for developing short-courses and computer demonstrations in pharmacokinetics and pharmacodynamic modeling. Dr Andersen is an author on over 180 papers and 30 book chapters. He has received many awards for professional contributions. These awards include the Stokinger Award (American Conference of Governmental Industrial Hygienists, 1988), the George Scott Award (Toxicology Forum, 1993), the Kenneth Morgareidge Award (International Life Sciences Institute, 1989), and the Frank R. Blood (1982) and Achievement Awards (1984) from the Society of Toxicology.

EDUCATION:

1971 Ph.D., Biochemistry and Molecular Biology, Cornell University, Ithaca, NY
1967 Sc.B., Chemistry, Brown University, Providence, RI

BOARD CERTIFICATIONS:

Certified in the Comprehensive Practice of Industrial Hygiene (1978). Certificate No. 1331.
Diplomate of the American Board of Toxicology (1981).

SOCIETY MEMBERSHIPS:

- Society of Toxicology
- American Academy of Industrial Hygiene
- American Conference of Governmental Industrial Hygienists; Associate Member
- Risk Assessment Section, Society of Toxicology
- Society of Risk Analysis

AWARDS:

George H. Scott Award of the Toxicology Forum for significant original contributions to the science of toxicology, 1993.

Best Industrial Hygiene related paper appearing in AIHA Journal or Applied Industrial Hygiene in 1989. Presented by the Michigan Society of Industrial Hygiene.

The Kenneth Morgareidge Award for Outstanding Research Contributions to the Science of Inhalation Toxicology, International Life Sciences Institute (ILSI), 1989.

Herbert Stokinger Award for Outstanding Contributions in Industrial Toxicology, American Industrial Hygiene Association, 1988.

Achievement Award, Society of Toxicology for Outstanding Contributions in Toxicology by a scientist less than 41 years of age. 1984

Paper of the Year, Inhalation Specialty Section, Society of Toxicology, 1985.

Award for Outstanding Professional Achievement, Affiliate Societies Council, Engineering and Science Foundation of Dayton, 1985.

Frank R. Blood Award - Society of Toxicology - 1982 (best paper in Toxicol. Appl. Pharmacol. for period 6/1980-5/1981).

Selection as an 'International Man of the Year', International Biographical Centre, Cambridge, England, 1992-1993.

Harry G. Armstrong Award for Scientific Excellence, AFAMRL, 1982.

DuPont Award, for excellence in teaching biochemistry; Cornell University, 1970.

Francis Wayland Scholar, Brown University, 1967.

Listed in American Men & Women of Science, 1995.

Listed in Who's Who in Science and Engineering, 1996.

Listed in Who's Who in America. 51st Edition 1997.

EDITORIAL BOARDS:

Member, Editorial Board, Food and Chemical Toxicology, 1991-1996.

Member, Editorial Board, Toxicology and Applied Pharmacology, 1985-1995.

Member, Editorial Board, Inhalation Toxicology, 1988-Present.

Member, Editorial Board, Human and Experimental Toxicology, 1994-Present.

Member, Editorial Board, Human and Ecological Risk Assessment, 1994-Present.

Associate Editor, Toxicology and Applied Pharmacology, 1982-1984.

CURRENT FACULTY APPOINTMENTS::

Adjunct Professor, Louisiana Tech University, Ruston, LA. 1996-2001.

Adjunct Professor in Medicine, Duke University, Duke University Medical Center, Durham, NC, 1 May 1994 - 30 June 1995

WORK EXPERIENCE:

1994-1999	The K.S. Crump Group, ICF Kaiser Consulting, Research Triangle Park, NC 27709, Vice-President
1993-1994	US Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC (Senior Scientist)
1992-1993	Duke University School of Medicine (Research Professor).
1989-1992	Chemical Industry Institute of Toxicology, Research Triangle Park, NC (Department Head, Risk Assessment and Senior Scientist)

1978-1989 Civil Service, Department of Defense, Toxic Hazards Division, Wright- Patterson AFB, Ohio (Director)

1971-1978 Active Duty, US Navy, Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson AFB, Ohio (Department Head)

OTHER POSITIONS:

Member, Committee on Toxicology, National Research Council, 1999-2000.

Chairperson, Expert Panel Evaluating EPA's Guidelines for Carcinogen Risk Assessment Using Chloroform and Dichloroacetate as Case Studies. ILSI, 1996-1997.

Co-Chairperson, Society of Toxicology, Task Force to Improve the Scientific Basis of Risk Assessment, 1996-1998.

American Board of Toxicology, 1991-1995.

American Conference of Governmental Industrial Hygienists (ACGIH) ACGIH Liaison to AIHA Unusual Workshift Committee, 1983-1984.

Threshold Limit Value Airborne Contaminants Committee, 1981-1984.

Safe Drinking Water Committee, National Academy of Sciences, Washington, D.C., 1984-1985.

Education Committee, Society of Toxicology, 1983-1985.

Awards Committee, Society of Toxicology, 1987-1989.

Scientific Advisory Panel, Chemical Industry Institute of Toxicology, 1986-1988.

Member, Pharmacokinetic Subcommittee, Safe Drinking Water Committee, National Academy of Sciences, 1986-1987.

DAVID J. BRUSICK, Ph.D., A.T.S.

VICE PRESIDENT, MAMMALIAN TOXICOLOGY
COVANCE LABORATORIES INC.
NORTH AMERICA

EDUCATION

NAS/NRC Postdoctoral Fellow 1970 -1971.

Ph.D., Microbial Genetics, Illinois State University, Normal, Illinois, 1970.

M.S., Genetics, Illinois State University, Normal, Illinois, 1965.

B.S., Biology, University of Illinois, Urbana, Illinois, 1963.

BACKGROUND

1997 - Present	Vice President, Mammalian Toxicology, Covance Laboratories Inc., Vienna, Virginia.
1995 -1997	Director CHNA Toxicology
1988 - 1995	Director, Corning Hazleton North America Toxicology, Corning Hazleton Inc., Vienna, Virginia.
1986 - 1987	Director, Molecular Toxicology Division, Hazleton Laboratories America, Inc., Kensington, Maryland.
1985 - 1986	Vice President. Biological Laboratories Division, Hazleton Biotechnologies, Kensington, Maryland.
1984 - 1985	Vice President, Biological Safety Evaluation Directorate, Litton Bionetics, Inc., Kensington, Maryland.
1981 - 1984	Vice President, Molecular Sciences Directorate, Litton Bionetics, Inc., Kensington, Maryland.
1974 - 1981	Director, Department of Molecular Toxicology, Litton Bionetics, Inc., Kensington, Maryland.
1971 - 1974	Assistant Professor of Microbiology, College of Medicine, Howard University, Washington, D.C.
1970 - 1971	National Academy of Sciences, National Research Council Postdoctoral Research Associate, Genetic Toxicology Branch. U.S. Food and Drug Administration, Washington, D.C.
1968 - 1970	Graduate Research and Teaching Assistant, Department of Biology, Illinois State University, Normal, Illinois.
1967 - 1968	Assistant Professor of Biology, Bridgewater College, Bridgewater, Virginia.

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1963 - 1967	Graduate Research and Teaching Assistant, Department of Biology, Illinois State University, Normal, Illinois.

ACADEMIC APPOINTMENTS

1981 - Present	Adjunct Associate Professor in the Department of Biological Sciences, George Washington University, Washington, D.C.
1985 - Present	Adjunct Associate Professor in the Department of Genetics and Human Genetics, Howard University, College of Medicine, Washington, D.C.

EXPERIENCE

Scientific Director, Corning Hazleton Inc., Vienna, VA. Manager of mammalian toxicology and pathology sciences.

Principal Investigator on mutagenicity testing contracts from agencies of the Federal government (e.g. EPA, FDA, NIEHS, NIOSH, DOD) and private sponsors.

Research experience in mutagenicity of chemical carcinogens and other environmental agents, carcinogen mechanisms. Research included *in vitro* and *in vivo* investigations.

Scientific Director of mutagenicity testing and molecular toxicology for Hazleton Laboratories worldwide.

Member of the editorial board of four scientific journals in genetics and toxicology.

Editor of *In Vitro* Toxicology, an international journal published by Mary Ann Liebert, Inc. (1988-1993).

Member of the two U.S. National Academy of Sciences Committees with Mutagenesis and Toxicology (Diesel Impact Committee and Toxicology Data Elements Committee). Chairman of a NRC subcommittee on DNA adducts.

Member of the International Commission for Protection Against Environmental Mutagens and Carcinogens (1986 - present). Chairman 1989 - 1995.

Past President of the U.S. Environmental Mutagen Society (1978).

Panel Member of the U.S.-Japan Environmental Mutagen Cooperative Program (1977-1979).

Councilor to the EMS Society.

Member of the Steering Committee for the EPA on the Geno-Tox Program for Genetic Testing Evaluation.

Member of NIH Study Section on Toxicology, 1992-1996.

Consultant to government agencies and private industrial firms regarding mutagenesis testing.

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Member of the Center for Alternatives to Animal Testing (CAAT), Technology Transfer Committee.

Board Member, Academy of Toxicological Sciences (1990-1993).

Board Member, Industrial *in Vitro* Toxicology Group (1989-present).

Secretary/Treasurer, Academy of Toxicological Sciences (1995-1996).

Associate Editor for Toxicological Sciences (1998 - present).

Sent By: COVANCE; 7037590892; Nov-9-98 5:15PM; Page 4/13

Rick D. Cardwell, Ph.D.

Ph.D., Fisheries (Aquatic Toxicology), University of Washington (1973)
Master of Science, Fisheries (Fish Physiology), Univ. of Washington (1968)
Bachelor of Science, Fisheries Science, Oregon State Univ. (1967)

Rick Cardwell is an aquatic biologist and ecotoxicologist by training with more than 25 years experience studying the effects of chemicals and habitat changes on aquatic life and wildlife. He has used a variety of tools for this purpose, including risk and impact assessment, field studies, monitoring, aquatic toxicity bioassays, computer modeling, and statistics. He has managed hundreds of projects in his career, several totalling more than $1.75 million in studies annually.

Project Experience (International)

Human Health and Ecological Risk Assessment for Copper Mine - PT Freeport Indonesia (1997)
Rick Cardwell is participating in a multi-disciplinary assessment of the risks posed by chemicals and sediments to people and their food supply downstream of a copper mine in Irian Jaya, Indonesia.

Strickland River Risk Assessment - Porgera Joint Venture, Porgera, Papua New Guinea
Rick Cardwell is managing a screening-level risk assessment of the risks posed to people and their fish and wildlife food supply downstream of a gold mine in the central highlands.

Third Party Peer Review of Risks Posed to Humans and Their Environment Downstream of Gold Mine -Papua New Guinea (1996)
Participated as member of third party, international team who reviewed the adequacy of existing environmental programs relative to potential risks posed by gold mining.

Risks Posed by Copper Mine Tailing in Marine Seafood- Lihir Island Copper Mine, Solomon Islands (1995)
Are the fish safe to eat? This is the question addressed in a human health risk assessment of fish and shellfish caught in the vicinity of a submarine discharge of effluent from a copper mine on Lihir Island. In addition to assessing risks, we estimated bioavailability and bioaccumulation of copper in marine food chains.

Risks Posed by Marine Outfall - Haifa Chemicals, Ltd., Israel (1994-1995)
Dr. Cardwell headed a project that evaluated the risks posed to marine life by heavy metals, pH and fluoride in effluent from a fertilizer plant in Israel. These included meetings with governmental officials, a screening-level risk assessment, evaluations of the chemical fate of effluent constituents, and evaluations of the effects posed by similar discharges elsewhere in the world.

Risk Assessments of Municipal Effluents, Sewer Overflows and Stormwaters - Sydney Water Corp., Sydney, Australia (1993-1997)
Conducted a series of human health and ecological risk assessments of various primary, secondary and tertiary-treated sewage, sewer overflows, and stormwaters in the Sydney, Australia metro area for the governing agency, Sydney Water Corporation. We assessed risks to people swimming and consuming fish and shellfish from local waters. Acute and chronic effects on aquatic life were also assessed. More than 100 metals, pesticides, and bacteria/viruses were evaluated, as were non-chemical stressors (suspended solids/turbidity, sedimentation, scour, and low dissolved oxygen).

Risk Assessment of Sewage Re-Use - Sydney Water Board, Sydney, Australia (1992)
Developed human health risk assessment of tertiary-treated sewage used as make-up water for a metal smelter in Sydney, Australia for the Water Board. Assessed risks to workers associated with exposure to metals, chlorinated organics (e.g., chloroform), and pathogenic bacteria/viruses in sewage.

Project Experience - (U.S.)

Literature Review Concerning the Fate and Transport and Bioavailability of Metals in the Aquatic Environment, International Council on Metals in the Environment (ICME)
Prepared a technical monograph synthesizing the literature concerning the fate, transport, bioavailability and bioaccumulation of metals in aquatic environments as it related to aquatic life.

Synthesis of Knowledge Concerning the Fate and Effects of Copper in the Environment - Kennecott Utah Copper (1994)
Copper is one of several metals whose environmental risks can be over-estimated if its biological availability is not defined. Accordingly, we reviewed and synthesized the scientific literature concerning the fate of copper in aquatic environments. Particular attention focused on its bioavailability, in water and sediments, and how bioavailability related to its bioaccumulation into -- and toxicity to -- aquatic organisms.

Development of Methodologies for Assessing Risks from Historic Copper Mining - ARCO Copper Mine, Butte, Montana (1992)
Retained by ARCO to assist multi-consultant team in developing ecological risk assessment methodologies for characterizing risks and identifying appropriate remediation for more than 20 riverine sites contaminated with wastes from historic mining operations near Butte, Montana.

Deciding When a River's Biological Health Has Been Restored Sufficiently: Historic Zinc Mine - Eagle River, Colorado (1993-present)
Conducting field studies of fish and aquatic invertebrates to define when the river had returned to biological health after years of impact due to acid rock runoff from historic zinc mining upstream. Analyzed factors affecting fish and invertebrates, including zinc, cadmium, and iron concentrations, stream flow, substrate composition.

Environmental Impact of Proposed Molybdenum Mine on Freshwater and Marine Life - U.S. Borax, Alaska (1983-1985)
Headed team of biologists who evaluated potential effects, as part of an environmental impact statement, of a proposed molybdenum mine situated with a U.S. national monument south of Ketchikan, Alaska. Evaluated impacts of sedimentation and water quality (especially heavy metals) on salmon in local streams and of marine organisms in the neighboring fjords.

Impacts of Antimony in Sediments and Surface Water Downstream of Antimony Smelter - Anzon, Inc., Laredo, Texas (1993)
Assessed impacts on aquatic life and human health uses from antimony in stream sediments and surface water. Conducted aquatic toxicity tests of antimony to establish site-specific water quality criteria, conducted sediment toxicity tests, analyzed dissolved vs. total recoverable antimony, and presented expert testimony. Prepared expert testimony in preparation for litigation.

Risks Posed by Metals, Arsenic and Selenium - Kennecott Utah Copper, Utah (1994-Present)
Risks posed by groundwater entering surface waters and contaminating the aquatic life consumed by various bird species was the question addressed in work for Kennecott Utah Copper, Inc. Additional assignments have involved development of water quality criteria for the Great Salt Lake, and revision of the national water quality criterion for selenium. A variety of metals (e.g., cadmium, copper) and metalloids (arsenic and selenium) were evaluated with respect to their bioaccumulation, bioavailability, and effects on shorebirds. Also conducted chronic tests with arsenic and selenium to evaluate toxicity and bioaccumulation in brine shrimp eaten by a variety of birds.

Site-Specific Water Quality Criteria, Lead Mines and Smelter - Missouri (1990)
Developed site-specific, surface water quality criterion for thallium, and attempted to develop site-specific criterion for cadmium and lead downstream of two lead mines and one smelter. The site-specific criterion for thallium, based on human health risks from consuming local fish, was three-times higher than the state standard.

Toxicity Identification Evaluations at Copper, Lead, Silver and Zinc Mines - ASARCO (1993-1994)
Conducted toxicity identification evaluations (TIE) of wastewaters from copper-silver and lead-zinc in Montana and Colorado, respectively. These TIEs included routine and customized toxicity testing and consulting in the causes of toxicity and methods for wastewater treatment.

Wildlife and Human Health Risk Assessments: Pit Lake Created from Gold Mining - Newmont Mining Co. (Formerly Santa Fe Pacific Gold Corp. , Nevada (1994-1995))
Parametrix assessed risks posed to wildlife and human health from exposure to the water, aquatic life, and plants colonizing a pit lake created via groundwater infiltration. Risks from several post-closure operations were assessed. Examined risks posed by metals, metalloids, and ions (Na, Mg, K, etc.).

Mercury in Estuarine Sediments - Lavaca Bay, Texas (1995-1998)
Conducted a risk-based remedial investigation/feasibility study of estuarine sediments contaminated with mercury in from Texas. Examined risks from mercury, polynuclear aromatic hydrocarbons, and polychlorinated biphenyls to freshwater and estuarine fish, invertebrates and wildlife (birds).

Metal Released from Lead Battery Recycling Site - Exide, Inc., Reading, Pennsylvania (1994-present)
Metals were released to the soil then to groundwater from recycling batteries at a site next to the Schuylkill River in Pennsylvania. Parametrix designed and conducted the chemical and ecological monitoring of potential adverse effects from lead and other heavy metals on river life.

Biological Impact Assessment of Arsenic - Elf Atochem North America, Bryan, Texas (1992-1996)
Designed and initiated a multi-year evaluation of the ecological impacts of historic arsenic releases in the metro Bryan-College Station, Texas area, including impacts to fish populations and invertebrates. Studies assessed arsenic concentrations, arsenic species, fish and invertebrate populations, and acute and chronic toxicity of surface waters and sediments.

Human Health Risks from Lead in Sediments and Surface Water - Hecla, Asarco, Coeur d'Alene Mining Corp. and Sunshine Mining Co. (1993)
Dr. Cardwell headed a team that assessed potential risks to human health from drinking water, eating dirt (children), and consuming sport-caught fish from the lower Coeur d'Alene River and Lake Coeur

d'Alene, Idaho, where lead, from historic lead mine tailings, frequently reached 10,000 ppm in the sediment (jig tails).

Site-Specific Water Quality Criteria: Metals - U.S. general
On behalf of the Electric Power Research Institute and Utilities Water Act Group, prepared comments and alternatives to methodologies proposed by both the U.S. EPA and Maryland for the derivation of site-specific water quality criteria for heavy metals and other toxic substances.

CURRICULUM VITAE

Charles H. Emerson, M.D., F.A.C.P. Telephone 508 856 3166

CURRENT POSITIONS - University of Massachusetts School of Medicine, Worcester, MA

Professor of Medicine

Professor of the Physiology Program

Associate Director: Endocrine Training Program

Associate Director: University of Massachusetts Medical Center Clinical Research Center

HOME ADDRESS

46 Richard Avenue
Shrewsbury, Massachusetts 01545

OFFICE ADDRESS

University of Massachusetts Medical Center
55 Lake Avenue North
Worcester, Massachusetts 01655

PERSONAL DATA

AMA Number: 051-01-67-023-8
Federal DEA Number BE3093351
Massachusetts Drug License Number ME0466917
UPIN D83087
Date of Birth: 1/6/41
Place of Birth: Ratlam, India
Marital Status: Married 3/18/1967
Children: Three

EDUCATION

High School Diploma 5/5/59
Kodaikanal School, Kodaikanal, India 1955-1959

B.S. Received 6/2/63
Randolph Macon College, Ashland, VA 1959-63

M.D. Received 6/4/67
University of Virginia Medical School, Charlottesville, VA 1963-67

POSTDOCTORAL TRAINING

Intern in Medicine
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
6/23/67 to 6/24/68

Resident in Medicine
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
7/1/68 to 6/30/70

Fellow in Endocrinology and Metabolism
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
7/1/70 to 6/30/71 and
7/1/73 to 6/30/74

LICENSURE

Inactive Licenses
6/23/67 - State of Virginia, License Number 18582
6/17/74 - State of Illinois, Certificate Number 36-49327

Active Licenses
3/31/80 - State of Massachusetts (Certificate 45591)
5/16/97 - State of Connecticut (Liscense No. 036013)

Specialty certification
6/21/72 - Internal Medicine, Certificate No 35149
10/18/77 - Endocrinology and Metabolism, Certificate No 35149

ACADEMIC APPOINTMENTS

7/1/68 to 6/30/70 - Assistant Instructor in Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

7/1/70 to 6/30/71 - Postdoctoral Fellow University of Pennsylvania School of Medicine

1/1/72 to 6/30/73 - Clinical Instructor in Medicine Medical College of Georgia, Augusta, Georgia

7/1/73 to 6/30/74 - Postdoctoral Fellow University of Pennsylvania School of Medicine

7/1/74 to 3/31/80 - Assistant Professor of Medicine, Abraham Lincoln School of Medicine (ALSM) of the University of Illinois, Chicago, Illinois

4/1/80 to 4/5/86 - Associate Professor of Medicine, University of Massachusetts School of Medicine, Worcester, MA

2/6/86 to present - Professor of Medicine, University of Massachusetts School of Medicine, Worcester, MA

12/14/87 to present - Professor of Medicine, University of Massachusetts School of Medicine, and Professor of the Physiology Program, University of Massachusetts Graduate School of Biomedical Sciences, Worcester, MA

PROFESSIONAL/ADMINISTRATIVE APPOINTMENTS

U.S. Army Medical Center, Fort Gordon, GA

7/6/71 to 6/30/73 - Chief, Endocrine Service and Staff Physician in Medicine

University of Illinois Hospital, Chicago, IL

7/1/74 to 9/1/78 - Staff Physician in Medicine VA West Side Hospital, Chicago, IL

7/1/74 to 9/1/78 - Staff Physician in Medicine and Nuclear Medicine

University of Massachusetts Medical Center, Worcester, MA

10/31/80 to 12/30/82 - Staff Physician in Medicine and Nuclear Medicine

1/1/83 to 8/1/83 - Staff Physician in Medicine and Nuclear Medicine Acting Chief, Division of Endocrinology and Metabolism, Department of Medicine

The Children's Hospital Medical Center, Boston, MA

10/18/89 to 10/18/93 - Visiting Staff (Research)

University of Massachusetts Medical Center, Worcester, MA

8/1/83 to present - Staff Physician in Medicine and Nuclear Medicine, Coordinator: Training Programs in Endocrinology, Metabolism and Diabetes

MEMBERSHIP IN HONORARY SOCIETIES

1963 - Chi Beta Phi Honorary Scientific Fraternity

1966 - Alpha Omega Alpha Honorary Medical Fraternity

1963 to 1967 - Florence Smith Scholar

CURRICULUM VITAE

Name: Joseph K. Haseman

Date and Place of Birth: July 20, 1943; Sheffield, Alabama

Citizenship: United States Marital Status: Married, two children

Education:

June, 1961 Graduated from High School (Valedictorian)
June, 1965 B.S. (Mathematics) cum laude, Davidson College
June, 1970 Ph.D. (Biostatistics), University of North Carolina (Chapel Hill)
Dissertation: The Genetic Analysis of Quantitative Traits Using Twin and Sib Data

Brief Chronology of Employment:

1966 - 1966 Summer employment as a statistician and computer programmer, Research Triangle Institute, Research Triangle Park, N.C.

1968 - 1970 Laboratory Instructor, Biostatistics Department, University of North Carolina, Chapel Hill, N.C.

1970 - date Research Mathematical Statistician, Biostatistics Branch,
National Institute of Environmental Health Sciences, P.O. Box 12233,
Research Triangle Park, N.C. 27709. Phone: (919) 541-4996. Fax: (919) 541-4311.

Professional Societies:

Biometric Society (ENAR), American Statistical Association (ASA), Society of Toxicology, Genotoxicity and Environmental Mutagen Society, Phi Beta Kappa

Honors and Professional Recognition

NIH Director's Award, 1983
Associate Editor, Shorter Communications, Biometrics, 1979-1984.
ASA Biopharmaceutical Section Executive Committee, 1987-1989.

Board of Editors, Environmental Health Perspectives, 1980-1997.
Editorial Board, Fundamental and Applied Toxicology, 1986-1992.
Elected as Fellow, American Statistical Association, 1989.
Distinguished Achievement Medal, American Statistical Association's Section on Statistics and the Environment, 1994.

Honored by Toxicologic Pathology for having one of the 10 most frequently cited articles in that journal over its 25 year history, 1997.

CURRICULUM VITAE

Curtis Dean Klaassen, Ph.D.

PLACE AND DATE OF BIRTH:

Fort Dodge, Iowa
November 23, 1942

MARITAL STATUS:

Married, two children

DEGREES:

1964 B.A. - Wartburg College (Biology)
1966 M.S. - University of Iowa (Pharmacology)
1968 Ph.D. - University of Iowa (Pharmacology)

CERTIFICATION:

1980 American Board of Toxicology
1992 The Academy of Toxicological Sciences (Honorary member)

ACADEMIC APPOINTMENTS:

1968-1970	Instructor of Pharmacology and Toxicology, University of Kansas Medical Center
1970-1974	Assistant Professor of Pharmacology and Toxicology, University of Kansas Medical Center
1974-1977	Associate Professor of Pharmacology and Toxicology, University of Kansas Medical Center.
1975	Guest Professor of Clinical Pharmacology, University of Bern, Bern, Switzerland, June-August
1977-pres	Head, Section on Toxicology, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center
1977-pres	Professor of Pharmacology and Toxicology, University of Kansas Medical Center
1978	Visiting Scientist, Department of Toxicology, Institute of Radiation and Environmental Research (GSF), Munich, Germany, March-August
1984-pres	Professor of Molecular Cytology, Institute of Investigative Cytology, Valencia, Spain
1986-1989	Associate Director, Environmental Health and Occupational Medicine Center, University of Kansas Medical Center
1989-1991	Interim Director, Environmental Health and Occupational Medicine Center, University of Kansas Medical Center

HONORS:

1. 1964 Magna Cum Laude, Wartburg College
2. 1965-1968 Public Health Service Predoctoral Fellowship Award, NIH

3. 1971-1976 Public Health Service Research Career Development Award, NIH
4. 1976 Achievement Award, Society of Toxicology
5. 1978 Alexander von Humboldt Fellow
6. 1982-1987 Burroughs Wellcome Scholar in Toxicology
7. 1982-1983 MASUA Honor Lecturer
8. 1982 KUMC Research Award
9. 1985 Distinguished Visiting Professor, New Mexico State University
10. 1985 KU-Higuchi Research Award (The Dolph Simons, Sr. Research Award)
11. 1986 Wartburg College Alumni Citation
12. 1987 Distinguished Visiting Professor, University of Toledo
13. 1993 Eugene Garfield in *Current Contents* (January 18, 1993) indicated that between 1980 and 1992, Curtis Klaassen:
    A. Published 115 peer-reviewed scientific publications on the study of xenobiotics.
    B. Had the 12th highest scientific impact (2,227 references) in the world in the study of xenobiotics.
    C. Had the 4th highest scientific impact in the United States in the study of xenobiotics.
    D. Had the greatest scientific impact in the world in the area of toxicology.
14. 1993 Educational Award, Society of Toxicology
15. 1993 Chancellor's Club Research Award, University of Kansas
16. 1994 Kenneth P. DuBois Award, by Midwest Regional Chapter of the Society of Toxicology
17. 1994 William P. Kinter Memorial Lectureship, Mount Desert Island Biological Laboratory, Maine
18. 1998 Founders Award, CIIT
19. 1998 John Doull Award, Central States Chapter of the Society of Toxicology

PROFESSIONAL SOCIETIES:

1. 1968 Sigma Xi
2. 1969 American Association for the Advancement of Science
3. 1970 Society of Toxicology
4. 1970 American Society of Pharmacology and Experimental Therapeutics
5. 1971 American Association for the Study of Liver Diseases
6. 1972 Society of Experimental Biology and Medicine
7. 1993 International Society for the Study of Xenobiotics (ISSX)
8. 1998 Founders Award, CIIT
9. 1998 John Doull Award, Central States Chapter of the Society of Toxicology

EDITORIAL BOARDS:

1. 1974-1998 *Journal of Pharmacology and Experimental Therapeutics*, Toxicology Field Editor
2. 1976-1978 *Chemico-Biological Interactions*, Editorial Board
3. 1977-pres *Journal of Pharmacological and Toxicological Methods*, Associate Editor
4. 1980-1990 *Toxicology and Applied Pharmacology*, Associate Editor
5. 1980-1983 *Hepatology, Editorial Board*

6. 1980-pres *Journal of Toxicology and Environmental Health*, Editorial Board
7. 1984-1993 *Xenobiotica*, Editorial Board
8. 1988-1989 *ISI Atlas of Science*: Pharmacology Advisory Editor
9. 1992-1998 *Chemico-Biological Interactions*, Editorial Board
10. 1993-1996 *Regulatory Toxicology and Pharmacology*, Editorial Board
11. 1996-pres *Current Protocols in Pharmacology*, Editorial Board
12. 1997-pres *Toxicological Sciences*, Editor-in-Chief

NATIONAL and INTERNATIONAL COMMITTEES:

Elected:

Society of Pharmacology and Experimental Therapeutics

1. 1976-1979 Executive Committee of the Drug Metabolism Division, American Society of Pharmacology and Experimental Therapeutics
2. 1977-1979 Treasurer of the Drug Metabolism Division, American Society of Pharmacology and Experimental Therapeutics

Society of Toxicology

1. 1979-1981 Education Committee, Society of Toxicology, Chairman 1980-1981
2. 1981-1984 Membership Committee, Society of Toxicology, Chairman 1983-1984
3. 1983-1986 Councilor, Mechanism Subsection, Society of Toxicology
4. 1983-1985 Councilor, Metals Subsection, Society of Toxicology
5. 1985-1987 Councilor, Society of Toxicology
6. 1988-1989 Vice-President Elect, Society of Toxicology
7. 1988-1990 Program Committee, Society of Toxicology, Chairman 1989-90
8. 1989-1990 Vice-President, Society of Toxicology
9. 1989-1991 Board of Publications, Society of Toxicology
10. 1989-1991 Finance Committee, Society of Toxicology
11. 1990-1991 President, Society of Toxicology
12. 1991-1992 Past-President, Society of Toxicology
13. 1991-1992 Awards Committee, Society of Toxicology, Chairman
14. 1991-1992 Ethics Committee, Society of Toxicology, Chairman
15. 1989-1993 Toxicology Education Foundation Board of Trustees, Vice President 1991-92, President 1992-93
16. 1992-1993 Nominating Committee, Society of Toxicology, Chairman
17. 1994-1995 Nominating Committee, Society of Toxicology
18. 1998-present Nominating Committee, Society of Toxicology

International Union of Toxicology

1. 1989-1992 Director, International Union of Toxicology (IUTOX)
2. 1992-1995 President, International Union of Toxicology (IUTOX)
3. 1995- Past President, International Union of Toxicology (IUTOX)

International Society of the Study of Xenobiotics

1. 1997- Councilor

Wartburg College

1. 1992-1995 Wartburg College Alumni Board

February 1998

CURRICULUM VITAE

Susan P. Porterfield, Ph.D.

Professor of Physiology and Endocrinology
Associate Dean for Curriculum
Office Address: CB-1104; CL-3150
Office Telephone: (706) 721-3217

Personal

Home Address:	714 Michaels Cr. Dr. Evans, GA 30809
Telephone:	860-8234
Date of Birth:	September 6, 1943
Place of Birth:	Plainfield, New Jersey
Citizenship:	U.S.A.

Education

High School: Worthington High School, Worthington, Ohio, graduated 1961
College: Miami University, Oxford, Ohio, 1961-1963
Ohio State University, Columbus, Ohio, 1963-1965; B.S. degree *Summa Cum Laude*; Physiology, Chemistry
Graduate Education: Ohio State University, Columbus, Ohio, 1965-1967; M.S. Degree 6/67; Physiology
Ohio State University, Columbus, Ohio, 1971-1973; Ph.D. Degree 12/73; Physiology

Academic Appointments

Research Associate, Ohio State University, 1967
Laboratory Instructor, Ohio Northern University, Ada, Ohio, 1969
Instructor, Ohio Northern University, Ada, Ohio, 1970-1973
Assistant Professor, Georgia Institute of Technology, Atlanta, Georgia, 1973-1976
Assistant Professor, Medical College of Georgia, Augusta, Georgia, 1976-1979
Associate Professor, Medical College of Georgia, Augusta, Georgia, 1979-1996
Professor, Physiology & Endocrinology, School of Graduate Studies, Medical College of Georgia, 1996-present
Associate Dean for Curriculum, Medical College of Georgia November 1994-present

Administrative Appointments

Associate Dean for Curriculum November 1994-present

Editorial Boards

Endocrine Reviews - 1992-1996

Outside Reviewer

Avline Reviewer
Journal of Nutrition
Endocrinology
Endocrine Reviews
New England Journal of Medicine
Environmental Health Perspectives
Journal of Clinical Endocrinology

Ad Hoc Grant Reviewer

National Science Foundation 1990
NIH - endocrine study section 1995, 1996
EPA 1996
Human Frontier Science Program (HFSP) 1996

Abstract Reviewer

Endocrine Society, 1987
American Physiological Society, 1984
Endocrine Society, 1997

Awards/Honors

Summa Cum Laude, Ohio State University
Member of *Phi Beta Kappa*
Recipient of a Muhlhaupt Fellowship (Ohio State), 1965-1966 and 1966-1967
President's Scholarship - Miami University
President's Award - Ohio State University
Participant - Honors Program, Miami University
Who's Who in the Southeast
Outstanding Faculty Award for Basic Science Teaching, 1993
Selected as a member of the National Board of Medical Examiners, Physiology Test-writing Committee Step I, 1994-present
Selected to serve as a member of the National Board of Medical Examiners, Physiology subject exam writing committee, 1994-present
Session Chairperson, National Meetings

1. American Physiological Society, Fall Meeting, Endocrinology & Metabolism, 1984
2. Endocrine Society, Thyroid Hormone Action, 1987
3. Developmental neurotoxicity of endocrine disrupters, 13th International Neurotoxicology Conference, 1995

4. Integration of clinical skills in basic science courses, Generalist Physician Initiative, 2nd Annual Program, Key Biscayne, FL
5. American Thyroid Association, Colorado Springs, CO, 1997

Invited faculty for the International Symposium on Advances in Perinatal Thyroidology at Longboat Key, FL, 1990. My participation was supported by Boots Pharmaceutical.

Invited faculty for the Learning Disorders Association/EPA, NIEHS and National Foundation for Brain Research sponsored conference "The Decade of the Brain", 1992. My participation was supported by the EPA.

Invited to serve as professional consultant to the EPA in the Learning Disorders Association Round-Table 1992, 1995.

Invited by Dr. Sumner Jaffee, Director for Research in Maternal and Child Health, NICHD, to participate and present in a workshop to develop research ideas on studying the impact of thyroid disorders on early fetal brain development. Conference was June 27, 28, 1994. My participation was supported by the NICHD.

Invited faculty for the Learning Disorders Association conference on "Thyroid Function and Learning Disabilities" to be held March 2, 1995. My participation will be sponsored by the Learning Disorders Association.

Invited speaker/participant, Conference on steroid hormones and the brain; Breckenridge, CO, March 31-April 4, 1995.

Invited consultant for the EPA; workshop on environmental toxins, thyroid hormones and brain development held in Raleigh on April 10-14, 1995

Invited speaker/participant for a conference on "Effects of Xenobiotics on Development of the Nervous System" held November 2-9, 1996 in Italy. My participation was be sponsored by NATO, and the Italian government.

Invited speaker/participant International Neurotoxicology Conference--Developmental neurotoxicity of endocrine disrupters, Hot Springs Ark, Oct. 1995, sponsored and supported by the EPA.

Selected as a participant at the Association of American Medical Colleges Professional Development Seminar for Senior Women in Medicine, Washington, D.C., March 25-27, 1995.

Selected as a participant at the Association of American Medical Colleges Executive Development Seminar for Associate Deans and Department Chairs, Ft. Lauderdale, FL, November 16-20, 1996.

Invited participant International Endocrine Disrupters Workshop sponsored by the Smithsonian Institution, the White House Office of Science and Technology Policy, United Nations Environment Program and United States Environmental Protection Agency. The workshop was January 23-24, 1997, in Washington, DC.

Invited speaker International Workshop on "Effects of endocrine disrupters in the environment on neuronal development and behavior--current knowledge, assessment, gaps." The workshop is sponsored by the German Environmental Protection Agency. The workshop was February 17-18, 1997 in Berlin, Germany.

Invited speaker – "Development, Function, and Teratology of the Thyroid", Development and Function of Endocrine and Immune Systems in Teratology, sponsored by the Teratology Society, San Diego, CA, June 20, 1998

Invited speaker -- "Xenobiotics and Thyroid Function"; The Role of Environmental Neurotoxicants in Developmental Disabilities, The 20th Rochester Conference on Environmental Toxicity, sponsored by the NIEHS, Rochester, NY September 23-24, 1998.

Invited speaker - "Perinatal Thyroid Function", NIH sponsored international conference on perinatal endocrinology, Nancy, France, September, 1998

Invited speaker - Learning Disabilities Association, Annual Meeting, Atlanta, GA, February, 1999.

Scientific and Professional Societies

Member Georgia Academy of Science
Member of American Association for the Advancement of Science
Member of the Endocrine Society
Member of the Society for Experimental Biology and Medicine
Member of the American Physiological Society
Member of the American Thyroid Association
Member of the Southeastern Society of Experimental Biology and Medicine

ROCHELLE W. TYL, Research Director, Center for Life Sciences and Toxicology

Professional Experience

Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC.

7/15/93 - present. Research Director, Center for Life Sciences and Toxicology. Establish and implement Center goals and objectives consistent with RTI's overall financial and research strategic planning; recommend, interpret, and implement RTI policies and procedures; establish guidelines and procedures for and manage allocation and utilization of Center facilities, equipment, and funding; initiate, plan, and implement operational plans and strategies for Center direction(s) and development, and staff professional development; play a major role in establishing and implementing marketing goals and activities for the Center; maintain high level of involvement in technical efforts of the Center, including establishment and implementation of objectives, goals, and procedures of projects, coordination and cooperation among senior staff in complex projects; contribute to and direct preparation and presentation of research reports to clients and the professional community, in addition to duties as described below. Direct supervision of six PhDs, one DVM, and four senior technical staff; Center is comprised of 43 staff.

5/1/91 - 7/14/93. Assistant Research Director, Mammalian Toxicology Program; Program Manager, Reproductive and Developmental Toxicology. Duties as described below.

5/1/90 - 4/30/91. Senior Program Director, Mammalian Toxicology Program; Program Manager, Reproductive and Developmental Toxicology. Duties as described below. In addition, supervise senior staff in general toxicology and adult and developmental neurotoxicology testing, work with higher management in policy and program development and implementation, building and renovation program for CLS.

6/15/89 - 4/30/90. Program Manager, Reproductive and Developmental Toxicology. Supervision of the staff of Developmental Toxicology, Developmental Neurotoxicology, Reproductive Toxicology, and Reproductive Endocrinology. Market and perform reproductive and developmental toxicity evaluations for governmental, industrial, and commercial clients according to appropriate governmental guidelines, FDA, EPA (FIFRA or TSCA), OECD, EPA Test Rules or FIFRA Data "Call-Ins," under Good Laboratory Practice regulations; design and implement research protocols in reproductive and developmental toxicology for various clients; plan, coordinate, and direct activities in program; prepare bids, protocols, final reports and manuscripts for refereed journals; evaluate personnel performance; direct supervision of four Ph.D. staff, two senior technical supervisory staff, and an administrative assistant. Program includes seven doctoral level staff, six supervisory staff and approximately 15 technical staff. Member of RTI Institutional Animal Care and Use Committee (IACUC), 1990 - present.

9/1/87 - 6/89. Assistant Director, Reproductive and Developmental Toxicology, Bushy Run Research Center (BRRC), Export, PA. Work with the Director and Associate Directors in planning, coordinating, and directing work activities at BRRC. Prepare equipment and personnel budgets and evaluate personnel performance. Supervise a postdoctoral scientist. Additional responsibilities as below.

7/1/83 - 8/31/87. Manager, Reproductive and Developmental Toxicology Section, Bushy Run Research Center (BRRC), Export, PA. Testing for developmental and reproductive toxicity of industrial chemicals by all routes of exposure in rats, rabbits, and mice for Union Carbide and other industrial clients. Supervise ten staff members, responsible for protocol development, contact with clients, writing SOPs, writing study reports and manuscripts for refereed journals, staff training, sponsor liaison for UCC

teratology studies done elsewhere, work under GLPs, FDA, EPA (TSCA), EPA (FIFRA), OECD guidelines, presentation of data to various professional groups.

5/83 - 6/30/83. Manager, Teratology Section and Senior Research Teratologist I, Research Triangle Institute, Research Triangle Park, NC. Research and testing in teratogenicity and reproductive toxicity of food additives, drugs, industrial chemicals, environmental pollutants, etc., in rats, mice, and rabbits for governmental and industrial clients. Supervise eight people, responsible for all facets of studies, client contact, protocol development, SOPs, writing reports and manuscripts for refereed journals, Institute and community service by lectures to professional and academic groups, work under GLPs, EPA, FDA guidelines.

7/81 - 5/83. Supervisor, Teratology Section and Senior Research Teratologist I, Research Triangle Institute, Research Triangle Park, NC. Research and testing in teratogenicity and reproductive toxicity of food additives, drugs, industrial chemicals, environmental pollutants, etc., in rats, mice, and rabbits for government and industrial clients. Supervise eight people, responsible for all facets of studies, client contact, protocol development, SOPs, writing reports and manuscripts for refereed journals, Institute and community service by lectures to professional and academic groups, work under GLPs, EPA, FDA guidelines.

7/78 - 7/81. Head, Teratology Section, Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC. Research and testing in teratogenicity and reproductive toxicity of priority commodity chemicals, toxicokinetics, and metabolism of commodity chemicals in pregnant animals, development of test battery for postnatal sequelae of *in utero* exposures.

9/68 - 6/78. Assistant Professor (1968-1973), Associate Professor (1973-1978), University of Connecticut, Southeastern Campus, Groton, CT. Teaching undergraduate courses in Biological Sciences, Genetics, Embryology, advising independent research students (undergraduate) and Master's thesis research students, direct own research, premedical student counseling, university committee memberships (tenured, 1974).

Education

A.B. cum laude, Biological Science, University of Connecticut, Storrs, CT, 1963
Ph.D., Developmental Genetics, University of Connecticut, Storrs, CT, 1968

Professional Societies

Teratology Society
Neurobehavioral Teratology Society
Society of Toxicology (SOT) Active Member
SOT, Reproductive and Developmental Toxicology Subsection Member
Middle Atlantic Reproduction and Teratology Association (MARTA)
North Carolina Chapter of SOT
Genotoxicity and Environmental Mutagenesis Society (GEMS, NC)
Allegheny-Erie Regional Chapter of SOT (1983-1989)

Honors

Phi Beta Kappa
Phi Kappa Phi
Sigma Xi
Gamma Sigma Delta

Certification

Diplomate, American Board of Toxicology (ABT), 1983; recertified in 1988, 1993, and 1998

CURRICULUM VITAE

Name	Kimber Littlepage White, Jr.
Social Security Number	225-76-9617
Date of Birth	May 2, 1950
Place of Birth	Boston, Massachusetts
Citizenship	United States
Family	Married Linda Runckel on June 16, 1973; Children: Kimberly Page, born December 20, 1979 and James Millard, born December 14, 1982
Home Address	2610 Olde Stone Road Midlothian, Virginia 23113 (804) 379-9848
Office Address	527 North 12th Street Strauss Immunotoxicology Research Laboratory Room 2011 Medical College of Virginia/Virginia Commonwealth University Richmond, Virginia 23298 (804) 828-6789 Phone (804) 828-5604 FAX
Military Service	United States Navy, 1968-1977
Rank:	Lieutenant
Qualifications:	Surface Warfare Officer Command Duty Officer Officer of the Deck Underway (Fleet) Engineering Officer of the Watch Combat Information Center Watch Officer
Awards:	Combat Action Ribbon Meritorious Unit Citation Vietnam Campaign Medal Vietnam Service Medal Letter of Commendation for Lifesaving
Vietnam Service:	1972-1973

CURRICULUM VITAE
Kimber Littlepage White, Jr.

Educational Background

Ferguson High School, Newport News, Virginia 1968

B.S., United States Naval Academy 1972
Annapolis, Maryland
Chemistry

Ph.D., School of Basic Sciences 1981
Department of Pharmacology and Toxicology
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia

Thesis Title: "Immunotoxicology of Chrysotile Asbestos"

Honors

NIH Predoctoral Fellow 1978 - 1981

Positions Held

5/1981-12/1982	Research Associate and Adjunct Assistant Professor Department of Pharmacology and Toxicology Medical College of Virginia/VCU
1/1983 - 8/1984	Assistant Professor Department of Microbiology and Immunology Medical College of Virginia/VCU
	Assistant Professor (Affiliate) Department of Pharmacology and Toxicology Medical College of Virginia/VCU
9/1984 - 7/1991	Assistant Professor Department of Biostatistics Medical College of Virginia/VCU
	Assistant Professor (Affiliate) Department of Pharmacology and Toxicology Medical College of Virginia/VCU
7/1986 - 7/1991	Assistant Professor (Affiliate) Biomedical Engineering Program Medical College of Virginia/VCU
7/1991 - 7/1995	Associate Professor Biomedical Engineering Program Medical College of Virginia/VCU

CURRICULUM VITAE
Kimber Littlepage White, Jr.

Positions Held (Continued)

Associate Professor (Affiliate)
Department of Pharmacology and Toxicology
Medical College of Virginia/VCU

7/1995 - present Associate Professor
Department of Pharmacology and Toxicology
School of Medicine
Medical College of Virginia/VCU

Associate Professor (Affiliate)
Biomedical Engineering Program
School of Engineering
Medical College of Virginia/VCU

Membership - Scientific, Honorary, and Professional Societies

Society of Toxicology - Immunotoxicology Subsection
Chairman, Methods Committee 1988-89, 1989-90
Councilor 1991-1993
- Risk Assessment Subsection
- National Capital Area Chapter

Immunotoxicology Discussion Group
Steering Committee Member 1991-present
The American Association of Immunologists
American Association for the Advancement of Science
Virginia Academy of Science

Invited Speaker or Invited Participant

1981 International Research and Development Corporation
Symposium on Immunotoxicology

1984 Environmental Protection Agency
Office of Pesticides and Toxic Substances

Immunotoxicology Discussion Group
Critical Review of Current Methodology

Northeast Chapter of Medicinal Chemists

1985 Environmental Protection Agency
Office of Pesticides and Toxic Substances

Northrop Services, Inc., Research Triangle Park

1986 Sterling-Winthrop Research Institute

CURRICULUM VITAE

R. Thomas Zoeller
Department of Biology
University of Massachusetts, Amherst, MA 01003
Tel. (413) 545-2088 /// *Fax.* (413) 545-3243
Email: tzoeller@bio.umass.edu

Academic Appointments:

Associate Professor with tenure, Biology Department, University of Massachusetts at Amherst, '96-Present
Associate Professor, Biology Department, University of Massachusetts at Amherst, '94-'96
Assistant Professor, Dept Anat. & Neurobiol., Univ. Missouri-Columbia Sch. Med. '88-'94
Research Associate, Laboratory of Neurochemistry, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD , '87-'88
Postdoctoral Researcher, Laboratory of Cell Biology, National Institute of Mental Health, NIH, Bethesda, MD '84-'87.
Research Assistant, Department of Zoology, Oregon State Univ. Corvallis, '80-'84

Education:

B.S., Indiana University, Bloomington. 1977 (Biology)
M.A., Oregon State University, Corvallis. 1979 (Endocrinology)
Ph.D., Oregon State University, Corvallis. 1983 (Neuroendocrinology)
Postdoctoral fellow, LCB, NIMH, NIH, Bethesda, MD
NRC Research Associate, LNC, NINDS, NIH, Bethesda, MD

Affiliations:

Member:
University of Massachusetts Center for Neuroendocrine Studies
UMass Neuroscience and Behavior Program
UMass Molecular and Cellular Biology Program
UMass Organismal and Evolutionary Biology Program
American Association for the Advancement of Science
Endocrine Society
Society for Neuroscience
Research Society on Alcoholism
International Brain Research Organization
International Society for Biomedical Research on Alcoholism

Honors:

Best Student Paper Award, American Society of Zoologists '82
Oregon State University Bayley Graduate Fellow, '82-'83.
Individual National Research Service Award (1984-1987), National Institute of Mental Health, NIH.
Individual Resident Research Associateship (1987-1988), National Institute of Neurological Disorders and Stroke. Awarded by the National Research Council of the National Academy of Sciences
Invited Participant, 2nd ORPRC Symposium on Primate Reproductive Biology: Neuroendocrine Aspects of Reproduction. Oregon Regional Primate Research Center, Beaverton, Oregon (1982).
Invited Speaker, "Reproductive Neuroendocrinology, New Approaches to Old Questions", San Antonio, TX, 27 December, 1990.
Invited Discussant, Ciba Foundation Symposium #168, "Functional Anatomy of the Neuroendocrine Hypothalamus", 8-10 October, 1991, Budapest, Hungary.
Invited Speaker and Chair, Session on "Thyroid Hormones and Brain Function" 1995 Steroid Hormone Workshop, Breckenridge, CO

Military Service

Drafted June 14, 1972, Honorable Discharge June 13, 1974.

Professional Reviews:

Hoc Reviewer for the following journals:
American Journal of Physiology, Brain Research, Molecular Brain Research, Developmental Brain Research, Development, Endocrinology, Molecular Endocrinology, General and Comparative Endocrinology, Histochemistry, Journal of Neuroendocrinology, Journal of Neuroscience, Journal of Experimental Zoology, Neuroscience Journal, Regulatory Peptides

Ad hoc Reviewer for the following agencies
National Science Foundation, US Veteran's Administration, Human Frontier Science Program Organization, EPA.

Member, Screening and Testing Workgroup of the Endocrine Disruptor Screening and Testing Advisory Committee.

Meetings Organized

Thyroid Hormones and Brain Function, a session at the 1995 Workshop on Steroid Hormones and Brain Function, Breckenridge, CO.
23rd New England Endocrinology Conference, Amherst, MA (September, 1995).

Research Support History:
Bayley Graduate Fellow (Oregon State Univ., 1983-1984)
Individual National Research Service Award (MH09104; 1984-1987)
Individual Fellow of the National Research Council (1987-1992; accepted only 1 year of 5). "Molecular Analysis of Neuroendocrine Peptide Gene Expression"
Graduate Research Council (UMC, 1988-1989; 4,645) "Molecular Analysis of Neuroendocrine Peptide Gene Expression"
Medical Research Council (UMC, 1989-1990; $25,000) "Neuroendocrine Peptide Gene Expression in Rat Brain"
American Heart Association (1990-1991; $30,000) "Cardiovascular Elements within the CNS: The Role of TRH Neurons" (T. Zoeller, PI).
Graduate Research Council, UMC (1990-1991; $3,340) "Central Regulation of Cardiovascular Function"
National Institutes of Health (RO1 AA 08887; 1991-1995; $281,300 direct) "Molecular Basis for Ethanol Induced Hypothyroidism" (T. Zoeller, PI) Note: I brought this grant with me from Missouri, but chose not to renew it as an alcohol grant (see statement of research direction). Rather, I have re-focused this work on an issue that this work revealed and have funded it through a separate mechanism (NSF IBN9514835).
National Institutes of Health (RO1 NS30178; 1992-1995; $411,925 direct) "IP3 metabolism and Ca++ homeostasis in cerebral ischemia (GY Sun, PI; T.Zoeller, 10% effort). Note: I have not maintained this collaboration after moving to UMass.
National Institutes of Health NRSA to NIAAA (1993-1996; $70,000 direct) "Ethanol and Hypothalamic-Pituitary-Thyroid Axis" (Sponsor for Dr. HC Scott). Note: Dr. Scott was not able to move with me to UMass for personal reasons. However, I continue to advise her on her work, and we have maintained a productive collaboration.
Graduate Research Council, Univ. Massachusetts (1994-1995; $5,000) "Identification of Thyroid Hormone-Regulation Genes in CNS Development". Note: This work has led directly to a collaboration with a group an UMass Worcester.
National Institutes of Health (RO1 AA10418; 1996-1999; $437,850, direct) "Molecular Mechanisms Underlying Fetal Alcohol Syndrome" (T.Zoeller, PI; Active).
National Science Foundation (IBN-9514835; 1996-1998; $80,000) "Functional Organization of Hypophysiotropic TRH Neurons" (T.Zoeller, PI; Awarded March 1, 1996, Active)

National Institutes of Health (ES083330; 1996-1999; $300,000 direct) "PCBs and Thyroid Hormone Action in Developing Cochlea" (T. Zoeller, PI; Active).

Appendix D

Conflict of Interest of Peer Reviewers

CONFLICT OF INTEREST

Each of the following Peer Reviewers signed a statement saying the following, "The undersigned hereby warrants that, to the best of their knowledge and belief, that no actual or potential organizational or personal conflicts of interests exist with respect to the perchlorates work assignment."

Dr. Melvin Andersen
Colorado State University

Dr. David Brusick
Covance Laboratories, Inc.

Dr. Rick Cardwell
Parametrix, Inc.

Dr. Charles Emerson
University of Massachusetts Medical Center

Dr. Joseph Haseman
National Institute of Environmental Health Sciences

Dr. Curtis Klaassen
University of Kansas Medical Center

Dr. Susan Porterfield
Medical College of Georgia

Dr. Rochelle Tyl
Research Triangle Institute

Dr. Kimber White
Medical College of Virginia

Dr. R. Thomas Zoeller
University of Massachusetts

Appendix E

Charge to External Peer Review Panel for Perchlorate Toxicity

1.0 Charge to External Peer Review Panel for Perchlorate Toxicity

1.1 Review of Individual Studies Initiated Since May 1997

For each study report assigned to you as a primary or secondary reviewer, please respond to the following questions:

1. Please comment on the strengths and weaknesses of the experimental design of the study. Are the questions being investigated in each study clearly identified? Are they important to enhancing the toxicological (ecotoxicological) characterization of perchlorate? Is the study design appropriate to answer the questions? Discuss all limitations in experimental design that would affect the ability to interpret the significance of study results. Also indicate areas in which insufficient information has been provided on the experimental design.

2. Please comment on any limitations in the conduct of the study which could decrease the relevance of study findings. For example, were the studies conducted in accordance with Good Laboratory Practices? Were there occurrences that necessitated a change in the protocol during the course of the study? If so, what impact did these changes have on the findings?

3. Please comment on the strengths and weaknesses of the statistical methodology(ies) used to evaluate study findings. What other statistical analyses, if any, should be performed?

4. Please comment on the strengths and weaknesses of the presentation of the investigations in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented in the report? Are the conclusions of the report supported by the data? Please explain.

5. Overall was the study as designed, performed and reported of sufficient quality for use for hazard characterization purposes? If so, indicate the extent to which it can be used for characterizing human health/ecotoxicological effects of ammonium perchlorate and the perchlorate ion. Do the findings provide information relevant to evaluating the sensitivities of specific subpopulations of exposed individuals and attendant effects (e.g., infants, hypothyroid individuals)?

6. For the studies that are not yet complete, are sufficient data available on experimental design, conduct and interim observations to derive meaningful conclusions? If so, what caveats, if any, should be placed on these conclusions? Or should all data from the study be evaluated following the conclusion of the study and development of the final study report?

1.2 Review of Toxicological Review Document

1.2.1 Effects of Concern to Human Health (all reviewers except Dr. Cardwell)

Please comment on the adequacy of the Toxicological Review document in presenting and evaluating the existing toxicology data base on ammonium perchlorate and the perchlorate ion relevant to effects on human health.

1. Have the key aspects of the protocols, conduct and results of each toxicology study been adequately described in the Toxicological Review document? Where limitations exist in study reports or published papers, have they been appropriately discussed in the Toxicological Review document? In what ways might the discussion of studies be improved?

2. Indicate the strengths and weaknesses of the analyses performed on the data in the Toxicological Review document first of specific toxicological studies and then of the overall toxicology data base on perchlorate. Has the document adequately evaluated the results of all relevant studies and the biological significance of the entire data base? Where inconsistencies appear to exist in the findings relevant to the hypothalamic-pituitary-thyroid axis within and between studies, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

3. Authors of the Toxicological Review document provided statistical analyses beyond those contained in the original study reports of recently completed studies. Where these statistical analyses were performed, were the appropriate methodologies used? Did they add to the overall understanding/relevance of the studies? Were the appropriate endpoints and/or time points used? Please explain.

4. Note any relevant references that have not been cited in the Toxicological Review document and their relevance to hazard characterization of ammonium perchlorate and the perchlorate ion.

1.2.2 Ecotoxicological Effects of Concern (Dr. Cardwell)

Please comment on the adequacy of the Toxicological Review document in presenting and evaluating the existing data base of ecotoxicological effects of ammonium perchlorate and the perchlorate ion.

1. Have the key aspects of the protocols, conduct and results of each study of ecotoxicological effects been adequately described in the Toxicological Review document? Where limitations exist in study reports or published papers, have they been appropriately discussed in the Toxicological Review document? In what ways might the discussion of studies be improved?

2. Indicate the strengths and weaknesses of the analyses performed on the data from individual studies of ecotoxicological effects and then of the overall data base on ecotoxicological effects of perchlorate. Has the document adequately evaluated the results of all relevant studies and the biological significance of the entire data base?

3. Note any relevant references on ecotoxicological effects of ammonium perchlorate or other perchlorate salts that have not been cited in the Toxicological Review document and their relevance to the characterization of ecotoxicological effects of these compounds.

1.2.3 Additional Issues Pertaining to the Toxicological Review Document (all peer reviewers)

1. Are there other sections of the document that could be improved? Please specify and note the revisions that would improve the document.

2. Is the document as currently written useful for the purpose of characterizing the human health/ecotoxicological effects of ammonium perchlorate and the perchlorate ion? If not, specify the nature and extent of changes that are needed.

1.3 Hazard Characterization

1.3.1 Development of Reference Dose (RfD) (all reviewers except Dr. Cardwell)

See the attached summary of the EPA guidelines for evaluating noncancer health effects of environmental chemicals through the development of reference doses (RfDs) and for evaluating carcinogenic effects through the development of cancer potency factors.

1. The Toxicological Review document developed no observed adverse effect levels (NOAELs) and/or lowest observed adverse effect levels (LOAELs) for most of the studies discussed in the document. Are the individual NOAELs/LOAELs appropriate given the totality of data from each study? Please explain.

2. It is general EPA policy to develop estimates of non-cancer toxicities of environmental chemicals by using the results on the most sensitive toxic endpoint from the group of toxicology studies that have been performed on the chemical. This serves as the basis for the reference dose(RfD), an estimate of a daily lifetime exposure without risk of deleterious noncancer effects during a lifetime. Given that all available data indicate that the thyroid organ is the most sensitive organ for perchlorate toxicity, it is important to assess the internal consistency of the overall data base. Comment on the use of a single study or the totality of data on thyroid toxicology as the basis for establishing an RfD.

3. The approach used in the Toxicological Review document for developing an RfD for perchlorate was to identify the principal study as the neurodevelopmental toxicity study in rats and the critical effect as the decrease in follicular lumen size and follicular cell

hyperplasia observed in pups on postnatal day 5 at the 0.1 mg/kg/day dose. Is this the appropriate selection? Is the designation of the 0.1 mg/kg/day dose as a "minimal" LOAEL an appropriate choice based upon the totality of the data? If not, specify a more appropriate approach to developing an RfD based upon the current toxicology data base.

4. The EPA position, as stated in the document entitled "Assessment of Thyroid Follicular Cell Tumors," is that in the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. This is considered to be a public health protective position where thyroid-pituitary disruptions are the sole mode of action, because rodents appear to be more sensitive to this carcinogenic mode of actions than humans. The available data base on the perchlorate ion indicates that disruption of the thyroid-pituitary axis is the most sensitive endpoint. Further, long-term treatment with perchlorate induced benign thyroid tumors in rats. Moreover, perchlorate appears not to be genotoxic based upon available data. Given the above EPA position and the toxicity data base on perchlorate, is the choice of an uncertainty factor of 3 for extrapolating potential differences in iodide inhibition between rodents and humans appropriate in the derivation of the RfD? If not, what should the uncertainty factor be for interspecies extrapolation?

5. Additional uncertainty factors of 3 each were used to account for using a minimal LOAEL as opposed to a NOAEL and to partially address intrahuman variability in pharmacodynamics, for data base deficiencies, and for accounting for intrahuman variation (sensitive subpopulations) in iodide uptake inhibition. Identify the strengths and limitations of using 3 as the value for each of these uncertainty factors. Would other values (e.g., 10 or 1) have been more appropriate? If so, specify those values and the reasons for their selection.

6. The Toxicological Review document concludes that the "RfD" is actually a harmonized oral human health risk estimate that will be protective for both noncancer health effects and cancer endpoints of perchlorate ion since the RfD is based upon reversible effects observed at dose levels below those at which thyroid tumors or neurodevelopmental effects were induced in the rat studies. Do the existing data support this position? Please explain.

1.3.2 Ecotoxicological Assessment (Dr. Cardwell)

1. Comment on whether the goals and objectives of this ecological screening analysis have been adequately described and to what extent these have been met.

2. Does the analysis support the summary and conclusions presented? Are relevant and important aspects of uncertainty are addressed sufficiently? Which aspects are not, and how could the discussion be improved?

3. Comment on whether the assays selected for evaluation in the ecological screening analysis can be reasonably expected to identify potential ecological effects of concern.

1.4 Further Testing Needs for Perchlorate

1.4.1 Toxicological Testing (all members of the peer review panel except Dr. Cardwell)

1. Were the experimental designs of the toxicity studies undertaken since May 1997 adequate to identify the potential hormone disrupting effects on development and reproductive performance due to thyroid function perturbations at low exposure levels? If not, specify more appropriate protocols.

2. Identify additional toxicology studies that would lead to a more complete toxicological characterization of ammonium perchlorate and the perchlorate ion. Provide information on the protocols that should be utilized.

3. Comment on the potential value added to these analyses by the development of a physiologically-based pharmacokinetic model to address species differences in inhibition of iodide uptake, perchlorate kinetics, and subsequent perturbations of the hypothalamic-pituitary-thyroid axis.

1.4.2 Ecotoxicological Testing (Dr. Cardwell)

1. Will the additional ecotoxicological studies currently underway be sufficient to characterize the ecotoxicological potential of ammonium perchlorate and the perchlorate ion? If not, explain what data needs will be unmet and describe further studies that should be considered, present the rationale for the studies, and provide overviews of the types of experimental designs that will be needed.

Appendix F

Copy of Written Comments Received by RTI from Outside Observers Before the Workshop

Kenneth S. Crump
K. S. Krump Group, Inc.

ICF KAISER

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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:

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

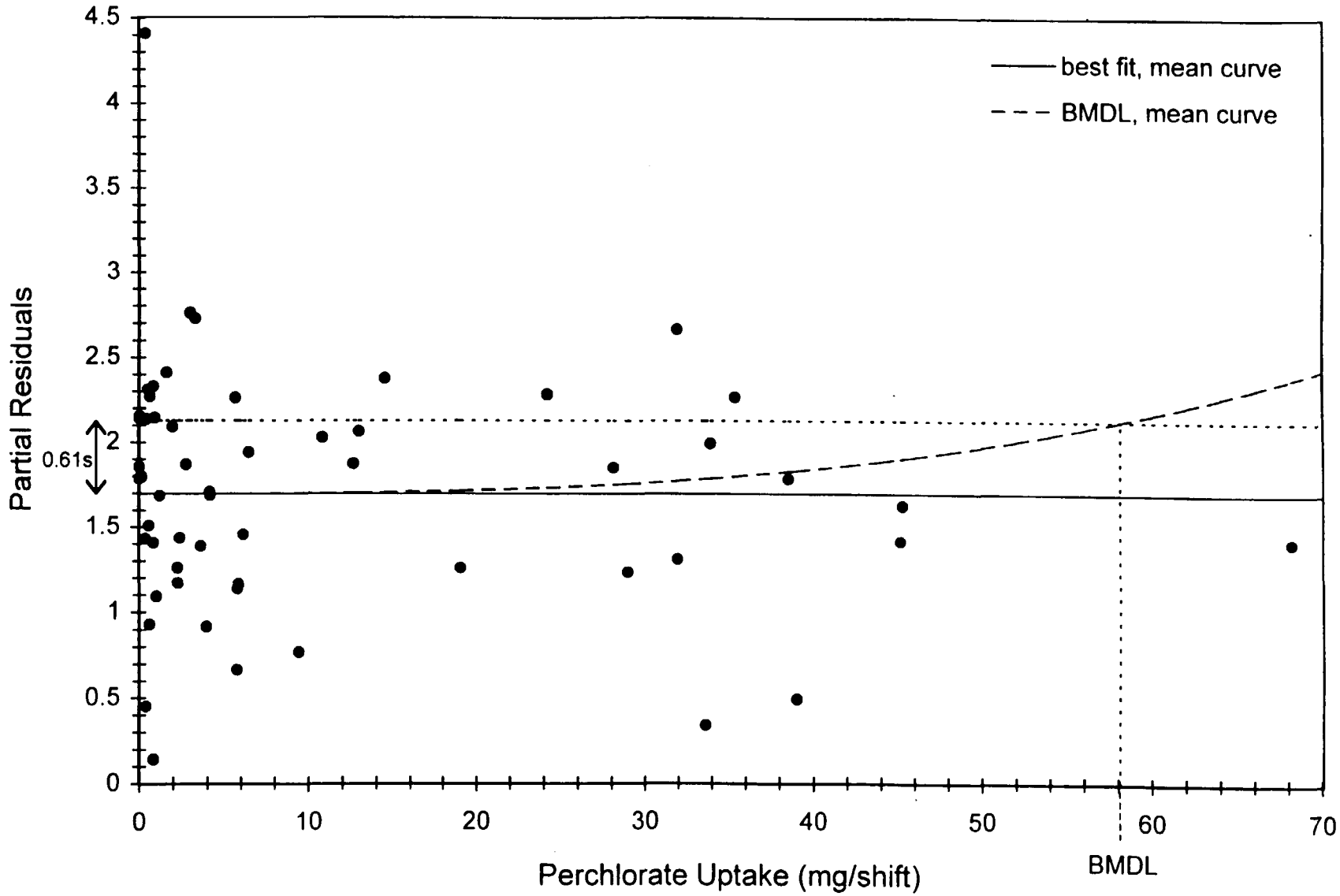
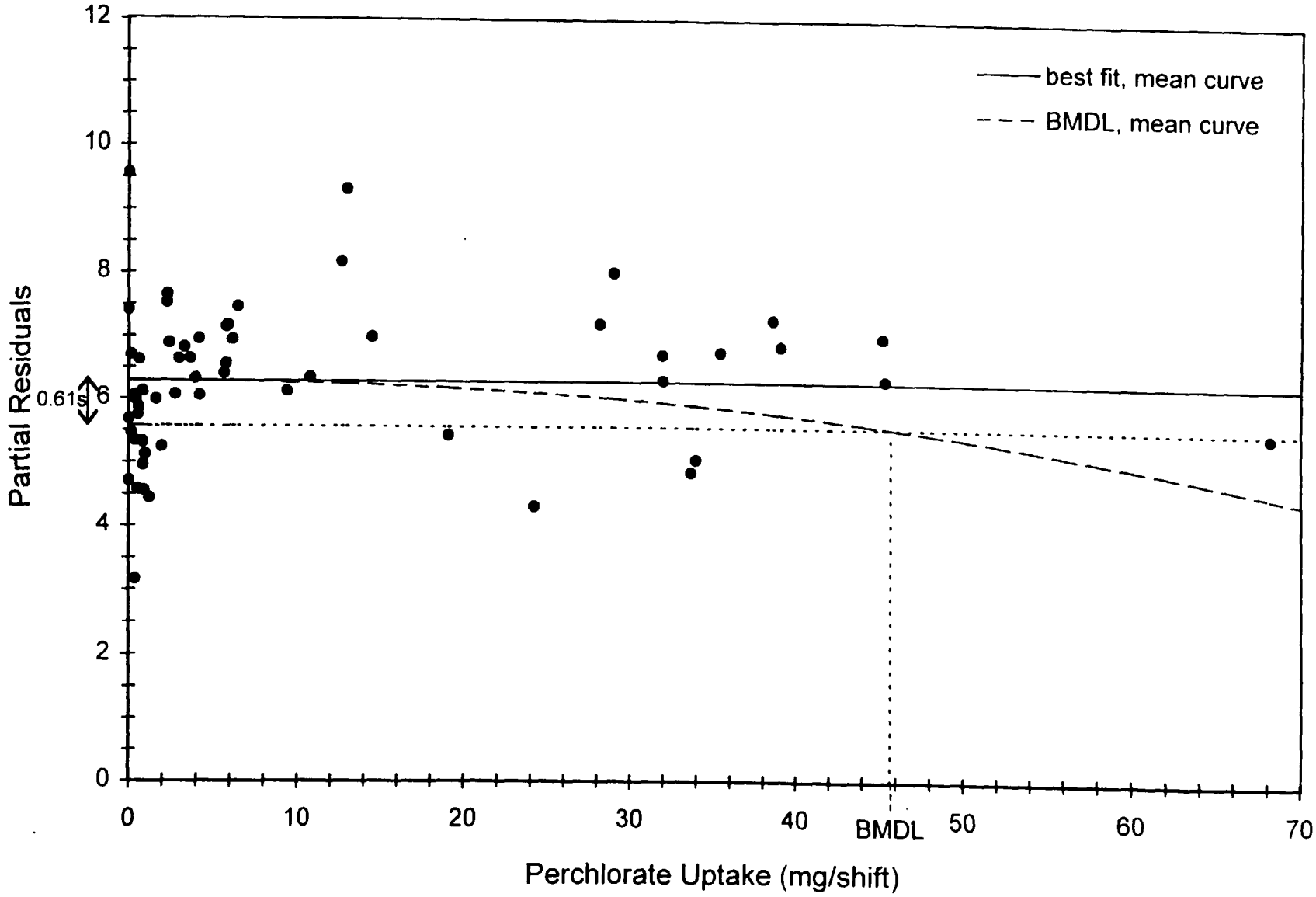


Figure 2: Residuals for FTI Fit by k-Power Model



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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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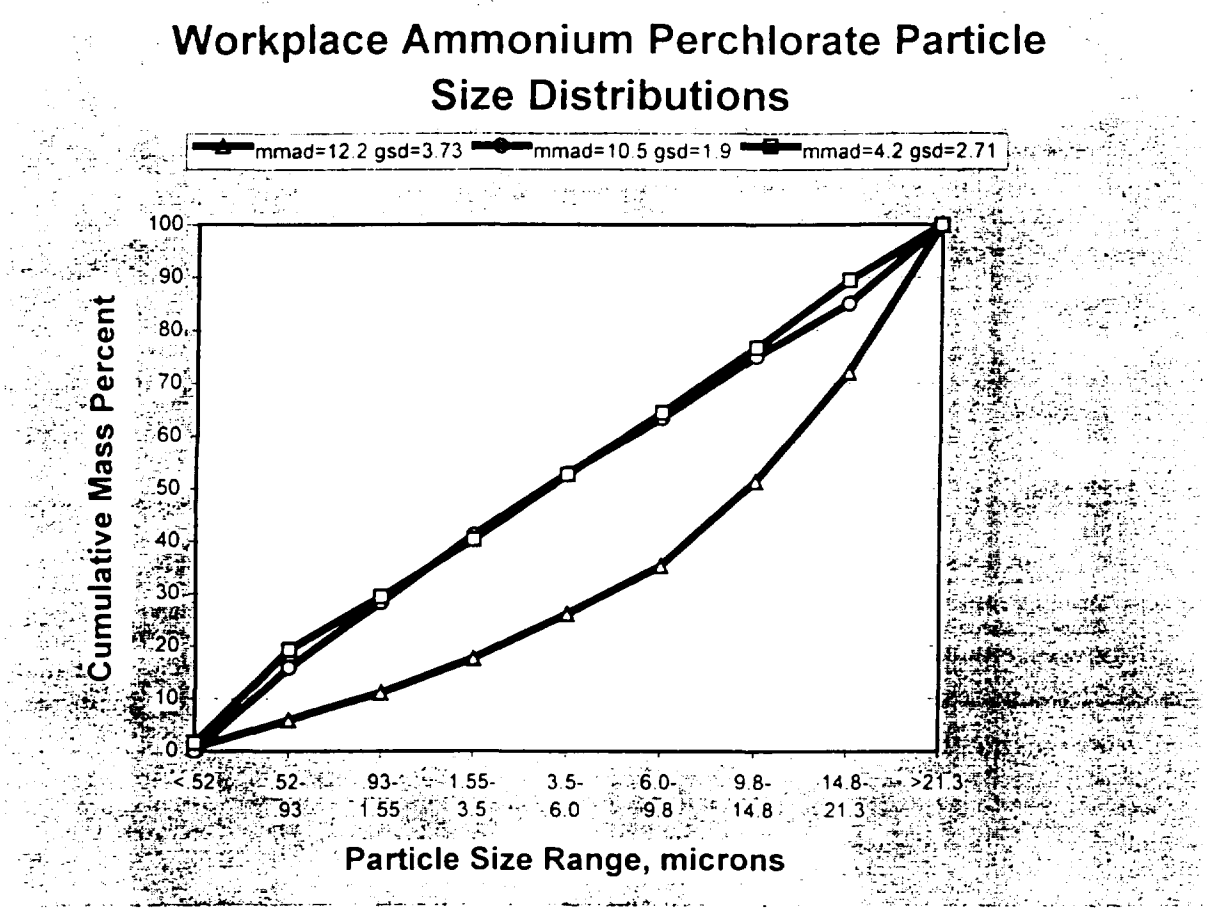

FIGURE 1 Particle size distributions of workplace dust representative of Lamm et al[4] (Cedar City Utah facility). Data collected by David Houck, CIH using a Graseby-Anderson Model 298 eight-stage Marple Cascade Impactor at 2 liters per minute flow rate for full shift - Jan 26-28, 1999.

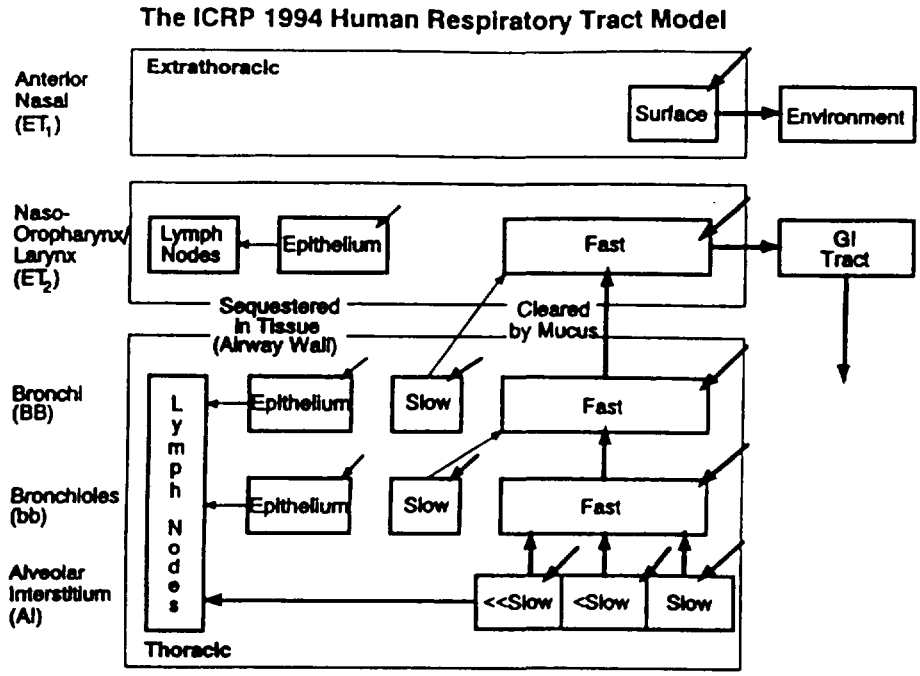

Figure 10-34. Schematic of the International Commission on Radiological Protection (ICRP66, 1994) model. Respiratory tract compartments in which inhaled particles may be deposited are illustrated. An explanation of clearance pathways, clearance rates, and subfractions of activity committed to different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

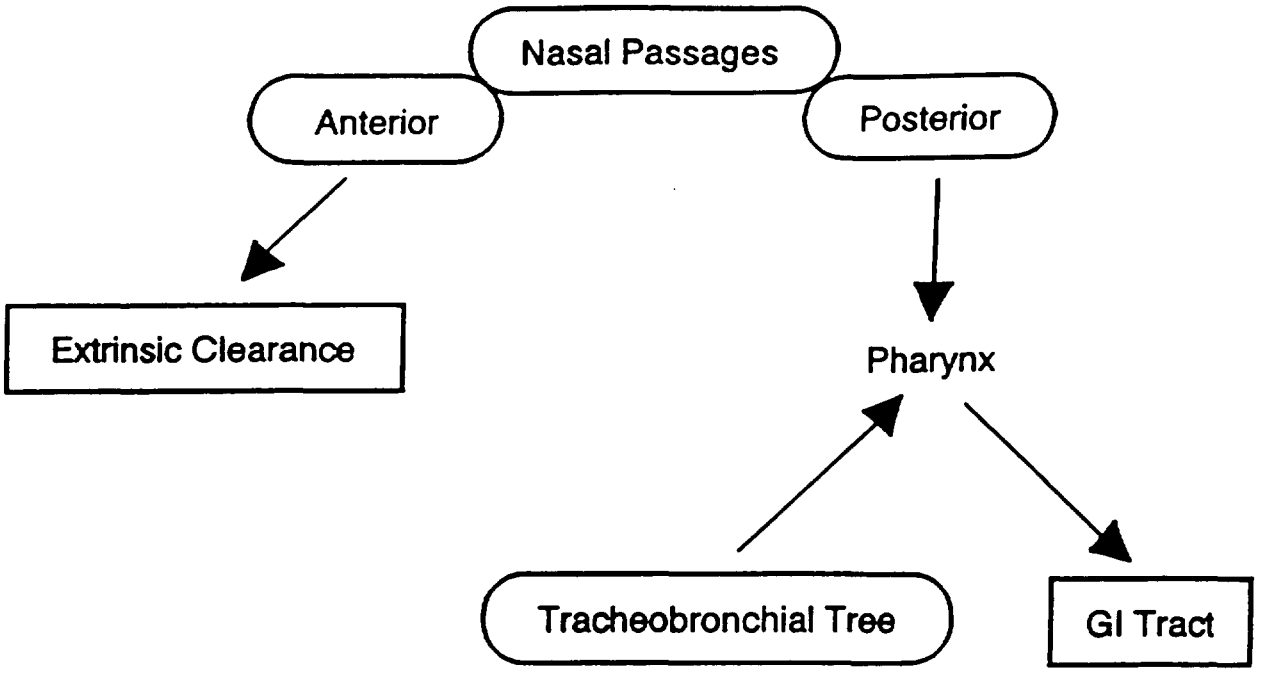

Figure 10-12. Major physical clearance pathways from the extrathoracic region and tracheobronchial tree.

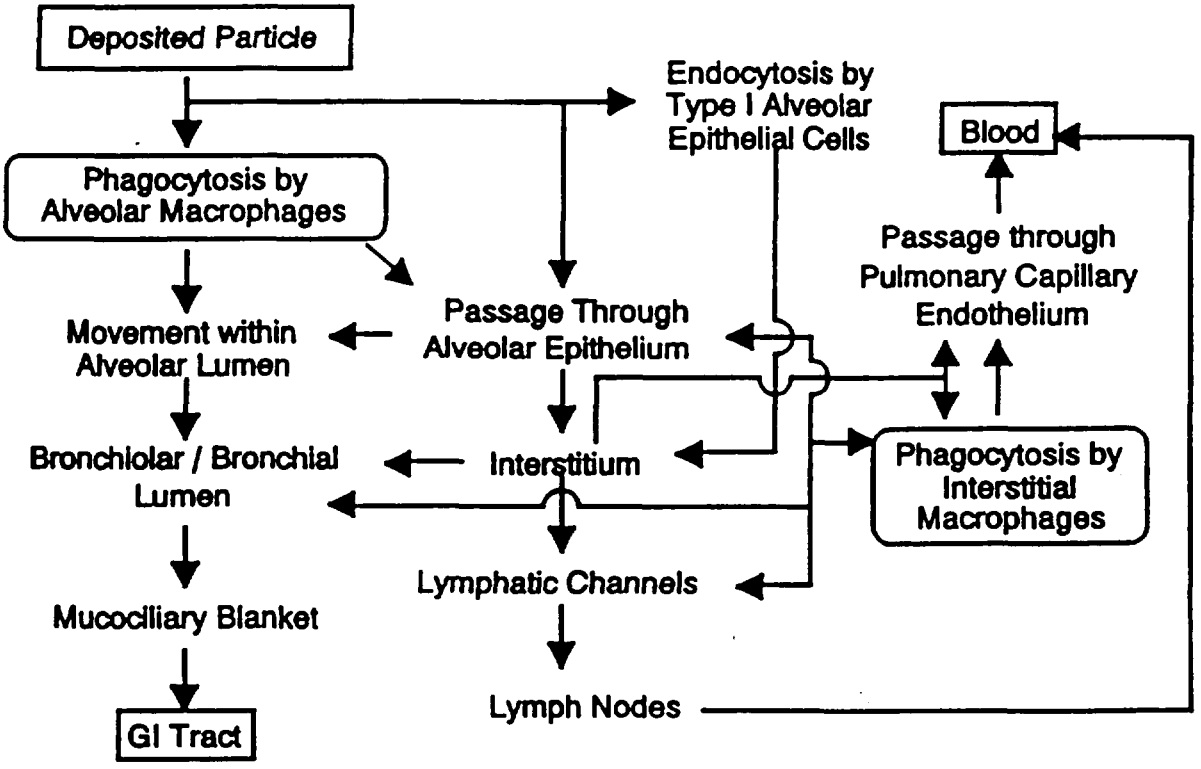

Figure 10-13. Diagram of known and suspected clearance pathways for poorly soluble particles depositing in the alveolar region.

Source: Modified from Schlesinger (1995).

William Stiteler
Syracuse Research Corporation

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.

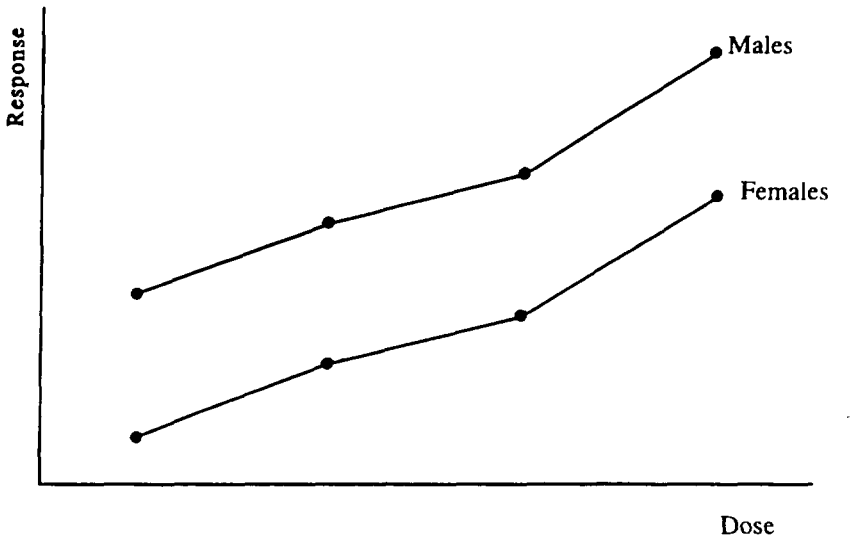

Figure 1 No Gender*Treatment Interaction

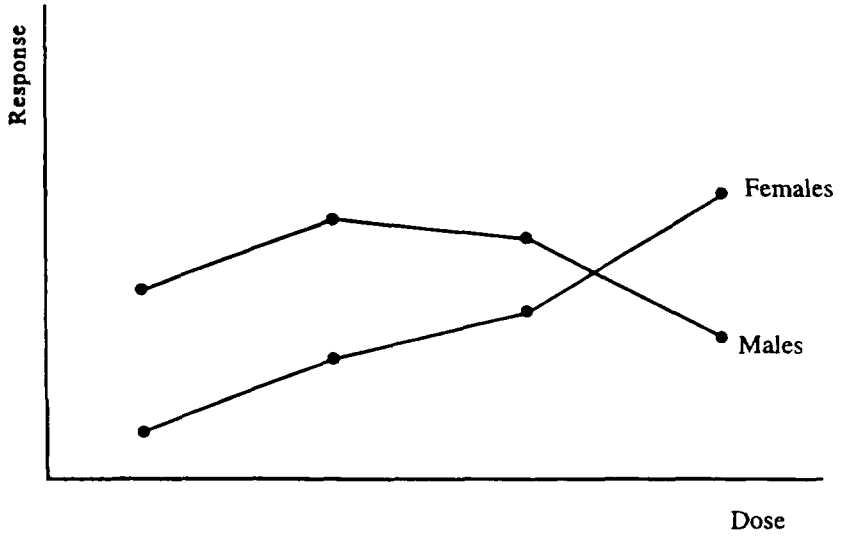

Figure 2 Gender*Treatment Interaction at Higher Doses

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January 29, 1999

Susan Goldhaber/Ella Darden
Research Triangle Institute
3040 Cornwallis Rd.
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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
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Steven Lamm
Consultants in Epidemiology and Occupational Health, Inc.

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
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3040 Cornwallis Rd.
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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]

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[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.

Acknowledgements

This paper is dedicated to Dr. Irving R. Tabershaw who taught me (SHL) the role of occupational medicine in assessing the effect of chemical exposures on human health and whose footsteps I have tried to follow for over twenty years.

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January 29, 1999

Susan Goldhaber/Ella Darden
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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

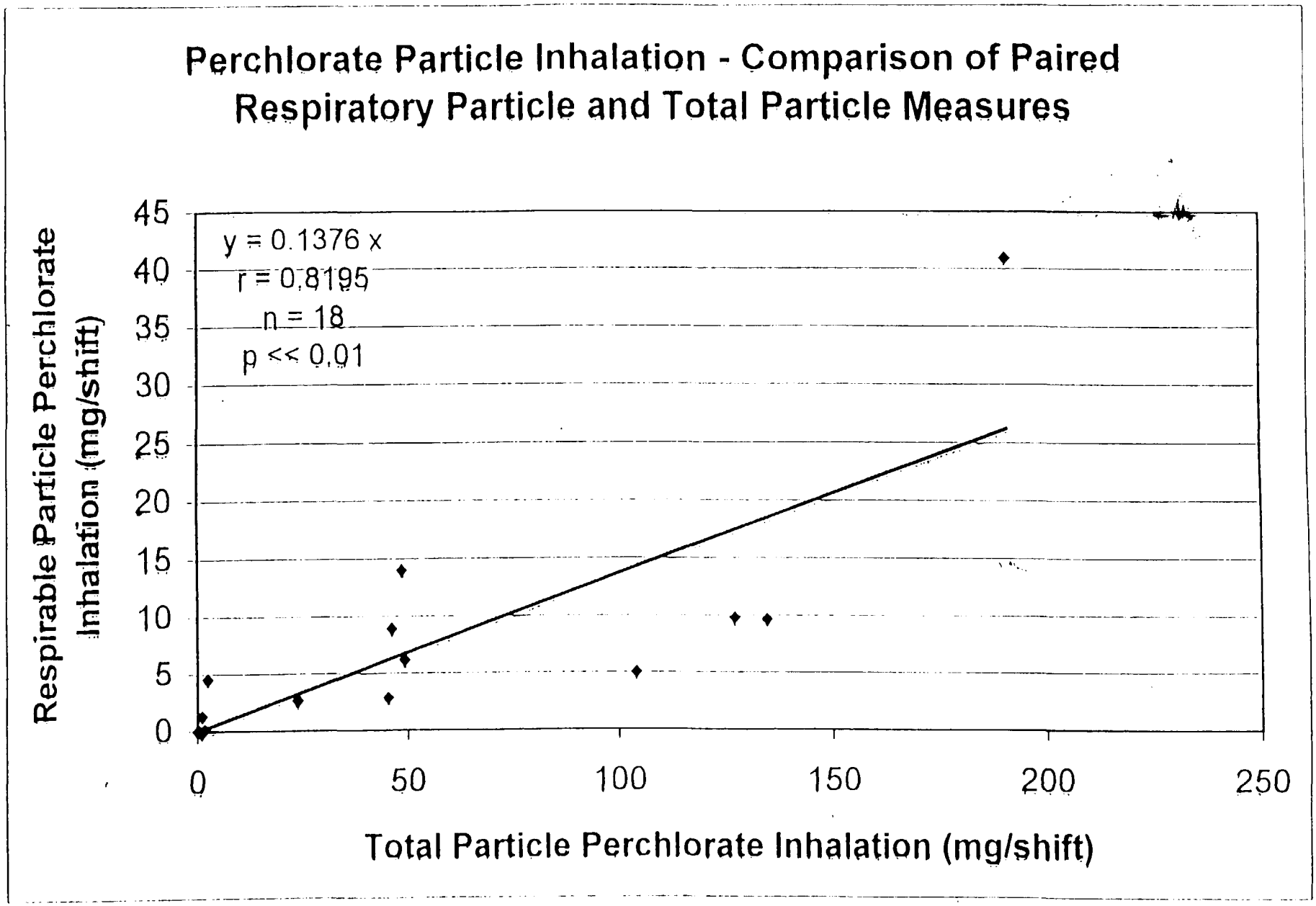
Perchlorate Particle Inhalation - Comparison of Paired Respiratory Particle and Total Particle Measures
y = 0.1376 x
r = 0.8195
n = 18
p << 0.01
Respirable Particle Perchlorate Inhalation (mg/shift)
Total Particle Perchlorate Inhalation (mg/shift)
0
5
10
15
20
25
30
35
40
45
0
50
100
150
200
250

Figure 2

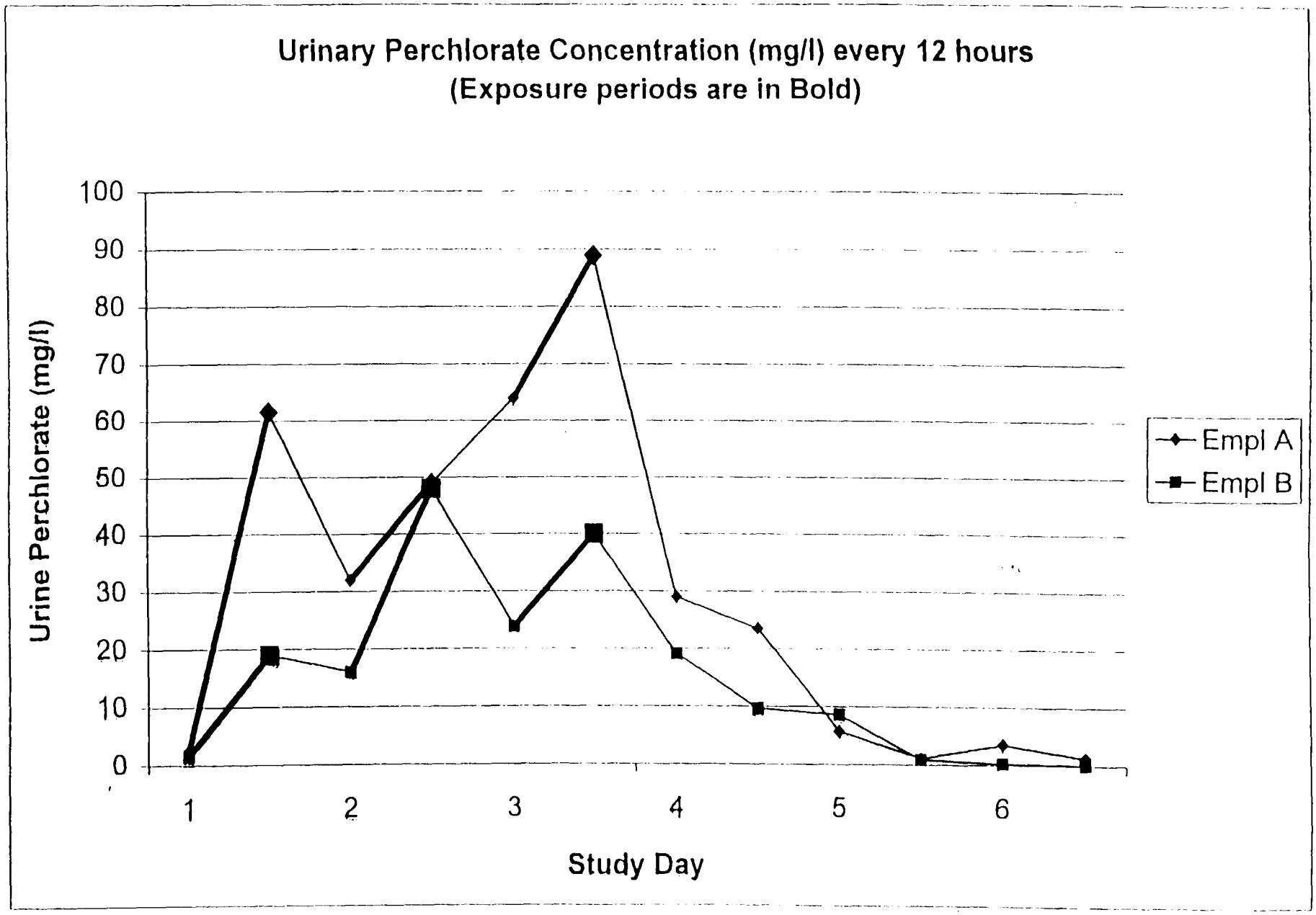
Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in Bold)
Urine Perchlorate (mg/l)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Study Day
Empl A
Empl B

Figure 3

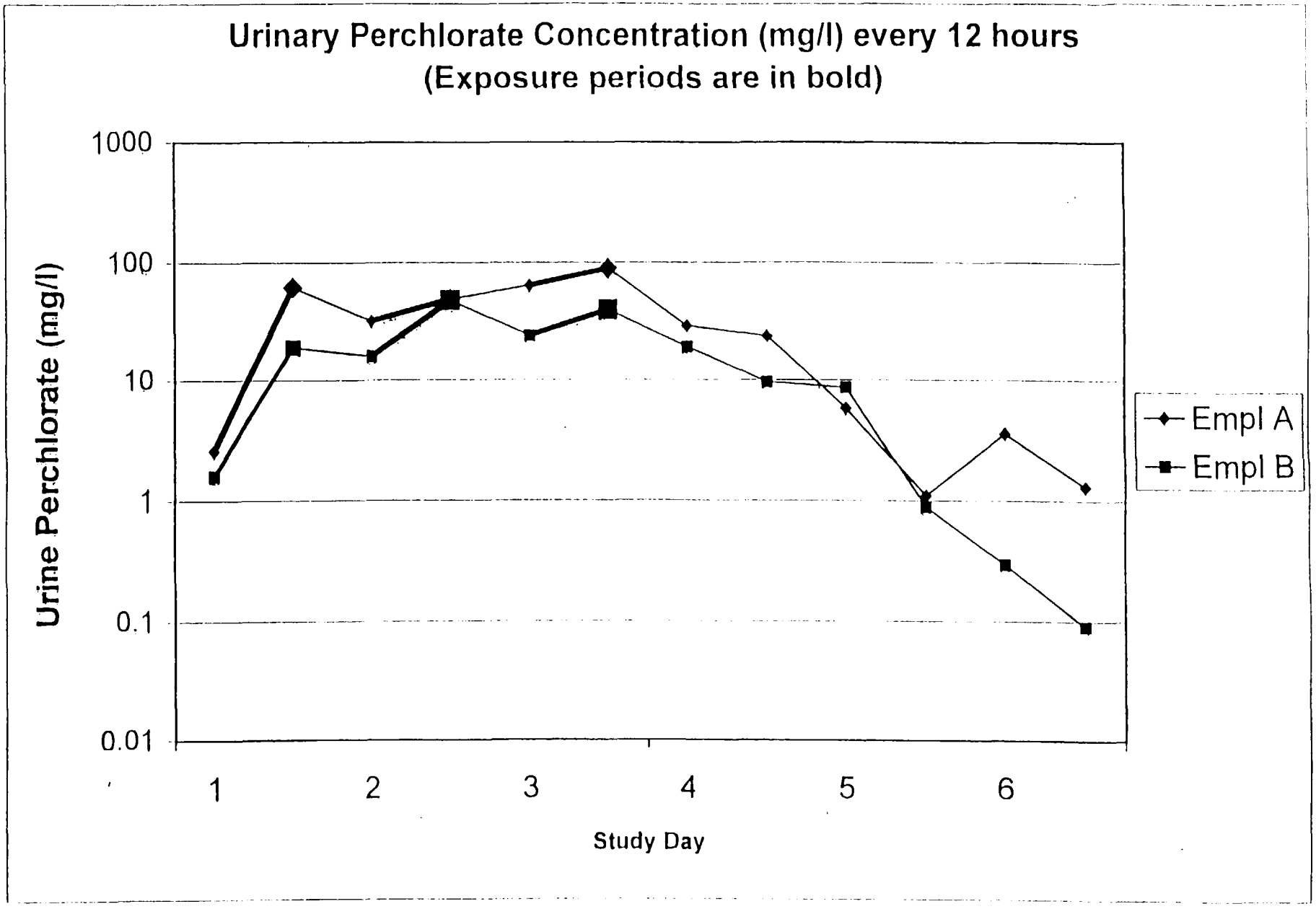
Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in bold)
Urine Perchlorate (mg/l)
1000
100
10
1
0.1
0.01
1
2
3
4
5
6
Study Day
Empl A
Empl B

Figure 4

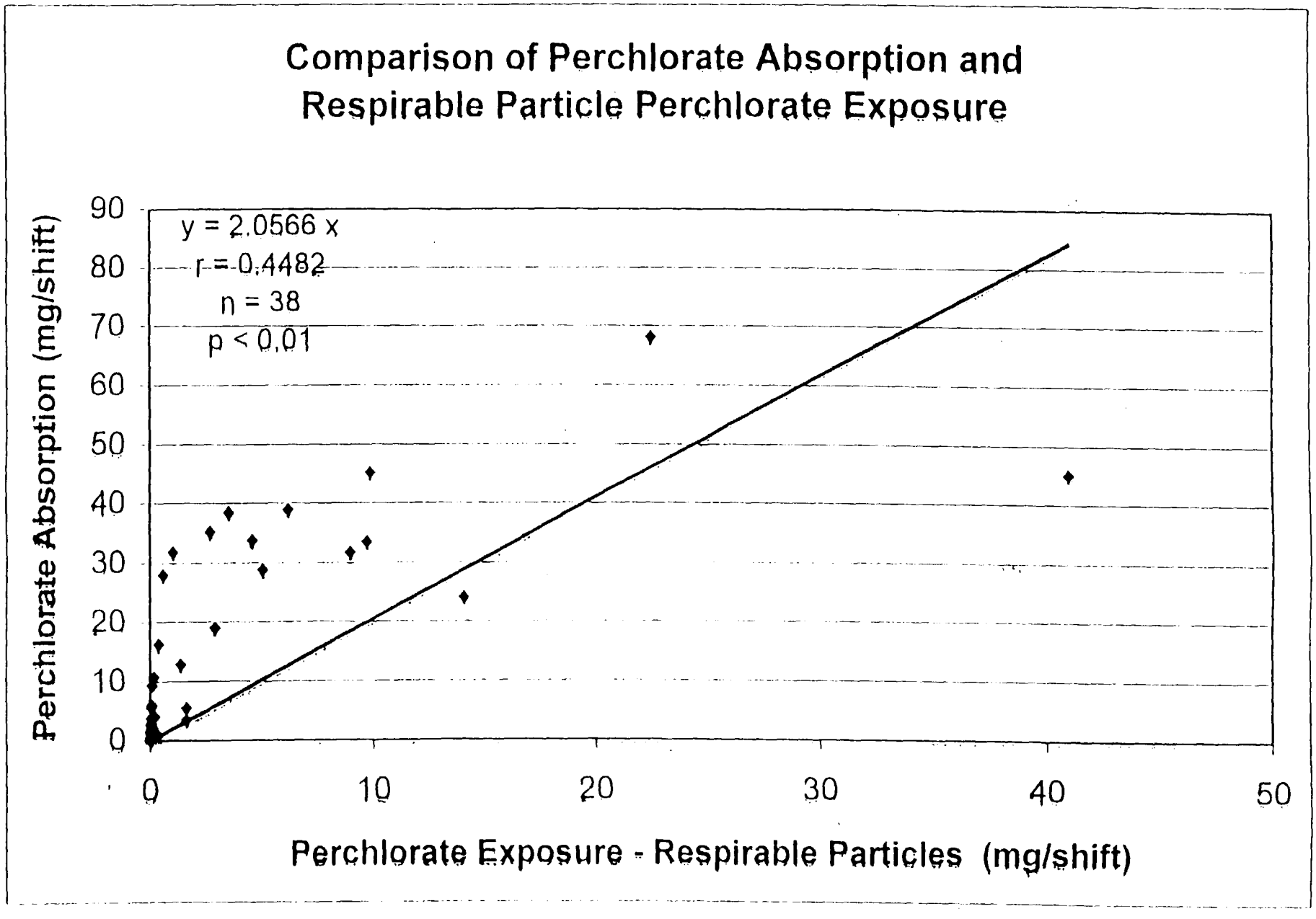
Comparison of Perchlorate Absorption and
Respirable Particle Perchlorate Exposure
y = 2.0566 x
r = 0.4482
n = 38
p < 0.01
Perchlorate Absorption (mg/shift)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Perchlorate Exposure - Respirable Particles (mg/shift)

Figure 5

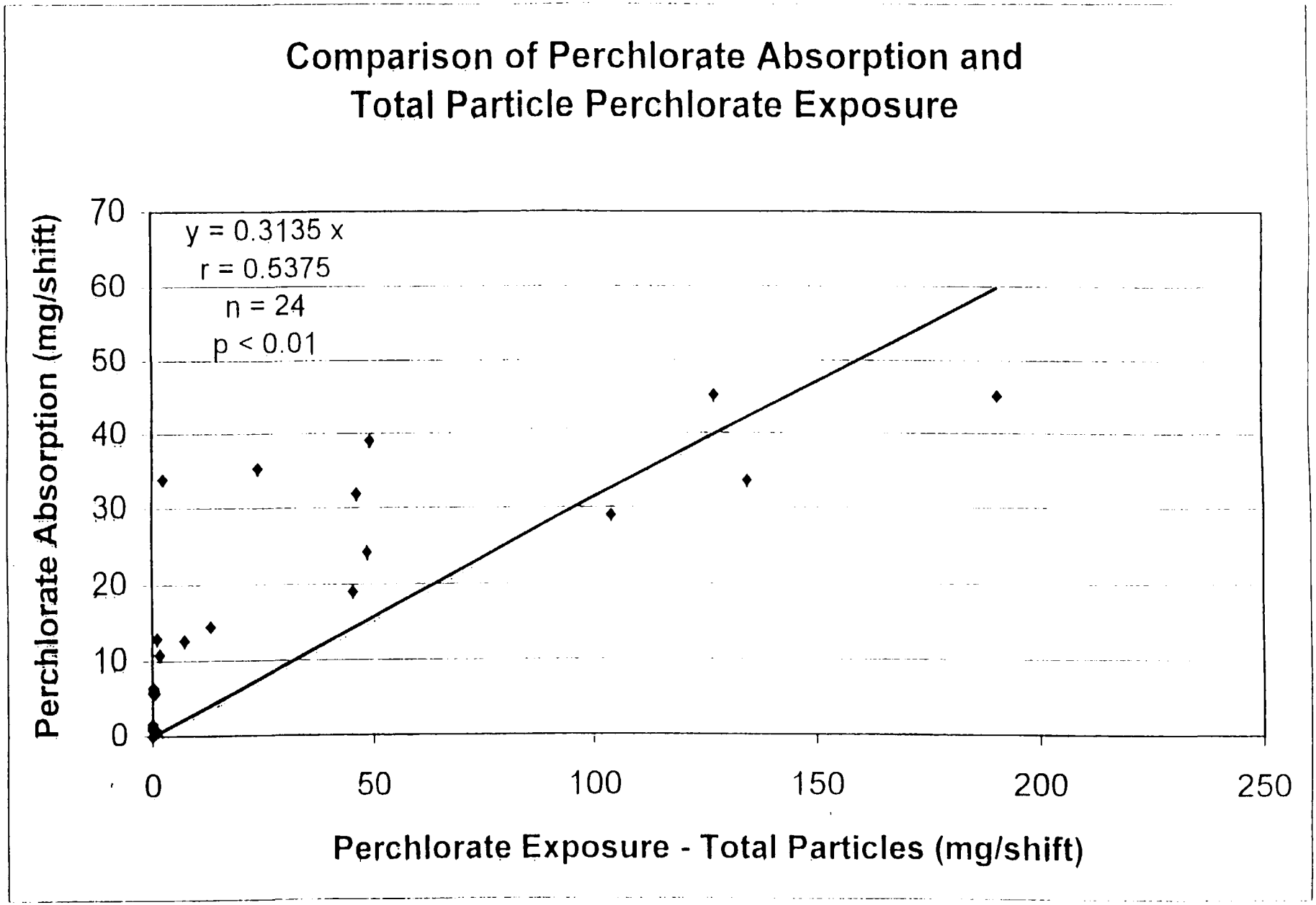
Comparison of Perchlorate Absorption and
Total Particle Perchlorate Exposure
y = 0.3135 x
r = 0.5375
n = 24
p < 0.01
Perchlorate Absorption (mg/shift)
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Perchlorate Exposure - Total Particles (mg/shift)

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.

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January 29, 1999

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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

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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.

References

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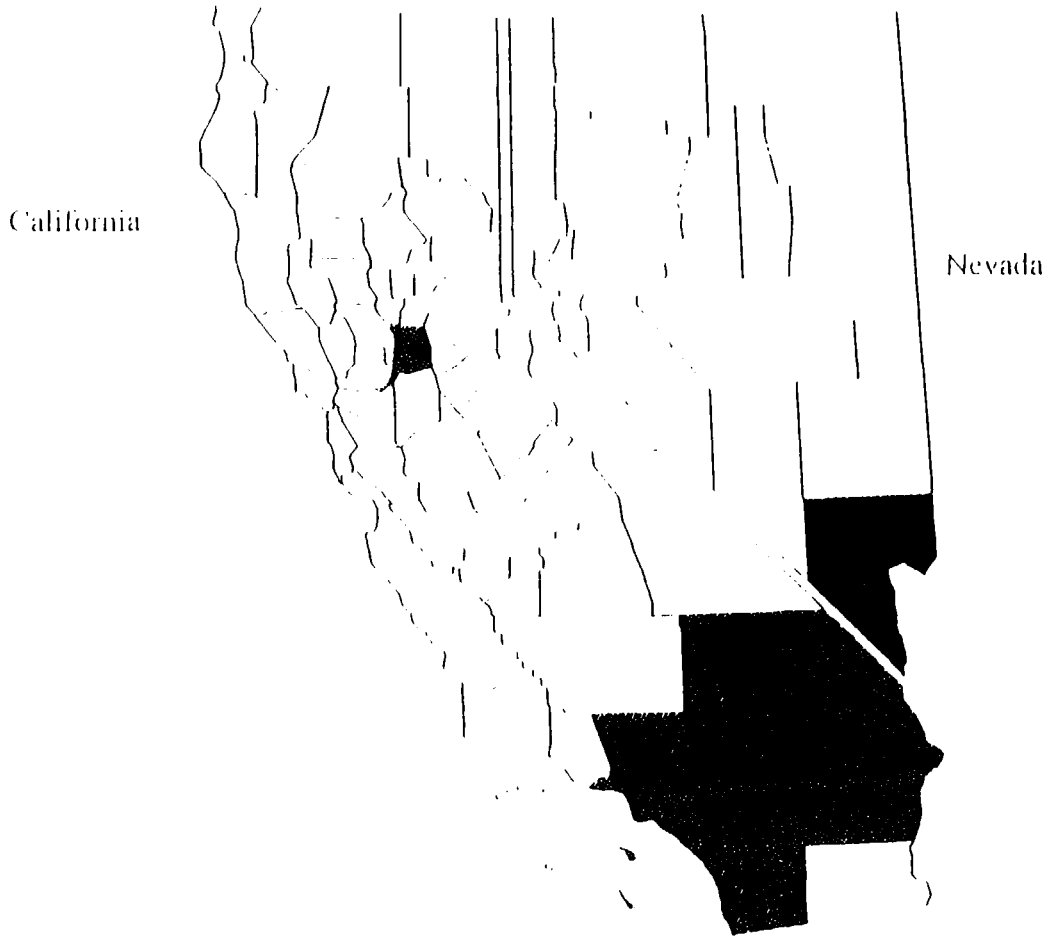
Table 1

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 adverse 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
Research Triangle Institute
P.O. Box 12194
Research Triangle Park, NC 27709-2914

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

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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]

[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)	0.036	One work shift (8 or 12 hours)	Greater cross-shift TSH changes in 12-hour shift workers than 8-hour shift workers. No change in other thyroid functions	Cross-sectional study
	Lifetime study (n=192 in control group, n=174 in exposed group)	Low cum. dose avg.=3.5 mg/kg, high cum. dose avg.=38 mg/kg	Avg. exposure =8.3 years	No significant effect on thyroid, bone marrow, liver, or kidney function	
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

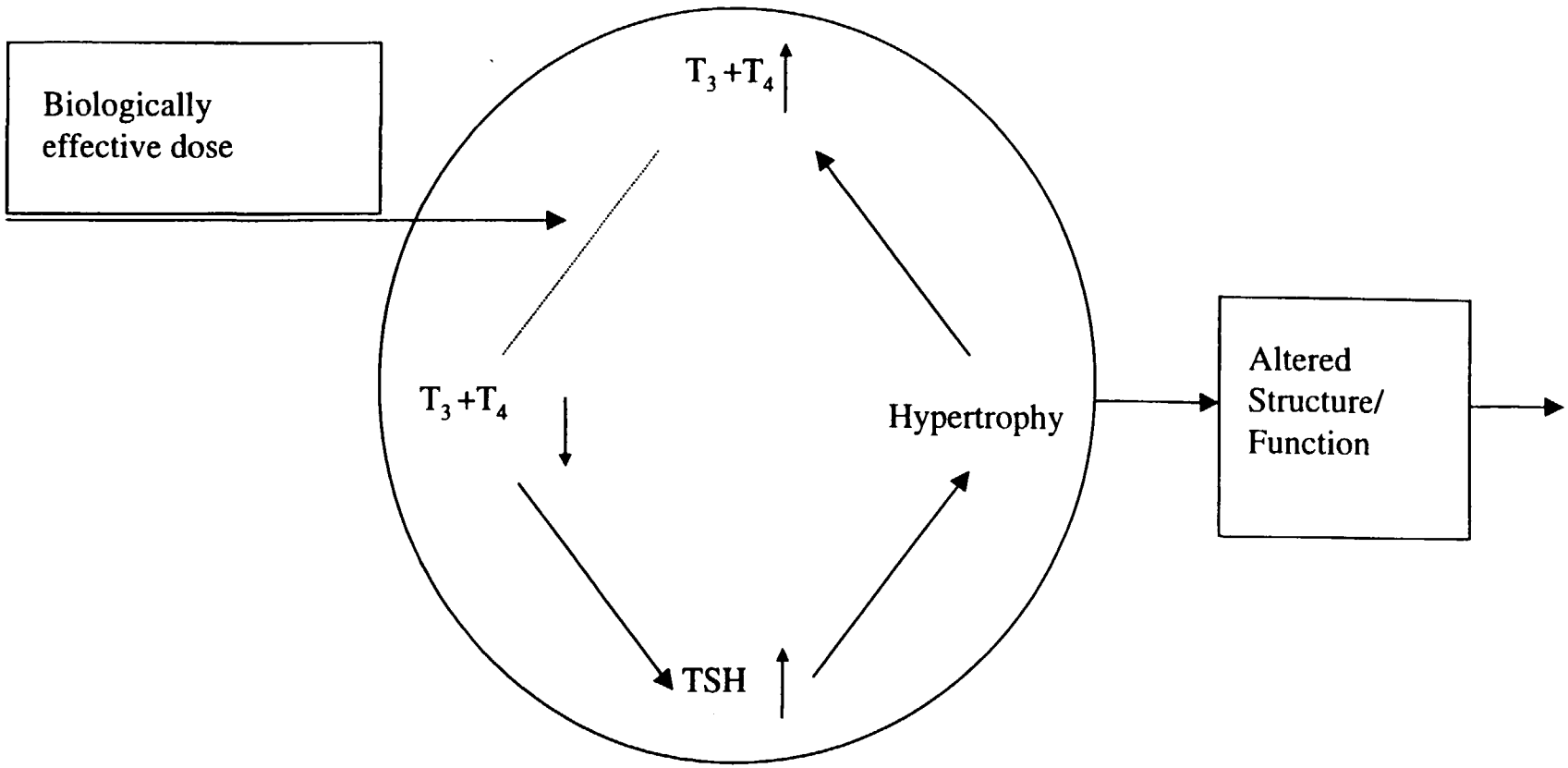
Estimated value(s) based on:

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 B. .ogical Homeostasis



Gay Goodman
Intertox

INTERTOX

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

Gay Goodman, Ph.D., D.A.B.T.

February 1, 1999

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."

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 2. EPA's hypothesis is that positive correlations for T3 *vs.* T4 and negative correlations for T3 (or T4)

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 1 mg/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.

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 differences are unlikely to be of consequence. With respect to T4 levels, the available data do not support the hypothesis that inter-strain differences in variability 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 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.

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 (Figure 2). 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

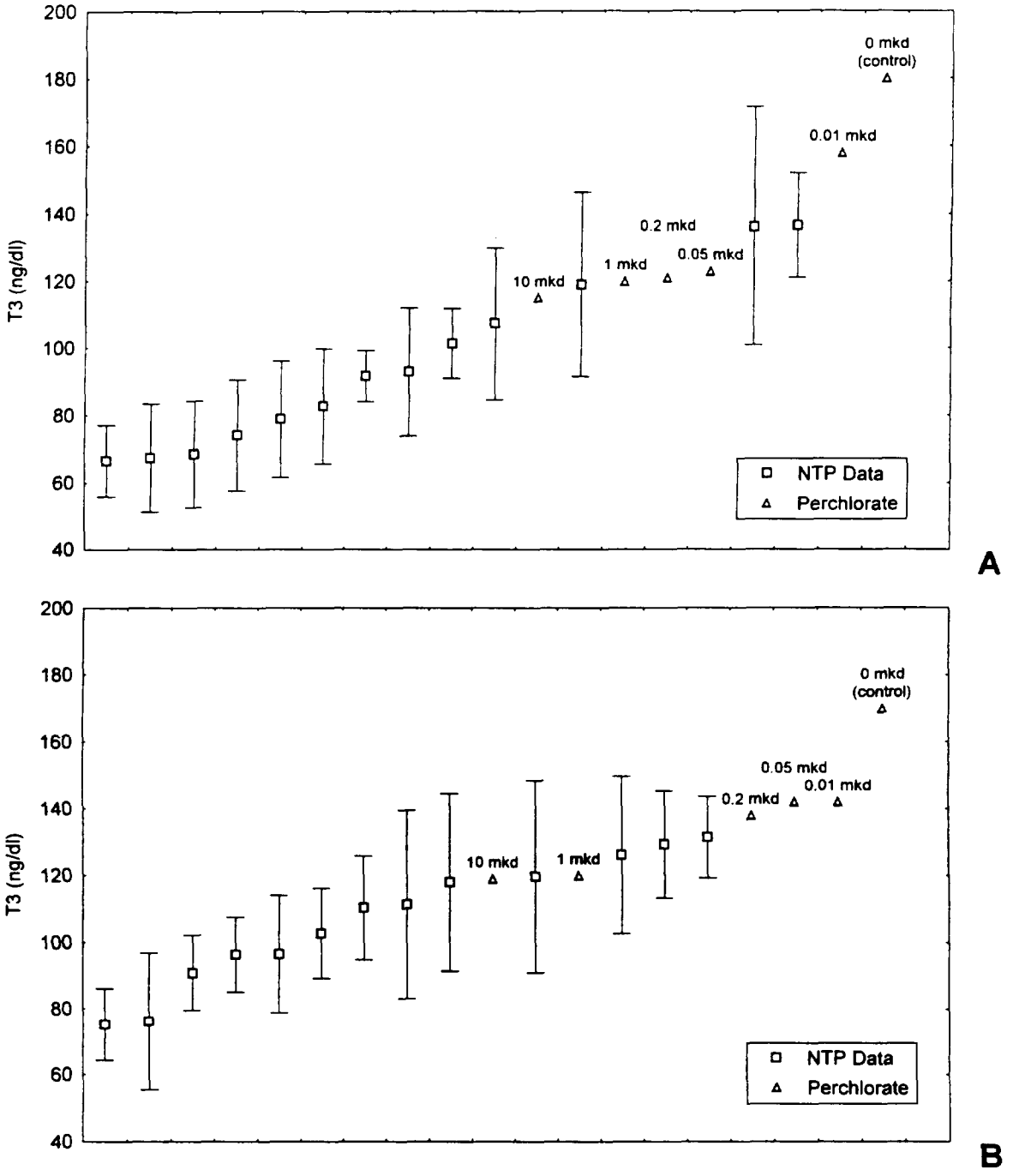

Figure 1. Serum total T3 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values 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.

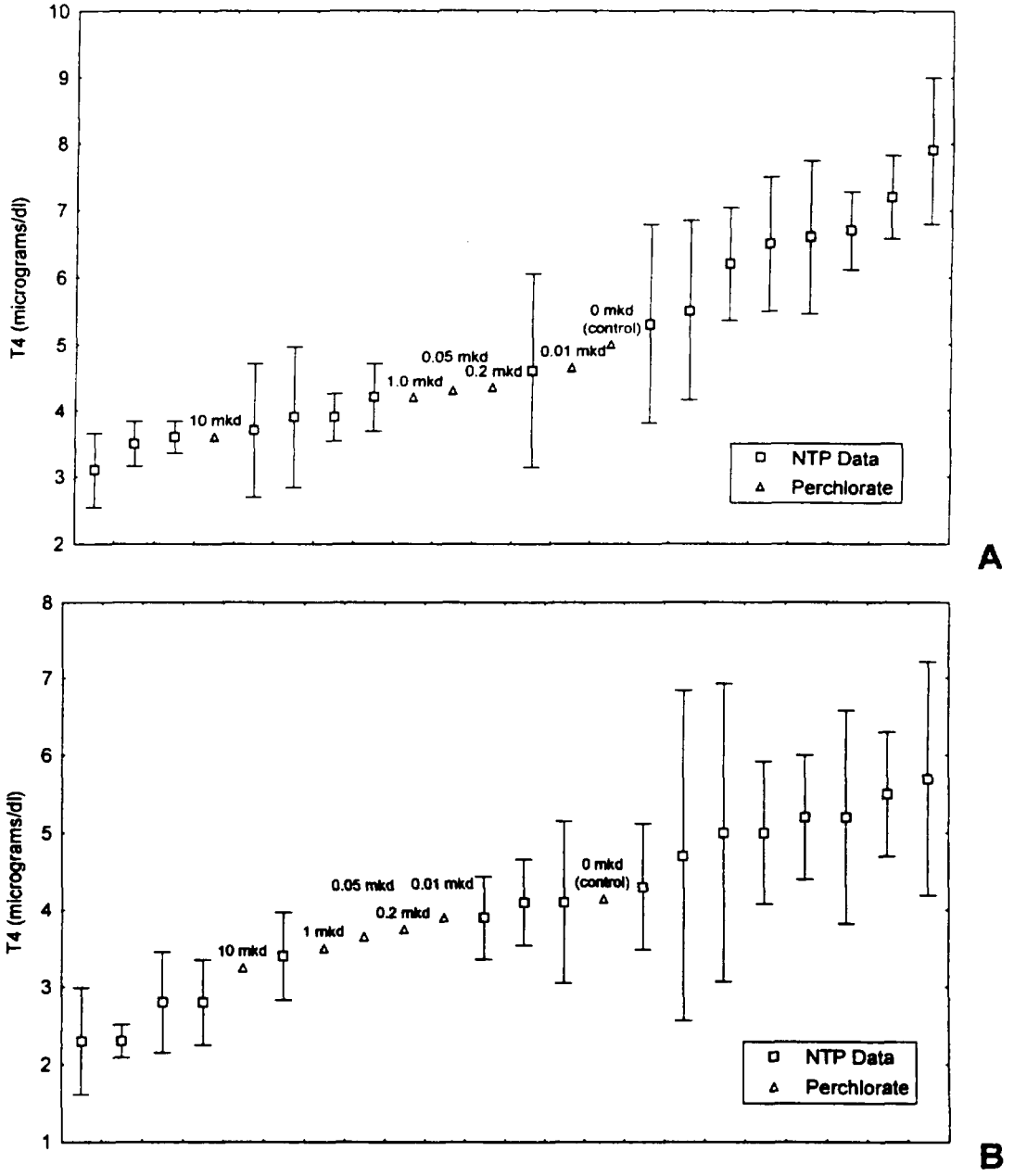

Figure 2. Serum total T4 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard deviations 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, DVM, PhD
Professor and Chairman

Appendix G

List of Outside Observers Giving Presentations

PRESENTATIONS FROM OBSERVERS

Michael Dourson
Toxicology Excellence for Risk
Assessment (TERA)
Cincinnati, Ohio

Larry Ladd
Community Representative
Aerojet Health Assessment Site Team
Rancho Cordova, California

John Gibbs
Kerr-McGee Corporation
Oklahoma City, Oklahoma

Steve Lamm
Consultants in Epidemiology and
Occupational Health, Inc.
Washington, D.C.

Kenneth Crump
K.S. Crump Group, Inc.
Ruston, Louisiana

Linda Ferguson
American Pacific Corporation
Las Vegas, Nevada

Barbara Beck
Gradient Corporation
Cambridge, Massachusetts

David Mattie
AFRL/HEST
Wright Patterson Air Force Base, Ohio

Gay Goodman
Human Health Risk Resources, Inc.
Seattle, Washington

Michael McClain
Jellinek, Schwartz and Connolly, Inc.
Arlington, Virginia

Richard Pleus
Intertox
Seattle, Washington

Dan Rogers
Wright-Patterson Air Force Base, Ohio

Deborah Keil
Medical University of South Carolina

Appendix H

Copy of Material Presented at the Meeting by Outside Observers

Larry Ladd
Aerojet Health Assessment Site Team

Larry Ladd

From: Dallas, John S. <jdallas@utmb.edu>
To: 'Larry Ladd' <lilladd@sprintmail.com>
Subject: RE: Congenital Hypothyroidism and Perchlorate Water Contamination
Date: Monday, February 08, 1999 7:46 AM

Dear Mr. Ladd,

It is true that the incidence of congenital hypothyroidism is higher in Hispanics than in Afro-Americans. In Texas, Hispanics tend to have an incidence around 1/3000 and Blacks around 1/15000-16000 (about a 5-6 fold difference). While it is clear that perchlorate can interfere with thyroid function, I know of no evidence that suggests that perchlorate alone can inhibit thyroid gland formation. Do the children you discuss in your letter have thyroid glands present in the appropriate location in the neck? I might expect them to have goiters, but they should all have thyroid glands that can be detected. Has there been an increase in acquired hypothyroidism or goiter detection in this area you reference? I'm not sure what else is in rocket fuel, but I would imagine there may be several other toxins, and whether or not any of these can, in association with perchlorate, cause permanent hypothyroidism may be unknown.

I have discussed your question with Dr. Lester Van Middlesworth (Memphis, TN). He is a world's expert on environmental toxins and thyroid function in animals. He has shown in rats that in utero exposure of perchlorate can cause fetal hypothyroidism with subsequent brain damage. However, the thyroid glands developed normally in the animals; the perchlorate blocked iodine uptake by the glands and led to hypothyroidism. Once the perchlorate exposure had been removed, the thyroid function reverted to normal in these animals, but the brain damage persisted.

I hope that this information is helpful to you. Good luck.

John Dallas

> ———
> From: Larry Ladd[SMTP:lilladd@sprintmail.com]
> Sent: Sunday, February 07, 1999 11:55 PM
> To: jdallas@UTMB.EDU
> Cc: Reserve Copy; Maryann Castaneda; Chris Anaya; Jane Williams
> Subject: Congenital Hypothyroidism and Perchlorate Water
> Contamination
>
> Dear Dr. Dallas,
>
> My name is Larry Ladd, and I'm a geographer serving as a community
> representative on the Aerojet Health Assessment Site Team (AHAST). AHAST
> is
> a committee of environmental regulators, Aerojet employees, and citizens
> who live next to the Aerojet Superfund Site. Our purpose is to
> investigate
> medical abnormalities that may be related to water contamination from the
> Aerojet sites in Rancho Cordova and Azusa, California. During the time
> period when Rancho Cordova's water supply became contaminated with 300 ppb

> perchlorate (1990-1996), our congenital hypothyroidism rate went up to
> 1/1300. A n of 4 cases of congenital hypothyroidism is of course not
> generalizable, but I did note that nationally there is a marked
> discrepancy
> between the Afro-American CH rate (1/30,000) and the Hispanic CH rate
> (1/2000). The reference I read suggested this difference was due to
> genetics, but in my initial search I have yet to find any evidence linking
> CH to a specific gene.
>
> I was wondering: Is there was a similar 15-fold distinction between Black
> and Hispanic CH rates in Texas? The reason I ask is that I am toying with
> the hypothesis that the high Hispanic CH rate is associated with
> perchlorate water contamination in the lower Colorado River, and the low
> Afro-American rate is partially due to the fact that soul food has no
> rocket fuel in it (e.g., preference for collared greens over Colorado
> River
> lettuce, watermelon over Colorado River cantaloupe, etc.). Any insights
> you have on this question that you can send my way would be deeply
> appreciated, especially if they come before the external peer review on
> perchlorate toxicology that will be held this Wednesday in San Bernardino,
> CA.
>
> Sincerely,
>
> Larry Ladd
>
> Community Representative
> Aerojet Health Assessment Site Team
> Rancho Cordova, California
>
For further information on perchlorate water contamination, see
http://www.zerowasteamerica.org/Perchlorate.htm

Trouble on Tap

What's In Sacramento's Drinking Water?

When Jake Parker developed a serious bone marrow infection last year, his mother, Lori, had no idea it might be linked to the water. The Parkers live next door to a Rancho Cordova well that was closed down in February due to the presence of 18 times as much perchlorate as the federal Environmental Protection Agency considers safe.

After he fell down in the driveway, Lori took 23-month-old Jake to the hospital for X-rays. Doctors didn't find anything, so they went home. But the problem did not go away.

"A week later, he was dragging his leg like a dead limb," Lori said. "He was pale. It was terrible."

Doctors finally found a bone marrow infection in Jake's shin. After many complications, and three months of antibiotics, he got better.

The Parkers say they have reason to suspect perchlorate caused their son's health problem. And they aren't the only ones. Last Friday, 100 Rancho Cordova residents filed suit against two local aerospace companies linked to the introduction of perchlorate into their groundwater.

In doses thousands of times higher than those found in Rancho Cordova's tainted wells, perchlorate can cause diseases of the thyroid, blood and bone marrow. If the substance turns out to be toxic in low doses over longer periods of time, it could be the most shocking environmental bombshell of the decade, said environmental activist Jane Williams.

Perchlorate is a chemical component of fireworks, rocket fuel and matches. The highly soluble chemical travels freely in groundwater, and no simple treatment method has been developed to remove it.

Since the closure of a number of Rancho Cordova wells last February, perchlorate has turned up all over the country. In Nevada, water flowing into Lake Mead has concentrations 100 times higher than the recommended safe levels. The lake was created by the damming of the Colorado River, which supplies drinking water to the Southwestern United States.

And perchlorate isn't the only toxin showing up in Sacramento-area water (see related stories). A plume of cancer-causing chemicals from the county dump is threatening domestic wells near Rancho Murieta. The gasoline additive MTBE has turned up in groundwater and wells wherever gas stations are located. Lead remains an ever-present threat to the safety of tap water. And the presence of nitrates has recently led to the closure of public drinking water wells in Davis.

Watch That Water

Chances are, the majority of Sacramentans are drinking safe water. But as residents like Larry Ladd have found out, sometimes it pays to start asking questions.

Ladd, a geographer who lives in Rancho Cordova, stumbled onto the perchlorate situation when investigating the boundary of the Folsom-Cordova school district split. He discovered that a toxic plume, the legacy of years of chemical dumping, was creeping southwestward through the groundwater from the nearby Aerojet General Corp. plant.

Under orders from the state, Aerojet has been filtering carcinogenic chemicals such as trichloroethylene (TCE) from the contaminated groundwater plume since the mid-1980s, and reinjecting the water into the aquifer just upstream from Rancho Cordova. But the treatment Aerojet uses to remove other chemicals doesn't remove perchlorate. As a result, the company continues to put water that is contaminated with up to 450 times the recommended safe concentration of perchlorate back into the ground at the rate of 8 million gallons per day.

Edie Cartwright, vice president of communications for Aerojet, said the company is doing what state officials have ordered it to do. "What do you do with 8 million gallons [of treated water] per day?" she said. "There's no immediate way to clean it up."

Although perchlorate is relatively new as a health concern, the chemical itself is certainly not new to Sacramento's groundwater. The chemical has been detected at 1,000 times the recommended safe concentration in Aerojet wells since the 1950s, and in public wells since at least the late 1970s. Aerojet's own labs found high levels of perchlorate in one of Rancho Cordova's public wells in 1993.

"The only people who really didn't know about it were the people who were drinking it," Ladd said.

State regulators overseeing the Aerojet cleanup were also in the dark because, as part of its state-mandated cleanup order, Aerojet was in charge of monitoring its own wells for perchlorate. It wasn't until about a year ago that regulators realized that Aerojet's testing method was sensitive only to a level of 400 parts per billion (ppb), far in excess of EPA's recommended safe dose of no more than 18 ppb.

Within a matter of weeks, the state Department of Health Services laboratories developed a much more sensitive test that detected perchlorate in public wells all over Rancho Cordova.

No one knows if chronic, long-term exposure to perchlorate is hazardous because that type of exposure has not been studied.

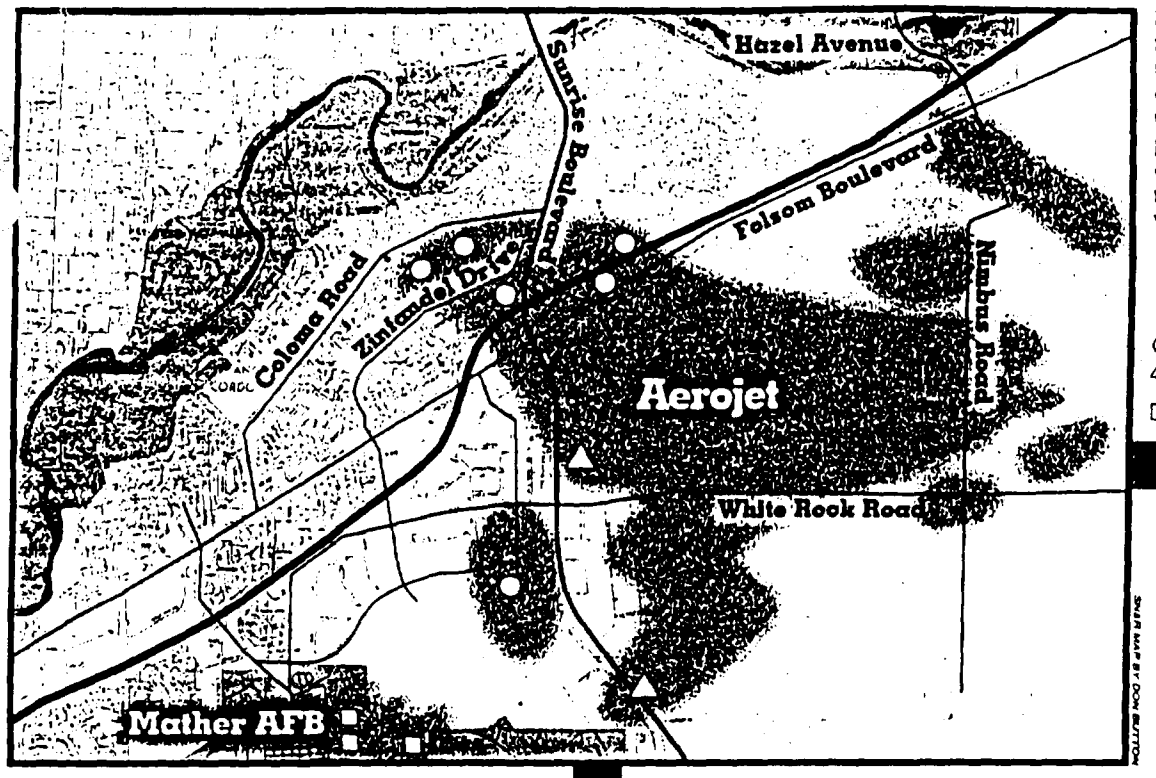
In high doses, perchlorate can cause some pretty nasty health problems. At 1,000 times the recommended safe dosage for drinking

Lori Parker believes the tap water in her neighborhood is the cause of her son Jake's medical problems.

BY AMY PARIS

PHOTOS BY LARRY DALTON

IS THIS THE DARK SIDE OF MEASURE O?



Shaded areas indicate places regional water officials consider to be affected by perchlorate. The contamination is moving southwestward, officials say.

○ Arden Cordova Wells
△ County Sunrise Service Area Wells
□ Mather Wells

No one knows if chronic, long-term exposure to perchlorate is hazardous because that type of exposure has not been studied.

water, it can cause fatal blood and bone marrow disorders. Because of its action as an endocrine disrupter, perchlorate might be linked to breast cancer or leukemia, according to Health Department toxicologist Marilyn Underwood. It can also interfere with development of the nervous system.

The chemical was used in the past as a treatment for Graves' disease, a thyroid disorder. Perchlorate inhibits the secretion of thyroid hormones, effectively slowing down the metabolism. People treated with perchlorate have reported stomach aches and skin rashes.

Almost nothing is known about the effects of perchlorate on children or developing fetuses. In general, children are more sensitive to chemical contaminants than adults.

Jake Parker isn't the only member of his family to have suffered unexplained health problems after drinking water from the tap. His 6-year-old sister has had many health problems, including anemia. And Lori Parker suffered two miscarriages in 1992. That same year, three other women on her street had miscarriages, she said.

Attorney Ed Masry filed suit Dec. 5 in Sacramento County Superior Court against Aerojet and McDonnell-Douglas, an aerospace company recently purchased by Boeing, as well as several local water utilities, on behalf of about 100 Rancho Cordova area residents complaining of health problems allegedly caused by the contaminated water. About 25 of the plaintiffs complain of thyroid conditions, while the remaining 75 have suffered various other afflictions, including cancer. The Parkers told SN&R they had not heard of the suit, but intended to contact the attorney.

Preliminary studies of hospital records that looked for unusual numbers of blood disorders, breast cancer, leukemia and babies born with severe thyroid problems among people living in areas affected by perchlorate have all turned up negative, according to Underwood. But the health department is a long way from isolating exactly who was exposed to the chemical, and even farther from determining what health effects were caused by that exposure, she said.

Perchlorate Politics

In the quest to establish a drinking water standard for perchlorate, the financial stakes are high.

The EPA decided in 1992 that it

(continued on next page)

North Sac: Got Lead?

Many Sacramentans may unknowingly be consuming hazardous levels of lead in their drinking water. In a random sampling of tap water, city of Sacramento water authorities found that many residents were drinking hazardous levels of lead, as much as nine times what federal authorities consider safe.

Two rounds of tap water testing in 1992 detected lead in 86 and 89 percent of households in the northern part of the city of Sacramento that get their water from a public well system. No follow-up testing has been done since then.

On the whole, Sacramento water samples were within EPA guidelines. But since lead contamination depends in some measure on the condition and chemical composition of each household's plumbing, residents should not assume their water is safe.

Lead is the leading environmental hazard for children, according to a recently released report from the National Resources Defense Council. Drinking water makes up about 20 percent of an average adult's exposure to lead and up to 85 percent for some bottle-fed infants.

People who live in houses built between 1982 and 1986 are at an increased risk for lead exposure because during those years, most copper pipes were soldered with lead. Brass faucets, which can legally contain up to 8 percent lead by weight, are leading contributors of lead to tap water.

Adding fluoride to water doubles its lead content by making the water more corrosive, according to EPA toxicologist Bill Marcus. Only a small number of Sacramentans in the Rosemont area have fluoridated water, but recent legislation has made countywide fluoridation an imminent possibility.

Lead makes you stupid, and that might be the nicest thing that it does. In addition to impairing mental and physical development, it can increase blood pressure and damage hearing, and at very high levels can cause anemia, kidney damage and mental retardation. Consumption of lead by pregnant women directly exposes the developing fetus, and can result in low birth weight and premature birth.

Running water for up to three minutes or until it becomes cold, especially the first time you use it in the morning, reduces lead levels by clearing stale water out from the pipes. Also, using cold water for cooking reduces lead exposure, since lead is more soluble in hot water. Boiling water does not remove lead.

The only way to find out for sure if lead is in your tap water is to have it tested by a state or EPA certified lab. Tests cost from $15 to $35, and the EPA Drinking Water Hotline (1-800-426-4791) can help you locate a certified lab in your area.

—A.P.

(continued from previous page)

did not have enough information about perchlorate to establish an enforceable drinking water standard, so instead it established a recommended safe dose of 4 parts per billion (ppb). By this time, Aerojet had already found almost 10,000 times this concentration in its own monitoring wells.

Aerojet and a group of businesses that manufacture and use perchlorate, fearing responsibility for an enormous and costly cleanup, formed the benignly named Perchlorate Study Group to pressure the EPA to relax its reference dose.

In 1995, the group got the EPA to expand the standard to 4-18 ppb, but considering they were shooting for much higher levels (at least 400 ppb), it was not the coup they had hoped for.

The EPA refused to reconsider the reference dose without conducting more studies. The Perchlorate Study Group and the Air Force—two groups that stand to bear the financial responsibility of cleanup—volunteered to fund studies to fill in the information gaps. The studies, which are already under way, could have a substantial effect on the determination of an EPA drinking water standard.

"The Air Force is funding a series of toxicity tests to convince the world that perchlorate is good for you," said Jane Williams, director of the California Communities Against Toxics. "There's an incestuous relationship there: They've been funding their own tests, hiring their own toxicologists, putting their own scientists on peer review panels. There is no public advocacy group watching the level changes. It's being done in the dark."

Taking scientific research as a hostage in the battle over cleanup costs is not unique to the perchlorate situation.

Larry Ladd and his daughter, Melody.

For example, last April, when federal government researchers invited Sacramento to participate in one of the most comprehensive groundwater studies ever undertaken, the city initially refused to participate. The reason: Officials wanted a guarantee they wouldn't be held liable for cleanup costs, should new contamination be found. City government changed its mind only when local media coverage incited a moderate amount of public outrage.

Since state regulators became aware of perchlorate in Sacramento area wells last February, a total of eight wells have been taken out of service, and three more were initially closed and have since reopened.

Hardest hit was Arden-Cordova Water Service, which shut down three wells showing levels from 140 to 320 ppb. Three more wells that are within the safe dosage range were initially taken offline and returned to service when the summer peak water demand came. Another well with levels within the safe range was put on standby in November.

Two wells in the county of Sacramento's Sunrise Boulevard service area are contaminated with 92 and 280 ppb, respectively. The wells are on standby, which means they kick in only in periods of high demand. One well pumped nearly 2 million gallons in May alone. The other pumped more than 120,000 gallons in August. Customers were notified of possible exposure.

In addition, two wells on Mather Air Force Base have been closed down, and a third is near the safety limit.

Anyone using private wells in the area of the plume (see map, previous page) should contact local health authorities, said Dave Lancaster of the Department of Health Services.

Water from the city of Sacramento, Fair Oaks and Folsom South Canal has not been contaminated.

A neighborhood served by Citizens Utilities is directly in the path of the plume, and the company has turned off one well because it detected trace amounts of perchlorate. The company has already been hit by a trichloroethylene-containing plume from Mather Air Force Base.

"The plume is moving our way, and we're watching it closely," said Robert Roscoe, managing engineer for Citizens Utilities. "It's like being tied to a railroad track and seeing a single light off in the distance. We'd not be doing a good job planning our water supply if we weren't planning for it."

Who Pays?

So far, Aerojet has paid an estimated $3 million-$5 million for numerous interconnections between water systems and additional storage reservoirs to reduce the level of perchlorate in local water.

Some officials say Aerojet considers it the Air Force's responsibility to pay the company back, at least in part, since the Air Force played a large role in perchlorate contamination. Edie Cartwright, vice president of communications for Aerojet, would only say that the company is in negotiations with the Air Force.

In addition, if the EPA's recommended dose is raised, local water purveyors might have to compensate Aerojet for work done on their systems.

"They picked up the cost for these projects, and if the safe level is actually above what's in the wells, they're probably going to negotiate for reimbursement," said John Coppola of the Sacramento County Water Resources Division.

But the real costs are not going to come from piecemeal system improvements. A treatment plant that will extract and treat water at the contamination site is being developed at an estimated cost of $210 million. It is scheduled to begin operation next April.

Until that time, under state orders, Aerojet will continue to reinject water containing perchlorate into the aquifer. Although the planned treatment is intended to stop the reintroduction of contaminated water, it will do nothing for the fringes of the plume, which have already spread over Rancho Cordova and are heading southwest. If EPA establishes a drinking water standard that is as low as the current recommended dose, water purveyors will have to find a way to treat individual wells. That technology is years away, Regional Water Quality Control Board officials say. Meanwhile the spread of perchlorate could threaten other wells and further tax the already burdened drinking water supply.

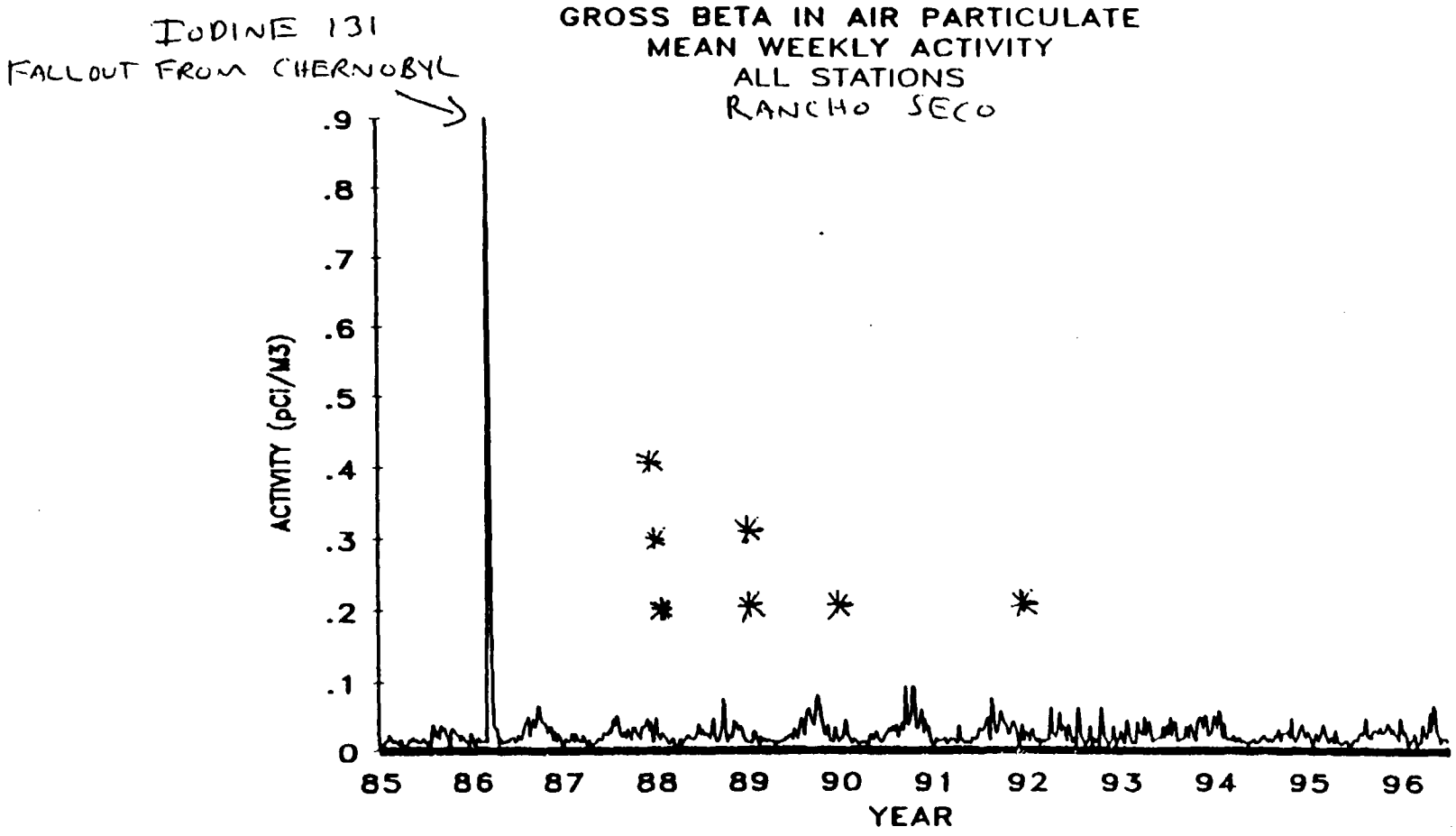
The Parkers, who have lived in Rancho Cordova for 11 years, aren't sticking around to see how it all turns out.

"We're putting our house on the market," Lori said. "We don't want to live here any more. It's scary being near Aerojet. Buffalo Creek—who knows what they might have washed through there? There have been a lot of unexplained health problems." □

CHERNOBYL - PERCHLORATE RESONANCE ?:

THYROID CANCER CASES IN RANCHO CORDOVA CENSUS TRACT WITH DRINKING WATER CONTAMINATED WITH PERCHLORATE AND NITROSODIMETHYLAMINE. EXPECTED FREQUENCY OF THYROID CANCER IN THE TRACT IS ONCE EVERY SEVEN YEARS.

FIGURE 5
GROSS BETA IN AIR PARTICULATE
MEAN WEEKLY ACTIVITY
ALL STATIONS
RANCHO SECO



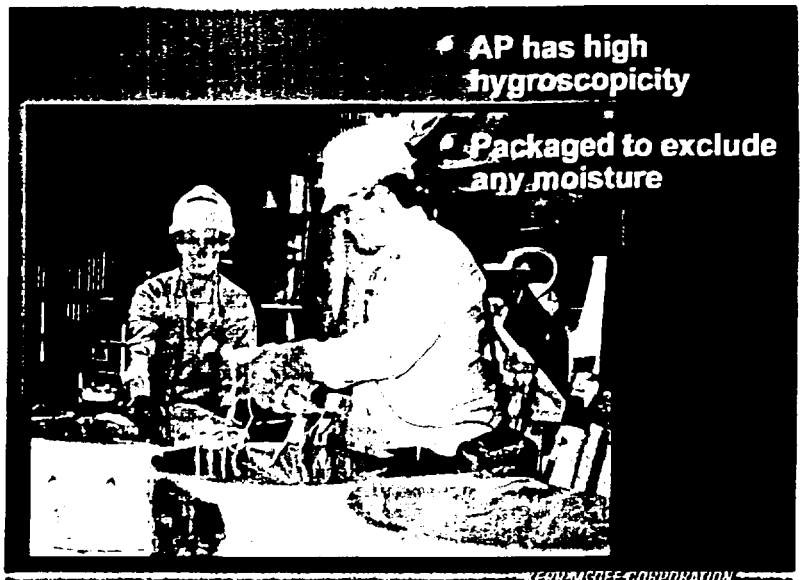
* = one thyroid cancer case

John Gibbs
Kerr-McGee Corporation

When Available, Human Health Data Should Be Used To Assess Human Health Risks

Perchlorate Production & Natural Occurence

- Both production facilities located in the desert
- Low humididy (<50%) necessary for handling dry product



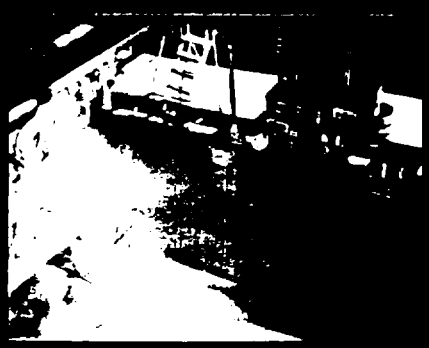
KERR-MCGEE CORPORATION

Workers at both of these production facilities have recently been studied and reported

- *Gibbs et al* - 1998 (Kerr-McGee - Nevada)
- *Lamm et al* - 1999 (WECCO - Utah)

KERR-MCGEE CORPORATION

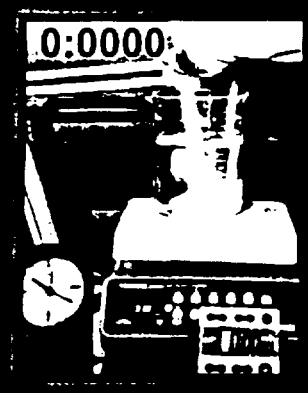
Perchlorate Exposure in the Workplace



- Primarily through the inhalation of dust
- All exposures well below OSHA PEL's

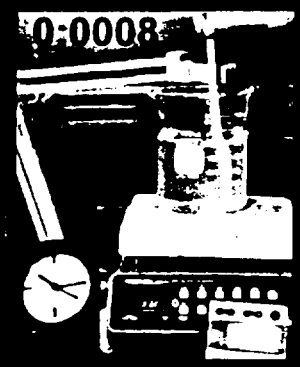
KERR-MCGEE CORPORATION

EPA questioned the validity of solubility assumptions in Gibbs et al



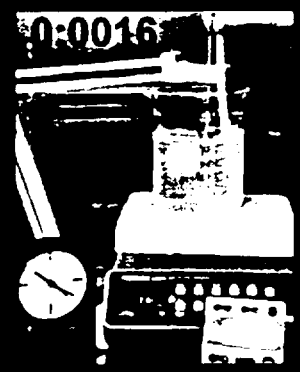
KERR-MCGEE CORPORATION

20 grams of ammonium perchlorate (large crystals)



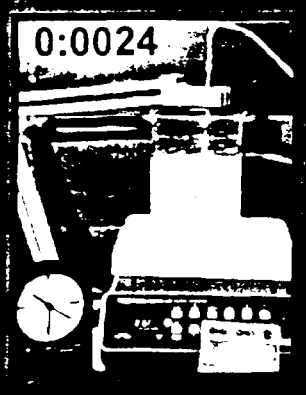
KERR-MCGEE CORPORATION

Mixed in 500 ml water



KERR-MCGEE CORPORATION

at 37°C

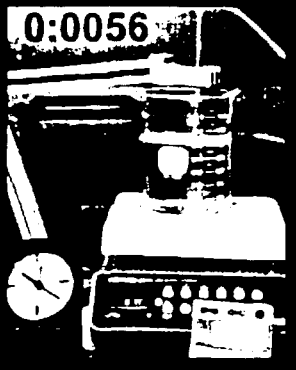


KERR-MCGEE CORPORATION

Completely dissolved in less than one minute

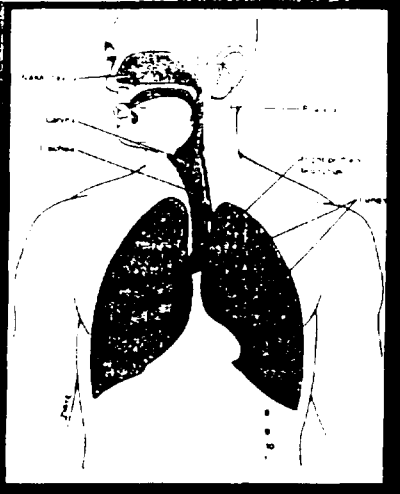
KERR-MCGEE CORPORATION

- Ammonium perchlorate is rapidly soluble in water
- Solubility is about the same as table salt at body temperature



KERR-MCGEE CORPORATION

EPA expressed concerns about particle size



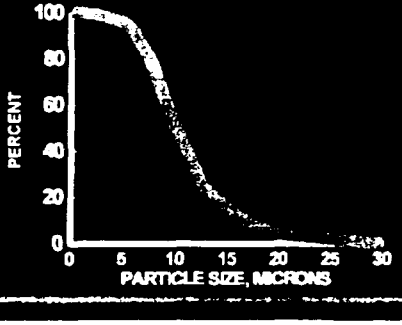
KERR-McGEE CORPORATION

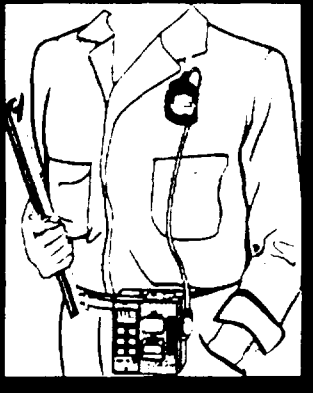
- Finished product is >100 micron particles
- Dust in the workplace is much smaller
- Most dust generated in the screening and cross-blending processes
- Fines removed from product (5% sent for recrystalli-zation)

KERR-McGEE CORPORATION

Exposure Assessment

- NIOSH method 0500
- Closely approximates thoracic dust





KERR-McGEE CORPORATION

Gibbs et al

- Assessed exposures to thoracic dust
- Estimated dose from exposure

Lamm et al

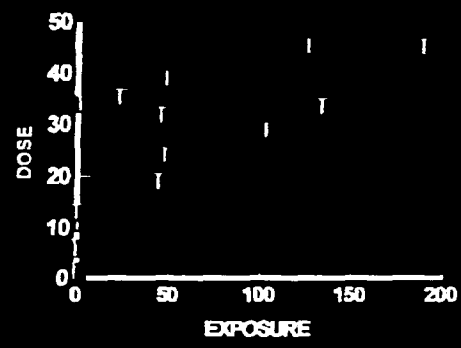
- Exposure assessment - both thoracic and respirable dust
- Particle size characterization (MMAD 4-12 microns)

Lamm et al

- Urinary perchlorate excretion rates
- Absorbed doses calculated

Lamm et al

- Strong exposure / dose relationship
- Validates exposure assumptions in Gibbs et al



Lamm et al & Gibbs et al

86 workers averaging 7 years exposure

- No thyroid effects
- No hematological effects
- No renal or hepatic effects

BMDL 0.6 - 0.8 mg/kg-day based on TSH & FTI

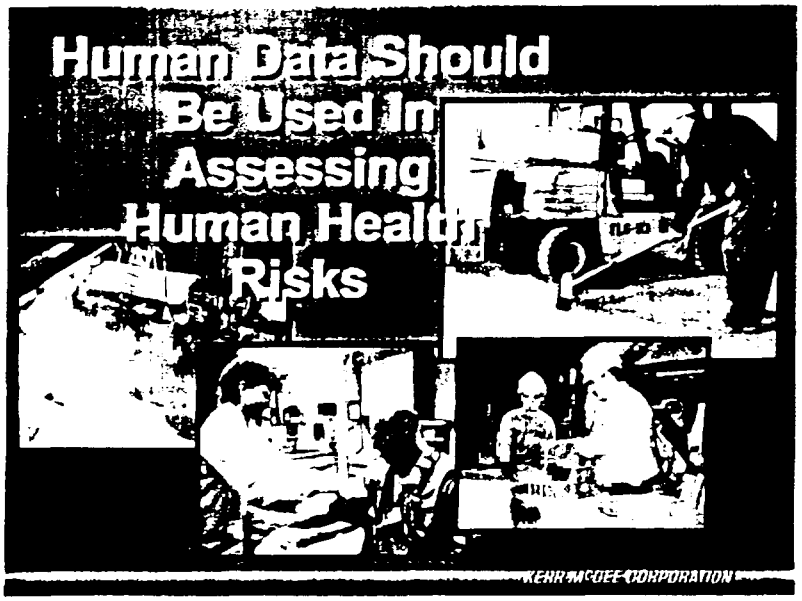
EPA Did Not Use These Studies In Developing RfD

Lam. et al

- Not yet accepted for journal publication
  - Accepted by JOE January 1999

Gibbs et al

- Concerns about dose estimates based on exposure
  - EPA should reconsider particle size, exposure measurements and solubility
  - Lam. et al confirmed exposure/dose relationship


Human Data Should
Be Used In
Assessing
Human Health
Risks
KERR-McGEE CORPORATION

Steve Lamm
Consultants in Epidemiology and Occupational Health, Inc.

Human Health Effects of Perchlorate

Reports prepared by Consultants in Epidemiology and Occupational Health, Inc.

Medical Literature Review
Occupational Study
Congenital Hypothyroidism Study

Sponsored by American Pacific Corporation

February 10, 1999
San Bernadino, California

Perchlorate Health Effects

- After nearly 50 years of clinical experience with perchlorate we have:
  - Clear understanding of effect level and limits of toxicity
    - Treatment levels at 200 (+) mg/day
    - Hematotoxicity at > 450 mg/day, usually 1,000 (+) mg/day
  - Its mechanism of action is well understood
    - Blockage of iodine uptake at the basolateral membrane, specifically, the Na-I symporter

Perchlorate Health Effects

- No change in thyroid hormone or TSH levels seen up to exposures of 70mg/day absorbed
- Grouped data yields NOAEL at 34mg/day
- Individual data yields BMDL of 44-58 mg/day (about 51mg/day)

Perchlorate Occupational Health Study (Lamm et al., 1999)

Study Population:

58 employees at WECCO, Cedar City, UT (July 15-17, 1998)

37 Perchlorate employees (35 males; 2 females; mean age 30; 40% employed > five years)

High	15 employees
Medium	8 employees
Low	14 employees

21 Azide employees (19 males; 2 females; mean age 35; 50% employed > 5 years)

Study Design:

Each worker studied across one of six twelve-hour shifts covering three consecutive days.

Added 2/22/99

Perchlorate Occupational Health Study Measures

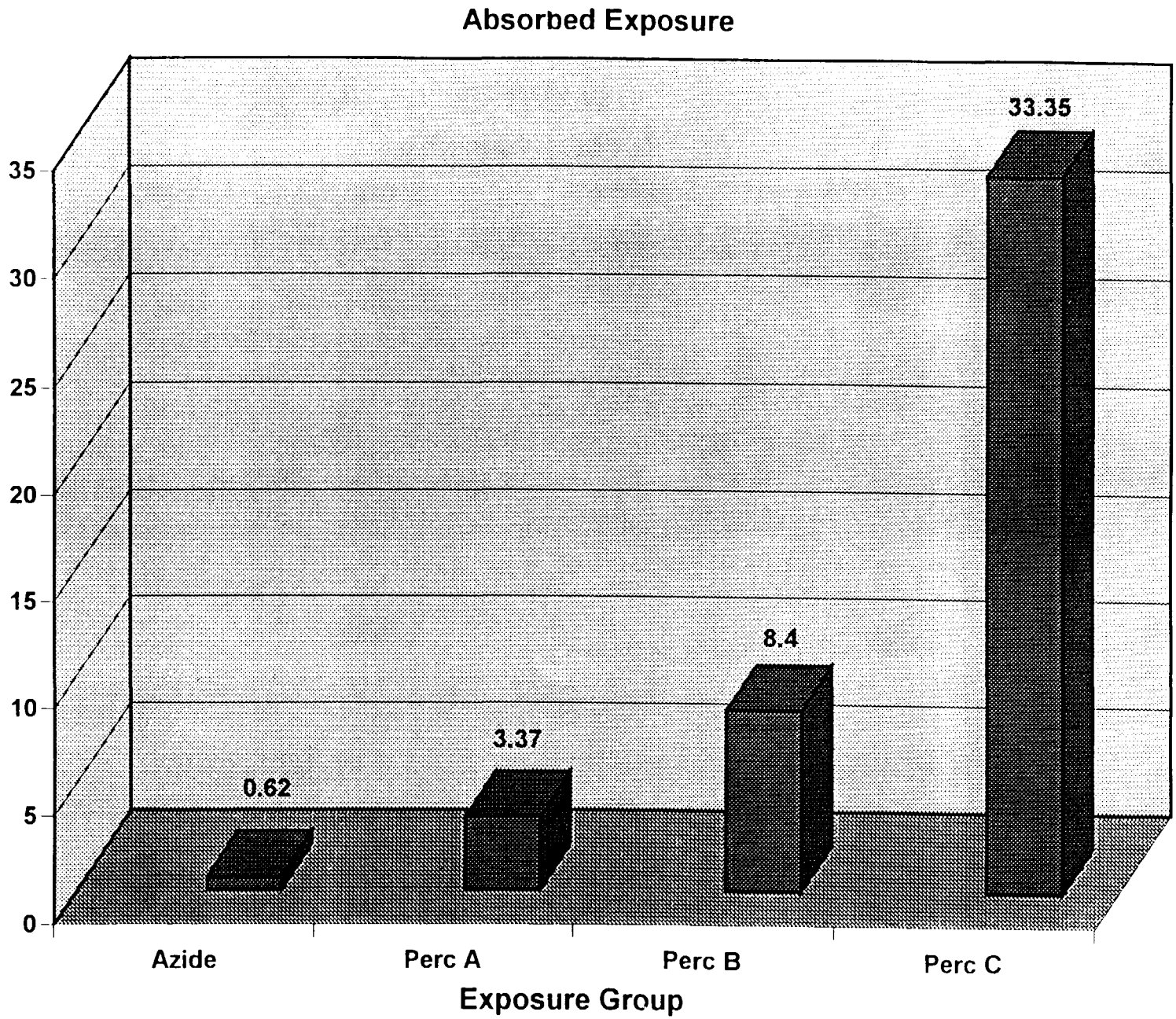
Exposure Measures:

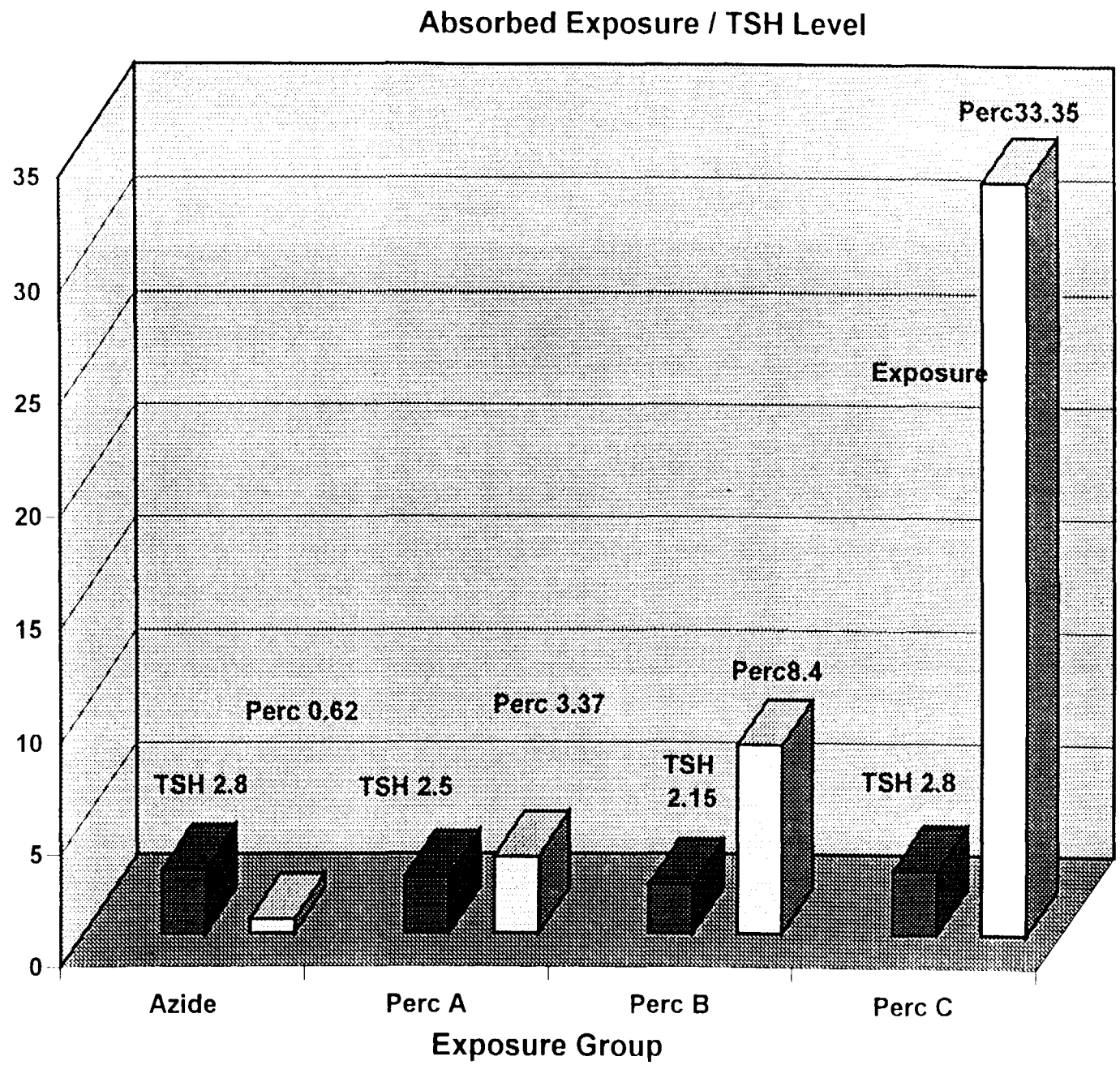
1. Pre-shift urine perchlorate (n=58) [mg/gm creatinine]
2. Cross-shift breathing zone respiratory (n= 38) and/or total (n=24) perchlorate particulate exposure [mg/day]
3. Particle size distribution [MMAD = 7.4 um]
4. Post-shift urine perchlorate (n=58) [mg/gm creatinine]
5. Absorbed perchlorate [mg/day]

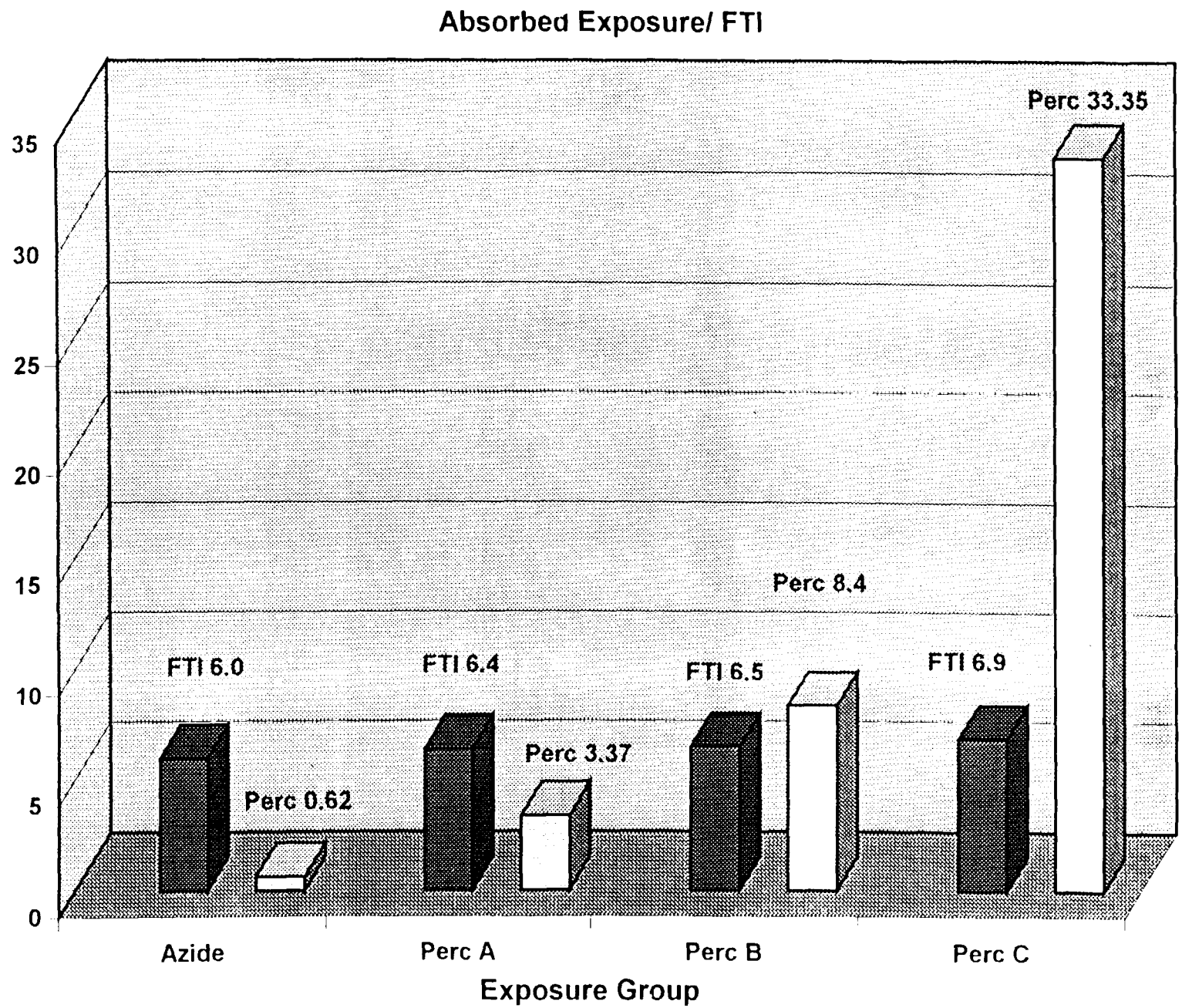
Outcome Measures:

1. Post-shift thyroid function tests (n=57)
   TSH, T_4, T_3, FTI, THBR, and Anti-TPO
2. Post-shift complete blood cell count (n=57)
   RBC, WBC, Platelets, Neutrophils, Lymphocytes, etc.
3. Post-shift blood chemistries (n=57)
   Liver function tests
   Renal function tests
   Metabolic status tests
4. Clinical thyroid examination (n=58)

Added 2/22/99


Absorbed Exposure
35
30
25
20
15
10
5
0
0.62
3.37
8.4
33.35
Azide
Perc A
Perc B
Perc C
Exposure Group

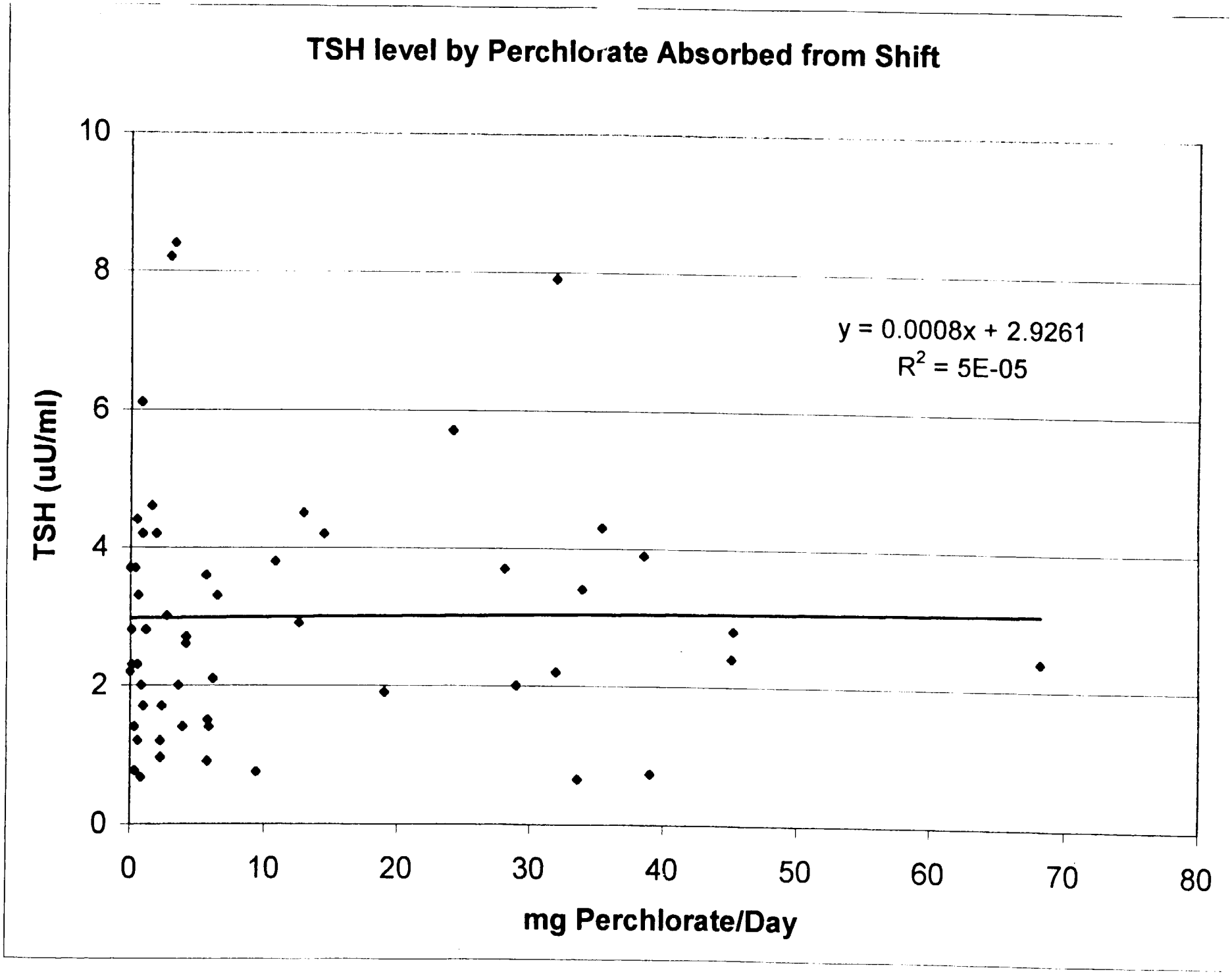

Absorbed Exposure / TSH Level
35
30
25
20
15
10
5
0
Perc33.35
Exposure
Perc8.4
Perc 0.62
Perc 3.37
TSH 2.8
TSH 2.5
TSH
2.15
TSH 2.8
Azide
Perc A
Perc B
Perc C
Exposure Group

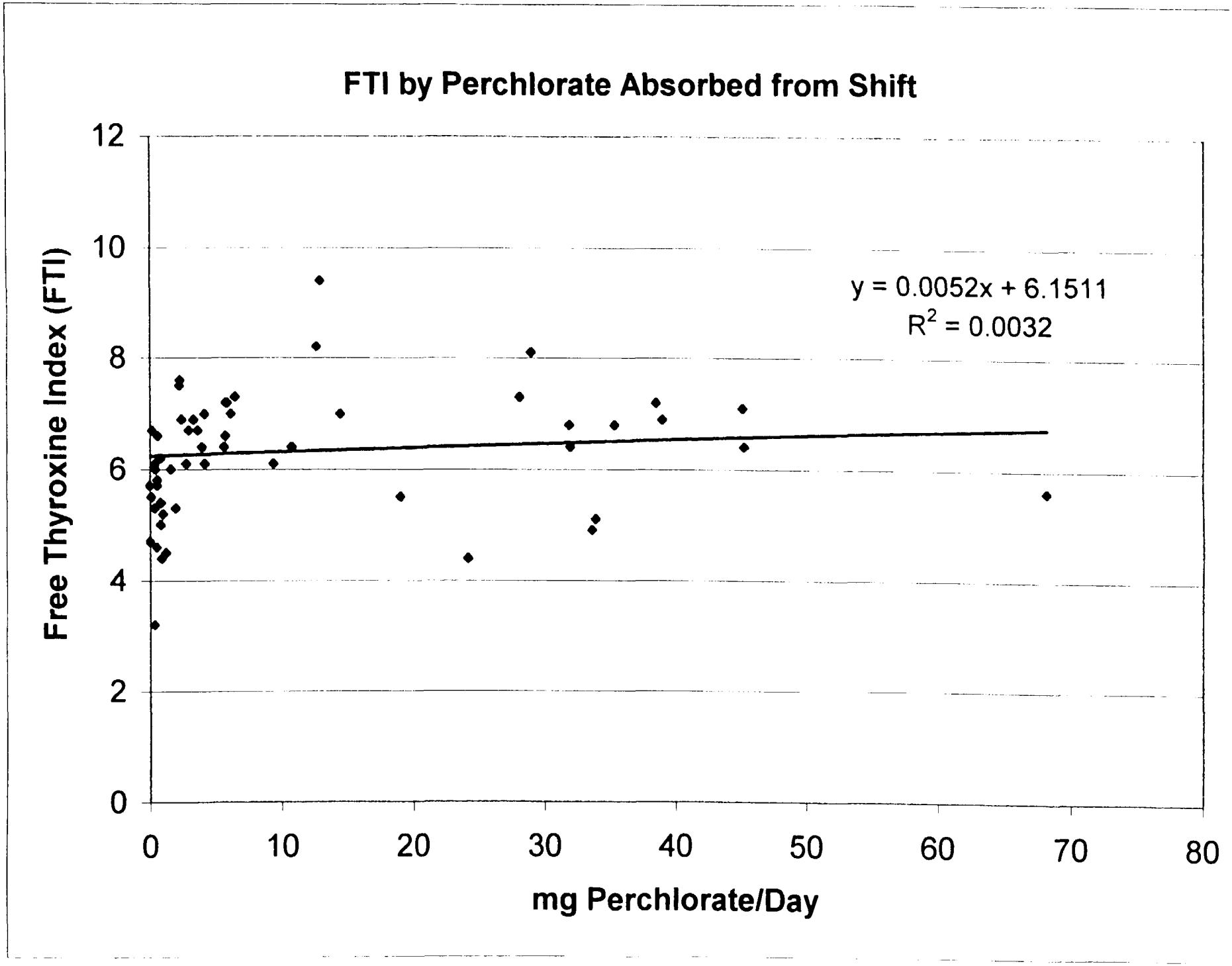

Absorbed Exposure/ FTI
35
30
25
20
15
10
5
0
Perc 33.35
Perc 8.4
FTI 6.0
FTI 6.4
FTI 6.5
FTI 6.9
Perc 3.37
Perc 0.62
Azide
Perc A
Perc B
Perc C
Exposure Group

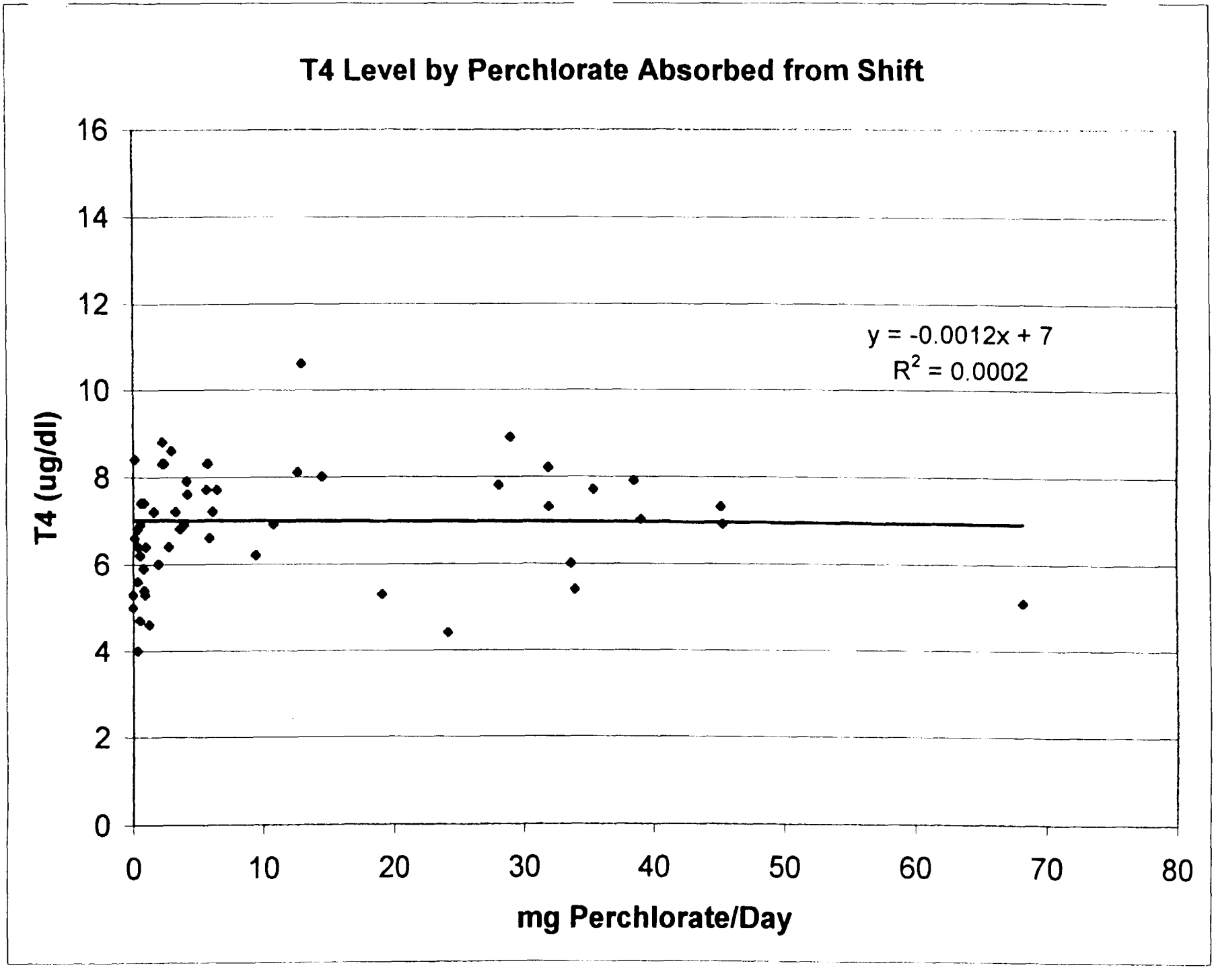
Perchlorate Absorption

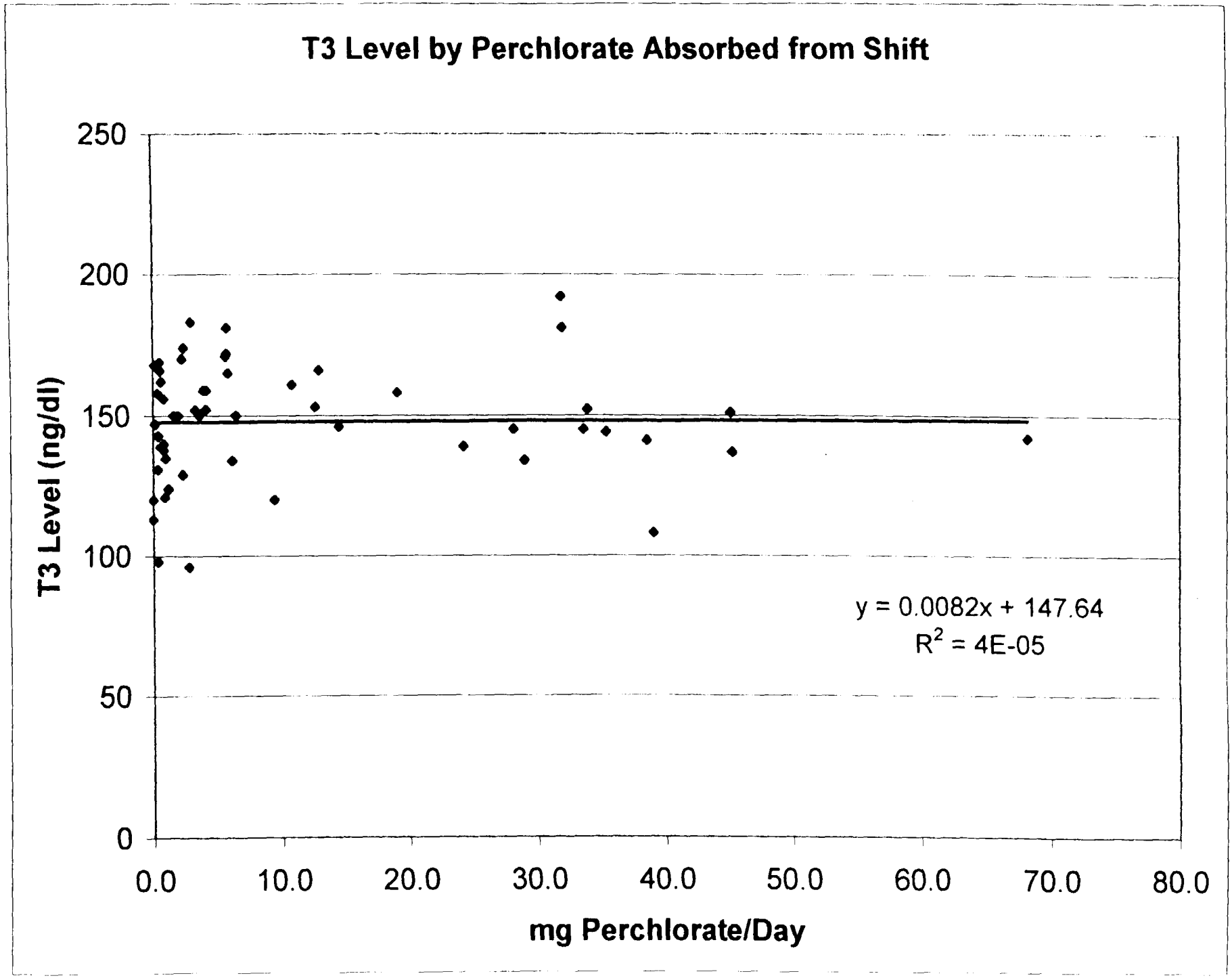
Expected Effects

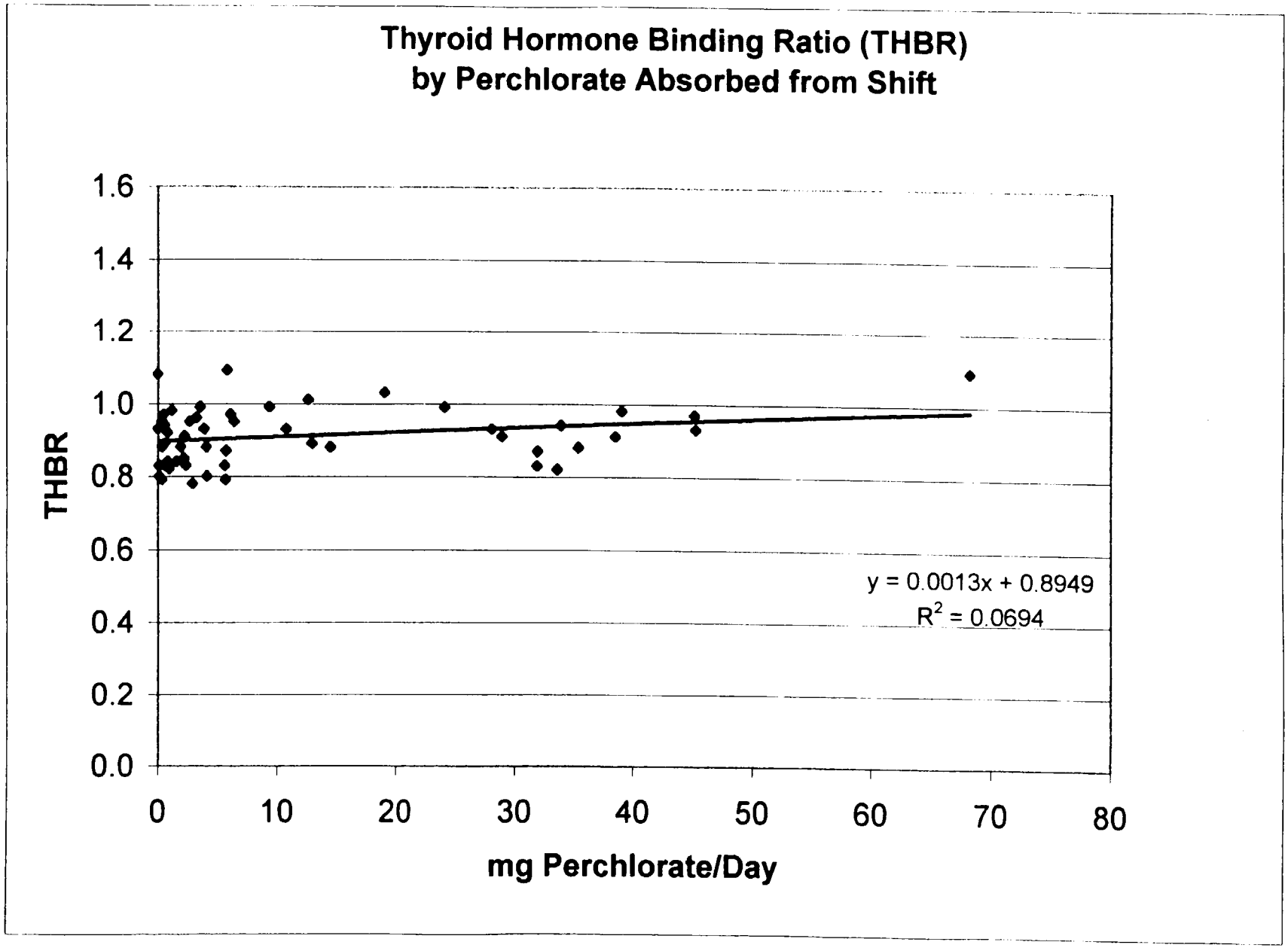
TSH	↑
FTI	↓
T_4	↓
T_3	↓
THBR	↓


TSH level by Perchlorate Absorbed from Shift
y = 0.0008x + 2.9261
R2 = 5E-05
TSH (uU/ml)
mg Perchlorate/Day
0
2
4
6
8
10
0
10
20
30
40
50
60
70
80


FTI by Perchlorate Absorbed from Shift
Free Thyroxine Index (FTI)
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
mg Perchlorate/Day
y = 0.0052x + 6.1511
R2 = 0.0032


T4 Level by Perchlorate Absorbed from Shift
y = -0.0012x + 7
R2 = 0.0002
T4 (ug/dl)
mg Perchlorate/Day
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70
80


T3 Level by Perchlorate Absorbed from Shift
250
200
150
100
50
0
T3 Level (ng/dl)
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
mg Perchlorate/Day
y = 0.0082x + 147.64
R2 = 4E-05


Thyroid Hormone Binding Ratio (THBR)
by Perchlorate Absorbed from Shift
THBR
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
mg Perchlorate/Day
y = 0.0013x + 0.8949
R2 = 0.0694

Perchlorate Absorption

Observed Effects
(0-70 mg/day)

TSH →

FTI →

T_4 →

T_3 →

THBR →

Perchlorate Absorption

Expected Effects		Observed Effects	
TSH	↑	TSH	→
FTI	↓	FTI	→
T_4	↓	T_4	→
T_3	↓	T_3	→
THBR	↓	THBR	→

Perchlorate Health Effects

- No evidence of increase in congenital hypothyroidism at recent environmental exposure levels.

California and Nevada
Seven Counties
(1996 – 1997)

Screened Newborns	691,208
Observed	249 cases
Expected	243 cases
Ratio	1.03 (0.90-1.16)
p-value	0.63

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

State	County	Newborns 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)
Nevada and California		691,208	249	242.9	1.02	(0.90-1.16)

* Expected numbers have been adjusted for Hispanic ethnicity.

2/9/99

Linda Ferguson
American Pacific Corporation

Comments to be presented to the External Peer Review Panel, Perchlorate Technical Workshop, San Bernadino, CA, February 10, 1999.

My name is Linda Ferguson, Vice President of American Pacific Corporation. Our headquarters are located in Southern Nevada. For approximately 30 years, and until 1988, we operated a manufacturing facility that produced ammonium perchlorate, not far from the present BMI complex of industrial facilities. At the current time, our principal operating facilities and offices are located in Southern Utah. We are the sole supplier of ammonium perchlorate in the U.S.

Before I continue, let me offer you our appreciation for agreeing to serve on such an important external review panel. I think we all agree that by making this process public and accessible to all, we have the best opportunity for a result that is reasonable and supportable. We especially appreciate the fact that you have taken time from your professional careers to participate.

We are a company of approximately 220 employees, 190 of whom live and work in Utah. Many of our Nevada employees live in Henderson and join us in our concern for the citizens of Clark County regarding the quality of our drinking water.

The Company has expended significant money in attempting to contribute to a full scientific review of perchlorates, so that the true significance can be understood, and public policy formulated, based on sound scientific knowledge. The company has tried to be very responsive to government agencies and we have therefore made extensive hydrogeologic evaluations of our former plant site in Henderson.

In the summer of 1998, American Pacific conducted and reported to EPA a human health study of our own production workforce. We found no evidence of thyroid problems, as reported by CEOH. Incidentally, our employees volunteered for the study and they are interested in the results of your work.

We believe it to be imperative that you give at least equal weight and consideration to the human health studies, some of which are still underway. The studies have been funded at great expense to our company and others. We believe these studies are essential in providing adequate information to protect our community and our employees.

American Pacific, both as a corporate citizen and as individual employees, resides in Clark County, Nevada. We have serious concerns that the public must be protected from any hazards; however, we have equally large concerns that the public not be subjected to undue alarm from

premature establishment of a value that was established without incorporation of available science. It is essential that the public be informed. However, publication of a reference dose that does not consider adequately all of the applicable and available science would create a number that may be subject to many revisions. Revisions cause public consternation and lower public trust both in governmental agencies and in corporations. It is essential that stakeholders in this process recognize the impact of their actions in establishing a reference dose.

The responsibilities faced by you as a peer review panel are critical. We wish to congratulate you on the contribution you are making as citizens by serving on the panel. We trust that you will be sensitive to the issues we and others have raised and award them due consideration. Thank you for your attention.

Referenced Studies

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

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

Recommendations to the US EPA Concerning the Derivation of a Reference Dose for Perchlorate

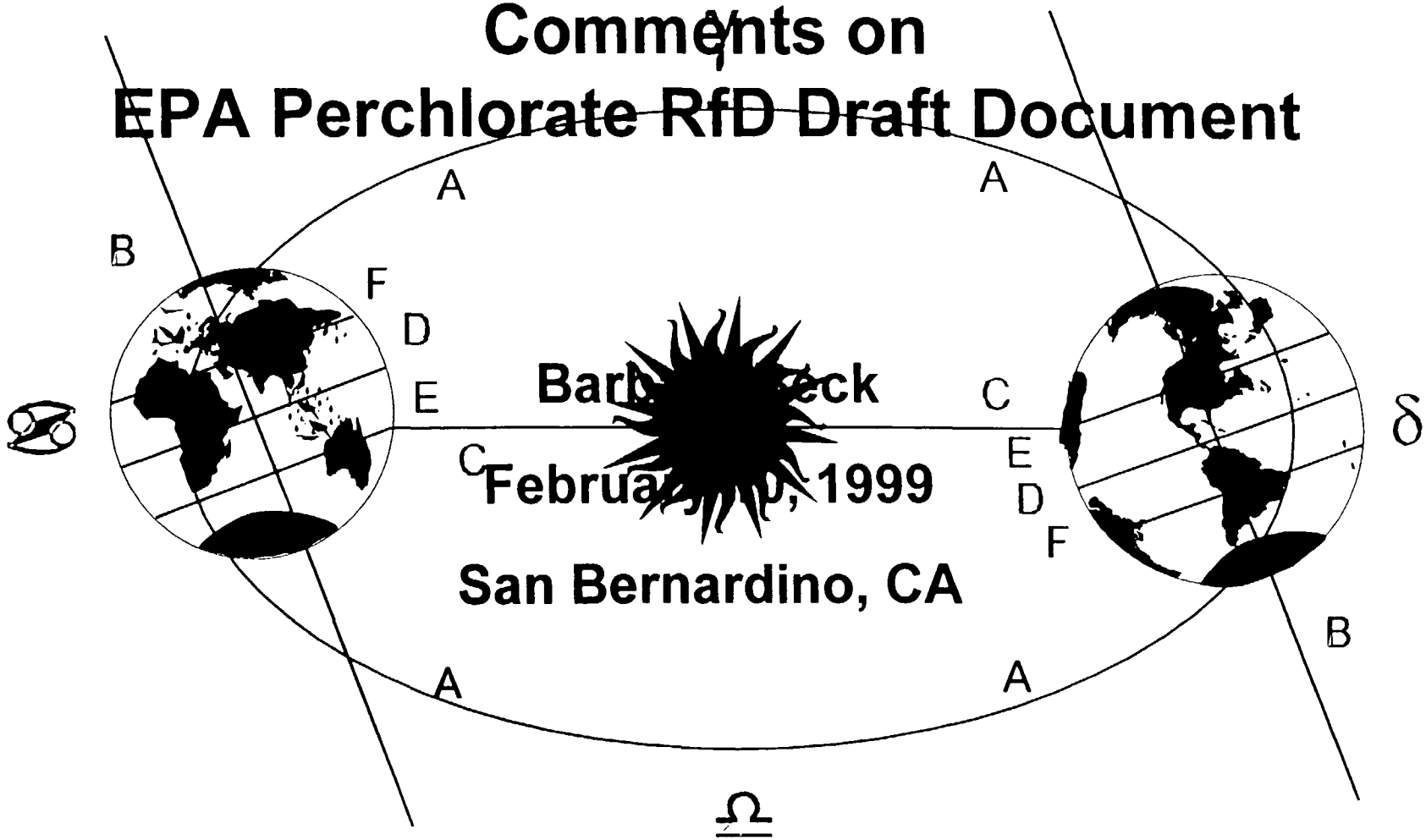
Evaluation of a Population with Occupational Exposure to Airborne Ammonium Perchlorate for Possible Acute or Chronic Effects on Thyroid Function - Gibbs, et al

The Effect of Low Dose Perchlorate on Thyroid Function - Braverman, PSG

Pharmacokinetic Study of Perchlorate Administered Orally to Humans - Brabant

Barbara Beck
Gradient Corporation

Comments on
EPA Perchlorate RfD Draft Document



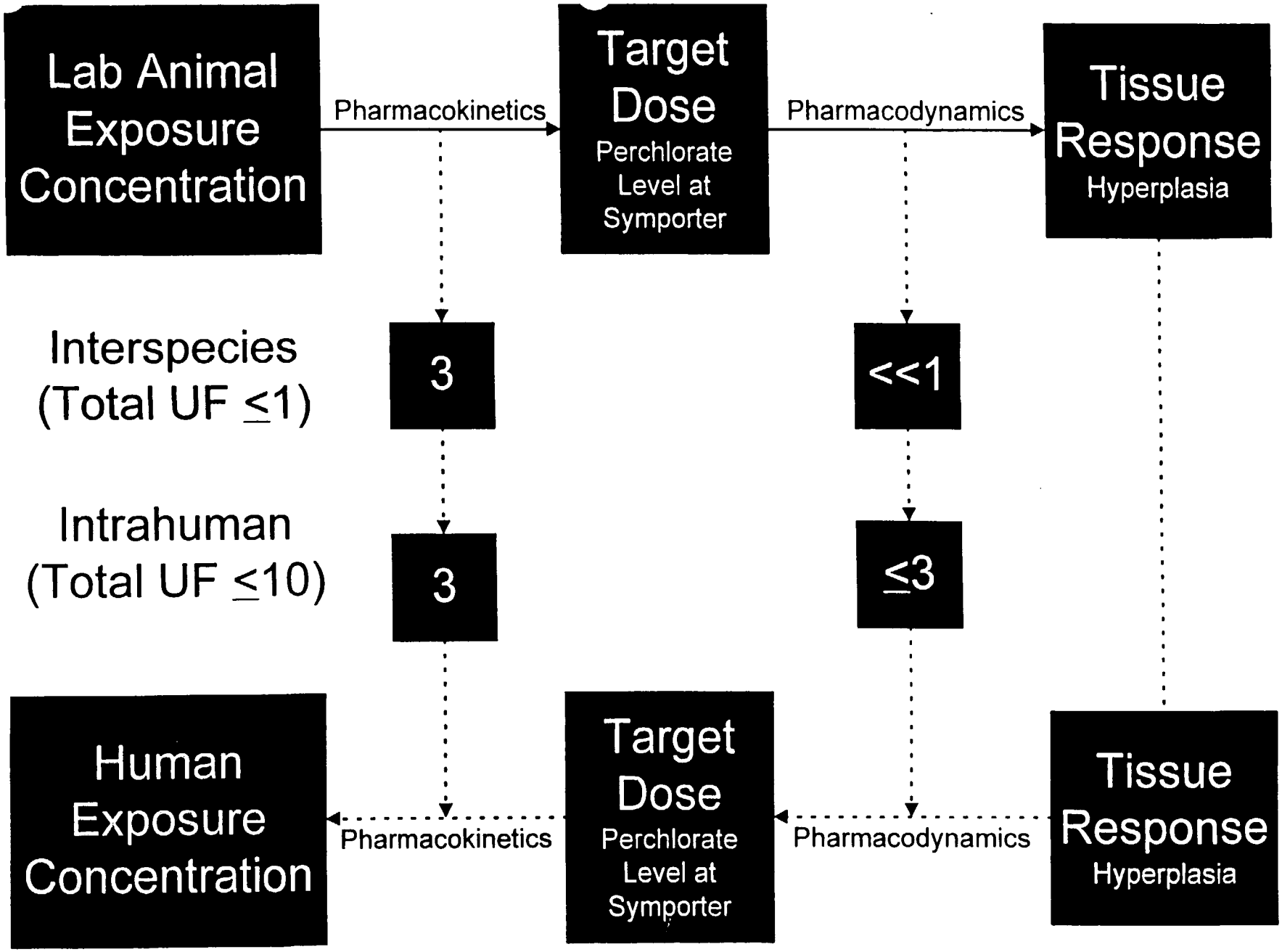
Barb[illegible]eck

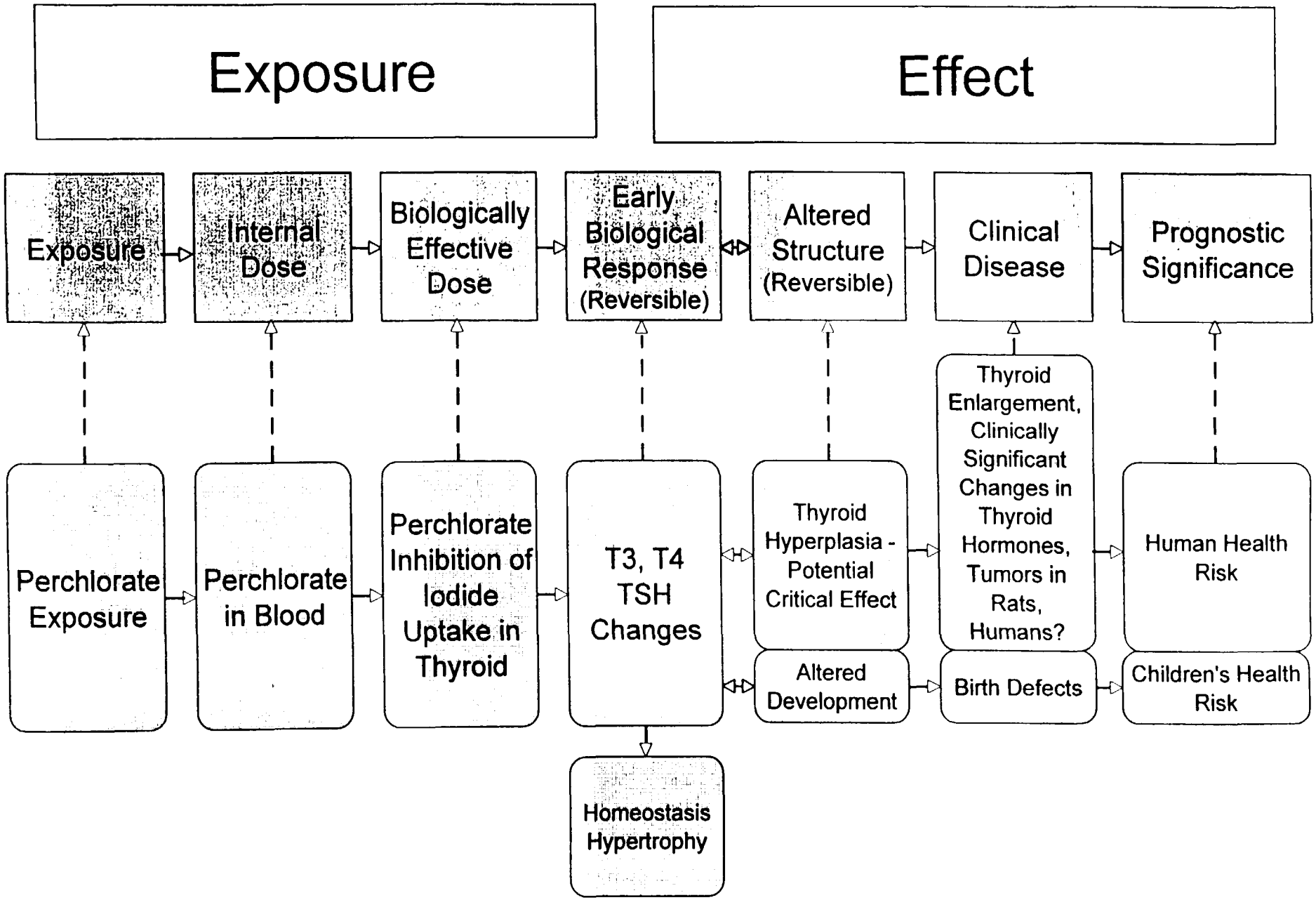
Februa[illegible], 1999

San Bernardino, CA

Gradient Corporation
An IT Company

Jellinek, Schwartz & Connolly, Inc.
An IT Company


Lab Animal Exposure Concentration
Pharmacokinetics
Target Dose
Perchlorate Level at Symporter
Pharmacodynamics
Tissue Response
Hyperplasia
Interspecies (Total UF ≤1)
3
<<1
Intrahuman (Total UF ≤10)
3
≤3
Human Exposure Concentration
Pharmacokinetics
Target Dose
Perchlorate Level at Symporter
Pharmacodynamics
Tissue Response
Hyperplasia


Exposure
Effect
Exposure
Internal Dose
Biologically Effective Dose
Early Biological Response (Reversible)
Altered Structure (Reversible)
Clinical Disease
Prognostic Significance
Perchlorate Exposure
Perchlorate in Blood
Perchlorate Inhibition of Iodide Uptake in Thyroid
T3, T4 TSH Changes
Thyroid Hyperplasia - Potential Critical Effect
Thyroid Enlargement, Clinically Significant Changes in Thyroid Hormones, Tumors in Rats, Humans?
Human Health Risk
Altered Development
Birth Defects
Children's Health Risk
Homeostasis Hypertrophy

Choice of the Critical Study and Critical Endpoint

- Rat neurodevelopment study:
  - PND5 data
  - Thyroid follicular-cell morphology changes at the lowest dose tested (0.1 mg/kg-day):
    - Increased cell height and/or diameter (*i.e.*, hypertrophy);
    - Decreased lumen size.
- Are the morphological changes merely histologically identifiable or are they indicative of a pathological process?

Physiological Significance of Follicular Cell Hypertrophy and Decreased Lumen Size

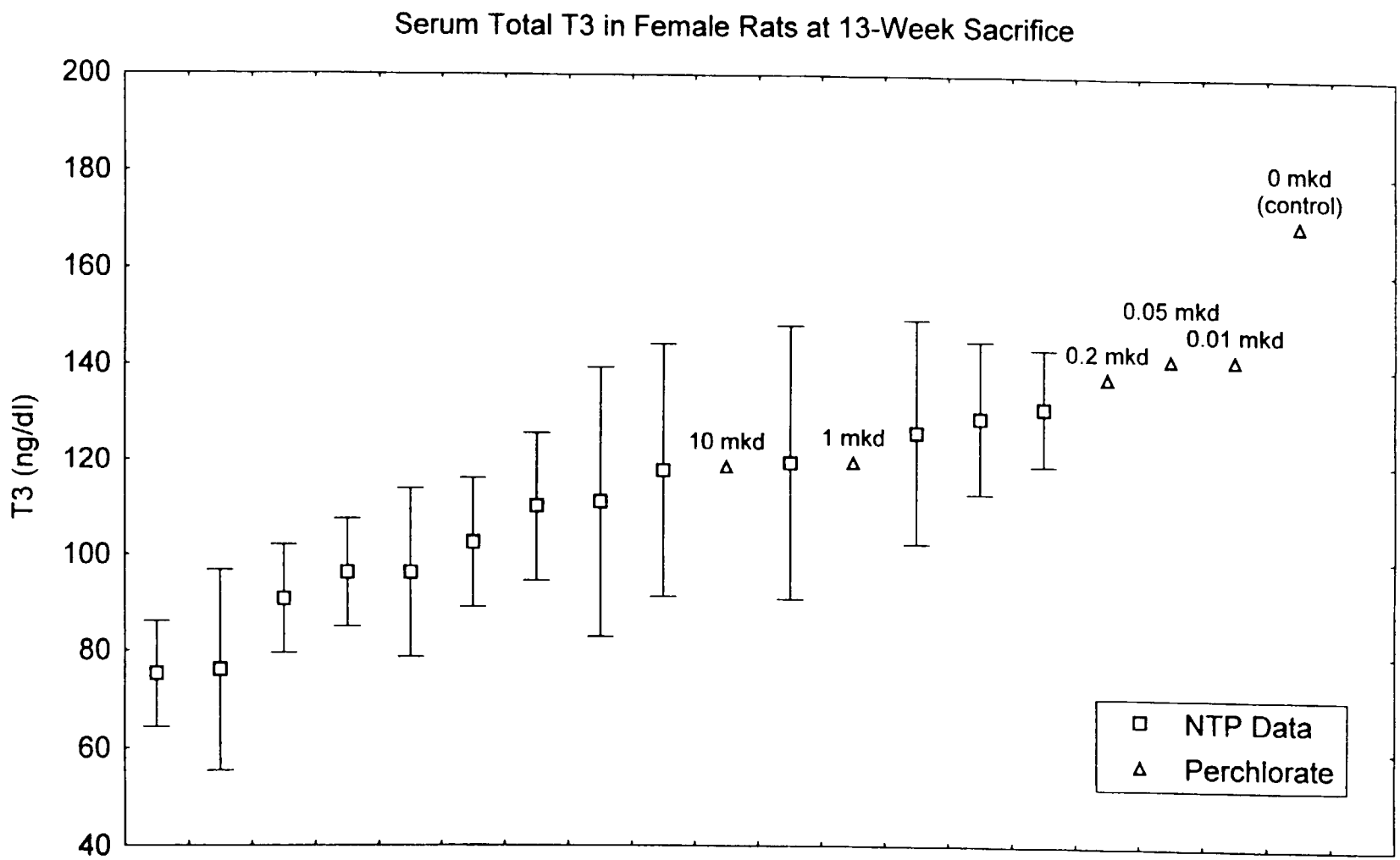
- Effects are reversible: No evidence for permanent cellular change found in rat pups exposed *in utero* and *via* breast milk to a maternal perchlorate dose as high as 10 mg/kg-day.
- No evidence offered by EPA/NCEA that such cellular changes, in and of themselves, fall outside the realm of physiologically normal thyroid morphology.

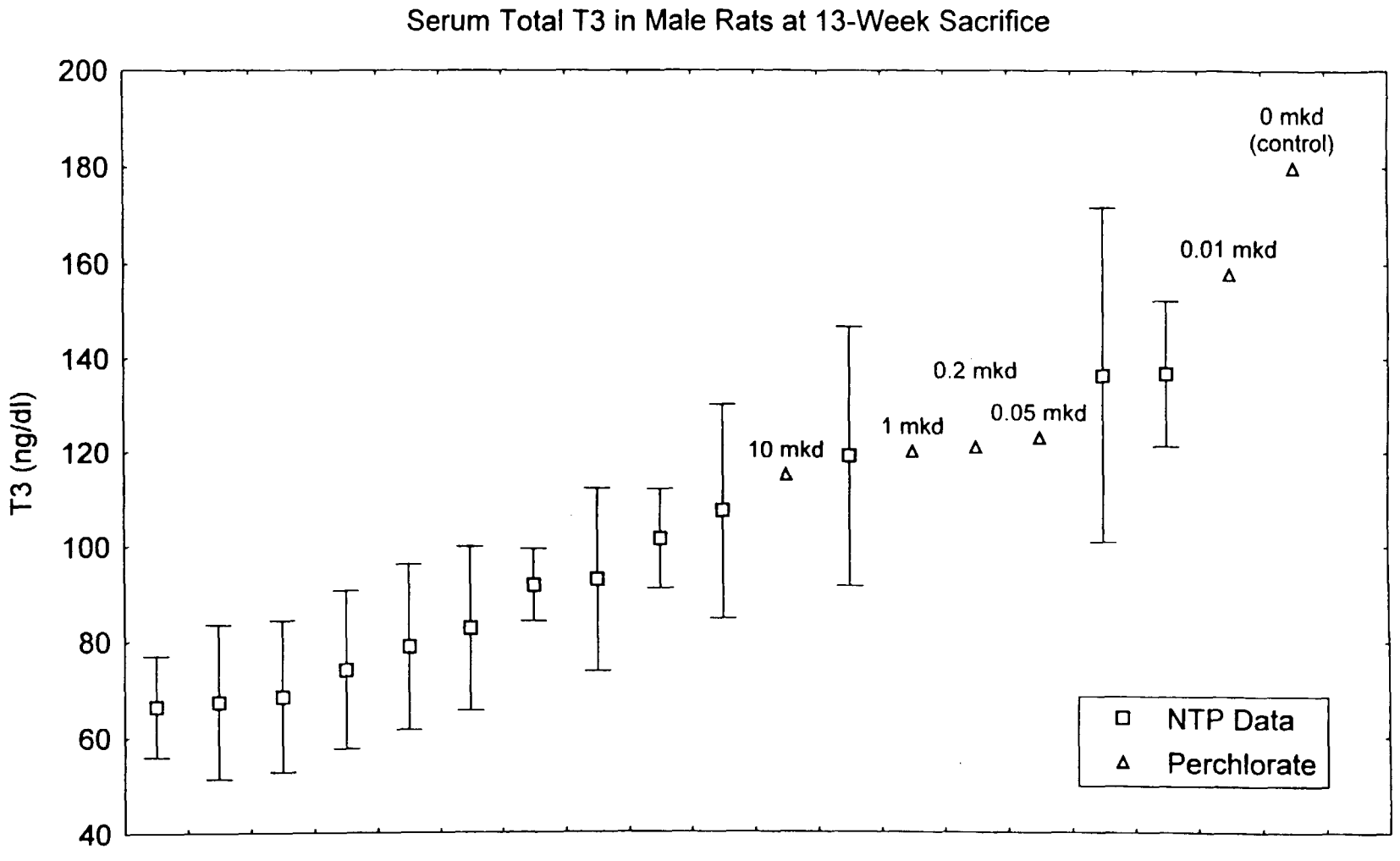
Table 1. Incidence Ratio of Any Evidence of Follicular-Epithelial-Cell Hypertrophy Among Rat Pups in the 1998 Neurodevelopmental Study of Perchlorate by Argus Laboratories, as Determined by Standard Histology

Time of Sacrifice	Control	Perchlorate Dose to the Dams (mg/kg-day)			
		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]

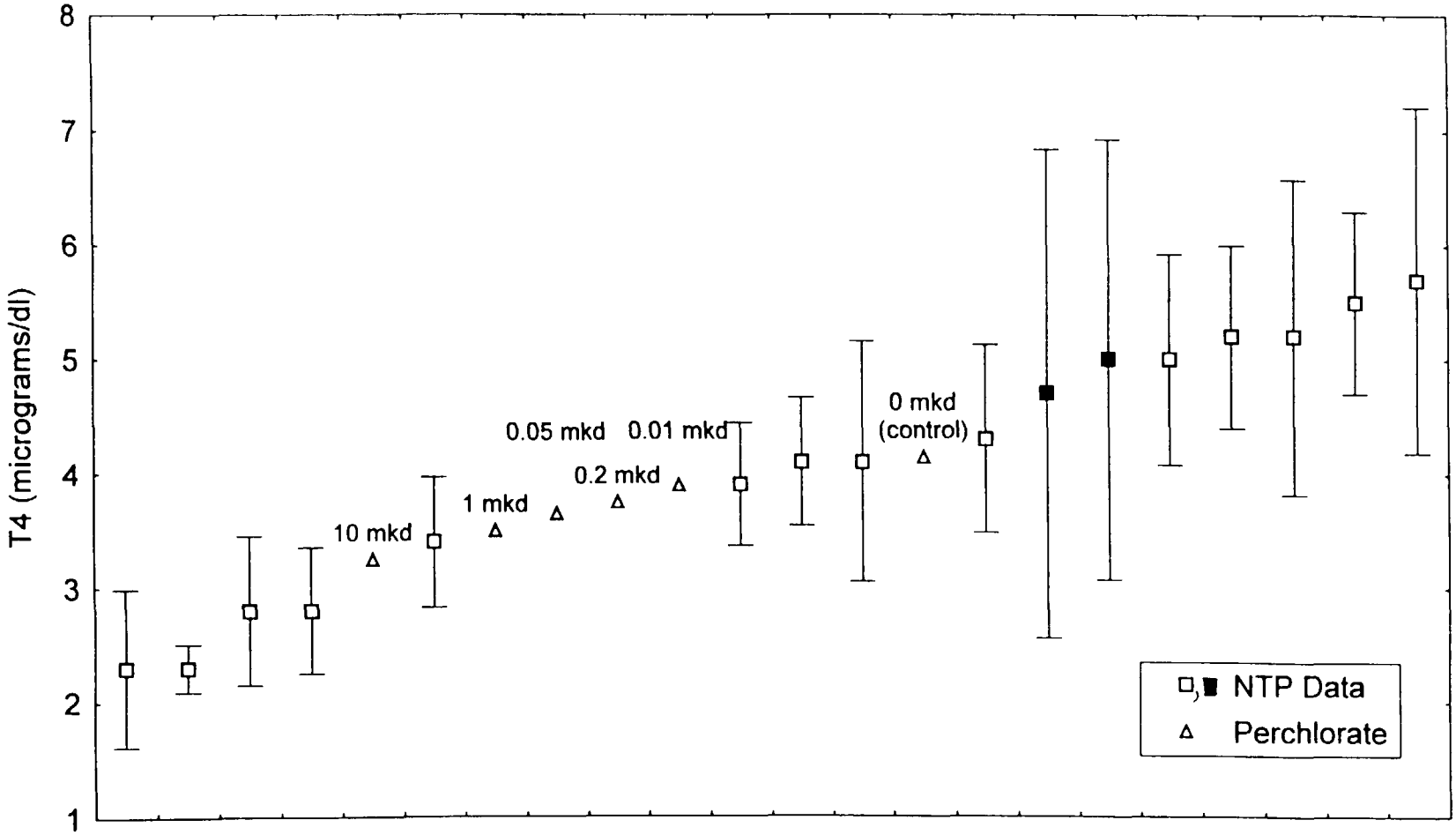
[a] The External Review Draft did not provide the absolute number of animals examined.

[b] Perchlorate exposure discontinued on PND10.


Serum Total T3 in Female Rats at 13-Week Sacrifice
T3 (ng/dl)
200
180
160
140
120
100
80
60
40
0 mkd
(control)
0.05 mkd
0.01 mkd
0.2 mkd
10 mkd
1 mkd
NTP Data
Perchlorate

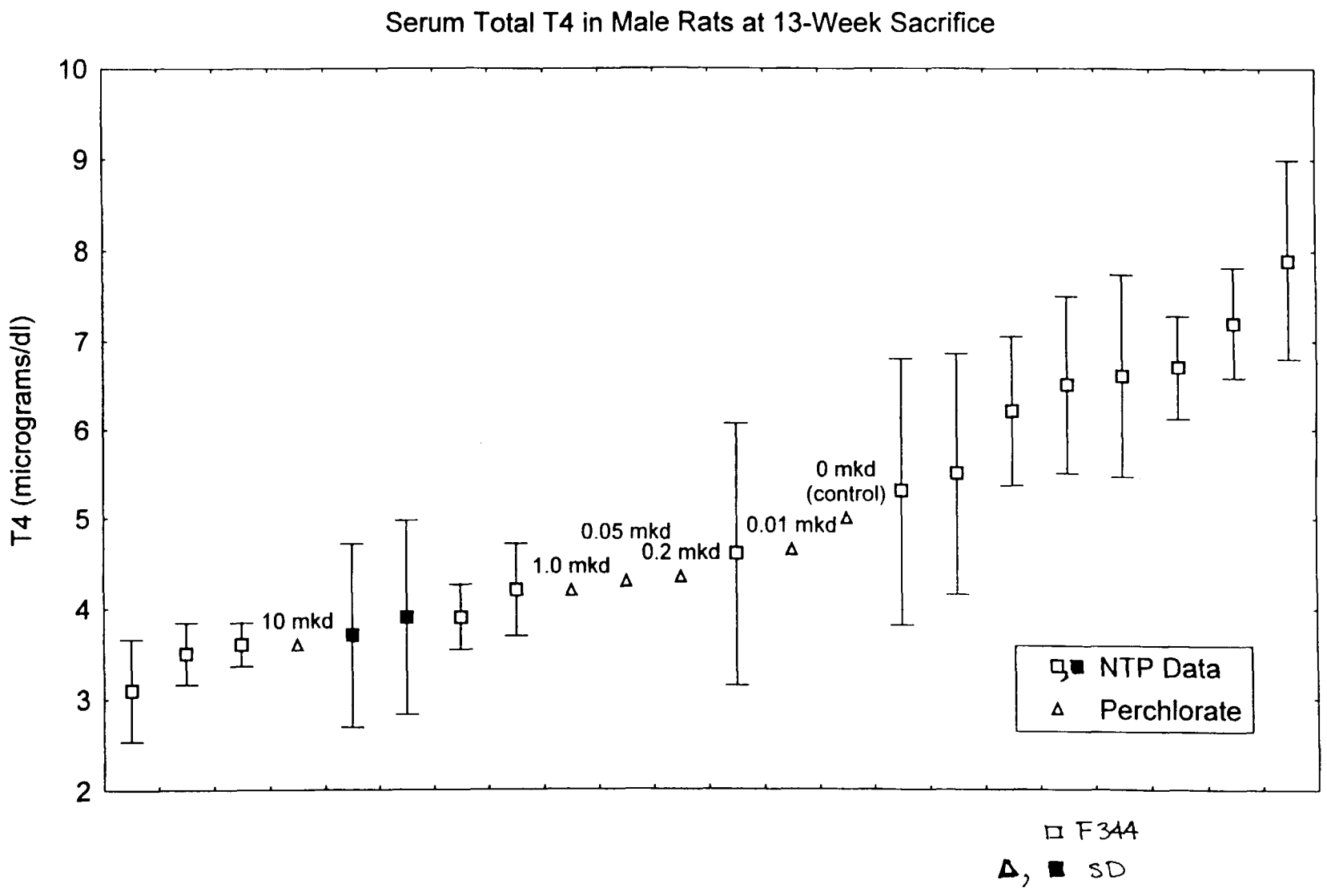

Serum Total T3 in Male Rats at 13-Week Sacrifice
T3 (ng/dl)
200
180
160
140
120
100
80
60
40
0 mkd
(control)
0.01 mkd
0.2 mkd
0.05 mkd
1 mkd
10 mkd
NTP Data
Perchlorate

Serum Total T4 in Female Rats at 13-Week Sacrifice



□ F344

Δ, ■ SD


Serum Total T4 in Male Rats at 13-Week Sacrifice
T4 (micrograms/dl)
10
9
8
7
6
5
4
3
2
10 mkd
1.0 mkd
0.05 mkd
0.2 mkd
0.01 mkd
0 mkd
(control)
NTP Data
Perchlorate
F344
SD

Correlation Analyses: EPA/NCEA Hypotheses

- Paired hormone comparisons
  - Expect positive correlations for T3 vs. T4;
  - Expect negative correlations for T3 vs. TSH;
  - Expect negative correlations for T4 vs. TSH.
- Paired hormone/histology comparisons
  - Expect positive correlations for TSH vs. histology;
  - Expect negative correlations for T3 vs. histology;
  - Expect negative correlations for T4 vs. histology.

Correlation Analyses

- In most cases, found statistically significant correlations of the expected sign.
- **Question:** Would the expected correlations be found if the high doses were removed from the analyses?
- **Answer:**
  - Not for T4 rank order vs. histology severity rating
  - Not for TSH rank order vs. histology severity rating
  - Not clear for T4 vs. TSH (evidence for and against)
  - Probably yes for T3 vs. T4.

Michael McClain
Jellinek, Schwartz and Connolly, Inc.

R. Michael McClain, PhD
Consultant in Toxicology
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Randolph NJ, 07859

February, 10, 1999

Ella Dardin
Research Triangle Institute
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Research Triangle Park, NC 27709-2914

RE: *Comments to the External scientific Peer Review Panel for the document entitled "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information.*

I was asked by the firm Jellinek, Schwartz and Connolly, Inc. to review and provide a scientific evaluation of the EPA document on Perchlorate Environmental Contamination and to briefly comment to the Peer Review Panel on what I consider to be some of the more important issues in establishing the RfD based on the information in the document.

EPA has done a thorough job in compiling the available information on perchlorate and in evaluating the more recent information concerning the effects of perchlorate on thyroid gland function in the rat, reproductive performance and development, neurobehavioral and immunologic effects. The document represents a sound scientific approach for perchlorate risk assessment.

The primary, if not only effect, of perchlorate is on thyroid gland function. By virtue of its ability to competitively inhibit the uptake of iodine by the thyroid gland the expected responses to altered thyroid gland function are observed. These include decreased circulating levels of thyroid hormone T3 and T4, a compensatory increase in TSH and compensatory morphological changes in the thyroid gland including increased weight and follicular cell hypertrophy and hyperplasia.

Consistent diagnostic criteria needs to be used in the histologic evaluation of the thyroid gland.

The morphological effects on the thyroid gland are important for evaluating the effects of chemical treatment. In the various studies however, no consistent criteria are provided for the diagnosis of follicular cell hypertrophy and hyperplasia. For example, in the Caldwell studies hypertrophy/hyperplasia appears to be recorded as a single diagnosis, in the 90 day rat study only hyperplasia at the high dose of 10 mg/kg/day is recorded although the written description mentions hypertrophy. In the neonatal study the subjective histologic diagnosis of hypertrophy is not consistent with the morphometric measurements. Also, in the text of the report, the term hyperplasia is used in a few places when hypertrophy was reported. For a diagnosis using consistent criteria, the thyroid glands should be reevaluated by a pathologist well versed in the histology of the rat thyroid gland. This and any morphometric examinations should be performed on a randomized blind basis.

As discussed in the EPA document there are marked species differences in thyroid gland function. The rat has an active thyroid gland, a high turnover of thyroid hormone and very little reserve capacity. As compared to the primates and many other species the follicular epithelium of the control rat is hypertrophied and the diagnosis for the rat is hypertrophy beyond that, that already exists. There is a great deal of variation in the rat thyroid gland with respect to the height of the follicular epithelium. Because of this and the normal variation, not all pathologists will record hypertrophy until the thyroid is stimulated to the point that hyperplasia is also evident.

Follicular cell hyperplasia in the presence of TSH is a more appropriate endpoint in establishing the RfD.

Follicular cell hypertrophy is indicative of an increased functional activity of the thyroid gland and should be regarded as a functional adaptive change and as such should not be considered an adverse effect. The EPA has used hypertrophy to set the RfD; however in my opinion follicular cell hyperplasia in the presence of increased TSH would be a more appropriate endpoint. Although these are also adaptive changes, this could be considered to be a response to altered thyroid gland function to a degree that is considered adverse.

Follicular cell hypertrophy and hyperplasia are distinctly different cellular events.

The proper histological diagnosis of hypertrophy and hyperplasia is also important because these are distinctly different cellular events. Hypertrophy is an increase in cell size indicating an increased functional activity. Hyperplasia is the result of cell division. Although hypertrophy precedes hyperplasia on the dose response continuum, hypertrophy does not necessarily progress to hyperplasia or in other words, hypertrophy is not necessarily a percussor lesion to hyperplasia. Hypertrophy can exist alone at a certain level of stimulation without necessarily progressing to hyperplasia. At a higher level of stimulation, hyperplasia, as a result of increased cell proliferation, a different cellular response, will be observed in addition to hypertrophy.

Extrapolation of thyroid function data in rats to risks for humans.

Because of the marked species differences in thyroid gland function between the rat and primates, I agree with the EPA that the extrapolation from rat data is conservative. In other words, the rat is more likely to overestimate than underestimate the risk of perchlorate to humans. Although all the data are not yet in and it is possible that the situation may change if the thyroids are reevaluated, I believe that the estimates are conservative. As far as the assessment of thyroid function is concerned, the Springborn 90 day rat study is better conducted than any of the others and should be the study used for the RfD.

Nevertheless there are a lot of people potentially exposed and it will be a long time before the environmental conditions improve. For the future or next steps, I believe that studies should be conducted in the non-human primate to ensure that the estimate of margin of safety is accurate. Also since potassium perchlorate is used as a human drug, I would explore the possibility of studies in humans at **low doses** (i.e. doses in the range of 1/10 to 1/100 the current clinically used doses for treatment of thyrotoxicosis in patients treated with the iodine containing antiarrhythmic agent amiodarone).

I appreciate the opportunity to comment on the EPA perchlorate document.

RICHARD PLEUS
INTERTOX

U.S. EPA's Failure to Consider Human Studies in the Provisional Development of the Perchlorate RfD

Richard C. Pleus, Ph.D.

INTERTOX

Seattle

Issues

- There is concern that the rat is more sensitive than the human to the effects of perchlorate.
- Clinical studies in humans are currently in progress; these will provide critical information regarding the dose-response for the effect of perchlorate on human thyroid function.
- In its December 31, 1998 document, U.S. EPA did not consider or even anticipate using human data.
- The release of the provisional RfD was driven by meeting a schedule rather than the pursuit of good science.

Did US EPA consider human studies in the development of the provisional RfD?

- The EPA document does not consider human data of any kind in the derivation of the RfD for perchlorate.
- Perchlorate was used as a therapeutic agent in the 1950s; there have been several clinical and occupational studies on the health effects of perchlorate.
- Two human studies are currently in progress; these could be important for
  - determining the dose-response for effects on thyroid hormones;
  - determining interspecies differences in sensitivity to the thyroidal and extrathyroidal actions of perchlorate, including any effects on hematology and blood chemistry.

Protocol of Braverman *et al.*

- Perchlorate given to 8 adult, male volunteers at a dose of 10 mg/day (~ 0.14 mg/kg/day);
- 14-day exposure;
- Four or five volunteers have completed the perchlorate exposure and iodide-uptake tests;
- Follow-up examinations for all volunteers to be completed by March 1, 1999.

Protocol of Brabant *et al.*

- Perchlorate given to seven adult volunteers per dose;
- Doses of 7, 70, or 840 mg/day (~ 0.1, 1, or 12 mg/kg/day, respectively);
- 14-day exposure;
- Study was expected to start January 1999;
- No results of perchlorate exposure at this time.

CONCLUSION

- Human studies currently in progress will provide dose-response information for the known thyroidal effects of perchlorate and any additional effects on blood chemistry and hematology.
- Data from the new human studies will provide information useful to the comparison of perchlorate sensitivity in laboratory animals and humans.
- U.S. EPA has not considered or anticipated using human data; rather, an enforced timetable has been driving the release of a provisional RfD.
- Let good science prevail.

Appendix I

Introductory Presentations by EPA at the Workshop

INTRODUCTORY PRESENTATIONS BY EPA

To begin the workshop, EPA presented background material on the perchlorate issue and risk assessment. Peter Grevatt, of EPA's Office of Solid Waste and Emergency Response, presented a brief overview of the perchlorate peer review process. This was followed by Kevin Mayer of EPA's Region IX who presented a local perspective on the perchlorate issue, including the history of the issue and areas of the country with perchlorate releases. William Farland of EPA's National Center for Environmental Assessment (NCEA) discussed the risk assessment process for perchlorate, including the development of a revised Reference Dose (RfD) and comprehensive characterization. Annie Jarabek, also of EPA's NCEA, presented a summary of EPA's mode-of-action approach to human and ecological risk assessment for perchlorate.

Kevin Mayer
EPA, Region 9

WELCOME to Region 9

Kevin Mayer
Superfund Program
U.S. EPA, Region 9

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Sacramento
San Francisco
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CALIFORNIA
Las Vegas
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Tucson
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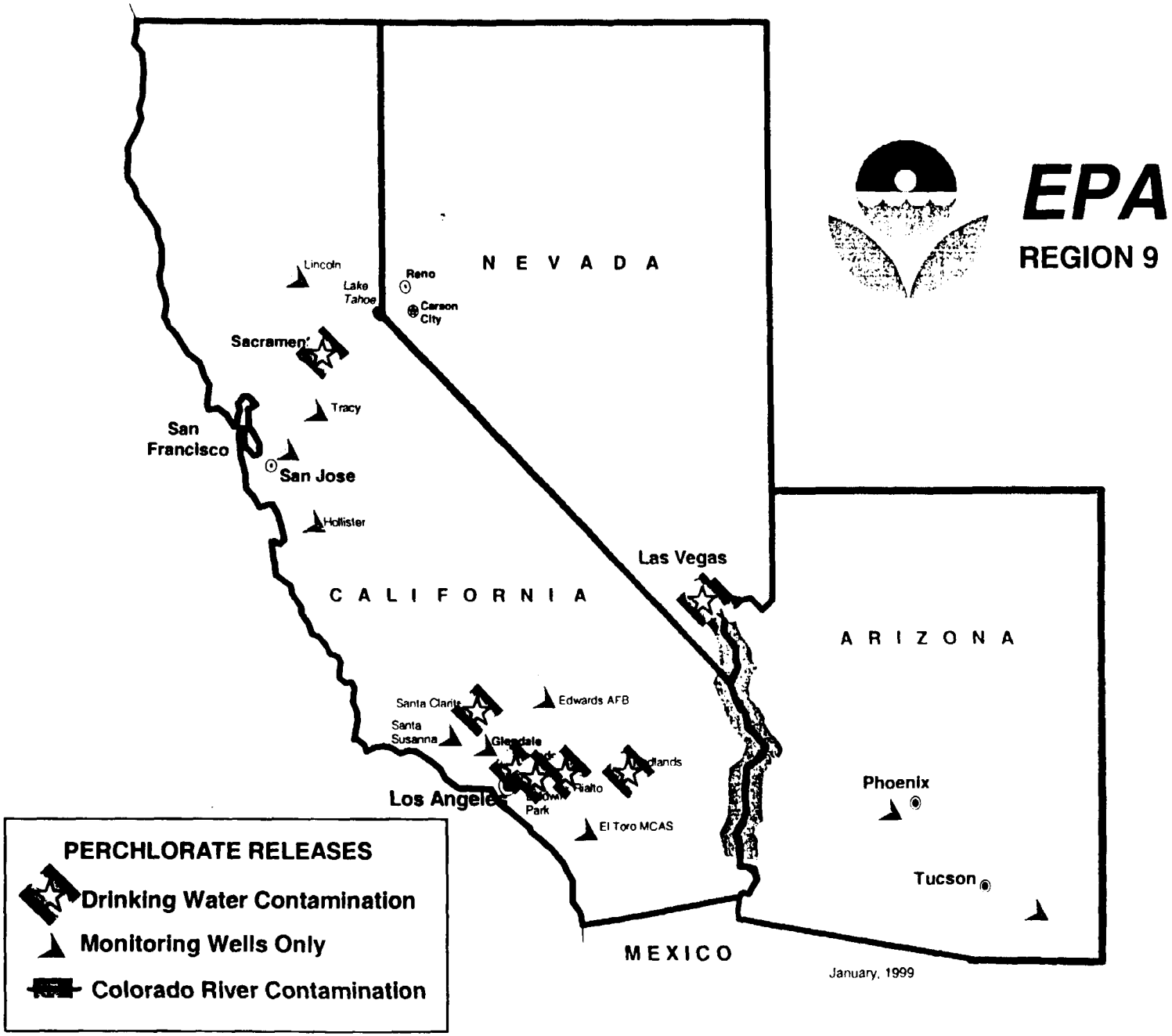
EPA
REGION 9


EPA
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Santa Clarita
Edwards AFB
Santa Susanna
Glendale
Rialto
Los Angeles
Park
El Toro MCAS
Phoenix
Tucson
PERCHLORATE RELEASES
Drinking Water Contamination
Monitoring Wells Only
Colorado River Contamination
January, 1999

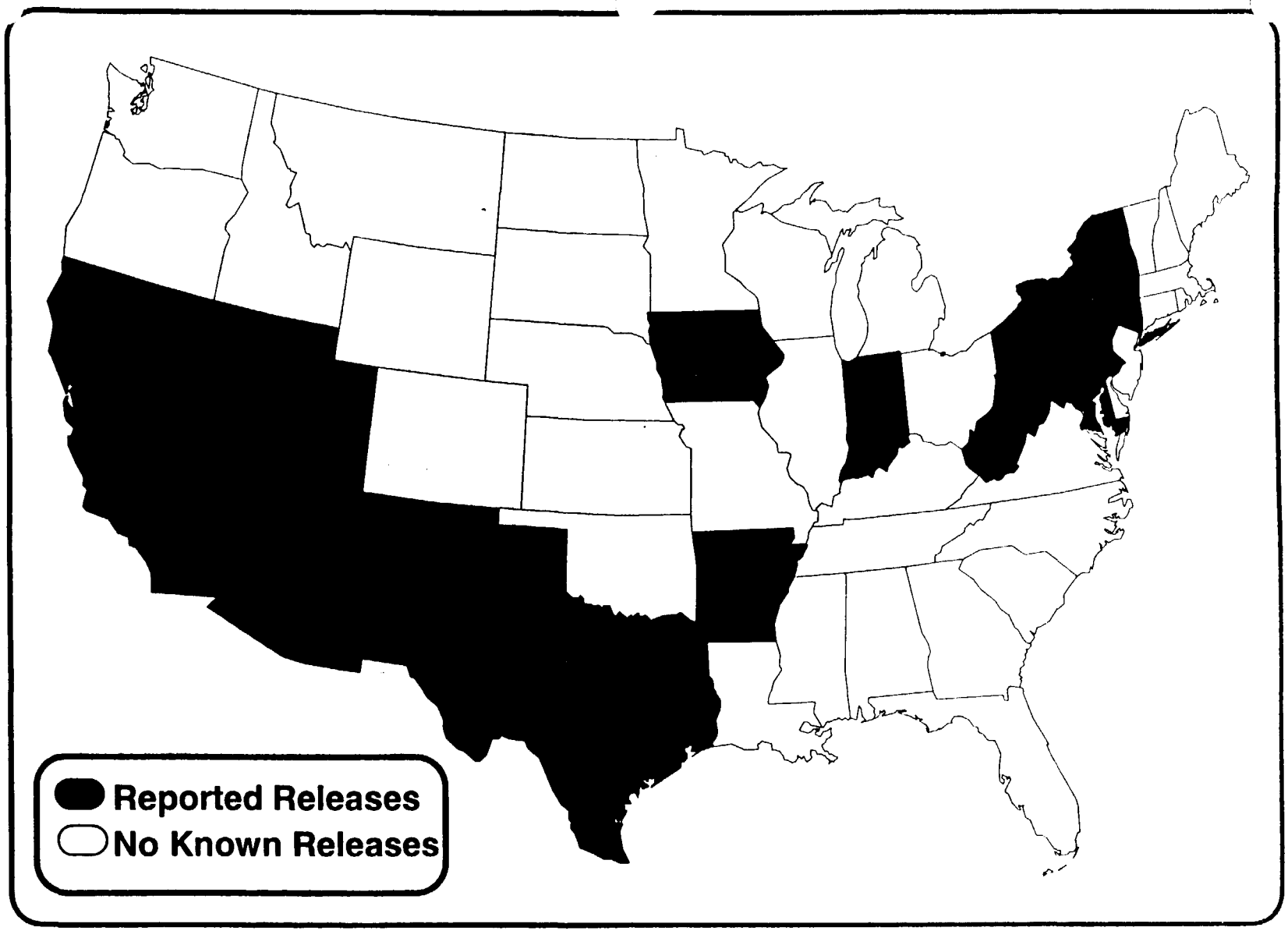
HISTORY - Before 1997

- 1980s - Aware of Perchlorate in CA, NV
- 1985-86 - San Gabriel Valley
- 1990s - Rancho Cordova (ppm)
- 1992-95 - Provisional Reference Dose (ppb range)
- 1997 - Analytical breakthrough

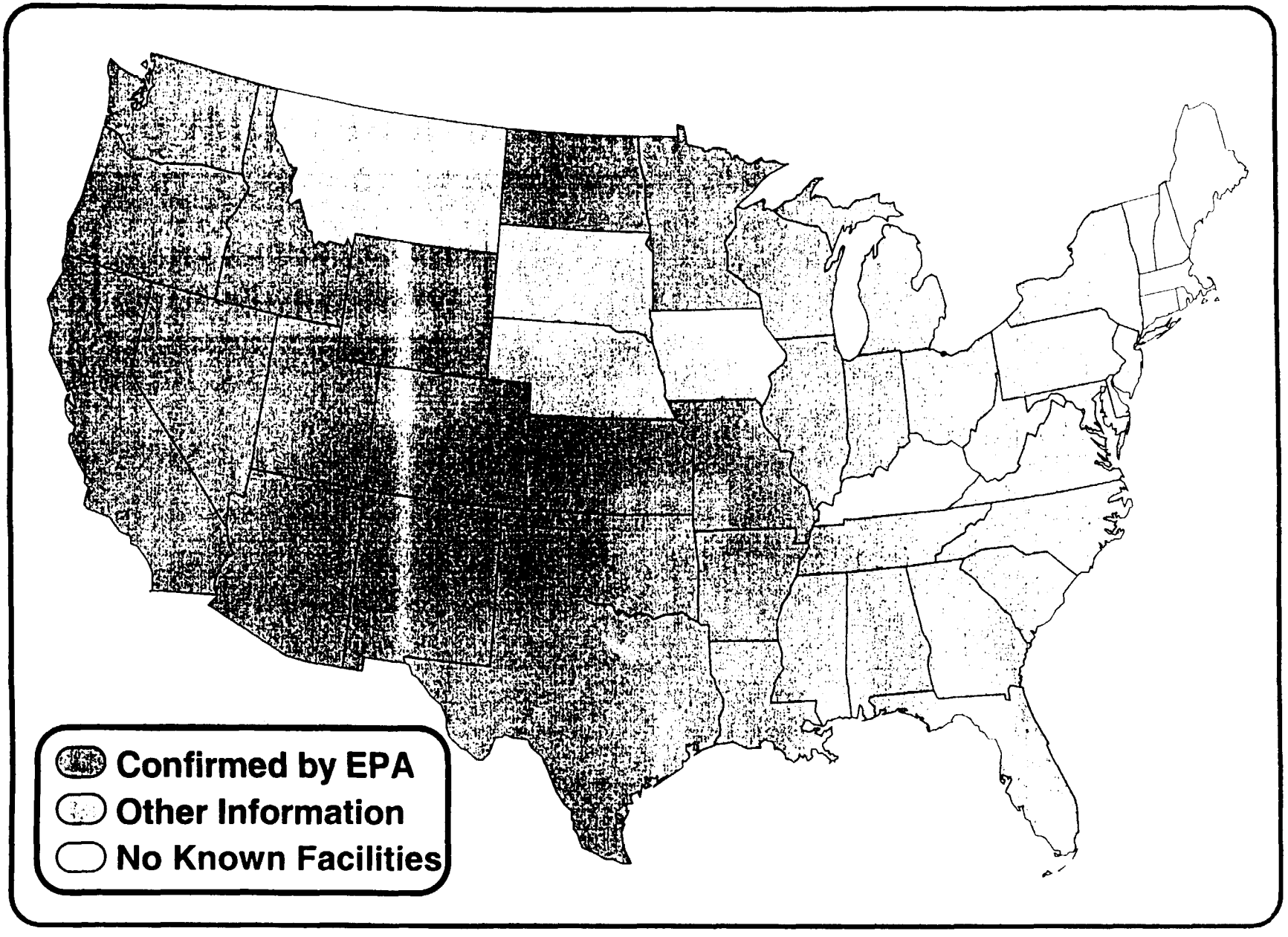
Agency for Toxic Substances and Disease Registry - ATSDR (January 21, 1986):

"...Given the proprietary nature of the laboratory method for quantification and the poor quality assurance results noted, the **data do not prove that perchlorate ion has actually been found**. If the presence of perchlorate ion is confirmed, the **scientific database** on this ion is **insufficient to generate either an acute or longer-term health advisory for drinking water**"

"... The minimal acute toxicity data available suggest that one or two ppm perchlorate ion would not represent an immediately acute and substantial threat to the public health. The ATSDR does not consider this level to be "safe" in the absence of experimental data.."



States with Environmental Releases of Perchlorate



States with Perchlorate Manufacturers or Users

Example Sites

- Southern CA - All 50 wells - Avg 25ppb, Range 4 ppb-130 ppb, std dev = 27
- River location - Avg 6 ppb, Range 4-[illegible] ppb
- Northern CA - 11 Public Water Supply wells (in 1997), Avg 125 ppb, Median 93, Range <4 ppb to 340 ppb, std dev = 125
- Southern CA - All 37 wells - Avg 126, Median 34 ppb, Range 4 to 1,100 ppb, std dev = 239

William Farland
EPA, NCEA

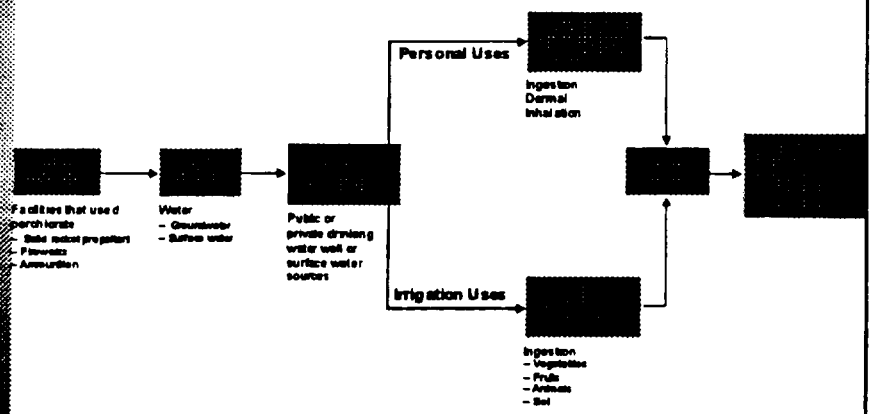
The Perchlorate Environmental Contamination Challenge: EPA ORD Assessment Strategy

William H. Farland, Ph.D.
Director
National Center for Environmental Assessment
U.S. EPA

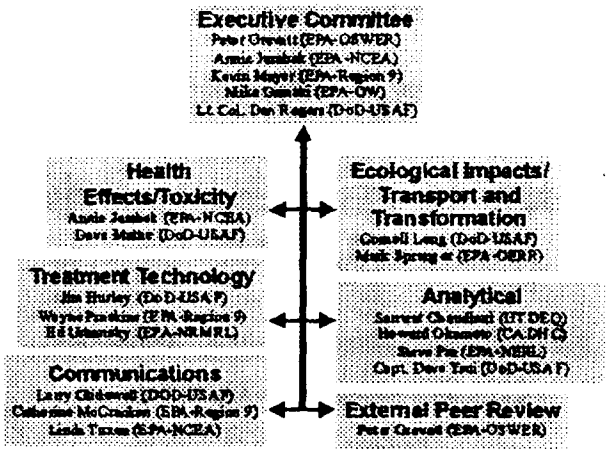
Perchlorate Peer Review Workshop
San Bernardino, California
February 10-11, 1999

I P S C

Comprehensive Characterization of Perchlorate Contamination



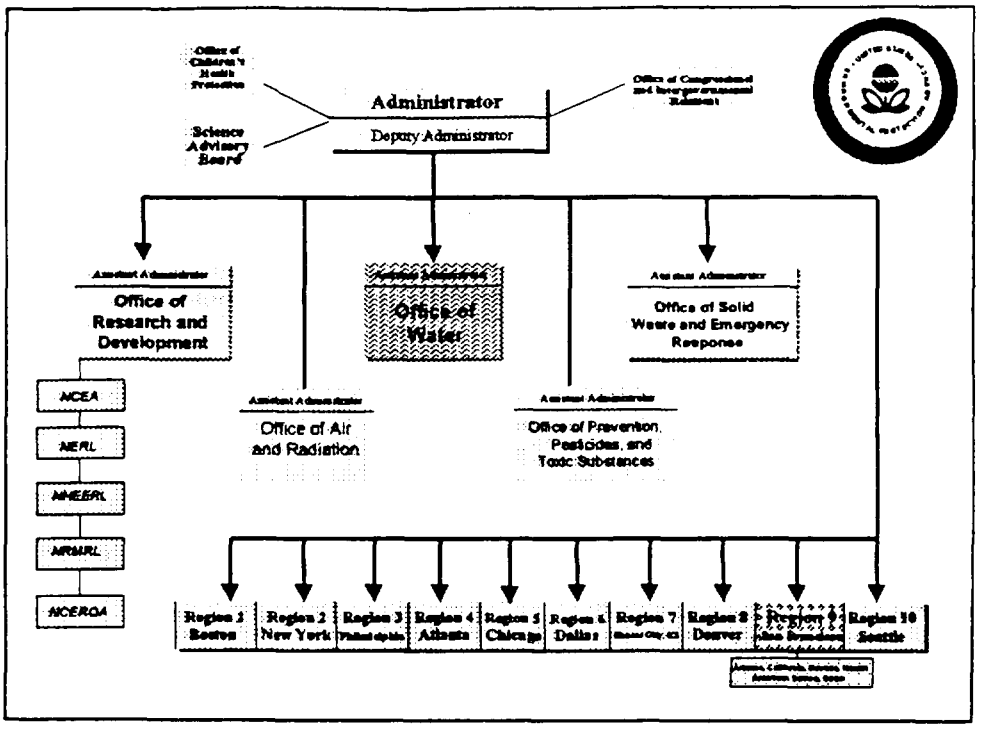
Interagency Perchlorate Steering Committee (IPSC)



The Perchlorate Contamination Challenge

Pro-Active Partnership

- Unprecedented timeframe
- Targeted expertise
- Commitment to continued improvement



The Perchlorate Contamination Challenge

Credible Science

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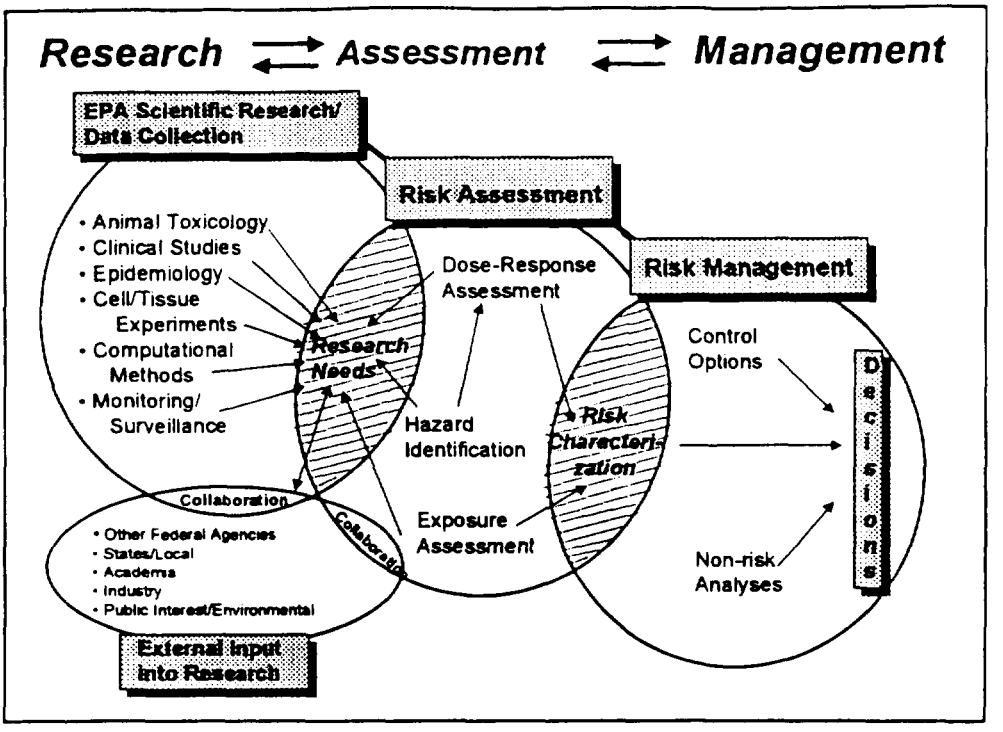
Credible Decisions

- Accurate risk characterization
- Appropriate management strategies



Recent Emphasis Focuses on the Development and Use of Better Data

"The quality of risk analysis will improve as the quality of input improves. As we learn more about biology, chemistry, physics, and demography, we can make progressively better assessments of the risks involved. Risk assessment evolves continually, with re-evaluation as new models and data become available."

"Science and Judgment in Risk Assessment" (National Research Council, 1994)

The Perchlorate Contamination Challenge:

An Integrated Approach

- Occurrence survey
- Stakeholder issues
- Health effects / toxicology
- Analytical methods (Detection Limit)
- Ecological impact / transport & transformation
- Treatment technology
- Technology transfer

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information

State-of-the-Science

- Human Health Assessment Based on Recent EPA Guidelines
- Harmonized Approach to Noncancer and Cancer Toxicity Based on Mode of Action
- Ecological Assessment Based on Recent EPA Framework
- Risk Assessment Recognized as an Iterative Process
- Internal and External Peer Review

Required for All Recommended Studies

- Good Laboratory Practice Standards EPA (40 CFR Part 792)
- Animal housing and care based on Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and Guide for the Use of Laboratory Animals (NIH Publication No. 96-03, 1996)

Standard Operating Procedures

- **Protocol review by expert panel**
- **EPA testing guidelines**
- **Standardized QA / QC process**

EPA Risk Assessment Guidelines

- Principles and procedures to frame the conduct of risk assessments
- Promote consistency and technical quality of scientific inferences
- Flexible, full consideration to all relevant scientific information case-by-case
- Revised as experience and scientific consensus evolve

EPA Risk Assessment Guidelines

- **Developmental toxicity (1991)**
  FR 56(234): 63798 - 63826
- **Proposed guidelines for carcinogen risk assessment (1996)**
  FR 61: 17960-18011.
- **Reproductive toxicity (1997)**
  EPA No. EPA/630/R-96/009a
  NTIS PB97-100093
- **Neurotoxicity (1998)**
  EPA No. EPA/630/R-95/001Fa
  NTIS PB98-117831
- **Thyroid follicular cell tumors (1998)**
  EPA/630/R-97-002

Technical Hazard Characterization

- Integrates the data analysis of all relevant studies into weight-of-evidence conclusions
- Presents the exposure conditions (route, duration, pattern, magnitude) under which effects are expressed
- Presents the agent's mode of action

Mode of Action Provides Important Insight to Characterization of Toxicity

- A chemical's influence on the molecular, cellular, and physiological functions in producing tumors
- Helps interpret the relevancy of experimental animal data
- Guides choice of appropriate dose-response procedure (linear, non-linear, mixed)
- Platform to harmonize approaches to cancer and noncancer toxicity

Toxicity Study Review and Revised Harmonized "RfD" Assessment

- Review of existing and new toxicity data in experimental animals and humans
- Hazard identification
- Dose-response evaluation
  - Noncancer
    - Designation of effect levels (mathematical modeling or NOAEL / LOAEL procedure)
    - UF assignment
    - Uncertainty characterization - confidence statements
  - Cancer
    - Genotoxic or indirect

Revised Harmonized Oral Human Health Benchmark ("RfD")

- Data across comprehensive array of endpoints to establish target tissue
- Mechanistically-motivated special studies to characterize critical dose-response relationships
- Harmonized nonlinear approach to both cancer and noncancer assessment based on mode of action
- Future refinements as required by new data

Screening Ecological Assessment

- Limited in scope due to the set of data that could be generated in short time frame
- Scope nevertheless responsive to stakeholder concerns regarding lettuce
- Problem formulation focused on selection of assessment endpoints, derivation of conceptual model, and analysis
- Analysis revealed uncertainties and research needs identified to guide next tier of testing

Assessment Development Process

- Internal peer review (December 1998)
- External peer review (February1999)
- Review of additional pending data
- Response / revisions subsequent to external peer review
- Additional external peer review
- Submit final revised assessment to Integrated Risk Information System (IRIS) process

Risk Assessment is an Iterative Process

Continued Improvement

- Provisional RfD (1992, 1995) - Superfund Technical Support Center, NCEA-Cin
- Revised Harmonized Human Oral Benchmark "RfD" (December 1998) - NCEA
- External Peer Review (1999)
- IRIS Consensus Review
- Refinements as required in the future

Comprehensive Characterization

- Health and ecotoxicology assessment required as focal points of integrated approach
- Contemporary with progress in other areas
- Risk characterization precluded by LACK of accurate exposure surveys
- Identification of research needs and recommendations will improve path forward

Annie Jarabek
EPA, NCEA

Perchlorate: Mode-of-Action Approach to Human and Ecological Assessment

Annie M. Jarabek
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Perchlorate Peer Review Workshop
San Bernardino, California
February 10-11, 1999

I P S C

EPA Perchlorate Risk Assessment Team

- **Randy Bruins** (NCEA Cin) Ecotoxicology
- **Harlal Choudhury** (NCEA Cin) Genl tox / risk assess
- **Eric Clegg** (NCEA W) Reproductive toxicology
- **Kevin Crofton** (NHEERL) Neurotoxicology
- **Vicki Dellarco** (OW) Genetic toxicology
- **Andrew Geller** (NHEERL) Neurotoxicology
- **Annie Jarabek** (NCEA RTP) Dosimetry / risk assess
- **Gary Kimmel** (NCEA W) Developmental toxicology
- **Mary Manibusan** (OW) Genl tox / risk assess
- **Ralph Smialowicz** (NHEERL) Immunotoxicology
- **Glenn Suter** (NCEA Cin) Ecotoxicology

Acknowledgements

- EPA: Clarence Callahan, Allan Marcus, Kevin Mayer, Martha Moore, Ed Urbansky
- NIEHS: Errol Zeiger
- WPAFB (and its contracted laboratories): Lt. Col. William Baker, Jeff Fisher, Dave Mattie, Latha Narayanan, Lt. Col. Dan Rogers, Kyung Yu
- Brooks AFB (and its contracted laboratories): Cornell Long and Ron Porter
- Perchlorate Study Group (PSG) [and its contracted laboratories and consultants]: Mike Girard, TERA

Unique Attributes

- Partnership to develop data base
- Both human and ecological risk assessments of available data
- Harmonized approach to noncancer and cancer toxicity based on mode of action

Outline

- Human Health Assessment
  - Derivation Procedures
  - Mode of Action
  - Development of testing strategy
  - Results, issues, research needs
- Ecological Screening Assessment
  - Approach
  - Results, issues, research needs

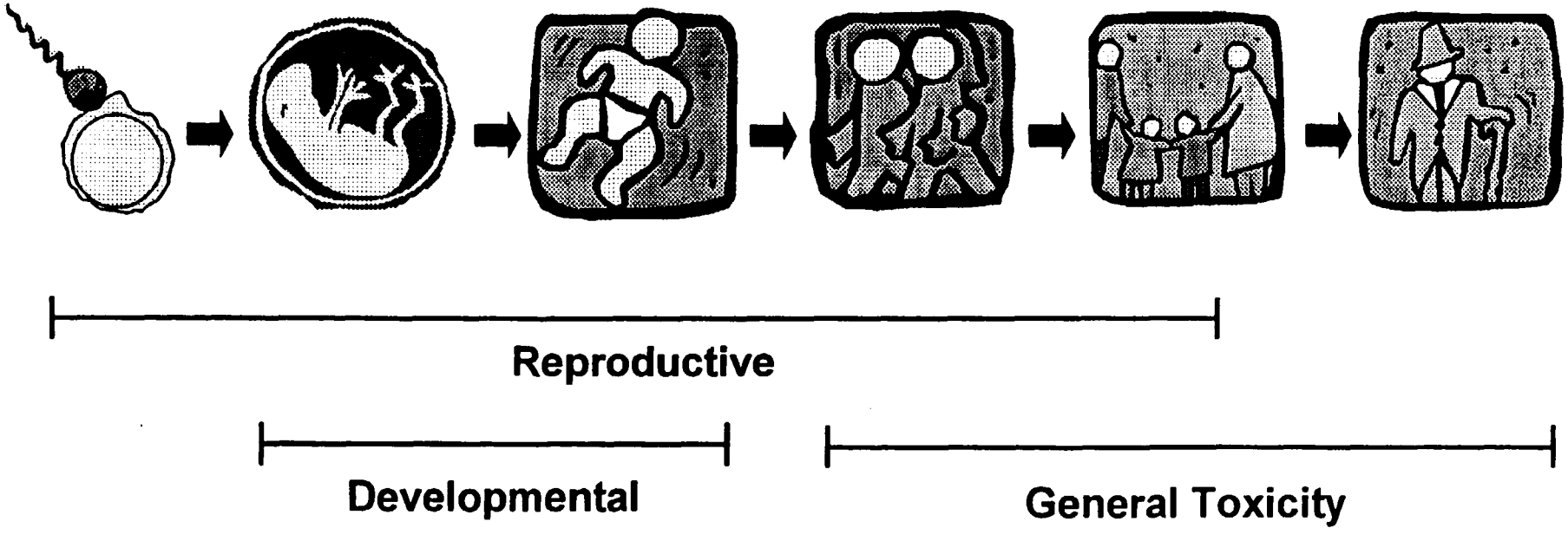
Outline

- Presentation of Data Received since December 1998
  - 1. Completed analyses
  - 2. Preliminary analyses
- Presentation of Ongoing Studies
  - 3. Pending data

Definition

An oral reference dose (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer health effects during a lifetime.

A High Confidence RfD is Based on Data that Addresses All Potentially Critical Life Stages.



Minimum Data Base for Derivation of an RfD

Mammalian Data Base**	Confidence	Comments
A. Two Chronic Oral Bioassays in Different Species B. One 2-Generation Reproductive Study C. Two Developmental Toxicity Studies in Different Species	High*	Minimum Data Base for High Confidence
One Subchronic Oral Bioassay	Low	Minimum Data Base for Estimation of an RfD

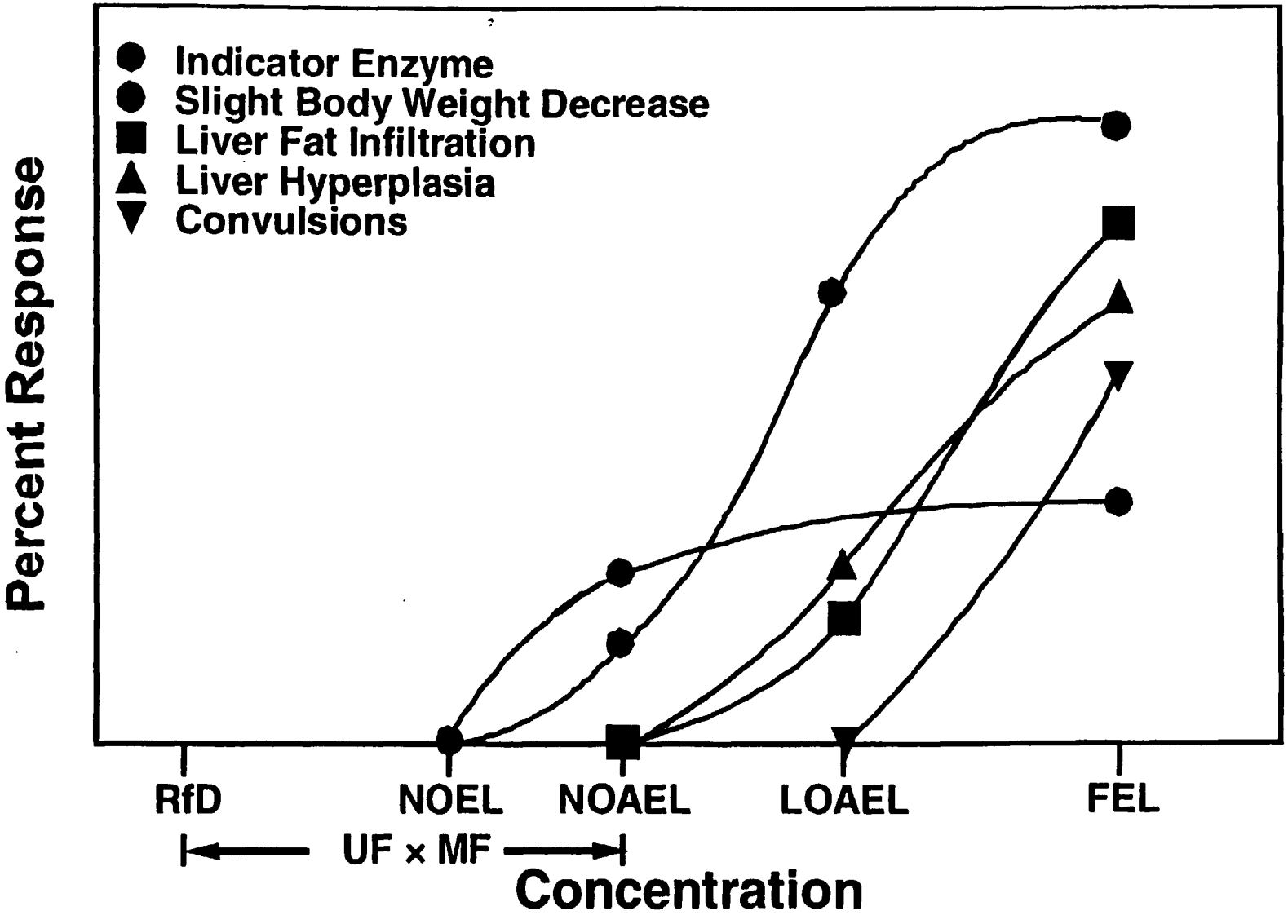
* Rationale is to address all potentially critical life stages

** Rationale is to use different species to evaluate variability in species sensitivity unless a particular laboratory animal model is more appropriate

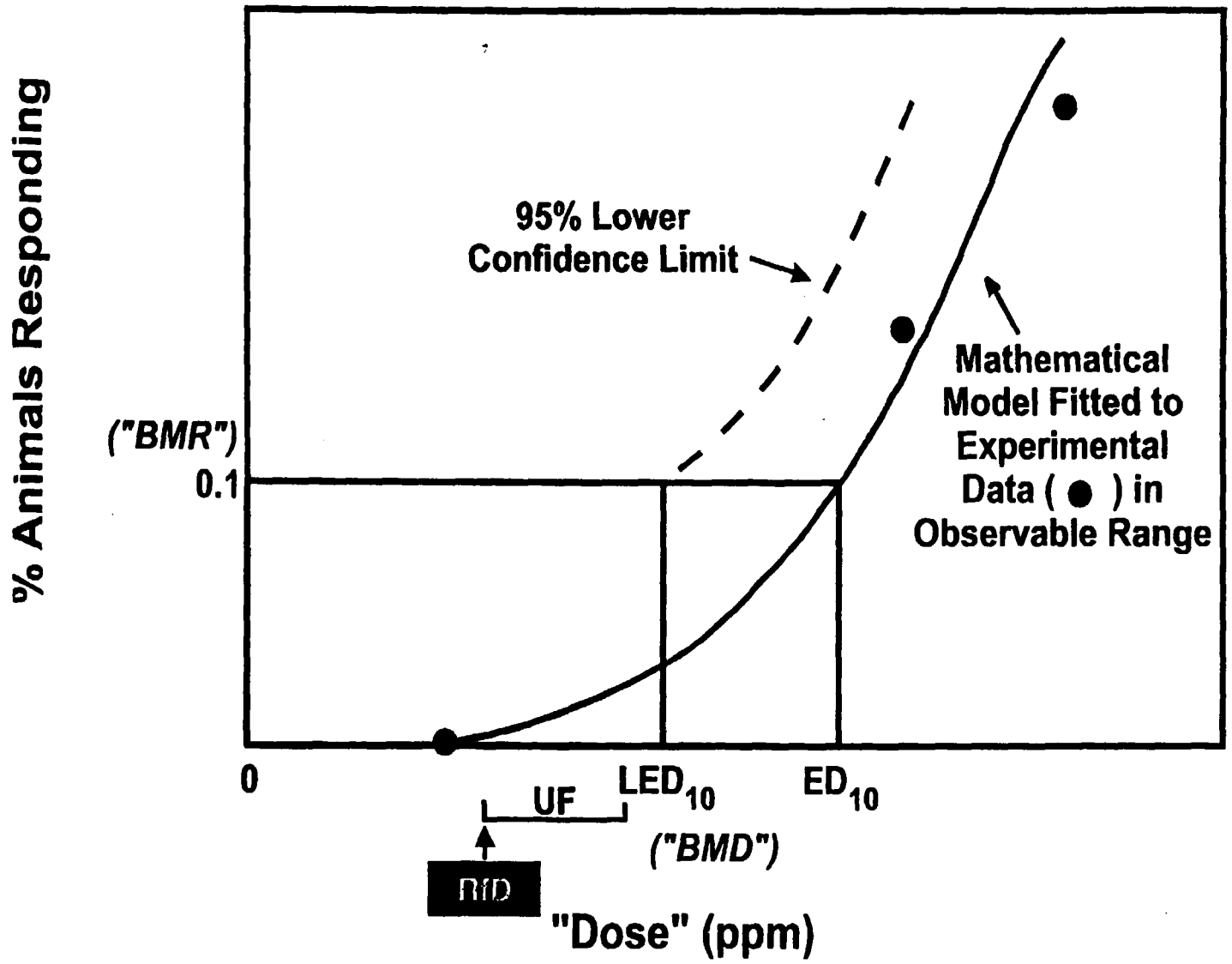
RfD Derivation

- Hazard identification and data array analysis
- Designation of effect levels (NOAEL, BMD)
- Choice of critical effect
- Dosimetric adjustment
- Application of uncertainty factors (UF)
- Characterization of uncertainty (confidence levels)

Data Array and Oral Reference Dose (RfD) Derivation



"Benchmark Dose" Approach to Dose-Response Analysis for Noncancer Endpoints



$$RfD = \frac{NOAEL^*[HED]}{UF \times MF}$$

Where:

NOAEL*[HED] =

The NOAEL or equivalent effect level obtained with an alternate approach (*), dosimetrically-adjusted to a human equivalent dose [HED].

UF =

Uncertainty factor(s) applied to account for the extrapolation required from the characteristics of the experimental regimen to the assumed human scenario, and

MF =

Modifying factor to account for scientific uncertainties in the study(ies) chosen as the basis for the operational derivation, e.g., poor statistical power or exposure characterization.

HAZARD ASSESSMENT

- Tumor data
- Mode of action related data (e.g., genotoxicity cell proliferation/death, physiological changes)
- Structural-activity relationships
- Toxicokinetic/dosimetry studies
- Toxicity and pathology findings
- Physical/chemical properties

TECHNICAL HAZARD CHARACTERIZATION

- Likelihood and conditions of human hazard
- Mode of action conclusion(s)

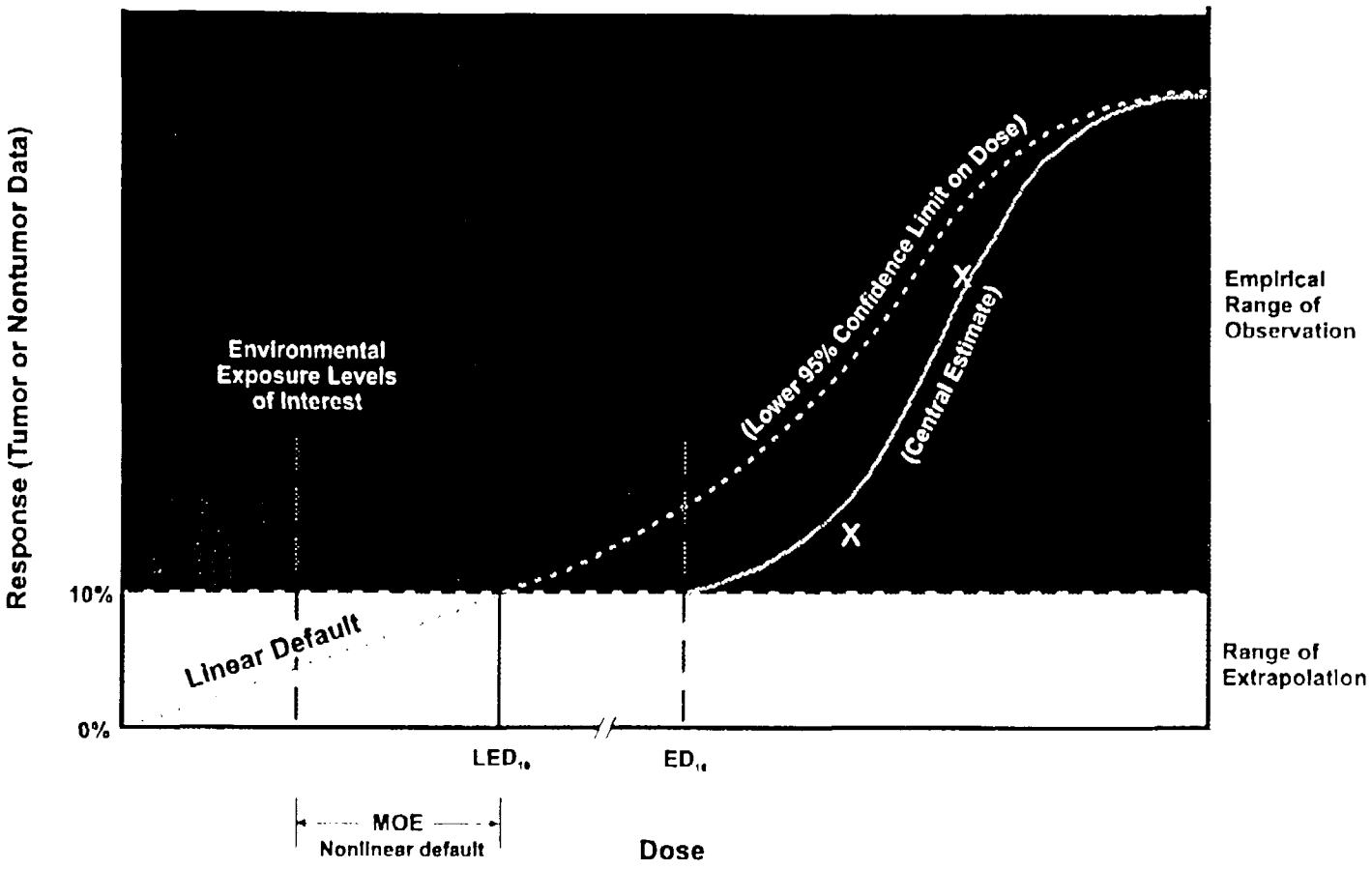
- Weight of evidence narrative and classification descriptors

Mode of action conclusion(s)

DOSE-RESPONSE ASSESSMENT

- Biologically based or case-specific models
- Linear default
- Nonlinear default
- Both linear and nonlinear defaults

Proposed EPA Cancer Guidelines
Extrapolation Approaches Based on Mode of Action



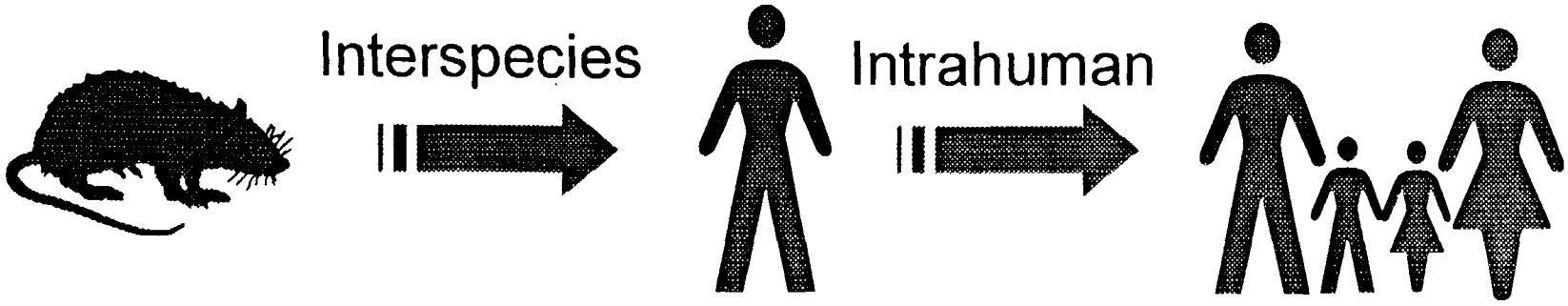
Factors for Uncertainties in Applied Extrapolations

10_H	Human to Sensitive Human
10_A	Experimental Animal to Human
10_S	Subchronic to Chronic Duration
10_L	LOAEL(HEC) to NOAEL(HEC)
10_D	Incomplete to Complete Data Base

Modifying Factor

MF Professional Assessment of Scientific Uncertainties of the Study and Data Base not Explicitly Addressed Above. Default for the MF is 1.0 e.g., applied for small sample size or poor exposure characterization.

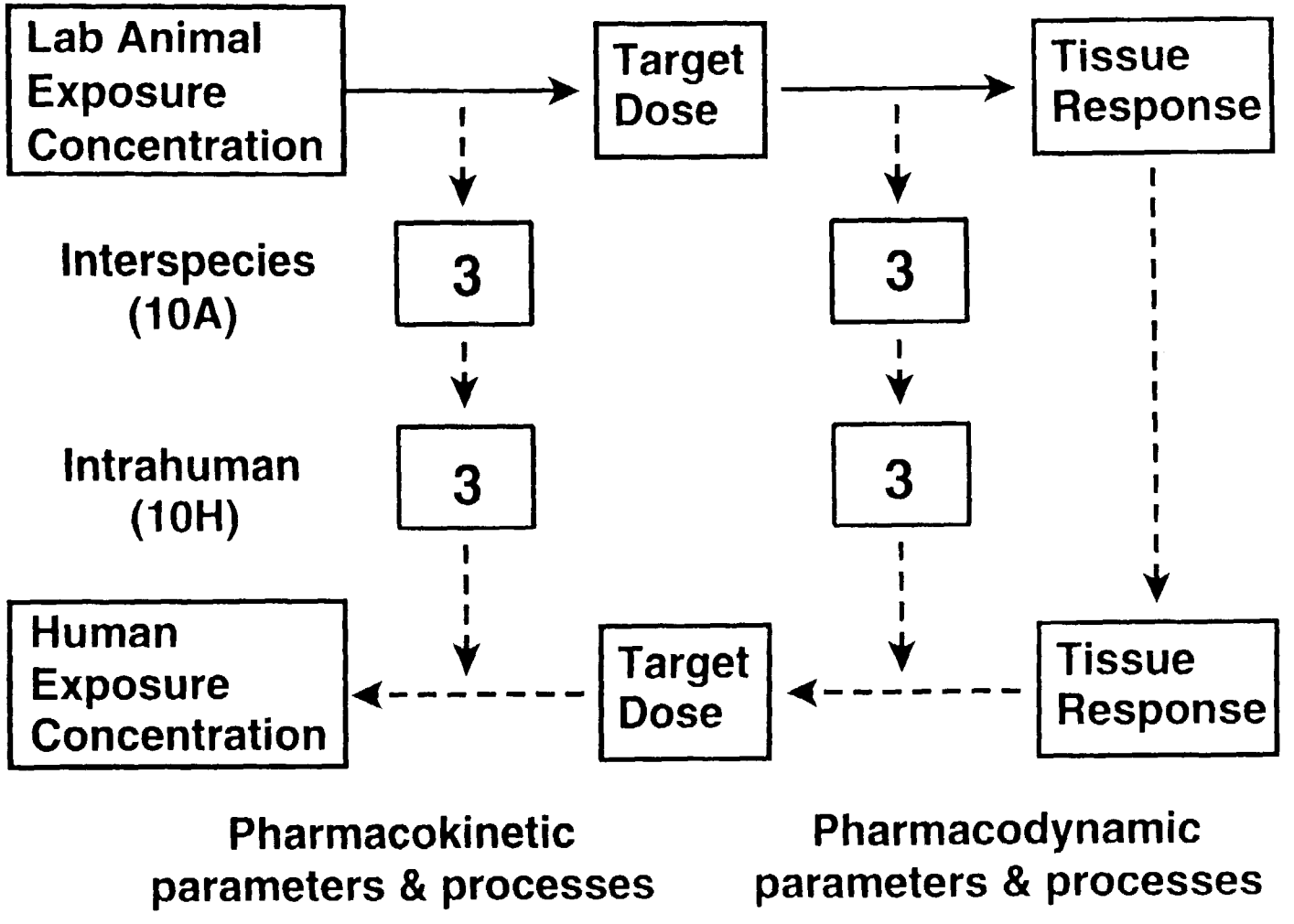
Extrapolation Uncertainties



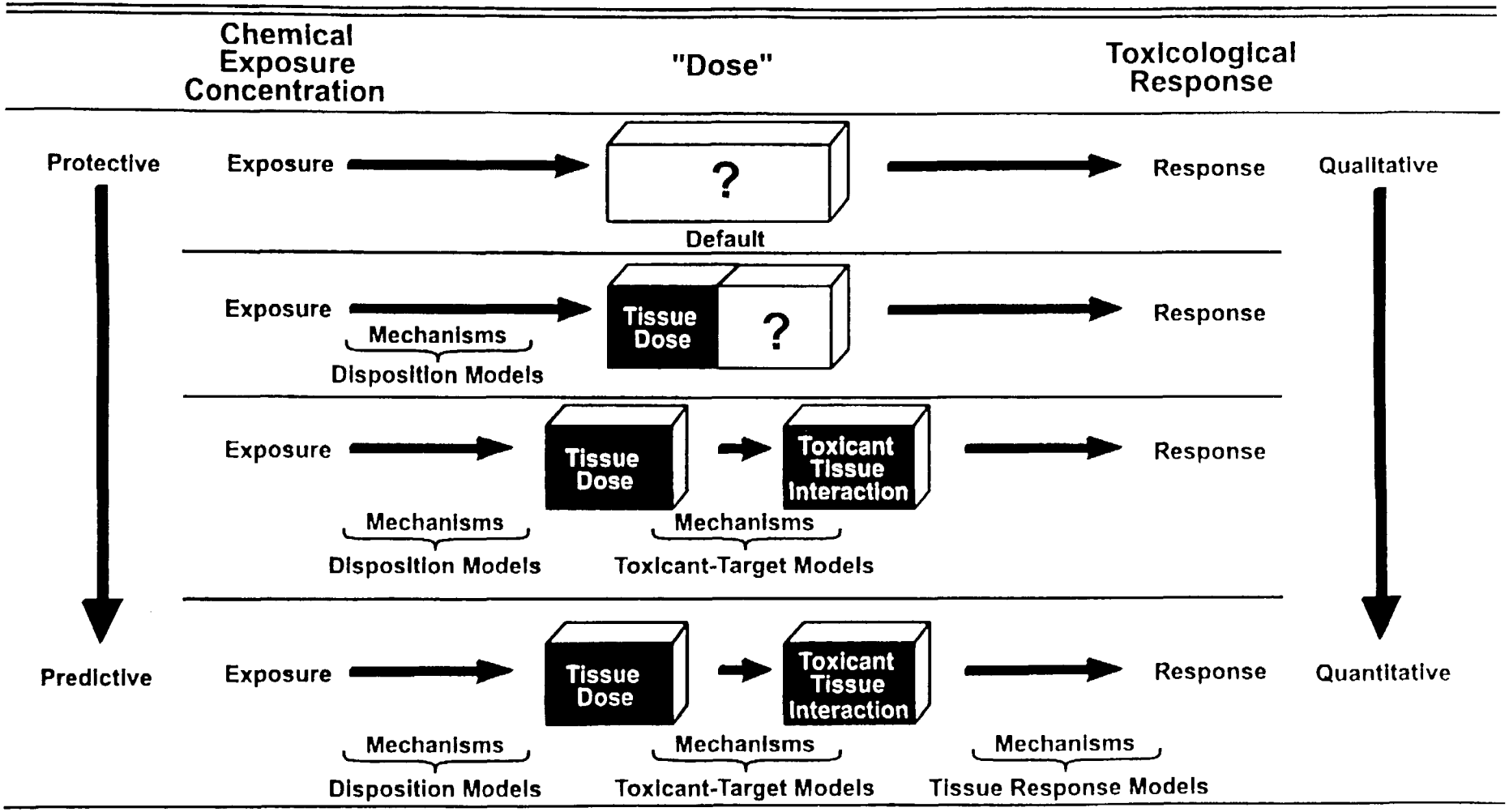
Rat to Human

Variability Across Humans

Schematic of Interspecies and Intrahuman UF Components Proposed for Perchlorate



Schematic Characterization of Comprehensive Exposure-Dose-Response Continuum and the Evolution of Protective to Predictive Dose-Response Estimates.



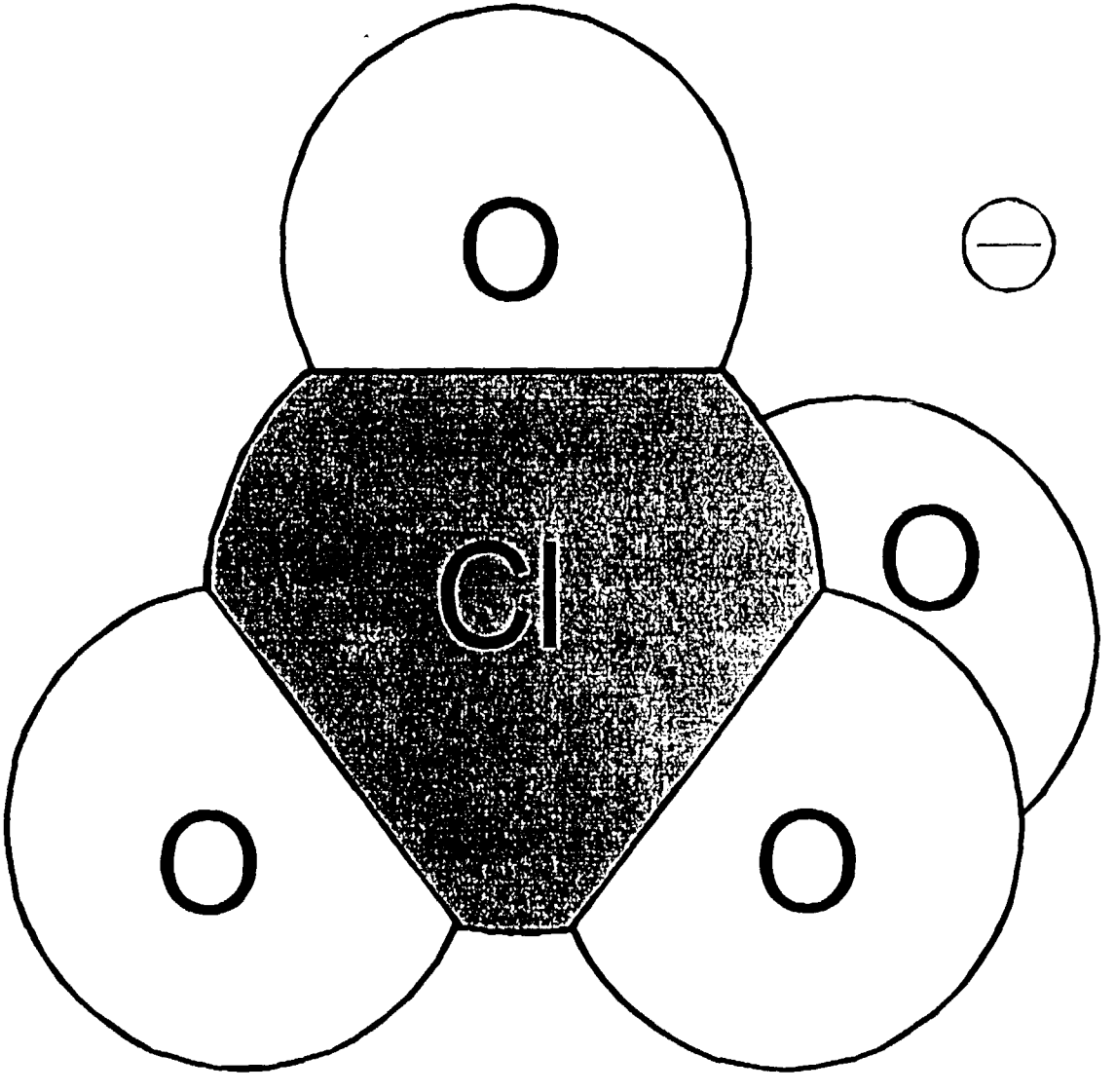
Provisional RfD Estimates

- **Principal study = Stanbury & Wyngaarden (1952)**
- **NOAEL = 0.14 mg/kg-day for 100% iodide release**
- **UF = 1000 (1992):**
  - intrahuman variability (10), less than chronic data (10), database deficiencies (10)
- UF = 300 (1995):
  - intrahuman variability (10), less than chronic data (10), database deficiencies (3)
- **Provisional drinking water action levels:**
  - **3.5 - 18 ppb based on 70 kg / 2 L water**

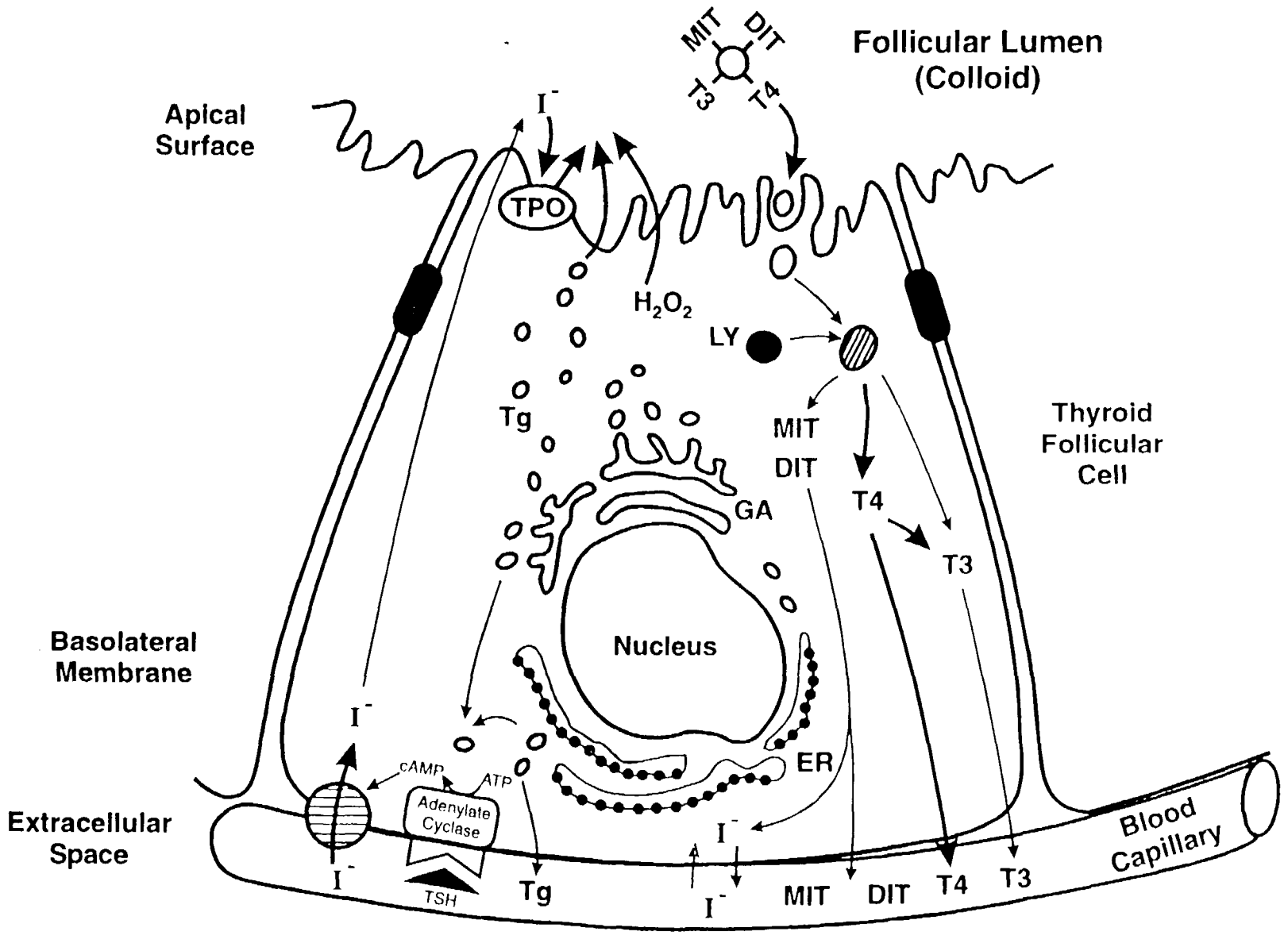
March 1997 TERA External Peer Review

- Same principal study, UF = 100:
  - intrahuman (3), subchronic to chronic(3), LOAEL to NOAEL (3), database deficiencies (3)
- Inadequate data base for RfD derivation
- Available mechanistic insights suggest special studies and synthesis strategy
- Eight (8) additional new categories of studies recommended

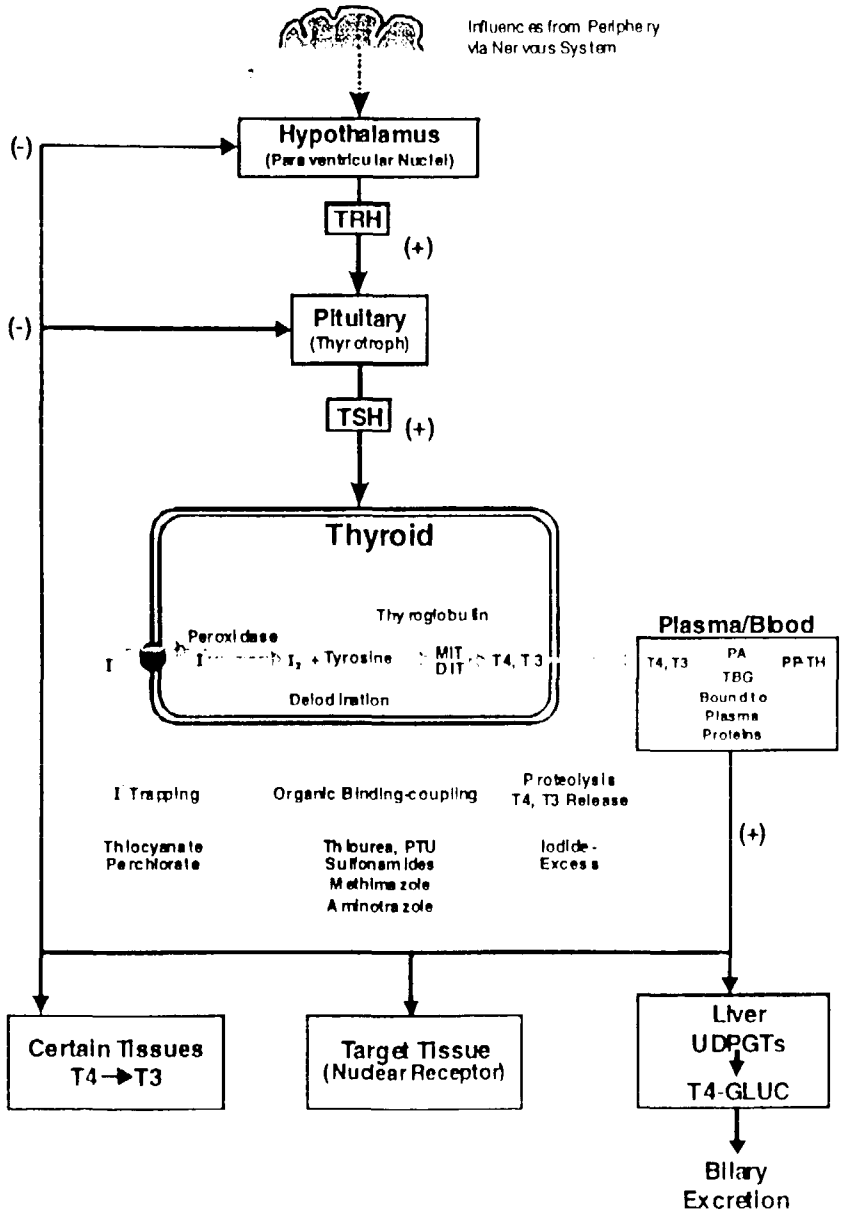
Chemical Structure of Perchlorate



Thyroid Hormone Biosynthesis and Secretion



Hypothalamic-Pituitary-Thyroid Axis and Feedback Mechanisms



INTERSPECIES AND INTRASPECIES DIFFERENCES IN THYROID STRUCTURE AND T3, T4, AND TSH HORMONES[a]

Parameter	Human	Rat
Thyroxine-binding globulin	Present	Essentially absent
T_4 Half-life	5 to 6 Days	0.5 to 1 Day
T_3 Half-life	1 Day	0.25 Day
T_4 Production rate/kg body weight	1 ×	10 × that in humans
TSH	1 ×	6 to 60 × that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		
Serum TSH	M = F	$M^d \leq 2 \times F^e$
Cancer sensitivity	F = 2.5 × M	M > F

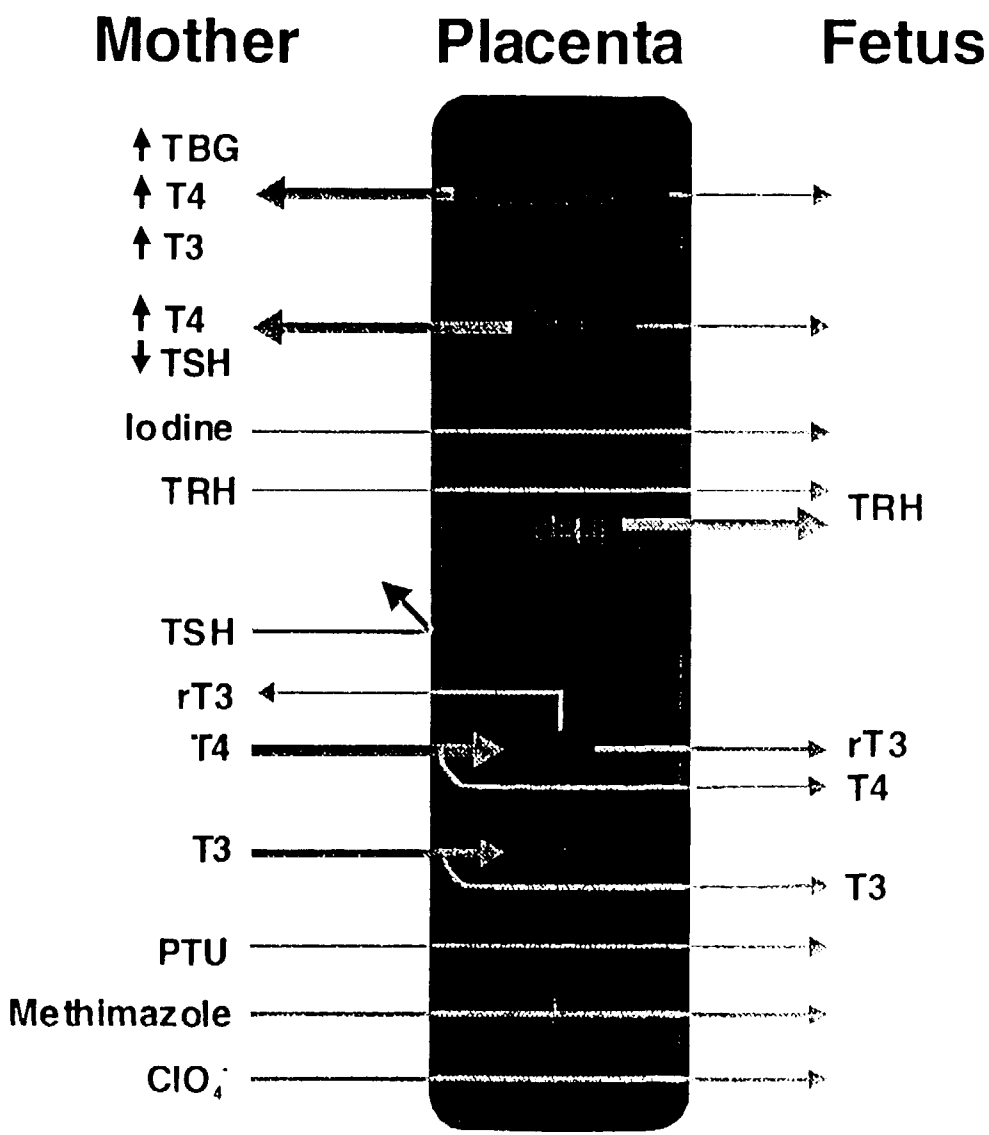
[a]M = male, F = female.

Source: U.S. Environmental Protection Agency (1998a).

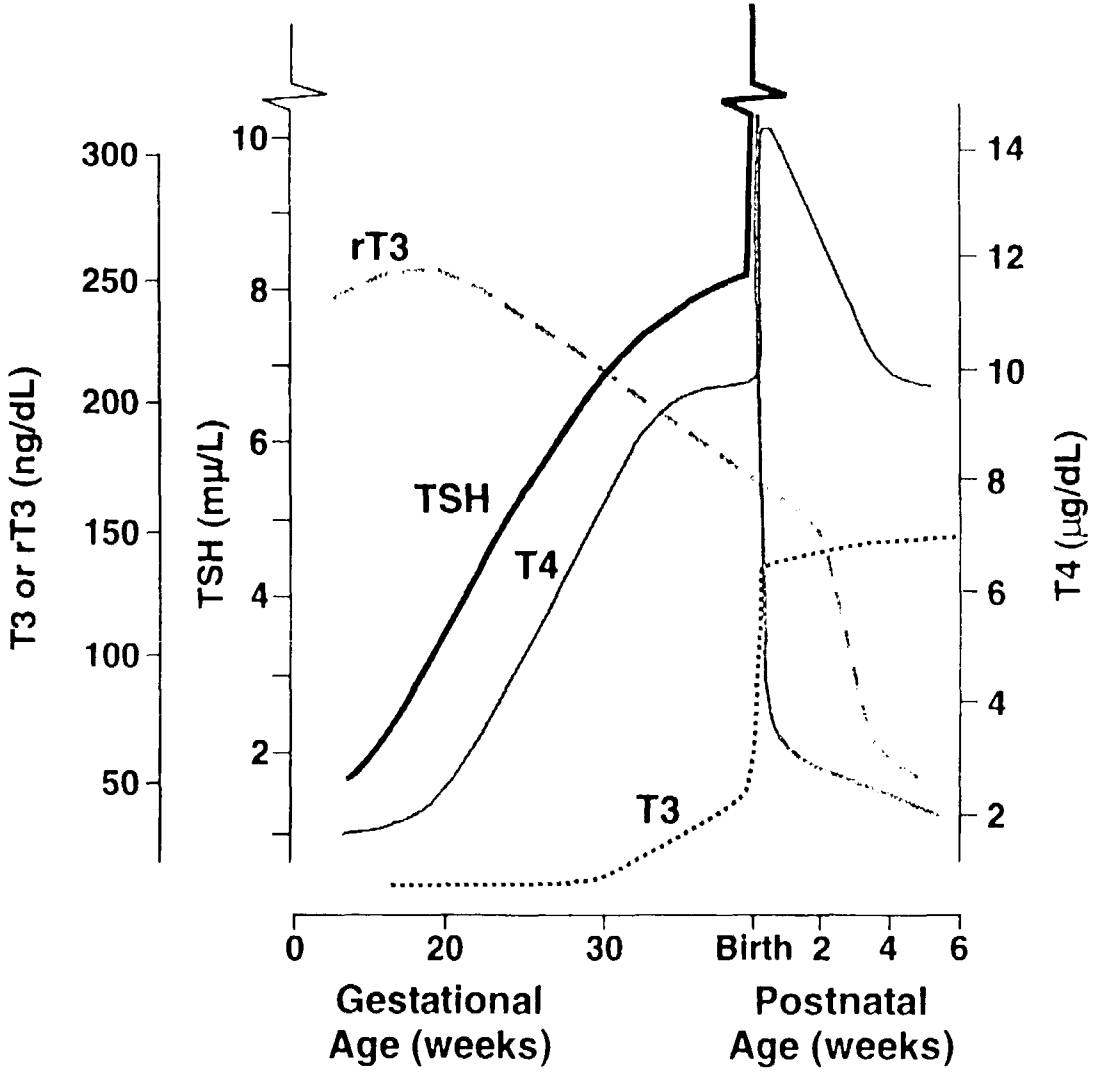
Main Symptoms and Effects of Hypothyroidism

Developmental *(Transient disruption leads to permanent effects.)*	Adult *(Transient disruption leads to transient effects.)*
• Delayed reflex ontogeny	• Run down, slow, depressed
• Impaired fine motor skills	• Sluggish, cold, tired
• Deaf-mutism, spasticity	• Dryness and brittleness of hair
• Gait disturbances	• Dry and itchy skin, constipation
• Mental retardation	• Muscle cramps
• Speech impairments	• Increased menstrual flow
	• Thyroid tumors in rodents

Role of Placenta in Human Thyroid Hormone Metabolism



Pattern of Change in Human Fetal and Neonatal Function Parameters During Pregnancy and Postnatal Periods



Mechanisms of Anti-Thyroid Mediated Neoplasia in Rodents

- DNA Directed:
  - X - rays
  - 131 I
  - Genotoxic chemicals
- Indirect
  - Partial thyroidectomy
  - Transplantation of TSH-secreting pituitary tumors
  - Iodide deficiency
  - Chemicals inhibiting iodide uptake
  - Chemicals inhibiting thyroid peroxidase
  - Chemicals inhibiting TH
  - Chemicals inhibiting conversion of T3 & T4
  - Chemical inhibiting hepatic thyroid hormone metabolism and excretion

Proliferative Lesions Thyroid Follicular Cells in Rodents

Morphologic Continuum →

Normal

Hyperplasia

Adenoma

Carcinoma

Significance in Risk Assessment

Deficiencies of 1997 Data Base

- Human Clinical Studies
  - Adult subjects
  - Typically subjects with thyroids altered by disease or other treatments
  - Few pregnant subjects
  - Acute or short-term exposure duration
  - Limited range of doses
- Laboratory Animal Studies
  - Limited range of doses
  - Dated
- Additional Target Tissues Suggested
  - Reproductive function
  - Immunotoxicity (aplastic anemia, leukopenia)

Mode of Action Provides Important Insight to Characterization of Toxicity

- A chemical's influence on the molecular, cellular, and physiological functions in producing tumors
- Prolonged depression of TH causes a feedback that leads to upregulation of TSH which leads to thyroid gland hyperplasia
- Genotoxic?

Existing Data Summary

- Target tissue appears to be the thyroid but available testing not comprehensive across endpoints
- Anti-thyroid effects would differ among adult versus developing fetus, children
- Anti-thyroid effects associated with benign neoplasia development in rats; a nonlinear process
- Genotoxicity not characterized
- Relevancy to human risk of rat tumors not established; presumed protective

Recommended Studies

- 90-Day subchronic bioassay
- Developmental neurotoxicity study
- Genotoxicity assays
- Mechanistic studies
- ADME - Absorption, Distribution, Metabolism and Elimination
- Developmental study
- 2-Generation reproductive toxicity study
- Immunotoxicity

Developmental Study in Rabbits (Argus, 1998c)

- 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day
- Developmental endpoints
  - Fetal NOAEL > 100 mg/kg-day
- Thyroid histopathology
  - Maternal NOAEL and LOAEL for hypertrophy at 1.0 and 10 mg/kg-day
- Maternal hormone analyses
  - EPA analysis designates LOAEL for T4 at 0.1 mg/kg-day
  - Lack of effect on T3 and TSH

2-Generation Reproductive Study (Argus, 1998b)

- 0, 0.3, 3.0 and 30 mg/kg-day (30/sex/group)
- Maternal organ weights
  - Thyroid increased at 3.0 and 30 in males and 30 mg/kg-day in females
  - Possibly also in pituitary
- Reproductive parameters
  - Hints of effects in the 0.3 mg/kg-day group in the mating, fertility, estrous cycle, ovarian weights

Preliminary Analyses

Analyses Submitted on 2/1/99 or 2/8/99

2-Generation Reproductive Study

- Thyroid weights and ratios
  - Stat sig in F1 pups males at 3.0 and 30 mg/kg-day; in females also at 0.3 mg/kg-day
- Histolopathology incidence and severity dose-related only in thyroid: hypertrophy, hyperplasia, decrease in colloid
  - P1 both sexes at all dose groups
  - F1 pups at 3.0 and 30 mg/kg-day
  - Require additional analyses

Analyses Submitted on 2/1/99 or 2/8/99

2-Generation Reproductive Study

- Hormone data for F0 and F1
  - No apparent trend
  - Require additional analyses
- Reproductive parameters (sperm morphology and estrous cyclicity)
  - Effects suggested in P1 not replicated in F1

- Final Audited Report due: 3/5/99

Immunotoxicity Studies at Medical University of South Carolina

(14-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"C, G, I, J, T, K"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured. Supplementary experiments were needed to acquire additional serum samples for hormone analysis or to repeat the NK assay.
"U, V"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and were challenged with listeria to assess **delayed type hypersensitivity**.
"H, F, M"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
SRBC Experiments	B6C3F1 female mice (two experiments of 30 mice each) were exposed to **90 days of AP** (0, 0.1, 3.0, or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 75, bled on day 79 to determine specific IgM antibody levels, and bled on day 90 to determine specific IgG antibody levels.

Immunotoxicity Studies at Medical University of South Carolina (90-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"A, D, N"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of immune, hematological, or thyroid parameters were measured. In experiments "A" and "D", thyroid histopathology was performed. Experiment "N" included a variety of other parameters: macrophage phagocytosis and nitrite production, NKh, assay organ weights and cellularities, flow cytometry, and serum for hormone analysis.
"B, E"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured.
"P"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
"Q"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with **B16F10 melanomas** on day 76.
"L"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with ***Listeria monocytogenes*** on day 86.
SRBC Experiments	B6C3F1 Female mice (1 experiment of 30 mice) were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0 or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 9 and bled on day 14 to determine specific IgM antibody levels.

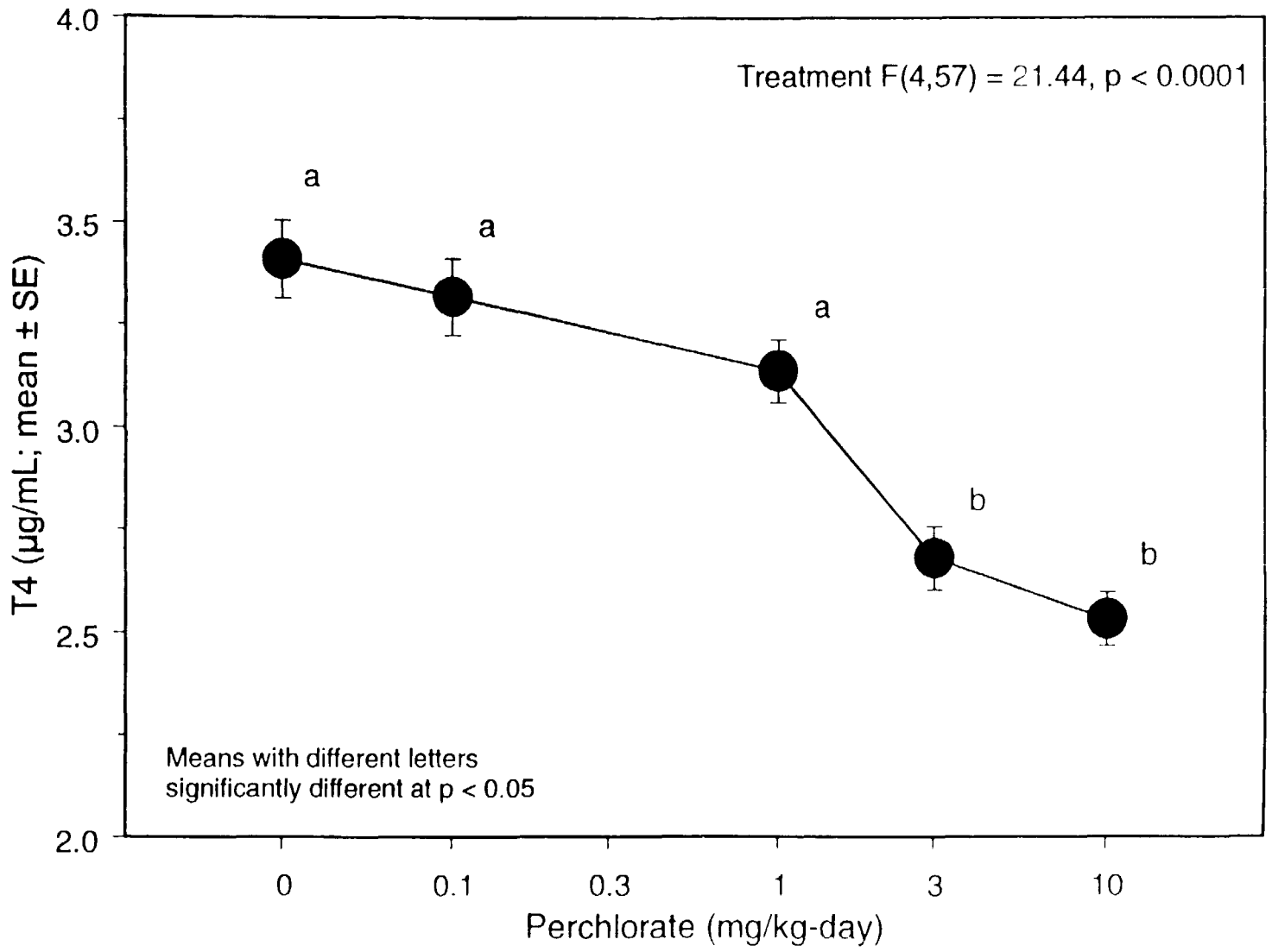
Preliminary Analyses

Analyses Submitted on 2/1/99 or 2/8/99

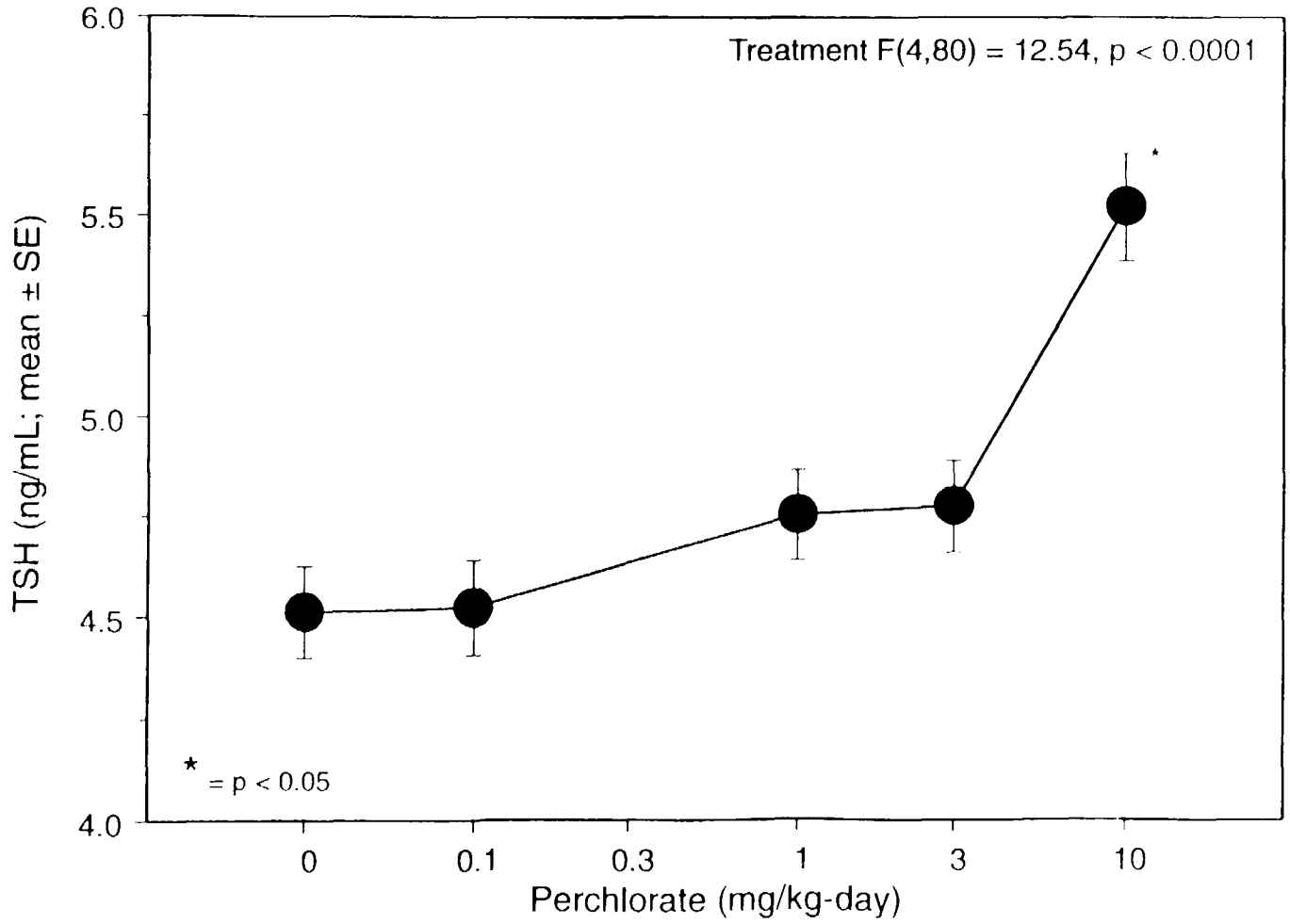
Immunotoxicity Studies

- Thyroid histopathology (Warren, 1999) in mice from 90-day (2 experiments) in immunotoxicity study @ MUSC
  - Control (0%) and 30 mg/kg-day high dose (100%); additional dose groups pending
  - Hypertrophy, hyperplasia, colloid depletion, congestion
  - Severity scores not provided
  - Likely useful to compare with 30 mg/kg-day dose of 2-generation reproductive
- 14-day and 90-day SRBC assays
  - Negative

Effects on Serum Total T4 in F1 Pups on PND5 (Data of Argus, 1998a)



Effects on Serum TSH Pups on PND5 (Data of Argus, 1998a)

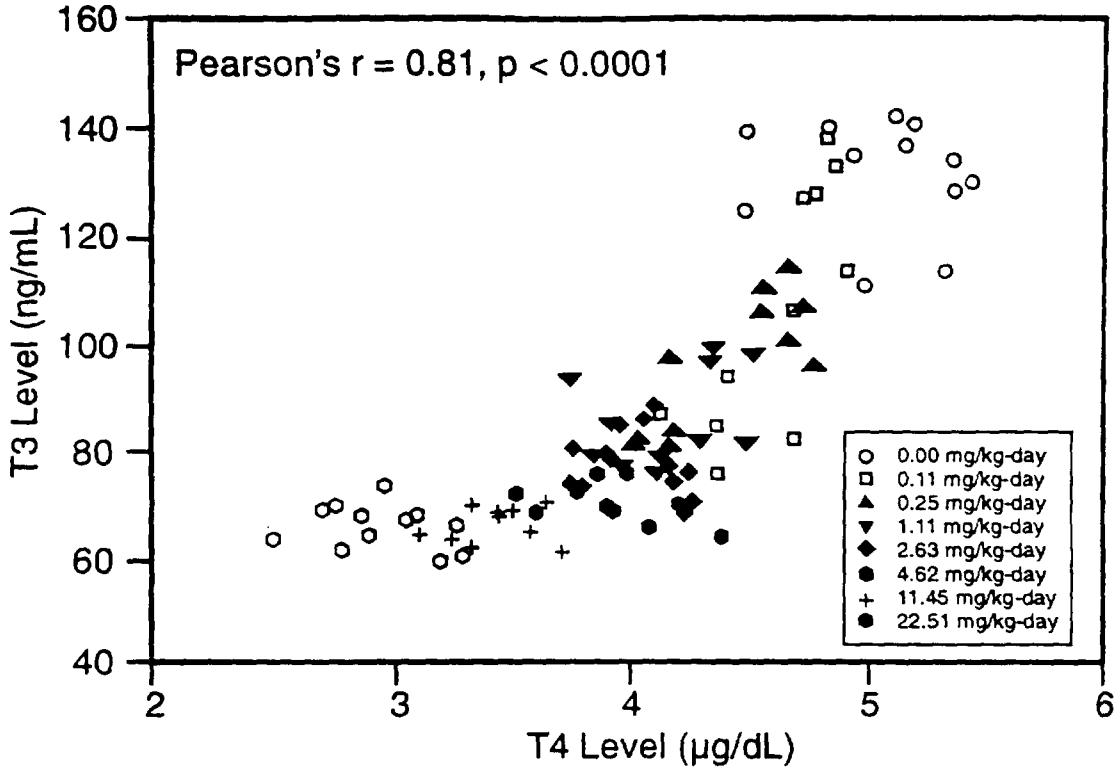


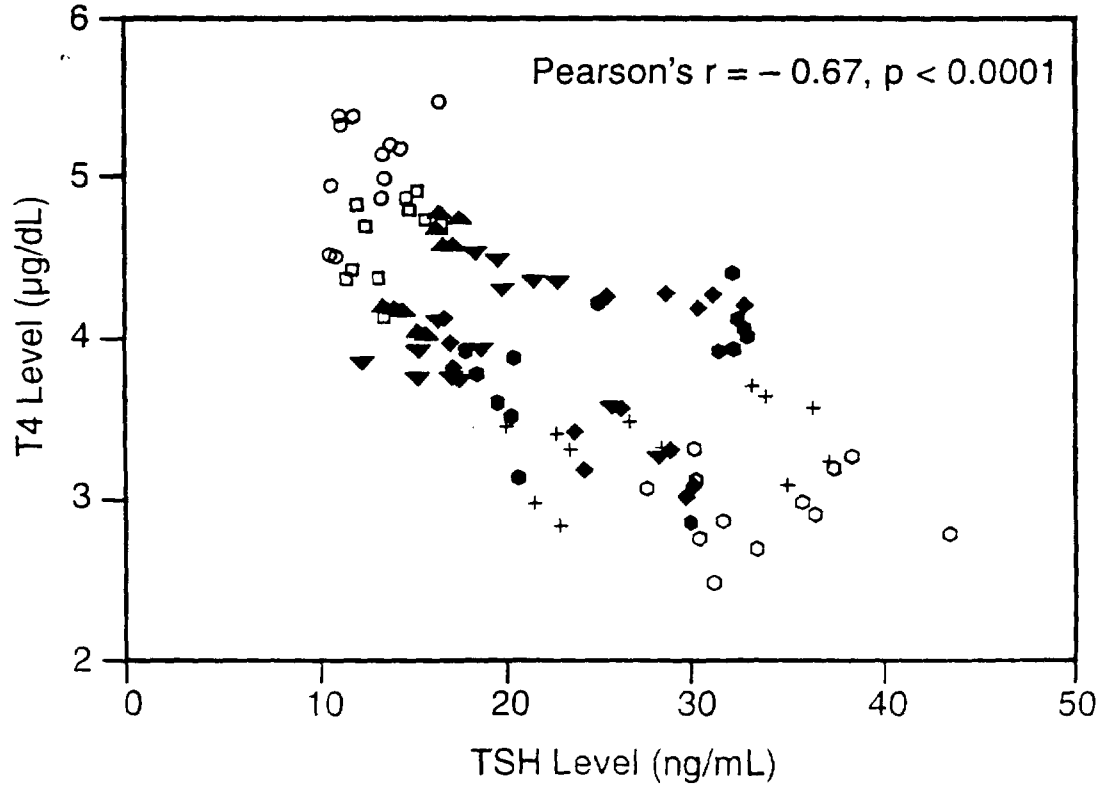
SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS

(Green cells designate NOAELs; Purple cells LOAELs; ✓ = dose tested)

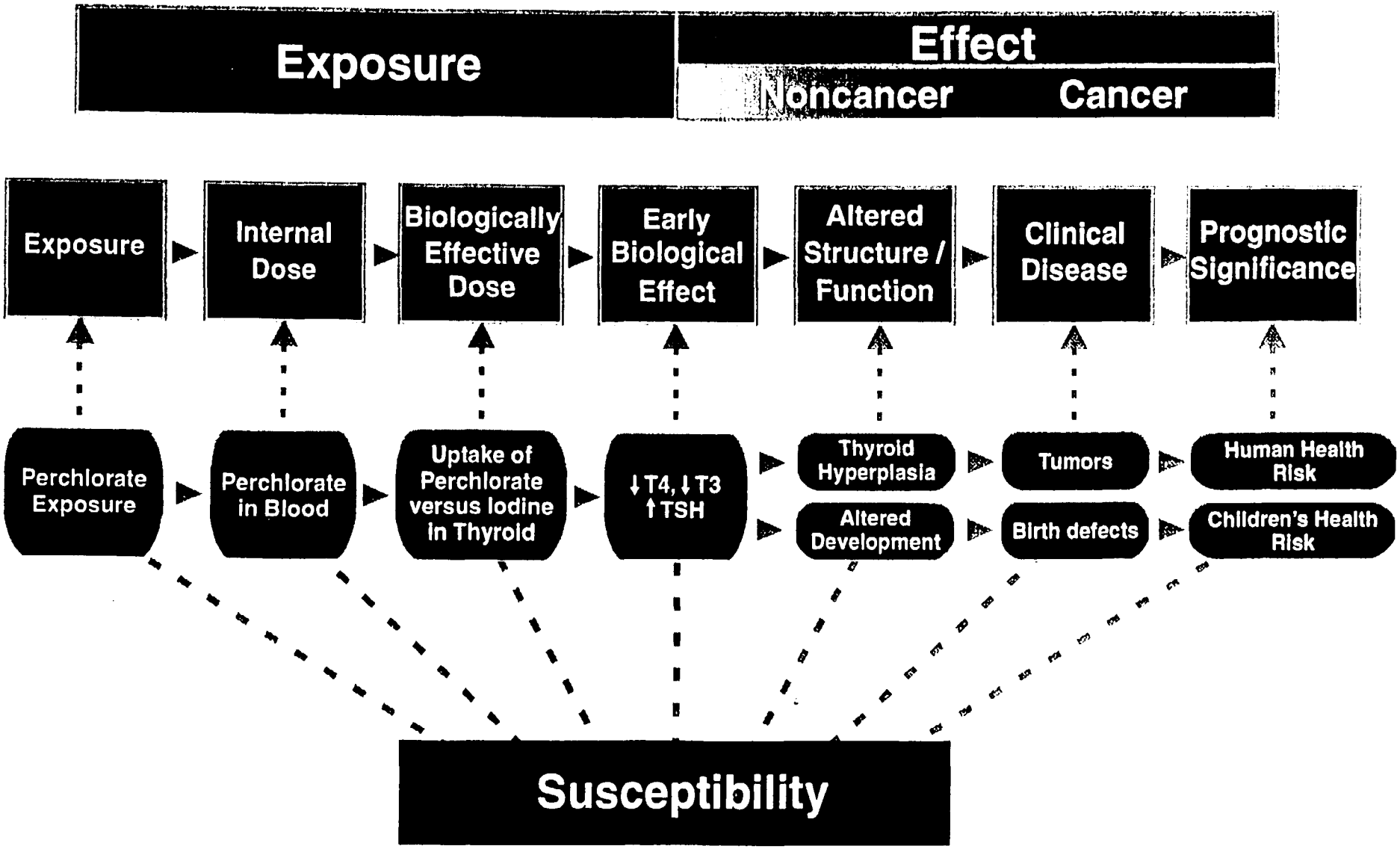
Study/Species	Dose Duration/ Age Tested	Sex	Effects	DOSES (mg/kg/day) 0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	T3	✓			F								
			rT3				M	✓					✓	✓	✓
			T4 hTG	✓											
			TSH	✓			F			M					
			Hist	✓											
							SH-FLS			MH-FLS					
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	T3	✓										M	
			T4	✓	✓	✓		✓			✓				
			TSH	✓	F	M									
			Hist	✓	✓	✓		✓			SH-FCH Thy-wt				
	90 days		T3	✓											
			T4	✓											
			TSH	✓	✓	M									
			Hist	✓	✓	✓		✓			SH-FCH Thy-wt				
	120 days		T3	✓							✓				
			T4	✓											
			TSH	✓		✓					✓			✓	
			Hist	✓		✓					✓			✓	

CORRELATIONS BETWEEN T3 AND T4 OR T4 AND TSH IN RATS FROM 14-DAY STUDY (CALDWELL ET AL., 1995)

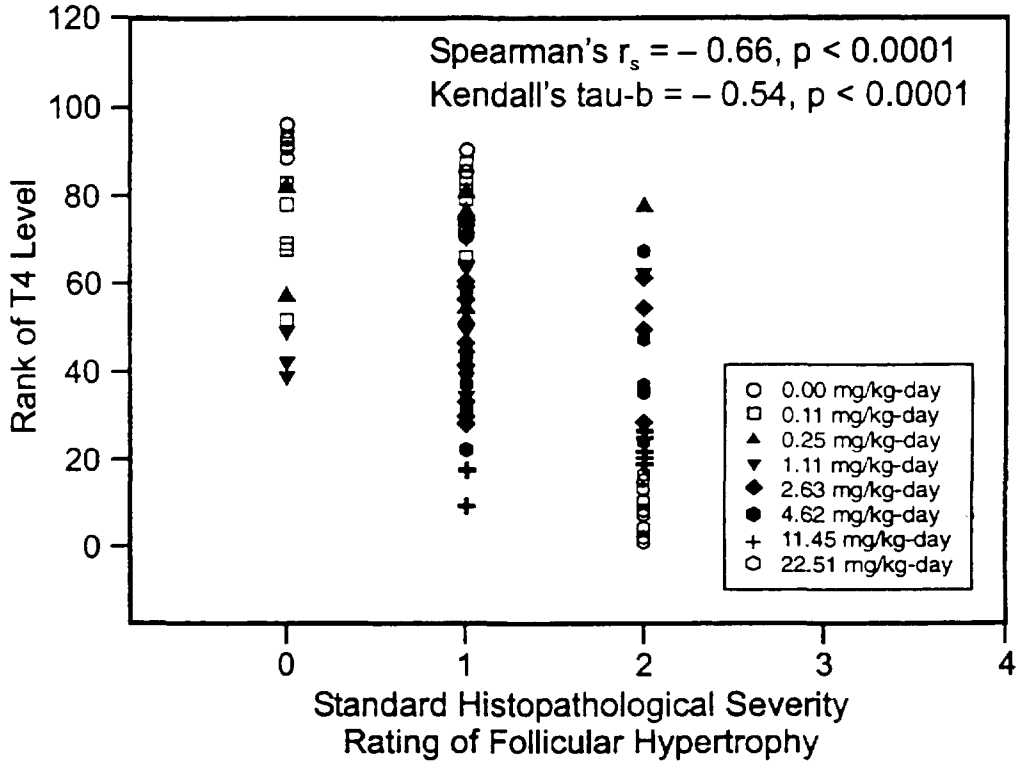


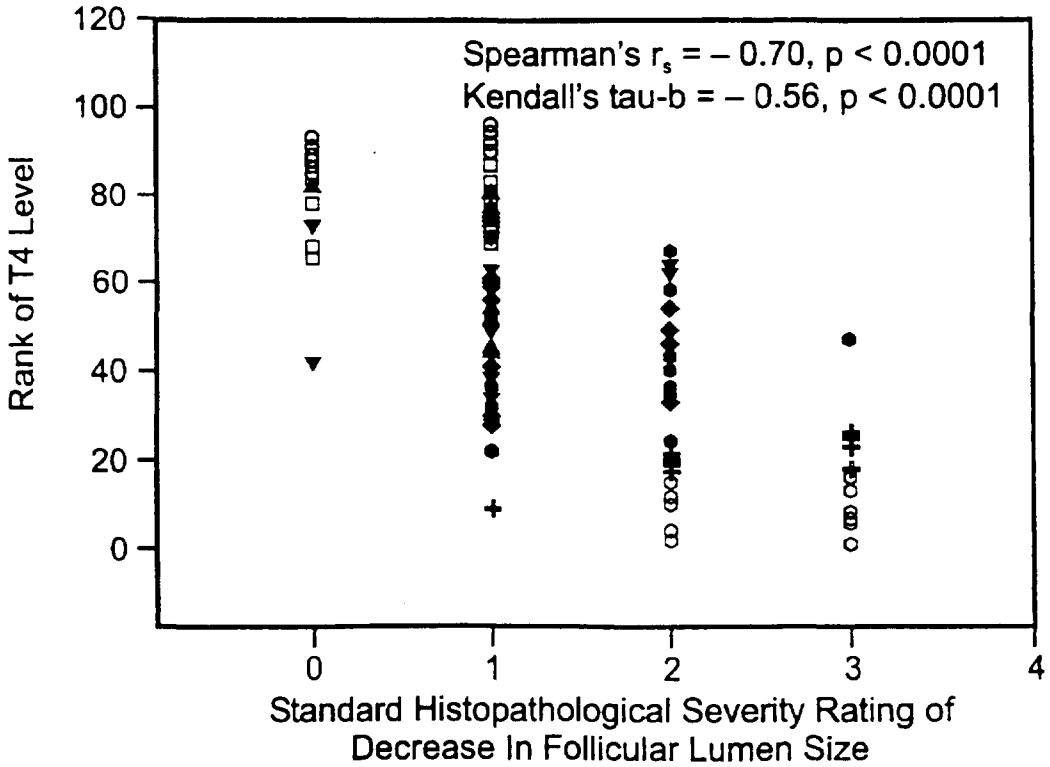


Proposed Mode-of-Action Model for Risk Assessment of Perchlorate

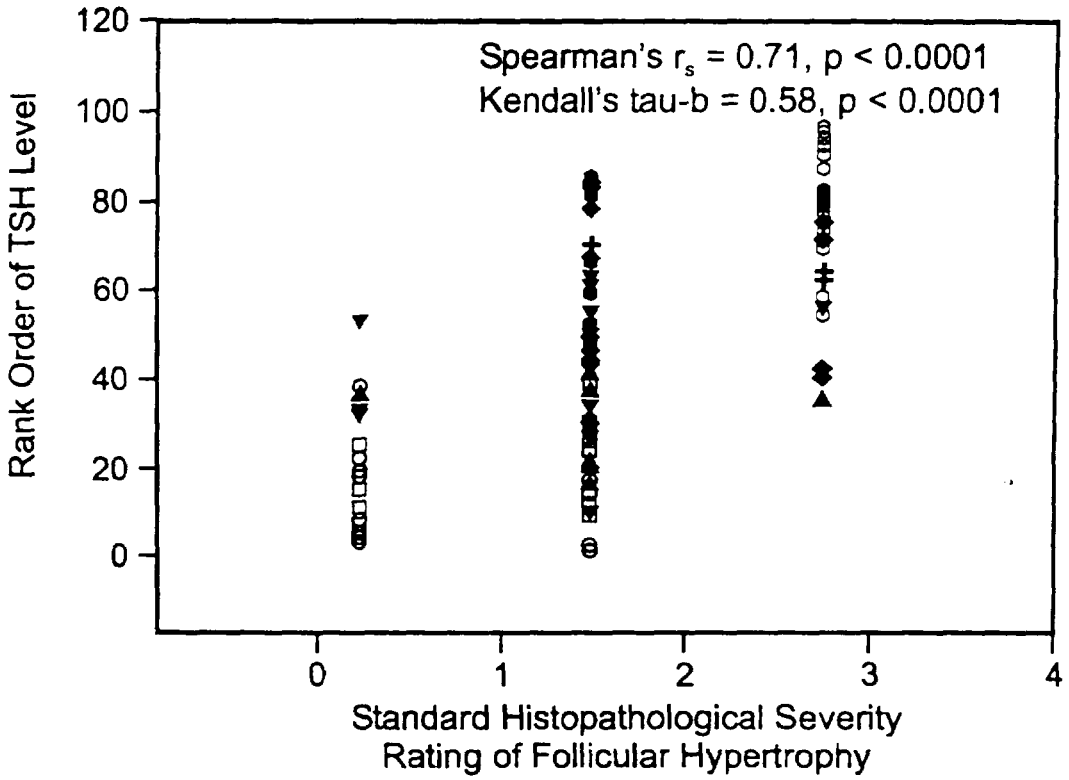


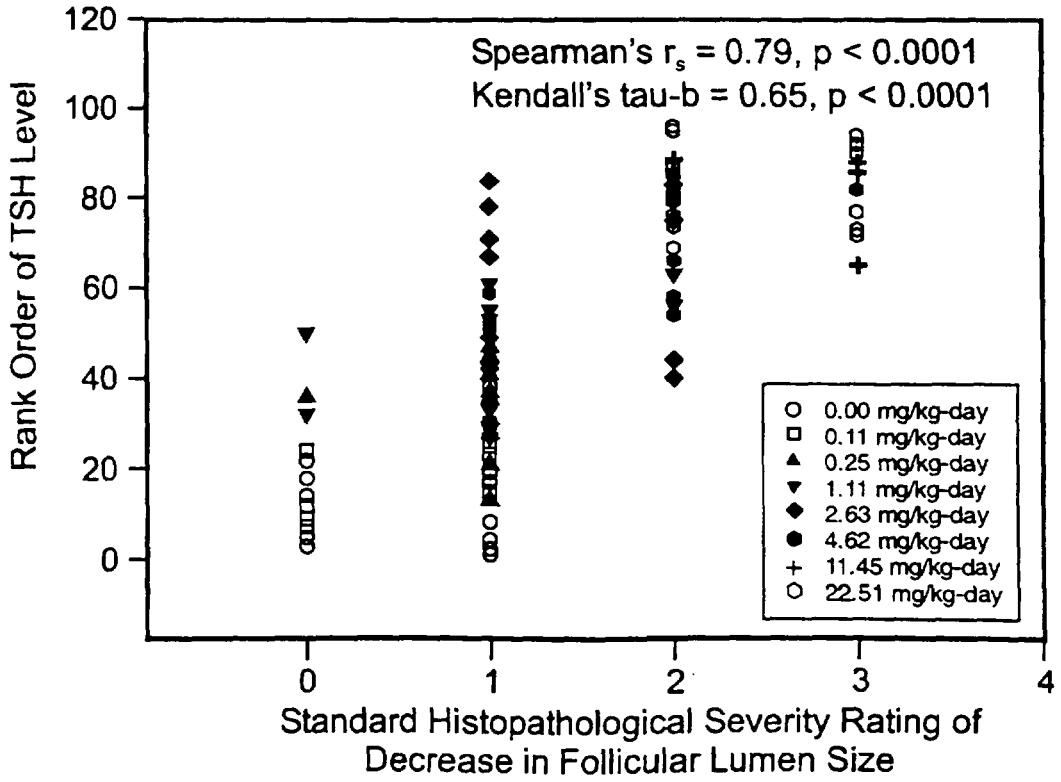
CORRELATIONS BETWEEN RANK ORDER OF 54 AND STANDARD HISTOPATHOLOGY SEVERITY RATING FOR LESIONS IN THYROIDS OF RATS FROM 14-DAY STUDY (CALDWELL ET AL., 1995)





CORRELATIONS BETWEEN RANK ORDER OF TSH AND STANDARD HISTOPATHOLOGY SEVERITY RATINGS FOR LESIONS IN THYROIDS OF RATS FROM 14-DAY STUDY (CALDWELL ET AL., 1995)





SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS

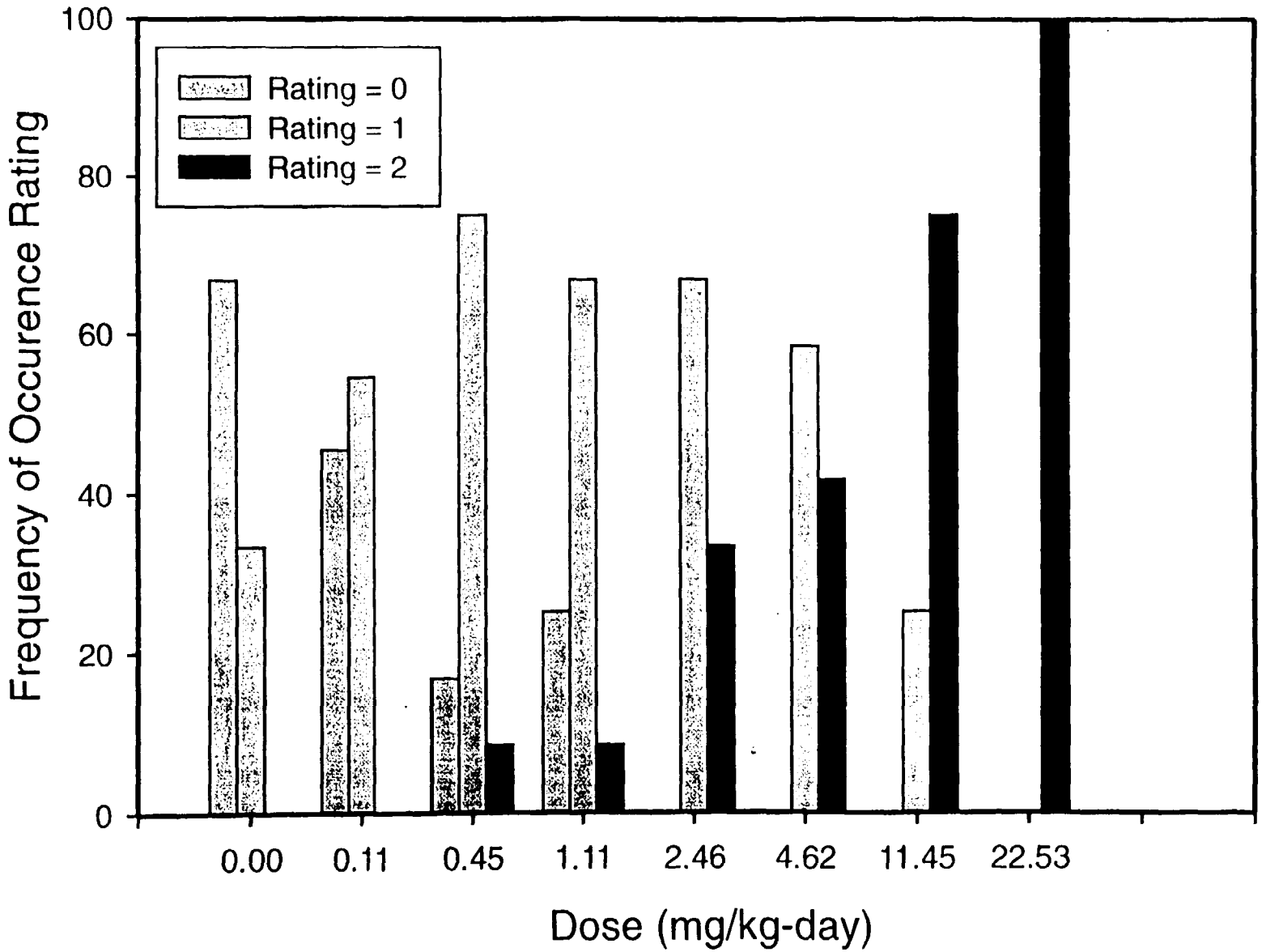
(Green cells designate NOAELs; Purple cells LOAELs; ✓ = dose tested)

Study/Species	Dose Duration/ Age Tested	Sex	DOSES (mg/kg/day)												
			Effects	0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	T3	✓											
			T4	✓											
			TSH	✓											
			Hist	✓			✓				SH TH				
	F1: PND5	M&F	T3	✓			✓								
			T4	✓			✓				✓				
			TSH	✓			✓				✓	✓			
			Hist	✓											
			Hist				✓				MH				
	F1: PND90	M&F	T3	ND							ND	ND		ND	
			T4	ND							ND	ND		ND	
			TSH	ND							ND	ND		ND	
			Hist[4]	✓							✓	20%		80%	

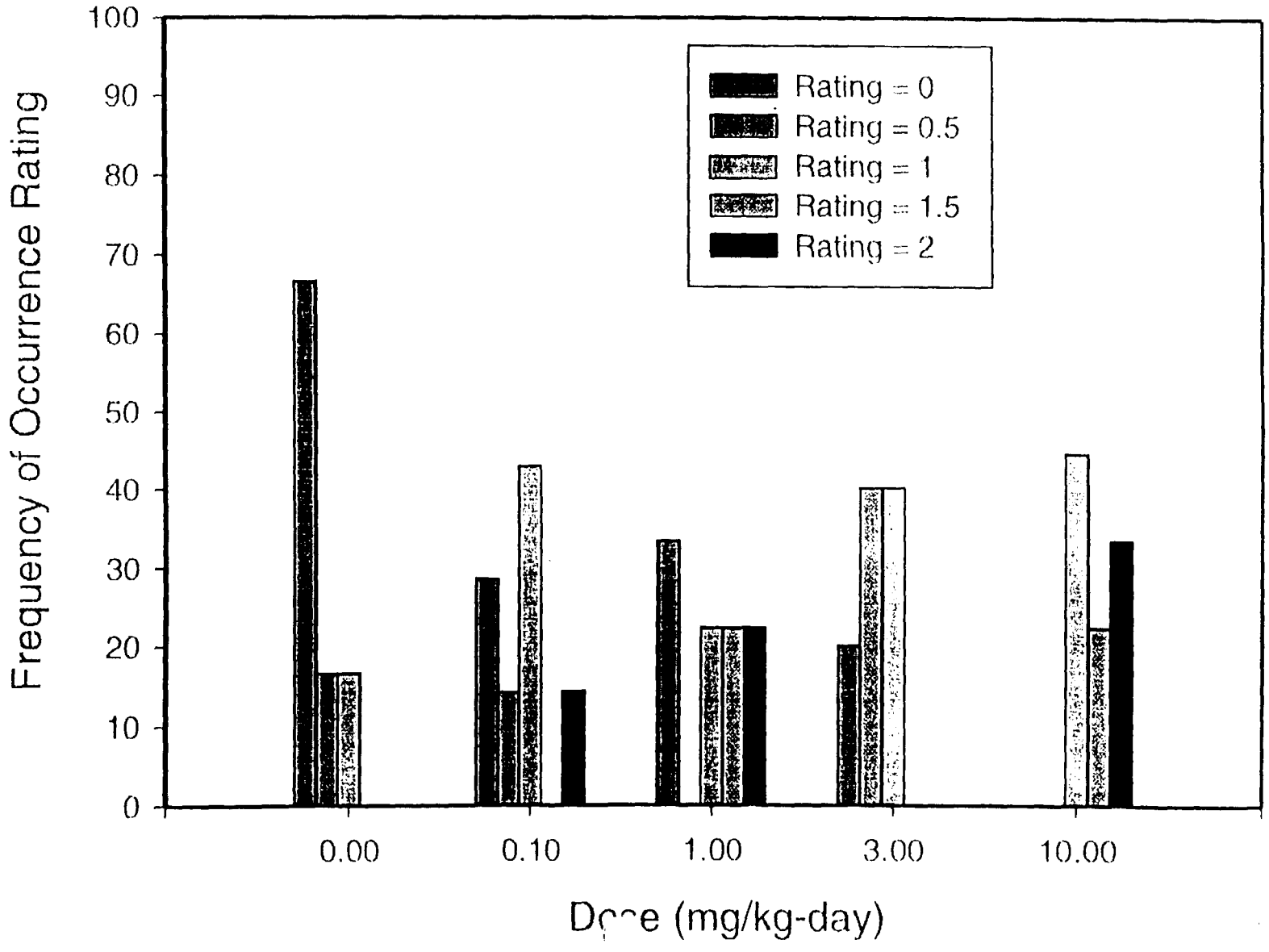
COMBINED INCIDENCE DATA AND AVERAGE SEVERITY SCORES FOR MALE AND FEMALE PND5 RAT PUPS FOR FOLLICULAR EPITHELIAL CELL HYPERTROPHY AND DECREASE IN FOLLICULAR LUMEN SIZE BASED ON STANDARD HISTOLOGY

		Perchlorate (mg/kg-day)				
Measure	Present	Control	0.1	1.0	3.0	10.0
Cell hypertrophy	Incidence[a]	3/12	8/12	9/12	8/12	12/12
	Severity[b]	0.33	0.84	1.08	0.83	1.42
Lumen Size	Incidence	6/12	10/12	10/12	11/12	12/12
	Severity	0.66	1.17	1.25	1.75	2.16

Frequency of Occurrence by Dose Group of Each Standard Histopathological Severity Rating for Follicular Epithelial Cell Hypertrophy in Rats of 14-Day Caldwell et al. (1995) Study



Frequency of Occurrence Per Litter By Dose Group of Each Standard Histopathological Severity Rating for Follicular Cell Hypertrophy in F1 Rat Pups on PND5 (Argus, 1998a; York, 1998a, b, c, d, e)

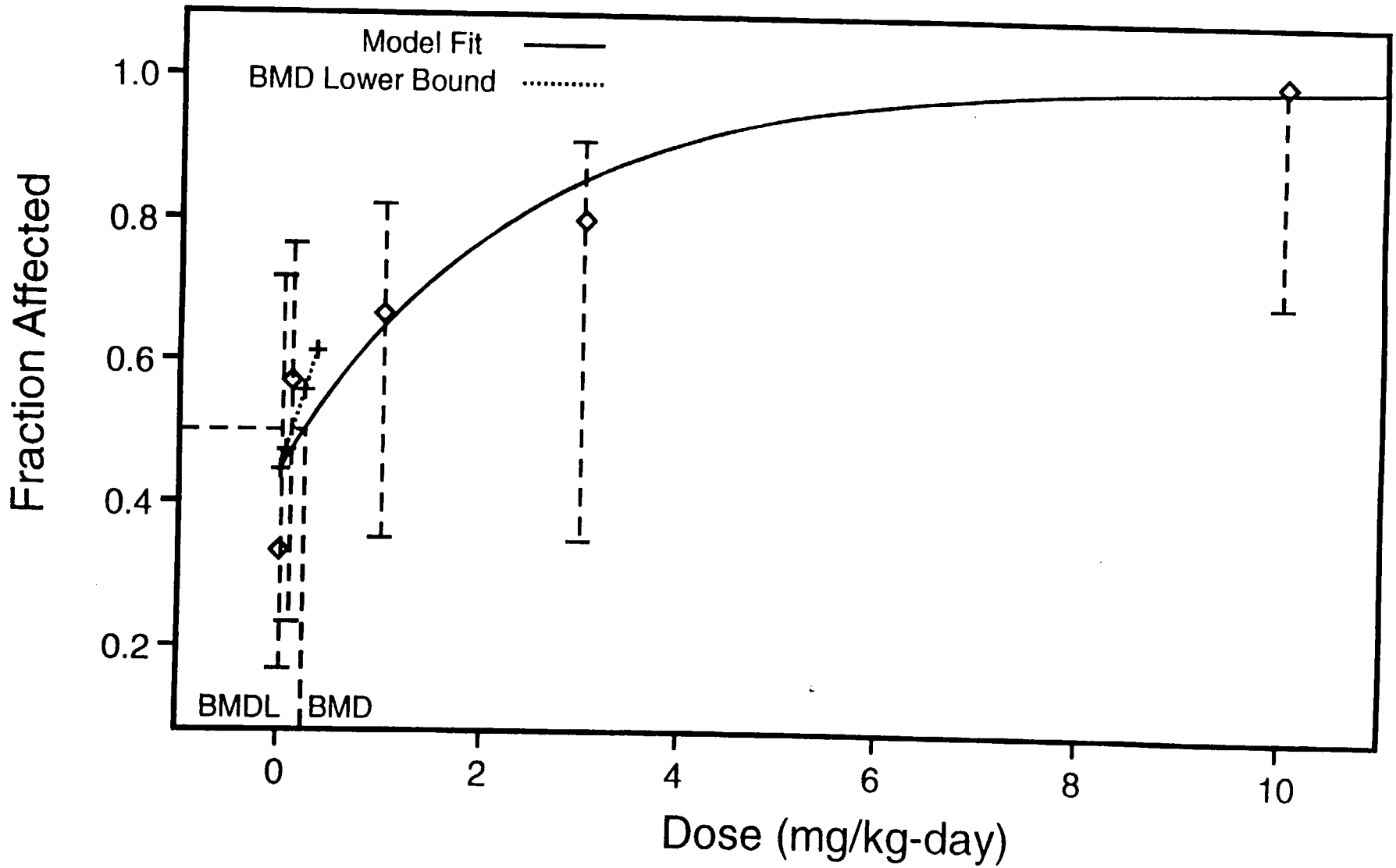


Benchmark Dose (BMD) and BMD 95% Lower Limit (BMDL) Estimates of the Incidence of Follicular Epithelial Cell Hypertrophy in the F1 Pups on PND5 From the Developmental Neurotoxicity Study (Argus, 1998a)

(Benchmark response based on 10% extra risk.)

Model	p of fit,df	BMD	BMDL	LOAEL	BMD: LOAEL	BMDL: LOAEL
Gamma	0.85, 3	0.234	0.10	0.1	2.34	1.0
Logistic	0.84, 3	0.35	0.27	0.1	3.5	2.7
Probit	0.84, 3	0.379	0.376	0.1	3.79	3.76
Quantal Linear	0.85, 3	0.234	0.10	0.1	2.34	1.0
Quantal Quadratic	0.74, 3	0.96	0.53	0.1	9.6	5.3
Weibull	0.85, 3	0.234	0.10	0.1	2.34	1.0

Model Fit to the Litter-by-Litter Incidence of Follicular Cell Hypertrophy (Standard Histopathology) in F1 Rat Pups on PND 5 (Argus, 1998a; York a, b, c, d, e)



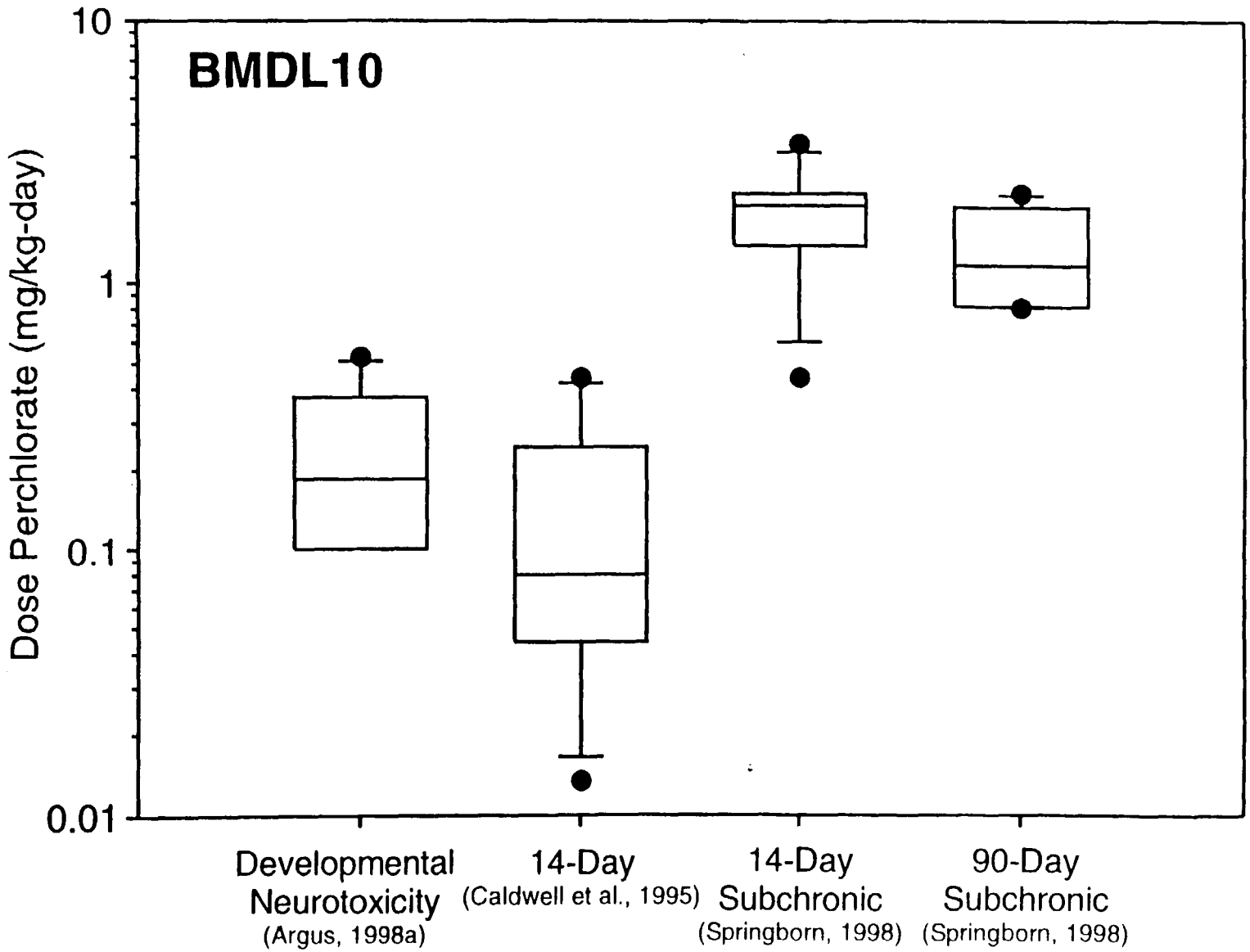
Preliminary Analyses

Analyses Submitted on 2/1/99 or 2/8/99

Neurodevelopmental Study

- Brain histopathology at the 3 mg/kg-day dose
  - Decrease in size of hippocampal gyrus (-12%) and caudate putamen (-7%); but no difference between control and high
  - Pending commentary on U-shaped awaits PBPK
- Nonparametric reanalysis of thyroid histopathology in pups on PND5
  - Exact tests reinforce concern for effect at 0.1 mg/kg-day; especially with small sample
- Litter-by-litter BMD analysis
  - BMD and BMDL virtually identical to Geller, 1998b

Lower Limit on 10% Response Benchmark Dose (BMDL) of Standard Histopathology in Rat Thyroids



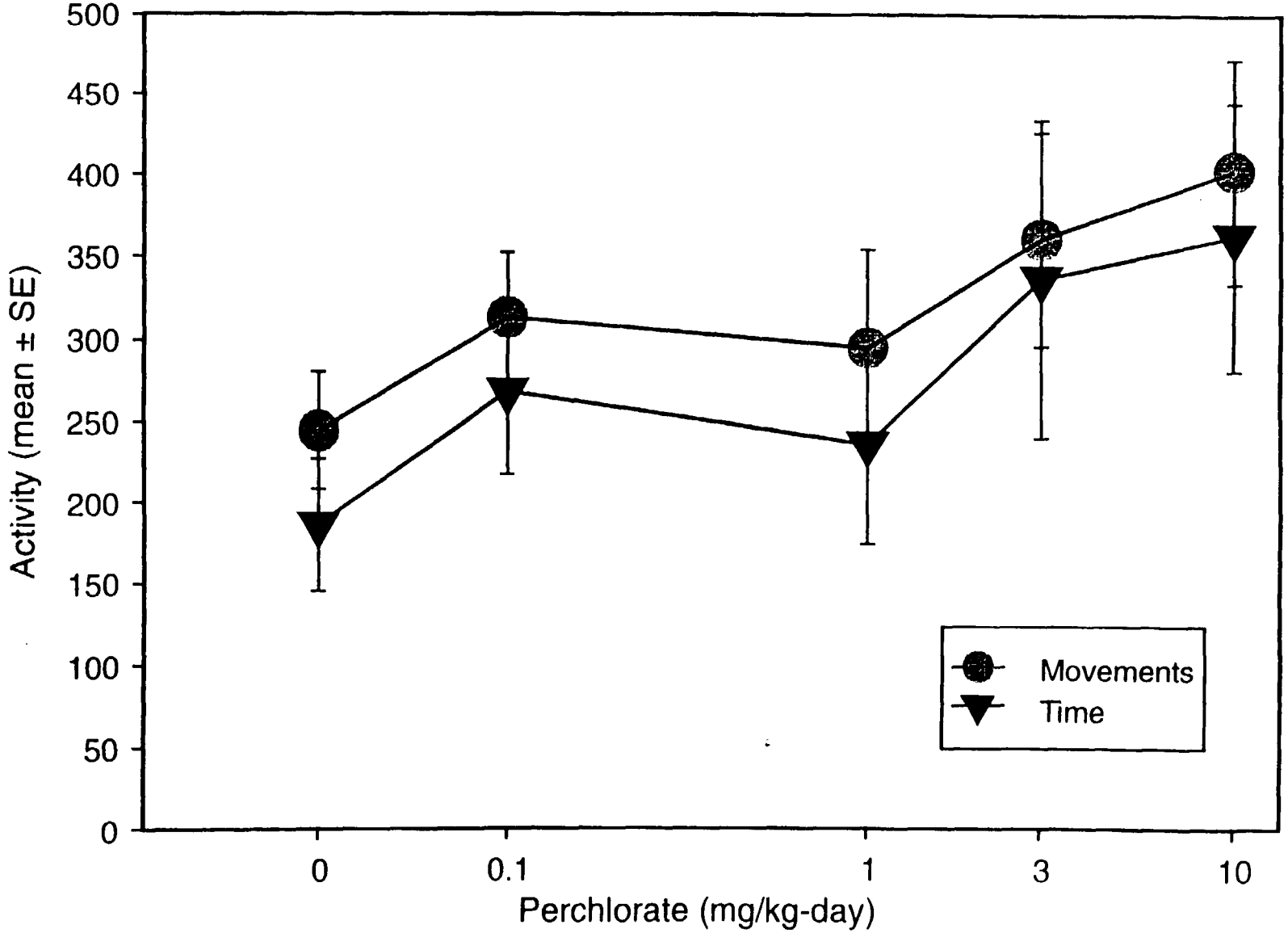
BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 14-DAY TIME POINT IN THE SPRINGBORN (1998) SUBCHRONIC STUDY

(Benchmark response based on 10, 20, and 40% changes from control value.)

Endpoint	Model	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	Power	0.203	1.16 0.0035	12.73 1.21	138.94 38.33	5.066	1.0
ln(T4)	Power	0.22	0.037	3.899	36.48		1.0
T3	Power	0.41	0.000033 —	0.207 —	129.39 0.129[a]	166.5	0.01[b]
ln(T3)	Power	0.35	Lower limit includes 0	0.000054[a]	43.16[a]		0.01[b]
TSH	Power	0.45	0.037 0.000076	0.326 0.005	2.89 0.36	12.616	0.01
ln(TSH)	Power	0.43	0.0015	0.098	6.587		0.01

[a]BMDL calculation failed at a number of values. This means BMDL value may not be accurate.
[b]LOAEL, not NOAEL.

Perchlorate Effects on Motor Activity in Male Rat Pups on PND 14 (Argus, 1998a)



Lower Limit on Benchmark Dose for Motor Activity

(Response Levels at Control + 10, 20, 30 or 40% of Control)

	p of Fit	+10% of Ctl	+20% of Ctl	+40% of Ctl	Ctl mean (std dev)	Estimated mean
Movement	0.72	1.04	2.08	4.17	244.5 (162.75)	273.04
Time	0.69	0.66	1.33	2.67	186.05 (184.78)	239.07

Interpretation Issues
Histopathology

- Standard histopathology versus morphometry on lumen size
- Four different pathologists across various studies
- Different severity rating scales
- No formal QA by second pathologist
- Pending Proposal: PWG of all histopathology

Interpretation Issues
Hormone Analyses

- Three different laboratories
- Considerable variability

- Pending study: Interlaboratory validation study

Pending Data

Pilot Study for Inter-Laboratory Variability of Hormone Analyses

- For T3, T4, TSH:
  - Obtain 3 standards (high, med, low) and three rat samples
  - Send to three labs as unknowns
  - Each lab runs standards using SOP & normal source for RIA kit / chemicals
  - AFRL/HEST will run the 6 samples using our source for kits plus source of kits / chemicals for the other two labs
  - All data will be sent to EPA or 3rd party for statistical analysis

Data Analyses

- EPA conducted extensive reanalysis of submitted data
  - Some too simplistic (e.g., lack of gender in the model
  - Some data were not analyzed (e.g., incidence data
  - A lot of data was subjected to benchmark analyses
- Future Needs
  - Some analyses and graphics need corrections per reviewer comments
  - New analyses on studies in progress

Evidence for Indirect Carcinogenic Antithyroid Mode of Action

- Demonstrated dose-dependent effect in both fetal and adult stages in thyroid follicular cell hypertrophy / hyperplasia, lumen size and colloid reduction
- Hormone (T3, T4, TSH) changes correlated with histopathology acorss a number of studies and time points
- Site of action clearly at symporter, possibly not only locus

Evidence for Indirect Carcinogenic Antithyroid Mode of Action

- Reversibility demonstrated in thyroid weight, hypertrophy, hyperplasia and hormones after 30-day recovery of 90-day studies in rats and mice; possibly in pups on PND10 and PND22
- Progression of lesions between 14-day and 90-day time points
- Genotoxicity battery negative

Completed Analyses

Genotoxicity Battery

- NTP repeat (Zeiger, 1999a) of mouse micronuclei assay by ip injection found perchlorate was not toxic or mutagenic at 125, 250 or 500 mg/kg-day. All animals died in the 1500 and 2000 mg/kg-day groups; 4/5 at 1000.
- Repeat of mouse lymphoma by BioReliance negative.
- EPA conclusion: Perchlorate is not mutagenic.

Completed Analyses

Genotoxicity Battery

- NTP repeat (Zeiger, 1999b) of Salmonella (Ames test) mutagenicity battery found perchlorate negative at doses of 100 to 10,000 μg / plate.
- 10% and 30% S-9 concentrations used from Aroclor induced hamster (HLI) and rats (RLI)
- Tester strains TA102, TA104, TA100, TA1535, TA97 and TA98

"RfD" Derivation

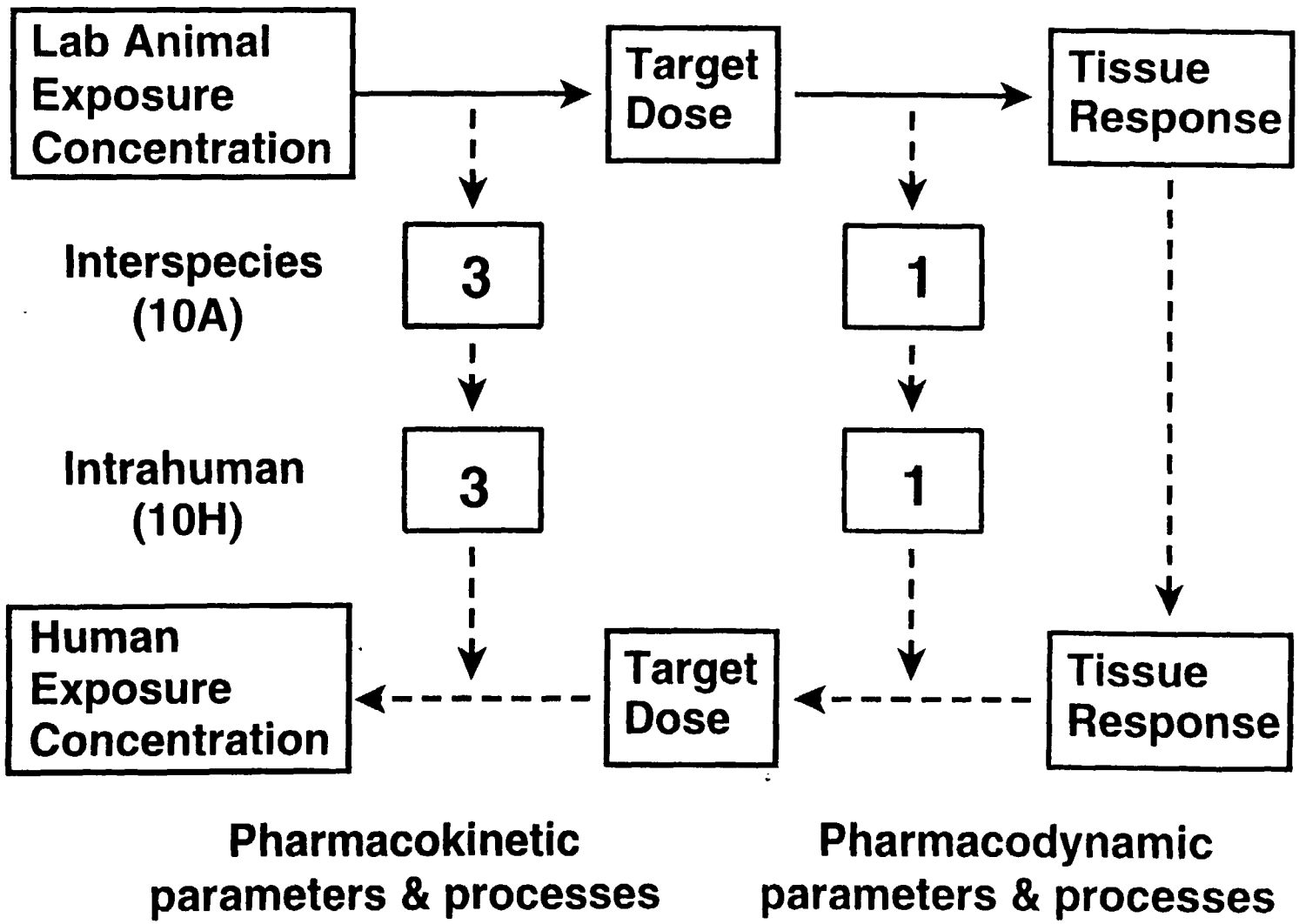
- LOAEL = 0.1 mg/kg-day
- Adjustment for molecular weight of ammonium perchlorate = 0.85
- Composite UF = 100
- "RfD" = 0.0009 mg/kg-day
- Confidence in study, database, and "RfD" is medium

Uncertainty Factors

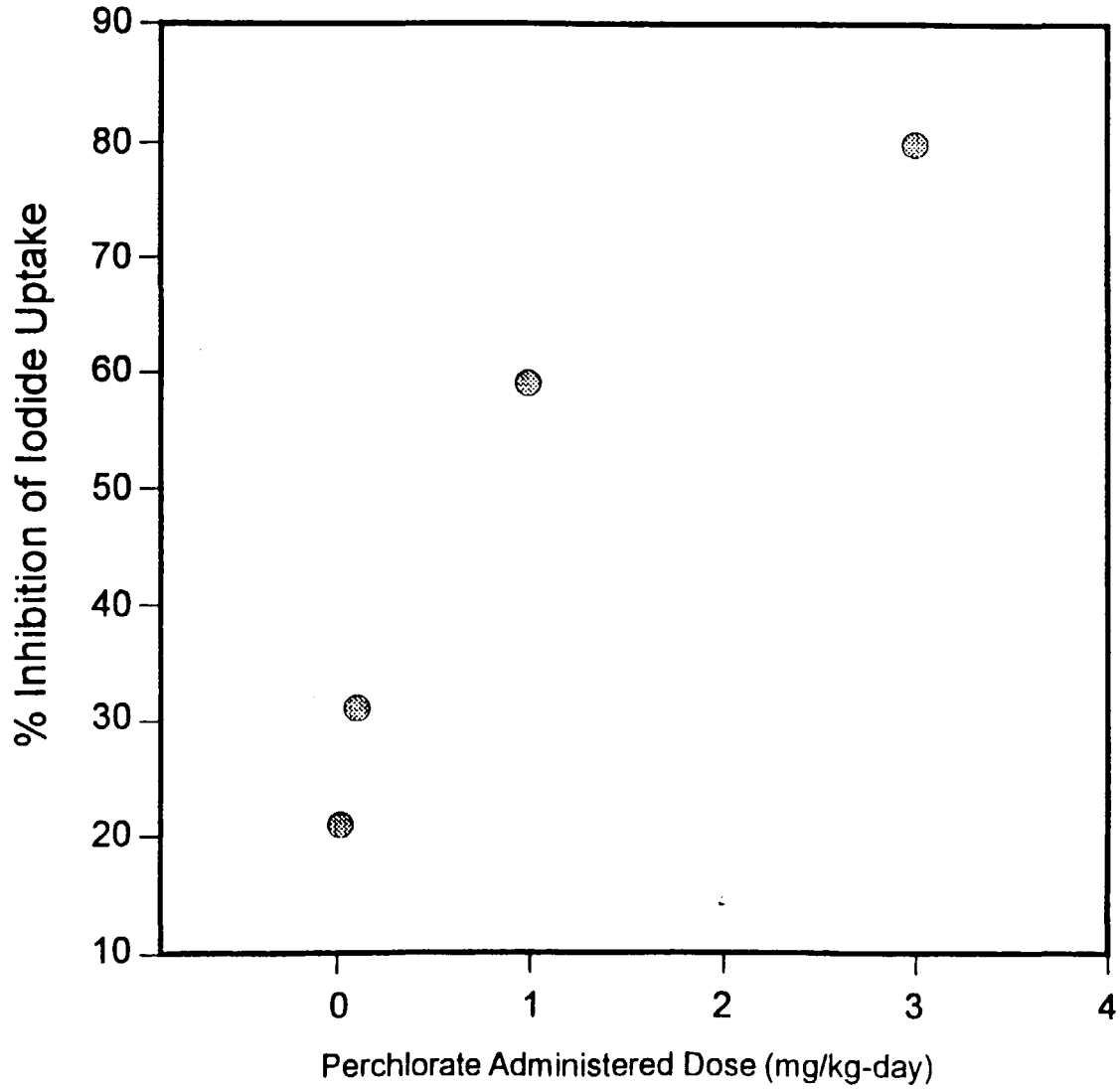
- Intrahuman: 3 for PK, PD partially covered by LOAEL to NOAEL factor
- Interspecies: 3 for PK
- LOAEL to NOAEL: 3 for minimal histopathology and concern over hormone data interpretation
- Data base deficiencies: 3 for lack of 2-generation reproductive and immunotoxity

- TOTAL COMPOSITE UF: 100

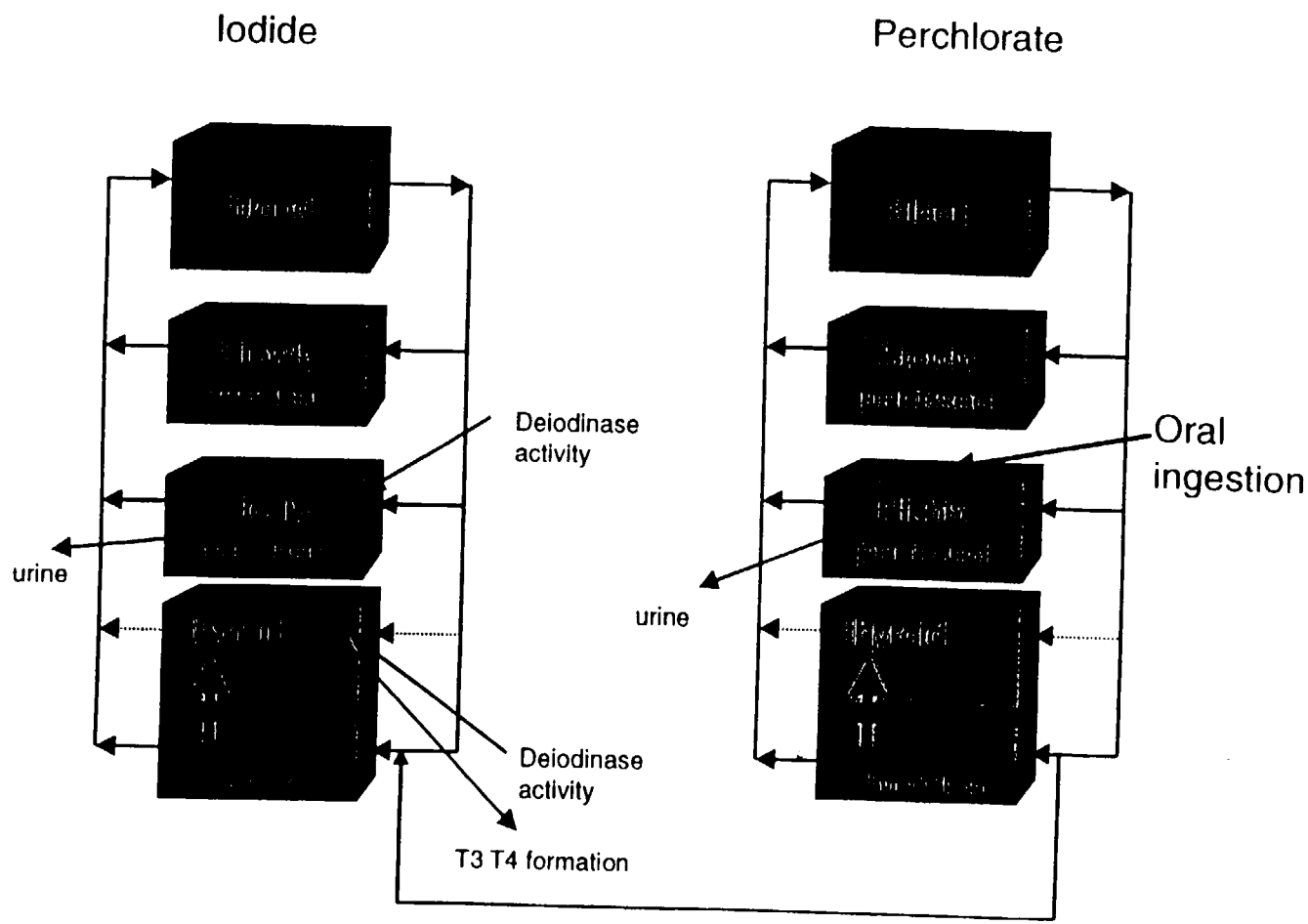
Schematic of Interspecies and Intrahuman UF Components Proposed for Perchlorate



Inhibition of Iodide Uptake by Oral Perchlorate Administration (Single 1p Dose)



Schematic of PBPK Model That is Under Development



Preliminary Analyses

Internal Dose Metric Correlations Using Data Submitted 2/5/99

- Meyer (1998) iodide uptake inhibition 9 hr after single iv dose
- Channel (1999) measured iodide uptake inhibition in rats dosed for 14 days by drinking water

Preliminary Analyses

Internal Dose Metric Correlations Using Data Submitted 2/5/99

- Caldwell 14-day study
  - Only 4 of 9 doses
  - Iodide uptake inhibition correlated better with T4 and T3 than did administered dose for single dose
  - TSH approximately same correlation for single dose
  - Worse with 14-day data than with administered
- Neurodevelopmental study
  - Only 4 of 5 doses
  - Correlations with administered or iodide inhibition significant and approximately similar for single dose
  - Correlations were worse with iodide inhibition than for administered dose for 14-day data

Preliminary Analyses

Internal Dose Metric Correlations Using Data Submitted 2/5/99

- Subchronic study
  - 3 of 6 doses tested
  - 14-day timepoint
    - Correlations for T3 and TSH marginally better with iodide uptake inhibition for single dose; T4 not
    - Correlations were worse with 14-day iodide uptake than for administered dose
  - 90-day timepoint
    - Correlations marginally higher with iodide uptake for single dose
    - Correlations were worse with 14-day iodide uptake than for administered dose

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Address dosimetry in dams, fetuses, and pups; repeat motor activity measures
- Timeline
  - Protocol development: 1 Mar 99
    - Submission to EPA as Consultative Letter
  - Protocol approval: 1 Apr 99
  - Begin studies: 1 May 99

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Pregnant Females / Fetuses
- Start dosing 2 wk prior to pregnancy
- Prenatal
  - Time points at GD 15 and 20
  - Determine inhibition of iodide uptake in the fetuses and dams
  - Measure T3,T4, TSH and perchlorate in blood
  - Thyroid histopathology

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Lactating Females / Pups
- Start dosing 2 wk prior to pregnancy
- Postnatal
  - Time points at PND 5, 10 and weening (PND 22)
  - Determine inhibition of iodide uptake in the pups and dams
  - Measure T3,T4, TSH and perchlorate in blood
  - Thyroid histopathology

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Pregnant Females / Fetuses
- Single dose
- Prenatal
  - Time points at GD 20
  - Determine inhibition of iodide uptake in the fetuses and dams
  - Measure T3,T4, TSH and perchlorate in blood

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Lactating Females / Pups
- Single dose
- Postnatal
  - Time point at PND 5
  - Determine inhibition of iodide uptake in the pups and dams
  - Measure T3,T4, TSH and perchlorate in blood

Pending Data

MOTOR ACTIVITY OF PUPS IN KINETIC STUDY

- Lactating Females/Pups
- Start dosing 2 wk prior to pregnancy
- Postnatal
  - Time point at PND 14
  - Conduct motor activity measurements to repeat data in Neurobehavioral Developmental Study
  - Pups then can be used for kinetic study time point at weening

Pending Data

HUMAN STUDY
Dr. Brabant (Medizinsche Hochschule) in Collaboration with AFRL/HEST

- Timeline
  - Conduct study: Feb and Mar 99
  - Data Analysis: Mar and Apr 99
  - Report Submission: May 99

Pending Data

HUMAN STUDY
Dr. Brabant (Medizinische Hochschule) in Collaboration with AFRL/HEST

- 14 day toxicity / kinetic study
- 3 doses, 7 male subjects per dose
- Each subject as own control
- Parameters to be measured
  - cytopuncture for PCR and iodide
  - body weight and thyroid ultrasound
  - CBC, T3, T4, TSH, Tg
  - iodide, perchlorate in blood and urine

Pending Data

HUMAN STUDY
Dr. Brabant (Medizinsche Hochschule) in Collaboration with AFRL/HEST

- Blood draws
  - prior to second dose on day one
  - daily on days 2-14 and days 1-3 post dosing
- Urine collection
  - 24 hour collection on days 1, 7, 14
  - 24 hour collection on days 1-3 post dosing

Pending Data

HUMAN STUDY
Dr. Braverman (Harvard Med School)
Funded by PSG

- 14 day toxicity study
- 1 dose level, 10 male subjects per dose
- Each subject as own control
- Parameters to be measured:
  - iodide^{123} in thyroid
  - body weight and physical exam
  - CBC, chem profile, urinalysis
  - TSH, T4 (serum and free), T3, Tg, TPO
  - iodide, perchlorate in blood and urine

Pending Data

HUMAN STUDY
Dr. Braverman (Harvard Med School)
Funded by PSG

- Blood draws
  - baseline
  - days 7, 14
  - amendment requested for day 15 at 2, 4, 8, 24 hrs after last dose
- Urine collection
  - 24 hour collection prior to days 1, 7, 14
  - 24 hour collection on days 1-5 post dosing (voluntary now - amendment requested)
- Amendment requested for 2 more doses

Analyses Submitted on 2/8/99

Human Studies

- Ecological Epidmiology Study (Lamm and Doemland, in press)
  - No evidence for increased incidence of congenital hypothyroidism in county and ethnic-specific data on newborns in NV and CA in 1996-1997
  - Limitations with respect to exposure estimates

Analyses Submitted on 2/8/99

Human Studies

- Cross-sectional Occupational Study of Workers Exposed via Inhalation (Lamm et al., in press)
- Ammonium Perchlorate Production workers (n = 37) in 3 categories (low, medium, high); sodium azide workers as controls (n = 21)
- Medical questionnaire, clinical chemistry, thyroid function, urine excretion pre- and post-shift for perchlorate, iodine, creatinine; CBC, Hg, HCT, MCV, MCH, MCHC

Preliminary Analyses

Analyses Submitted on 2/8/99

Human Studies

- Measures of respirable and total particles were highly correlated. MMAD for high exposure group (n =15) of 7.4 um with a geometric standard deviation of 1.8
- Absorption demonstrated by urinary excretion
- No evidence for thyroid or effects on other parameters
- Quantitative route-to-route extrapolation may be possible using these data

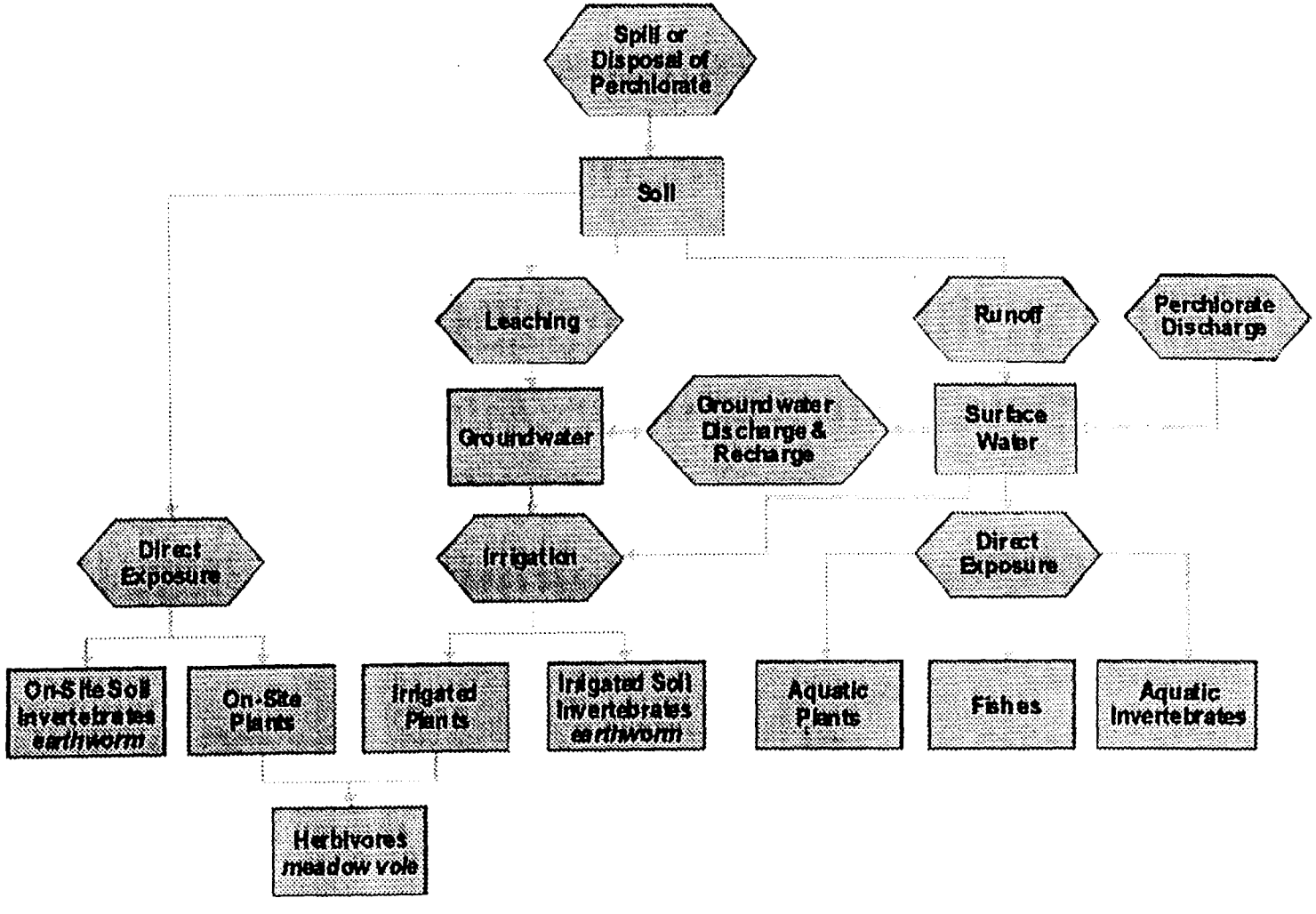
Ecological Screening Assessment

- Limited in scope due to database
  - IPSC report including literature search and screening battery
  - LC50 aquatic chronic toxicity testing
  - Frog Embryo Teratogenesis Assay: Xenopus (FETAX)
  - Phytotransformation study
- Scope responsive to stakeholder concerns

Ecological Screening Assessment

- Problem formulation focused on selection of assessment endpoints
  - Fish community richness and productivity
  - Aquatic invertebrate community richness and productivity
  - Aquatic plant productivity
  - Soil invertebrate community richness and productivity
  - Terrestrial plant productivity
  - Population productivity of herbivorous wildlife

Conceptual Model of Exposure of Ecological Receptors to Perchlorate



Ecological Screening Assessment

- Secondary acute value of 5 mg/L (as perchlorate) to be protective of aquatic organisms with 90%
- Quartile inhibitory concentrations for terrestrial plant growth in soil and sand at 78 mg/kg (293 mg/L) and 41 mg/kg (160 mg/L). A factor of 10 applied for interspecies variance to arrive at screening benchmark of 4 mg/kg.
- Limited data on invertebrates; conservative estimate at 1 mg/kg.
- A factor of 10 applied to human health risk LOAEL to obtain screening benchmark of 0.01 mg/kg-day for herbivores

Ecological Screening Assessment
Uncertainties and Research Needs

- Accurate exposure information
- Accurate linkage between biologically effective dose and degree of perturbations in hormones and neurobehavioral
- Data on bioaccumulation
  - Aquatic biota
  - Terrestrial vascular plants
- Effects on nondaphnid invertebrates and dietary exposure of birs and herbivorous or litter-feeding invertebrates
- Fate and transport in irrigated soils

Ecological Studies Under Consideration

- Screening animal exposure studies
  - Texas Tech (funded 99-00)
- Additional plant exposure studies are planned under the direction and management of Brooks Air Force Base
- Further plant studies are under consideration to be jointly funded between the USAF and EPA

Additional Proposed Studies

- Development of Analytical Methodologies for Plant Tissues
- Analytical Support and Pathway Analysis
- Plant Tissue Cultures to Define Plant Effects
- Mass Balances to Define Reaction Pathways and Kinetics
- Identification of Enzyme Systems
- ELISA to Identify Perchlorate Adaptable Plants
- Plant Selection and Evaluation
- Soil Chemistry
  - Sorption
  - Competitive Uptake
  - Growth Impacts
- Pilot Treatability Investigation
- Field-Scale Water Balance
- Plume Fate and Transport
- Development of Transgenic Plants
- Evaluation of Perchlorate Content in North American Fertilizers

Further Needs

- Evaluate how the proposed studies will further the recommended research needs
- Prioritize the studies for funding
- Establish appropriate management documentation
- Obtain funding
- Start studies

Conclusions

- Hormone and histology resutls support proposed mode of action model
- Uncertainties to be better addressed by pending studies
- Require recommendations on path forward to final document

Appendix J

Additional Analysis from EPA's National Center for Environmental Assessment

Table 1. Data Analyses Provided in February 1, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Final genetox assays a) Repeat of Salmonella battery plus 2 additional strains by NTP b) Repeat of mouse micronuclei assay by NTP c) Repeat of mouse lymphoma by BioReliance	Final — Memos and revised text to document provided.	David Brusick
2. Brain histopathology at the 3 mg/kg-day dose from the Argus (1998a) neurodevelopmental study	Preliminary pending recommendations at peer review.	Tom Zoeller
3. Nonparametric Reanalysis of thyroid histopathology in pups on PND5 from the Argus (1998a) neurodevelopmental study	Preliminary — Provided in response to request by Joe Haseman to correct some data entries and to extend analysis with more exact procedures	Joe Haseman Susan Porterfield Tom Zoeller
4. Hormone data for F0 and F1 generation in 2-generation reproductive study (Argus, 1998b).	Preliminary — These particular data are audited but the overall final report and data have not been audited nor released. Analysis represents alternative approach suggested by Joe Haseman.	Tom Zoeller Joe Haseman Susan Porterfield
5. Reproductive parameters (sperm morphology and estrous cyclicity) from F1 generation in 2-generation reproductive study (Argus, 1998b).	Preliminary — These particular data are audited but the overall final report and data have not been audited nor released.	Rochelle Tyl
6. Sheep red blood cell (SRBC) assays from 90-day experiments in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
7. Thyroid histopathology in mice from immunotoxicity studies	Preliminary — Data are audited but additional dose levels required for EPA to evaluate dose response	Tom Zoeller Susan Porterfield

Table 2. Data Analyses To Be Provided in February 8, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Occupational cross-sectional study of workers exposed via inhalation and an epidemiological study	Preliminary — Manuscripts submitted as accepted on 1/22/99. EPA analysis not complete.	Susan Porterfield Tom Zoeller Charles Emerson
2. Sheep red blood cell (SRBC) from 14-day experiment (repeat) in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
3. 14-day repeated dose pharmacokinetic study	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen
4. Correlations between percent of iodide uptake inhibition and hormone perturbations using single dose and repeated 14-day dose PK studies	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen Tom Zoeller

February 1, 1999 EPA Assessment Submission

Attachment #1
Final Genetox Review

A. Final NTP Salmonella battery
B. Repeat of Mouse Micronuclei assay by NTP
C. Review of A and B by EPA (Dellarco memo)
D. Repeat of Mouse Lymphoma by BioReliance
E. Review of D by EPA (Moore memo)
F. Revised section of document

ATTENTION PANEL MEMBER(S):

DAVID BRUSICK

January 28, 1999

NOTE TO: Annie Jarabek
FROM: Vicki Dellarco
RE: Review of the NTP Mutagenicity Studies on Ammonium Perchlorate

I have reviewed both the Ames assay and the mouse bone marrow micronucleus assay on ammonium perchlorate conducted under the auspices of the National Toxicology Program. Negative results were found in both assays. I find the protocols and the results from these tests to be acceptable. Furthermore, these recent studies confirm and reinforce the negative findings reported by another laboratory from these assays. I will revise the assessment document on perchlorate accordingly to reflect these new and important findings.

(NTP, 1999a)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: January 11, 1999

From: Errol Zeiger, Environmental Toxicology Program, NIEHS

Subject: Ammonium Perchlorate MN Summary Test Results

To: Annie Jarabek, National Center for Environmental Assessment, EPA

Male B6C3F1 mice were treated i.p with 125, 250, 500, 1000, 1500, and 2000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Five mice per group were injected daily for 3 consecutive days, and were sacrificed 24 hrs after the last injection. Their femoral bone marrow was removed and the polychromatic erythrocytes (PCE) scored for micronuclei (MN). All testing and scoring were done under code.

All animals in the 1500 and 2000 mg/kg groups died after the first i.p. injection, and 4/5 animals in the 1000 mg/kg group died after the second i.p. injection; the fifth animal was sacrificed and not scored for MN. All animals in the 125, 250, and 500 mg/kg groups survived the treatment; 2000 PCE's were scored per animal for MN.

The test data were analyzed statistically and have been entered into the NTP genetic toxicity database. No increases in MN-PCE were seen at any of the test doses, and the trend test was not positive. The positive control yielded a significant increase. No bone marrow toxicity was seen, as indicated by the percent PCE. The following table summarizes the results of the test.

mg/kg	mean MN cells/ 1000 PCE ± S.E.M.	pairwise p*	%PCE
0	3.00 ± 0.57		46.6
125	3.10 ± 0.40	0.4490	51.7
250	3.20 ± 0.56	0.3996	55.6
500	2.10 ± 0.29	0.8956	49.2
pos** 15	19.60 ± 2.03	0.0000	56.5

trend test p = 0.903

* p value for pairwise comparison against the solvent (0 dose) control
** positive control, cyclophosphamide

The results of this study are consistent with those reported in the Perchlorate Study Group report (Study No. 6100-001). In that study, which used gavage administration, the highest dose that could be scored was 1000 mg/kg.

(NTP, 1999b)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: January 13, 1999

From: Errol Zeiger, Environmental Toxicology Program, NIEHS

Subject: Ammonium Perchlorate Salmonella Summary Test Results

To: Annie Jarabek, National Center for Environmental Assessment, EPA

The results of the NTP's Salmonella mutagenicity testing of Ammonium perchlorate are attached. The values presented are the means and standard errors of the mean, of triplicate plates.

The chemical was dissolved in water and tested using the preincubation procedure at doses from 100 to 10,000 µg/plate, without metabolic activation (NA), and using S-9 liver homogenates from Aroclor induced hamster (HLI) and rats (RLI). Two different concentrations of S-9 were used, 10% and 30%. The tests without metabolic activation (NA) were performed twice. Salmonella tester strains TA102, TA104, TA100, TA1535, TA97, and TA98 were used. "Pos" is the positive control.

Ammonium perchlorate was not toxic or mutagenic under the conditions of this test.

Although there were a number of differences between the NTP protocol and that used by the Perchlorate Study Group report (Study No. 6100-001), the conclusions of both tests are the same.

AMMONIUM PERCHLORATE

(LAB: SRI SOLVENT: H20 PROTOCOL: PREINC)

DOSE	TA102 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	163	12.1	205	13.7	312	14.8	269	10.5	274	29.3	281	8.4
100.000	182	8.2	207	25.0	319	17.1	270	21.1	302	9.5	275	11.0
333.000	174	4.5	220	14.2	316	10.5	257	16.5	306	14.7	262	5.8
1000.000	161	3.3	232	7.8	287	25.2	265	15.3	296	9.0	276	10.4
3333.000	182	6.0	216	9.5	291	10.0	240	18.7	271	7.3	265	23.7
10000.000	176	10.2	190	31.2	317	7.1	256	16.3	280	24.8	270	4.1
POS	751	27.7	739	17.1	1182	17.9	1049	44.5	1043	52.0	942	18.1

DOSE	TA104 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	247	12.7	317	20.7	436	12.4	334	15.7	422	23.6	334	17.3
100.000	280	9.7	341	17.6	439	18.7	344	21.5	404	16.7	310	11.3
333.000	254	26.3	318	18.8	374	60.4	373	8.7	426	8.2	344	17.6
1000.000	250	17.7	326	7.2	426	15.1	385	9.3	451	13.2	350	21.6
3333.000	272	15.1	338	19.3	424	12.7	351	13.7	413	18.6	344	27.6
10000.000	254	12.5	341	17.8	442	15.9	341	19.0	450	9.8	331	12.3
POS	847	25.7	843	28.0	1200	25.9	1260	12.5	962	18.6	1225	33.9

DOSE	TA100 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	155	5.2	128	2.2	125	13.7	173	4.6	126	7.3	147	3.8
100.000	152	3.2	121	6.5	128	0.6	161	10.0	131	3.5	161	3.8
333.000	155	3.5	124	4.0	132	4.9	155	13.8	122	3.3	151	8.1
1000.000	163	4.7	128	13.8	133	4.5	164	3.0	133	6.2	148	6.0
3333.000	147	7.2	132	4.8	121	2.6	172	8.6	135	11.9	159	2.6
10000.000	157	14.1	126	6.1	119	4.9	170	5.5	126	2.9	146	4.9
POS	928	7.2	937	18.8	629	9.2	722	12.4	540	14.8	657	20.5

DOSE	TA1535 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	12	2.2	9	1.8	16	3.2	9	1.8	15	1.5	11	1.5
100.000	13	0.6	9	1.8	13	3.5	10	0.7	16	0.9	15	2.7
333.000	10	1.3	14	1.9	11	0.9	14	3.2	9	2.1	12	0.3
1000.000	10	1.2	10	1.5	13	0.6	12	0.9	11	0.7	11	0.0
3333.000	13	3.3	12	1.2	10	1.5	12	0.9	12	1.7	13	1.9
10000.000	10	0.6	10	1.9	12	1.9	9	0.9	10	1.7	8	0.0
POS	835	18.2	856	11.6	152	8.7	131	8.4	137	8.7	110	6.7

DOSE	TA97 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	121	3.7	135	16.4	178	12.9	168	14.6	157	13.7	158	14.9
100.000	130	9.3	141	9.1	182	7.0	183	3.4	167	4.9	179	6.2
333.000	131	16.8	132	7.9	170	4.0	172	7.0	153	10.5	174	14.5
1000.000	140	7.4	161	3.7	162	6.4	191	3.1	149	8.1	168	5.8
3333.000	134	5.5	164	8.5	153	12.7	192	0.9	154	16.5	173	13.8
10000.000	124	4.0	122	6.3	177	12.4	131	9.9	143	11.3	167	5.2
POS	508	20.7	553	21.5	513	183.0	592	13.0	656	10.0	517	8.2

DOSE	TA98 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	22	4.1	24	3.0	25	3.8	17	1.8	27	3.8	24	1.3
100.000	17	1.7	29	5.9	35	3.3	22	2.1	32	3.2	23	2.4
333.000	17	0.9	21	3.8	26	2.8	20	2.5	29	0.9	24	3.0
1000.000	23	2.3	24	0.3	23	1.2	21	4.6	22	0.7	21	2.0
3333.000	18	3.5	21	1.5	30	2.0	17	3.8	35	3.8	20	3.1
10000.000	18	4.4	26	3.3	27	4.8	20	4.4	29	2.6	19	0.3
POS	355	17.6	362	7.7	543	12.9	545	16.9	466	18.2	536	45.1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK
NORTH CAROLINA 27711

MEMORANDUM

DATE: January 29, 1999

SUBJECT: Analysis of Perchlorate

FROM: Martha M. Moore, Chief (MD-68) Martha Moore
Genetic & Cellular Toxicology Branch

TO: Vicki Dellarco, (MD7509C)
Office of Pesticides Programs
Annie Jarabek, (MD-52)
Toxocologist

I have reviewed the mouse lymphoma data generated in the repeat analysis of perchlorate and based on this information, I am confident that the data are sufficient to determine the chemical to be nonmutagenic both with and without S9 activation. While I am a little concerned that the background mutant frequency is too low, particularly in the without S9 experiment, this data set looks overall to be very good. It is internally very consistent. The problems that were observed in the data generated by the first laboratory are not present in the data from this laboratory. The issue of low background mutant frequency relates to whether the laboratory is adequately quantitating all of the mutants. I think that the mutant colony sizing curves that are included in the data provides sufficient evidence that the laboratory is quantitating mutants properly.

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BIORELIANCE CORPORATION
14920 BROSCHART ROAD
ROCKVILLE, MARYLAND 20850-3349 USA
PHONE: 301.738.1000 • FAX: 301.738.1036

January 27, 1999

Mr. Michael F. Girard
Perchlorate Study Group Representative
Highway 50 and Aerojet Road
Building 20019/Department 0330
Rancho Cordova, CA 95813-6000

Dear Mr. Girard:

Enclosed please find the original of the final report for the BioReliance study G98BA06.702, *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay), which was performed using your test article: Ammonium perchlorate. Also enclosed is the Response to Audit Comments.

Should you require additional information or have questions, please call Dr. Richard San at (301) 738-1000, extension 2222.

Sincerely,

Diane Gray
Secretary
Toxicology Testing Services

Enclosures

cc: Michael L. Dourson, Ph.D., DABT
Toxicology Excellence for Risk Assessment
4303 Hamilton Avenue
Cincinnati, OH 45223

Annie Jarabek (phone: 919-541-4847)
USEPA/NCEA
Progress Center
Catawba Building
3200 Highway 54
Research Triangle Park, NC 27709

R. San
P. Smith
Study file

Response to Audit Comments

Test Article ID: Ammonium perchlorate
MA Study No.: G98BA06.702
Report Type: Draft to Final

All changes requested by the Sponsor have been incorporated into the final report.

RS 1/27/99

FINAL REPORT

Study Title

***In Vitro* Mammalian Cell Gene Mutation Test**
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Test Article

Ammonium perchlorate

Authors

Richard H. C. San, Ph.D.
Jane J. Clarke, B.A.

Study Completion Date

January 27, 1999

Performing Laboratory

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

Laboratory Study Number

G98BA06.702

Sponsor

Perchlorate Study Group
Highway 50 and Aerojet Road
Building 20019/Department 0330
Rancho Cordova, CA 95813-6000

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STATEMENT OF COMPLIANCE

Study G98BA06.702 was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the US EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Regulations, the Japanese GLP Regulations and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

> The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility.
>
> Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
>
> The stability of the test or control article under the test conditions has not been determined by the testing facility.

Richard H. C. San, Ph.D.
Study Director

1/27/99
Date

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QUALITY ASSURANCE STATEMENT

Study Title: *IN VITRO* MAMMALIAN CELL GENE MUTATION TEST

Study Number: G98BA06.702

Study Director: Richard H. C. San, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 04 DEC 98, TO STUDY DIR 04 DEC 98, TO MGMT 04 DEC 98
PHASE: Protocol Review

INSPECT ON 15 DEC 98, TO STUDY DIR 15 DEC 98, TO MGMT 17 DEC 98
PHASE: Dilution of test and/or control material

INSPECT ON 20 JAN 99-21 JAN 99, TO STUDY DIR 21 JAN 99, TO MGMT 22 JAN 99
PHASE: Draft Report

INSPECT ON 27 JAN 99, TO STUDY DIR 27 JAN 99, TO MGMT 27 JAN 99
PHASE: Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Diane B. Madsen 1-27-99

Diane B. Madsen, B.S.
QUALITY ASSURANCE

DATE

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

FINAL REPORT

Sponsor:	**Perchlorate Study Group** **Highway 50 and Aerojet Road** **Building 20019/Department 0330** **Rancho Cordova, CA 95813-6000**
Study Monitor:	**Michael F. Girard** **Perchlorate Study Group Representative**
Scientific Advisor:	**Michael L. Dourson, Ph.D., D.A.B.T.** **Toxicology Excellence for Risk Assessment**
Performing Laboratory:	**BioReliance** **9630 Medical Center Drive** **Rockville, MD 20850**
Test Article I.D.:	**ammonium perchlorate**
Test Article Lot No.:	**05006CQ**
Test Article Purity:	**99.999% (Provided by Sponsor)**
BioReliance Study No.:	**G98BA06.702**
Test Article Description:	**white, crystalline solid**
Storage Conditions:	**room temperature; protected from light and moisture**
Test Article Receipt:	**November 16, 1998**
Study Initiation:	**December 2, 1998**
Laboratory Manager:	**Jane J. Clarke, B.A.**

Study Director: Richard H. C. San, Ph.D. 1/27/99 Date

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SUMMARY

The test article, ammonium perchlorate, was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9. The preliminary toxicity assay was used to establish the dose range for the mutagenesis assay. The mutagenesis assay was used to evaluate the mutagenic potential of the test article.

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in DMSO at 500 mg/mL, the maximum concentration tested.

In the preliminary toxicity assay, the maximum concentration of ammonium perchlorate in treatment medium was 5000 µg/mL. No visible precipitate was present at any concentration in treatment medium. Selection of dose levels for the mutation assay was based on reduction of suspension growth relative to the solvent control. Substantial toxicity, i.e., suspension growth of ≤50% of the solvent control, was not observed at any concentration with or without S9 activation.

Based on the results of the preliminary toxicity assay, the doses chosen for the mutagenesis assay ranged from 50 to 5000 µg/mL for both the non-activated and S9-activated cultures. No visible precipitate was present at any concentration in treatment medium. No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10^6 clonable cells over that of the solvent control. There was not a dose-response trend. Toxicity in the cloned cultures, i.e., total growth of ≤50% of the solvent control, was not observed at any doses without activation but was observed with S9 activation at doses of 4000 and 5000 µg/mL.

The trifluorothymidine-resistant colonies for the positive and solvent control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

Under the conditions of this study, test article ammonium perchlorate was concluded to be negative in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells.

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, ammonium perchlorate, was received by BioReliance on November 16, 1998 and was assigned the code number 98BA06. The test article was characterized by the manufacturer as a white powder, which should be stored in a cool dry place. Its purity was given as 99.999%. Upon receipt, the test article was described as a white, crystalline solid and was stored at room temperature, protected from light and moisture.

The vehicle (solvent) used to deliver ammonium perchlorate to the test system was DMSO (CAS 67-68-5) obtained from Fisher.

Methyl methanesulfonate (MMS), CAS 66-27-3, lot # 09419LR, expiration date 5/01, was supplied by Aldrich Chemical Company and was used as the positive control for the non-activated test system at stock concentrations of 1000 and 2000 µg/mL. 7,12-Dimethyl-benz(a)anthracene (7,12-DMBA), CAS 57-97-6, lot # 85H0296, expiration date 1/99, was supplied by Sigma Chemical Company and was used at stock concentrations of 250 and 400 µg/mL as the positive control for the S9-activated test system.

MATERIALS AND METHODS

Test System

L5178Y cells, clone 3.7.2C, were obtained from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, NC. Each lot of cryopreserved cells was tested using the agar culture and Hoechst staining procedures and found to be free of mycoplasma contamination. Prior to use in the assay, L5178Y cells were cleansed of spontaneous $TK^{+/-}$ cells by culturing in a restrictive medium (Clive and Spector, 1975).

Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor-1254, 500 mg/kg, five days prior to sacrifice. The S9 was batch prepared and stored at ≤-70°C until used. Each bulk preparation of S9 was assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 was mixed with the cofactors and Fischer's Medium for Leukemic Cells of Mice with 0.1% Pluronics (F_0P) to contain 250 µL S9, 6.0 mg nicotinamide adenine dinucleotide phosphate (NADP), 11.25 mg DL-isocitric acid and 750 µL F_0P per mL of S9-activation mixture and kept on ice until used. The cofactor/F_0P mixture was filter sterilized and adjusted to pH 7.0 prior to the addition of S9. The formulation of the activation mixture is based on information from Turner *et al.* (1984). The final concentration of S9 in the treatment medium was 10%.

Solubility Test

A solubility test was conducted to select the solvent. The test was conducted using one or more of the following solvents in the order of preference as listed: distilled water, dimethyl sulfoxide, ethanol and acetone. The test article was tested to determine the solvent, selected in order of preference, that permitted preparation of the highest soluble or workable concentration, up to 500 mg/mL (the highest concentration tested).

Preliminary Toxicity Assay

The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation.

Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to $3x10^5$ cells/mL after 24 hours only. Cultures with less than $3x10^5$ cells/mL were not adjusted. Toxicity was measured as suspension growth relative to the growth of the solvent controls.

Mutagenesis Assay

The mutagenesis assay was used to evaluate the mutagenic potential of the test article. L5178Y mouse lymphoma cells were exposed to the solvent alone and at least eight concentrations of test article in duplicate in both the absence and presence of S9. Positive controls, with and without S9-activation, were tested concurrently.

Treatment of the Target Cells

The mutagenesis assay was performed according to a protocol described by Clive and Spector (1975). Treatment was carried out in conical tubes by combining 6 x 106 L5178Y/TK+/- cells, 4 mL F0P medium or S9 activation mixture and 100 µL dosing solution of test or control article in solvent or solvent alone in a total volume of 10 mL. A total of at least eight concentrations of test article were tested in duplicate. The positive controls were treated with MMS (at final concentrations in treatment medium of 10 and 20 µg/mL) and 7,12-DMBA (at final concentrations in treatment medium of 2.5 and 4.0 µg/mL). Treatment tubes were gassed with 5±1% CO2 in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C.

The preparation and addition of the test article dosing solutions were carried out under amber lighting and the cells were incubated in the dark during the exposure period. After the treatment period, the cells were washed twice with F0P or F0P supplemented with 10% horse serum and 2 mM L-glutamine (F10P). After the second wash, the cells were resuspended in F10P, gassed with 5±1% CO2 in air and placed on the roller drum apparatus at 37±1°C.

Expression of the Mutant Phenotype

For expression of the mutant phenotype,. the cultures were counted using an electronic cell counter and adjusted to $3x10^5$ cells/mL at approximately 24 and 48 hours after treatment in 20 and 10 mL total volume, respectively. Cultures with less than $3x10^5$ cells/mL were not adjusted.

For expression of the $TK^{-/-}$ cells, cells were placed in cloning medium (C.M.) containing 0.23% granulated agar. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or V.C. (viable count). Each flask was prewarmed to 37±1°C, filled with 100 mL C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was decanted. The cells were then diluted in C.M. to concentrations of $3x10^6$ cells/100 mL C.M. for the TFT flask and 600 cells/100 mL C.M. for the V.C. flask. After the dilution, 1.0 mL of stock solution of TFT was added to the TFT flask (final concentration of 3 µg/mL) and both this flask and the V.C. flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed and 33 mL of the cell suspension was pipetted into each of three appropriately labeled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 30 minutes. The plates were then incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for 10-14 days.

Scoring Procedures

After the incubation period, the V.C. plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with ≥10% total relative growth. The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm. The rationale for this procedure is as follows: Mutant L5178Y $TK^{-/-}$ colonies exhibit a characteristic frequency distribution of colony sizes. The precise distribution of large and small TFT-resistant mutant colonies appears to be the characteristic mutagenic "finger-print" of carcinogens in the L5178Y $TK^{+/-}$ system (Clive *et al.*, 1979; DeMarini *et al.*, 1989). Clive *et al.* (1979) and Hozier *et al.* (1981) have presented evidence to substantiate the hypothesis that the small colony variants carry chromosome aberrations associated with chromosome 11, the chromosome on which the TK locus is located in the mouse (Kozak and Ruddle, 1977). They suggested that large colony mutants received very localized damage, possibly in the form of a point mutation or small deletion within the TK locus, while small colony mutants received damage to collateral loci concordant with the loss of TK activity.

Evaluation of Results

The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 10^6 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding V.C. plates and multiplying by the dilution factor ($2x10^{-4}$) then multiplying by 10^6. For simplicity, this is described as: (Average # TFT colonies / average # VC colonies) x 200 in the tables.

In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on sound scientific judgement; however, the following criteria are presented as a guide to interpretation of the data (Clive *et al.*, 1995):

- The result was considered to induce a positive response if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level.

- A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level.

- Test articles producing fewer than 55 mutants per 10^6 clonable cells over the background level were concluded to be negative.

Criteria for a Valid Test

The following criteria must be met for the mutagenesis assay to be considered valid:

Negative Controls:

The spontaneous mutant frequency of the solvent control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%.

Positive Controls:

At least one concentration of each positive control must exhibit mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).

Test Article-Treated Cultures:

A minimum of four analyzable concentrations with mutant frequency data will be required.

Archives

All raw data, protocol, and a copy of all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at:

BioReliance
14920 Broschart Rd.
Rockville, MD 20850

RESULTS AND DISCUSSION

Solubility Test

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in DMSO at 500 mg/mL, the maximum concentration tested.

Preliminary Toxicity Assay

The results of the preliminary toxicity assay are presented in Table 1. The maximum dose tested in the preliminary toxicity assay was 5000 µg/mL. No visible precipitate was present at any dose level in treatment medium. The osmolality of the solvent control was 447 mmol/kg and the osmolality of the highest soluble dose, 5000 µg/mL, was 462 mmol/kg. Suspension growth relative to the solvent controls was 89% at 5000 µg/mL without activation and 72% at 5000 µg/mL with S9 activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 50 to 5000 µg/mL for both the non-activated and S9-activated cultures.

Mutagenesis Assay

The results of the mutagenesis assay are presented in Tables 2 through 5. Colony size distributions are presented in Figures 1 and 2. No visible precipitate was present at any dose level in treatment medium. In the non-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL were cloned and produced a range in suspension growth of 61% to 98%. In the S9-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL were cloned and produced a range in suspension growth of 14% to 80%.

No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed in the non-

activated or S9-activated systems. The total growths ranged from 69% to 92% for the non-activated cultures at concentrations of 1000 to 5000 µg/mL and 13% to 85% for the S9-activated cultures at concentrations of 1000 to 5000 µg/mL.

The TFT-resistant colonies for the positive and solvent control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, ammonium perchlorate was concluded to be negative.

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TABLE 1

PRELIMINARY TOXICITY ASSAY USING ammonium perchlorate

Test Article Concentration (µg/mL)	Cell Concentration (X10^6)[a] Day 1	Day 2	Suspension Growth Total[b]	% of Control[c]
WITHOUT ACTIVATION				
Solvent 1	0.921	1.343	13.7	
Solvent 2	0.915	1.297	13.2	
.5	0.901	1.362	13.6	101
1.5	0.923	1.357	13.9	103
5	0.863	1.373	13.2	98
15	0.827	1.392	12.8	95
50	0.872	1.323	12.8	95
150	0.926	1.282	13.2	98
500	0.862	1.359	13.0	97
1500	0.895	1.281	12.7	95
5000	0.732	1.469	11.9	89
WITH S-9 ACTIVATION				
Solvent 1	0.663	1.292	9.5	
Solvent 2	0.650	1.333	9.6	
.5	0.686	1.368	10.4	109
1.5	0.693	1.379	10.6	111
5	0.700	1.349	10.5	110
15	0.652	1.307	9.5	99
50	0.663	1.341	9.9	103
150	0.647	1.316	9.5	99
500	0.661	1.333	9.8	102
1500	0.606	1.413	9.5	99
5000	0.507	1.224	6.9	72

Solvent = DMSO

1 and 2 are duplicate cultures

[a] - Cultures containing $<0.3x10^6$ cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / $0.3x10^6$ cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)	TFT Colonies Counts				Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent 1	22	21	24		22 ±1	191	178	170	180	±9	25		
Solvent 2	20	17	25		21 ±3	179	140	172	164	±17	25		
Mean Solvent Mutant Frequency= 25													
1000 A	27	17	15		20 ±5	149	176	148	158	±13	25	0	83
1000 B	13	21	24		19 ±5	157	156	131	148	±12	26	1	84
2000 A	28	26	14		23 ±6	168	155	159	161	±5	28	3	81
2000 B	18	22	19		20 ±2	172	181	169	174	±5	23	-2	92
3000 A	25	25	25		25 ±0	172	158	163	164	±6	30	5	90
3000 B	24	24	26		25 ±1	153	177	181	170	±12	29	4	85
4000 A	19	14	14		16 ±2	190	179	197	189	±7	17	-8	86
4000 B	31	20	24		25 ±5	205	184	207	199	±10	25	0	92
5000 A	17	24	25		22 ±4	195	183	157	178	±16	25	0	69
5000 B	24	34	32		30 ±4	203	188	189	193	±7	31	6	69
Positive Control - Methyl Methanesulfonate (µg/mL)													
10	106	139	159		135 ±22	114	110	114	113	±2	239	214	48
20	106	127	119		117 ±9	35	40	46	40	±4	582	557	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 3

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.438	1.362	21.8		180		
Solvent	2	1.446	1.270	20.4		164		
1000	A	1.229	1.397	19.1	90	158	92	83
1000	B	1.301	1.425	20.6	98	148	86	84
2000	A	1.236	1.333	18.3	87	161	94	81
2000	B	1.311	1.306	19.0	90	174	101	92
3000	A	1.200	1.492	19.9	94	164	96	90
3000	B	1.187	1.367	18.0	85	170	99	85
4000	A	1.131	1.318	16.6	79	189	110	86
4000	B	1.135	1.323	16.7	79	199	116	92
5000	A	1.062	1.193	14.1	67	178	104	69
5000	B	1.068	1.085	12.9	61	193	113	69
Positive Control - Methyl Methanesulfonate (µg/mL)								
10		1.232	1.134	15.5	74	113	66	48
20		1.081	0.884	10.6	50	40	23	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Cultures containing <0.3x10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)	TFT Colonies Counts			Mean	VC Colonies Counts			Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent 1	21	28	30	26 ±4	150	130	157	146 ±11	36		
Solvent 2	24	34	50	36 ±11	163	178	163	168 ±7	43		
Mean Solvent Mutant Frequency= 40											
1000 A	24	30	18	24 ±5	186	208	166	187 ±17	26	-14	80
1000 B	15	19	28	21 ±5	158	157	181	165 ±11	25	-15	85
2000 A	35	35	25	32 ±5	190	173	153	172 ±15	37	-3	65
2000 B	21	24	26	24 ±2	199	170	207	192 ±16	25	-15	82
3000 A	35	38	33	35 ±2	192	165	169	175 ±12	40	1	58
3000 B	28	22	34	28 ±5	186	176	186	183 ±5	31	-9	64
4000 A	38	32	33	34 ±3	169	172	150	164 ±10	42	2	41
4000 B	33	28	34	32 ±3	183	185	175	181 ±4	35	-5	42
5000 A	38	34	47	40 ±5	135	137	142	138 ±3	57	18	13
5000 B	+	40	50	45 ±4	191	162	173	175 ±12	51	12	21
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)											
2.5	135	129	136	133 ±3	120	129	137	129 ±7	207	168	65
4	171	166	189	175 ±10	130	111	99	113 ±13	309	270	42

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

+ - Culture lost to contamination

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 5

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK+/- MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.291	1.443	20.7		146		
Solvent	2	1.315	1.503	22.0		168		
1000	A	1.150	1.129	14.4	68	187	119	80
1000	B	1.225	1.256	17.1	80	165	105	85
2000	A	1.014	1.114	12.5	59	172	110	65
2000	B	1.060	1.218	14.3	67	192	122	82
3000	A	0.919	1.088	11.1	52	175	112	58
3000	B	0.845	1.248	11.7	55	183	116	64
4000	A	0.685	1.108	8.4	40	164	104	41
4000	B	0.642	1.083	7.7	36	181	115	42
5000	A	0.293	0.919	3.1	14	138	88	13
5000	B	0.412	0.866	4.0	19	175	112	21
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)								
2.5		1.087	1.397	16.9	79	129	82	65
4		0.949	1.188	12.5	59	113	72	42

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Cultures containing <0.3x10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

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Figure 1

Colony Size Distribution in the Absence of Metabolic Activation

(Positive Control Compared with Solvent Control)

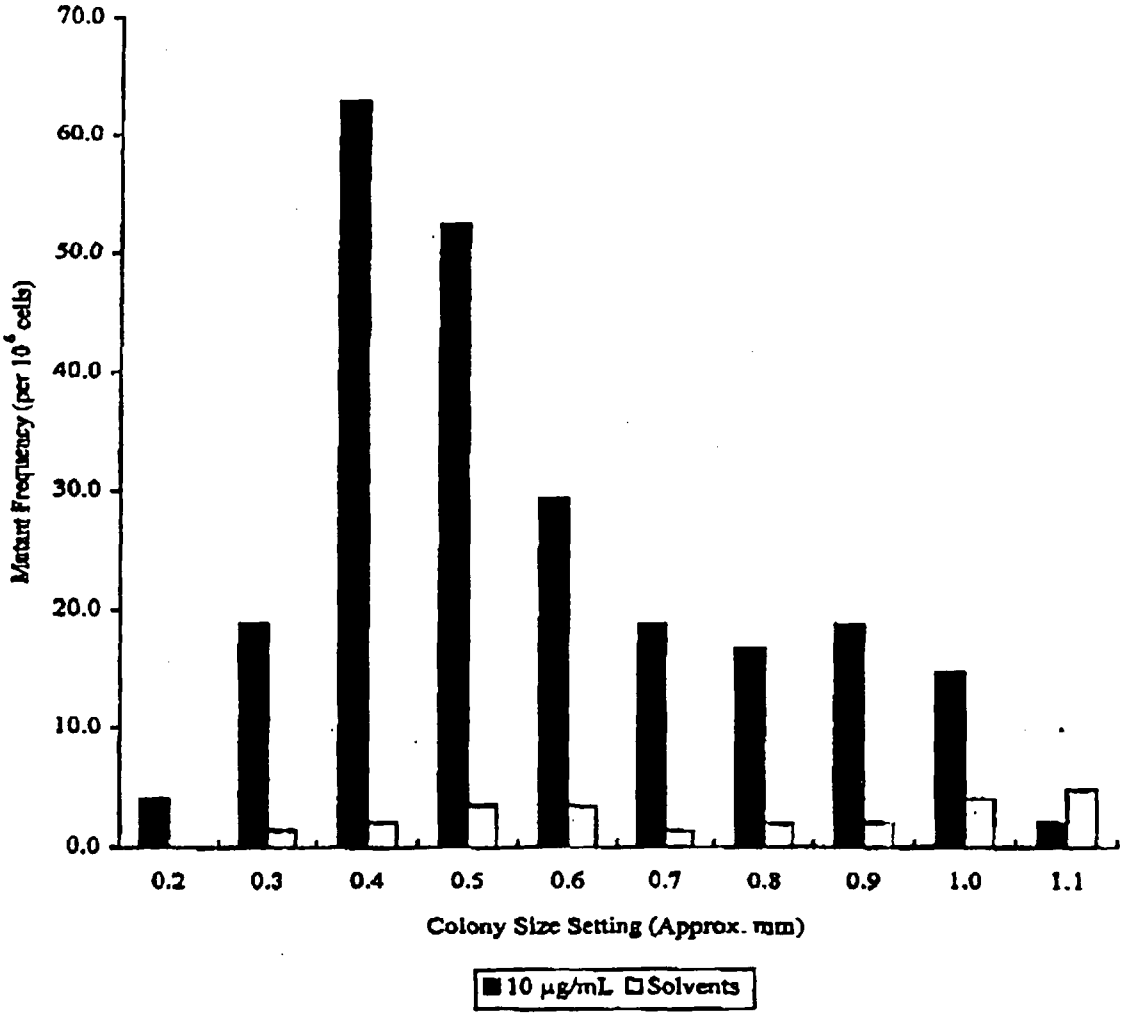

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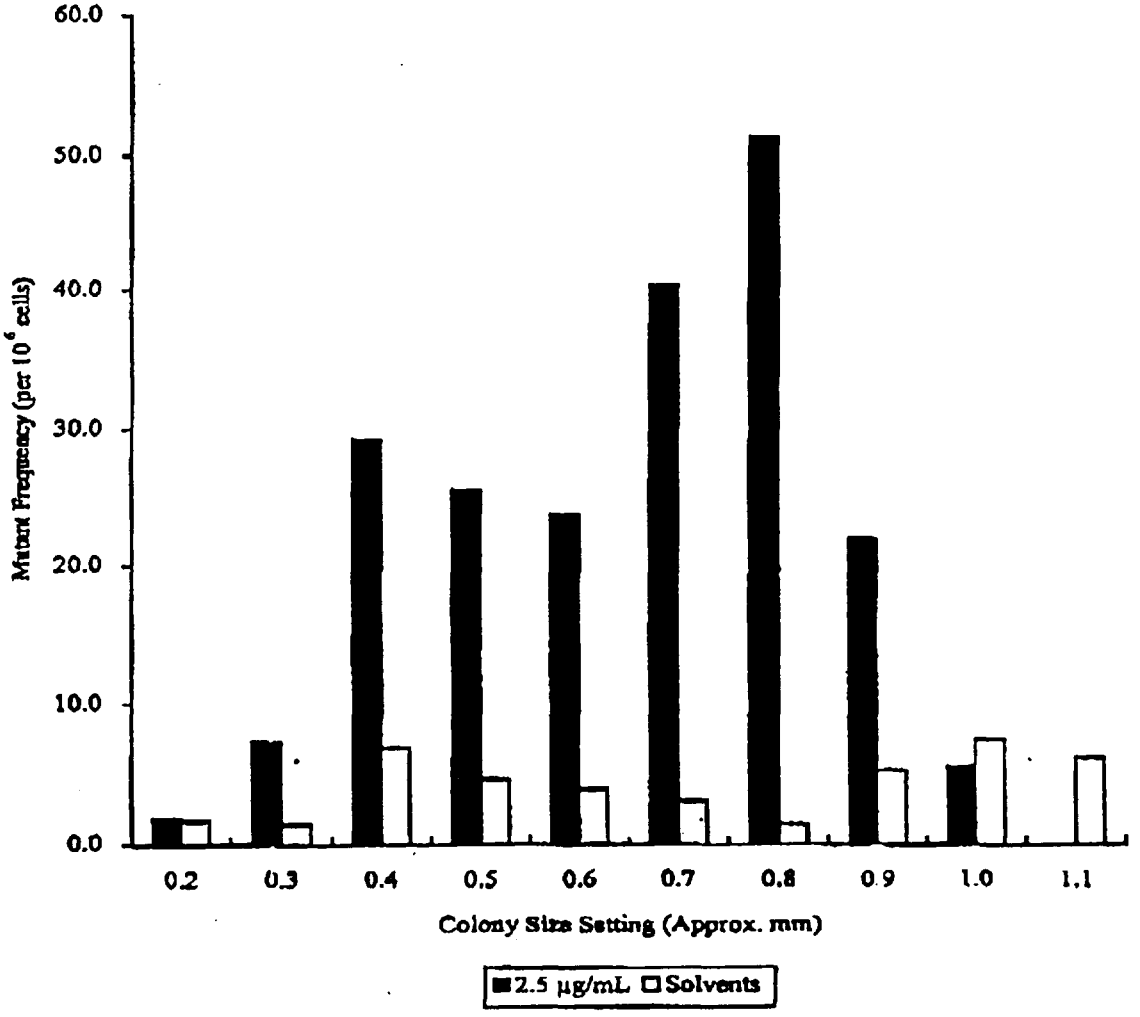
Figure 2

Colony Size Distribution in the Presence of Metabolic Activation

(Positive Control Compared with Solvent Control)



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APPENDIX I

Historical Control Data

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Mouse Lymphoma Historical Control Data

1995-1997

	Non-activated			S9-Activated		
	Solvent Control	20 µg/mL MMS	10 µg/mL MMS	Solvent Control	4.0µg/mL DMBA	2.5µg/mL DMBA
Mean MF	35.7	655.3	336.0	58.0	453.2	269.8
SD	10.3	293.3	128.5	18.6	158.5	95.1
Maximum	68.0	2400.0	729.0	100.0	1029.0	1048.0
Minimum	20.0	198.0	128.0	28.0	222.0	141.0

Solvent control (Fischer's medium, distilled water, saline, DMSO, ethanol, acetone or vehicle supplied by Sponsor)

MMS Methyl methanesulfonate
DMBA Dimethylbenz(a)anthracene
MF Mutant frequency per 10^6 clonable cells
SD Standard deviation

APPENDIX II

Study Protocol

QA 12-4-98
APPROVED

In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

1.0 PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells.

2.0 SPONSOR

2.1	Name:	**Perchlorate Study Group**
2.2	Address:	Highway 50 and Aerojet Road Building 20019/Department 0330 Rancho Cordova, CA 95813-6000
2.3	Study Monitor:	Michael F. Girard Perchlorate Study Group Representative Telephone: (916) 355-2945 Telefax: (916) 355-6145
2.4	Scientific Advisor:	Michael L. Dourson, Ph.D., DABT Toxicology Excellence for Risk Assessment 4303 Hamilton Ave. Cincinnati, OH 45223 Telephone: (513) 542-7475 Telefax: (513) 542-7487
2.5	Sponsor Project #:	NP

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1	Test Article:		**Ammonium perchlorate**
3.2	Controls:	Negative:	Test article solvent (or vehicle)
		Positive:	Methyl methanesulfonate (MMS) 7,12-dimethylbenz(a)anthracene (DMBA)

3.3 Determination of Strength, Purity, etc.

Unless alternate arrangements are made, the testing facility at BioReliance will not perform analysis of the dosing solutions. The Sponsor will be directly responsible

for determination and documentation of the analytical purity and composition of the test article, and the stability and strength of the test article in the solvent (or vehicle).

3.4 Test Article Retention Sample

The retention of a reserve sample of the test article will be the responsibility of the Sponsor.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Toxicology Testing Facility BioReliance
4.2	Address:	9630 Medical Center Drive Rockville, MD 20850
4.3	Study Director:	Richard H. C. San, Ph.D.

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 12/7/98

5.2 Proposed Experimental Completion Date: 1/19/99

5.3 Proposed Report Date: 1/28/99

6.0 TEST SYSTEM

L5178Y/TK$^{+/-}$ mouse lymphoma cells are heterozygous at the normally diploid thymidine kinase (TK) locus. L5178Y/TK$^{+/-}$, clone 3.7.2C, were received from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, North Carolina. Each freeze lot of cells has been tested and found to be free of mycoplasma contamination. This system has been demonstrated to be sensitive to the mutagenic activity of a variety of chemicals.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The mammalian mutation assay will be performed by exposing duplicate cultures of L5178Y/TK$^{+/-}$ cells to a minimum of eight concentrations of test article as well as positive and negative (solvent) controls. Exposures will be for 4 hours in the presence and absence of an S9 activation system. Following a two day expression period, with daily cell population adjustments, cultures demonstrating 0% to 90% growth inhibition will be cloned, in triplicate, in restrictive medium containing soft agar to select for the mutant phenotype. After a 10 to 14 day selection period, mutant colonies will be enumerated. The mutagenic potential of the test article will be measured by its ability to induce TK$^{+/-}$ → TK$^{-/-}$ mutations. For those test articles demonstrating a positive response, mutant colonies will be sized as an indication of mechanism of action.

7.1 Selection of Solvent

Unless the Sponsor has indicated the test article solvent, a solubility determination will be conducted to measure the maximum soluble concentration in a variety of solvents. Solvents compatible with this test system, in order of preference, include, but are not limited to, culture medium or distilled water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5) and acetone CAS 67-64-1). The solvent of choice will be that solvent, selected in order of preference, that permits preparation of the highest soluble stock concentration, up to a maximum of 500 mg/ml.

7.2 Dose Selection

In the preliminary toxicity test, L5178Y/TK$^{+/-}$ cells will be exposed to solvent alone and to at least nine concentrations of test article, the highest concentration being the lowest insoluble dose in treatment medium but not to exceed 5000 µg/ml. The pH of the treatment medium will be adjusted, if necessary, to maintain a neutral pH in the treatment medium. The osmolality of the highest soluble treatment condition will also be measured. After a 4-hour treatment in the presence and absence of S9 activation, cells will be washed twice with F_0P (Fischer's Media for Leukemic Cells of Mice with 0.1% Pluronics) or $F_{10}P$ (F_0P supplemented with 10% horse serum and 2mM L-glutamine) and cultured in suspension for two days post-treatment, with cell concentration adjustment on the first day.

Selection of dose levels for the mutation assay will be based on reduction of suspension growth after treatment in the preliminary toxicity test. Unless specified otherwise by the Sponsor, the high dose for the mutation assay will be that concentration exhibiting approximately 100% growth inhibition. The low dose will be selected to exhibit 0% growth inhibition. For freely soluble, non-toxic test articles, the highest concentration will be 5000 µg/ml. For relatively insoluble, non-toxic test articles, the highest concentration will be the lowest insoluble dose in treatment medium but not to exceed 5000 µg/ml. In all cases, precipitation will be evaluated at the beginning and at the end of the treatment period using the naked eye (ICH, 1996).

7.3 Route and Frequency of Administration

Cell cultures will be treated for 4 hours by way of a vehicle compatible with the system, both in the presence and absence of metabolic activation. This technique of administration has been demonstrated to be effective in the detection of chemical mutagens in this system.

7.4 Exogenous Metabolic Activation

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The source of S9 will be adult male Sprague-Dawley rats induced by a single injection of Aroclor 1254 at a dose level of 500 mg/kg body weight five days prior

to sacrifice. The S9 will be batch prepared and stored frozen at approximately -70°C until used.

Immediately prior to use, the S9 will be thawed and mixed with a cofactor pool to contain 11.25 mg DL-isocitric acid, 6 mg NADP, and 0.25 ml S9 homogenate per ml in F_0P. The S9 mix will be adjusted to pH 7.

7.5 Controls

7.5.1 Negative Control

The solvent (or vehicle) for the test article will be used as the negative control.

7.5.2 Positive Controls

Methyl methanesulfonate (MMS) will be used at two concentrations of 10 and 20 µg/ml as the positive control for the non-activated test system. For the S9-activated system, 7,12-dimethylbenz(a)anthracene (DMBA) will be used at two concentrations of 2.5 and 4.0 µg/ml.

7.6 Preparation of Target Cells

Prior to use in the assay, L5178Y/TK$^{+/-}$ cells will be cleansed to reduce the frequency of spontaneously occurring TK$^{-/-}$ cells. Using the procedure described by Clive and Spector (1975), L5178Y cells will be cultured for 24 hours in the presence of thymidine, hypoxanthine, methotrexate and glycine to poison the TK$^{-/-}$ cells.

L5178Y/TK$^{+/-}$ cells will be prepared at 1 x 10^6 cells/ml in 50% conditioned $F_{10}P$ and 50% F_0P. If cultures are to be treated with more than 100 µl of test article dosing solution, the cell concentration may be adjusted.

7.7 Identification of the Test System

Using a permanent marking pen, the treatment tubes will be identified by the study number and a code system to designate the treatment condition and test phase.

7.8 Treatment of Target Cells

Treatment will be carried out in conical tubes by combining 100 µl dosing solution of test or control article in solvent or solvent alone, 4 ml F_0P medium or S9 activation mixture with 6 x 10^6 L5178Y/TK$^{+/-}$ cells in a total volume of 10 ml. A minimum of eight concentrations of test article will be tested in duplicate. All pH adjustments will be performed prior to adding S9 or target cells to the treatment medium. Volumes of test article dosing solution in excess of 100 µl may be used if required to achieve the target final concentration in treatment medium. Treatment

tubes will be gassed with 5±1% CO_2 in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C. The preparation and addition of the test article dosing solutions will be carried out under amber lighting and the cells will be incubated in the dark during the 4-hour exposure period.

7.9 Expression of the Mutant Phenotype

At the end of the exposure period, the cells will be washed twice with F_0P or $F_{10}P$ and collected by centrifugation. The cells will be resuspended in 20 ml $F_{10}P$, gassed with 5±1% CO_2 in air and cultured in suspension at 37±1°C for two days following treatment. Cell population adjustments to 0.3 x 10^6 cells/ml will be made at 24 and 48 hours.

7.10 Selection of the Mutant Phenotype

For selection of the trifluorothymidine (TFT)-resistant phenotype, cells from a minimum of five non-activated and five S9-activated test article concentrations demonstrating from 0% to 90% suspension growth inhibition will be plated into three replicate dishes at a density of 1 x 10^6 cells/100mm plate in cloning medium containing 0.23% agar and 2-4 µg TFT/ml. For estimation of cloning efficiency at the time of selection, 200 cells/100mm plate will be plated in triplicate in cloning medium free of TFT (viable cell (VC) plate). Plates will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 for 10-14 days.

The total number of colonies per plate will be determined for the VC plates and the total relative growth calculated. The total number of colonies per TFT plate will then be determined for those cultures with ≥10% total growth. Colonies are enumerated using an automatic counter; if the automatic counter cannot be used, the colonies will be counted manually. The diameters of the TFT colonies from the positive control and solvent control cultures will be determined over a range of approximately 0.2 to 1.1 mm. In the event the test article demonstrates a positive response, the diameters of the TFT colonies for at least one dose level of the test article (the highest positive concentration) will be determined over a range of approximately 0.2 to 1.1 mm.

7.11 Independent Repeat Assay

Verification of a clear positive response will not be required (OECD Guideline 476, ICH 1997). For equivocal and negative results, the Sponsor will be consulted regarding the need for an independent repeat assay.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

8.1 Negative Controls

The spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent (or vehicle) control group must be greater than 50%.

8.2 Positive Controls

At least one concentration of each positive control must exhibit mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).

8.3 Test Article-Treated Cultures

A minimum of four analyzable concentrations with mutant frequency data will be required.

9.0 EVALUATION OF TEST RESULTS

The cytotoxic effects of each treatment condition are expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency for each treatment condition is calculated by dividing the mean number of colonies on the TFT-plates by the mean number of colonies on the VC-plates and multiplying by the dilution factor (2×10^{-4}), and is expressed as TFT-resistant mutants per 10^6 surviving cells.

In evaluation of the data, increases in mutant frequencies which occur only at highly toxic concentrations (i.e., less than 10% total growth) are not considered biologically relevant. All conclusions will be based on sound scientific judgement; however, the following criteria are presented as a guide to interpretation of the data (Clive *et al.*, 1995):

- The result will be considered to induce a positive response if a concentration-related increase in mutant frequency is observed and one or more dose levels with 10% or greater total growth exhibit mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level.

- A result will be considered equivocal if the mutant frequency in treated cultures is between 55 and 99 mutants per 10^6 clonable cells over the background level.

- Test articles producing fewer than 55 mutants per 10^6 clonable cells over the background level will be concluded to be negative.

10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used in the generation and analysis of data.

Results presented will include, but not be limited to:

- test substance: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.
- solvent/vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- cell type used, number of cultures, methods for maintenance of cell cultures
- rationale for selection of concentrations and number of cultures
- test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period, selective agent
- method used to enumerate numbers of viable and mutant colonies and the number of colonies in each plate
- dose-response relationship, if applicable
- distribution of the mutant colony diameter for the solvent and positive controls and, when the test article induces a positive response, for at least one dose level of the test article (the highest positive concentration)
- positive and solvent control historical data

11.0 RECORDS AND ARCHIVES

Upon completion of the final report, all raw data and reports will be maintained in the archives of BioReliance, Rockville, MD in accordance with the relevant Good Laboratory Practice Regulations.

12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), July 1997, and with the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, S2A document recommended for

adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202. April 24, 1996.

This study will be performed in compliance with the provisions of the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.

Will this study be submitted to a regulatory agency? yes

If so, to which agency or agencies? U.S. EPA, U.S. DOD

Unless arrangements are made to the contrary, unused dosing solutions will be disposed of following administration to the test system and all residual test article will be disposed of following finalization of the report.

13.0 REFERENCES

Aaron, C.S., Bolcsfoldi, G., Glatt, H.-R., Moore, M., Nishi, Y., Stankowski, L., Theiss, J. and Thompson, E. (1994) Mammalian cell gene mutation assays working group report. Mutation Research 312:235-239.

Clive, D., Bolcsfoldi, G., Clements, J., Cole, J., Homna, M., Majeska, J., Moore, M., Muller, L., Myhr, B., Oberly, T., Oudelhkim, M., Rudd, C., Shimada, H., Sofuni, T., Thybaud, V. and Wilcox, P. (1995) Consensus agreement regarding protocol issues discussed during the mouse lymphoma workshop: Portland, Oregon, May 7, 1994. Environ. Molec. Mutagen. 25:165-168.

Clive, D. and Spector, J.F.S. (1975) Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Research 31:17-29.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202. April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. Federal Register 62:16026-16030, November 21, 1997.

Moore, M.M., Clive, D., Howard, B.E., Batson, A.G. and Turner, N.T. In situ analysis of trifluorothymidine-resistant (TFT) mutants of L5178Y/TK$^{+/-}$ mouse lymphoma cells. (1985) Mutation Research 151:147-159.

OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), July 1997.

14.0 APPROVAL

Michael F. Girard Sponsor Study Monitor	11/19/98 Date
Michael L. Dourson, Ph.D., DABT Sponsor Scientific Advisor	11-10-98 Date
Richard H.C. San, Ph.D. BioReliance Study Director	12/2/98 Date

If submission to Japanese Regulatory Agency is indicated in section 12.0, BioReliance management will sign.

David Jacobson-Kram, Ph.D., DABT BioReliance Study Management	Date

5.3 GENOTOXICITY ASSAYS

ManTech Environmental Technology, Inc., performed a battery of three genotoxicity assays (*Salmonella typhimurium*/microsome mutagenesis assay [Ames assay], the mouse lymphoma cell mutagenesis assay [L5178Y-TK test], and the in vivo mouse bone marrow micronucleus induction assay) with ammonium perchlorate to help determine its potential for various interactions with DNA and to gain insight on its possible carcinogenicity (ManTech Environmental Technology, Inc., 1998). To confirm the findings of ManTech Environmental Technology, the EPA requested the National Toxicology Program to also evaluate ammonium perchlorate in the Ames assay and the mouse bone marrow micronucleus test (NTP, 1999a). The sponsor (PSG) also had the mouse lymphoma assay repeated (BioReliance, 1999).

Ammonium Perchlorate was evaluated in the Ames assay (*Salmonella typhimurium*/ microsome mutagenesis assay), which is a well-defined assay for detection of carcinogens/ mutagens. It measures the reversion from a his$^-$ (histidine independent) state induced by chemicals that cause base-pair changes or frameshift mutations in the genome of the organism (i.e., it measures for point mutations [e.g., substitution, addition, or deletion of one or a few DNA base pairs within a gene]). In this assay, bacteria are exposed to the test chemical with and without a metabolic activation system (Arochlor 1254-induced rat liver S9 with co-factors). The mutagenicity is evaluated by the increase in the number of revertant colonies. The L5178Y mouse-lymphoma assay is another short term in vitro assay to detect both point mutations and structural chromosomal changes. The in vivo mammalian micronucleus test detects the damage of chromosomes or of the mitotic apparatus caused by a clastogenic chemical in bone marrow cells (polychromatic erythrocyte [PCE] stem cells) of treated animals. Micronuclei are believed to be formed from chromosomes or chromosome fragments left behind during anaphase of mitosis. The induction of micronuclei indicates changes in either chromosome structure or number in bone marrow cells. ManTech Environmental Technology, Inc., performed this assay in Swiss-CD-1 mice and the NTP used B6C3F1 mice (NTP, 1999a). The micronucleus assay also was performed as part of the 90-day bioassay in Spraque-Dawley rats (Springborn Laboratories, Inc., 1998). This is an adequate series of tests to determine the mutagenic and clastogenic

(chromosomal breaking) potential of an agent. It should be noted that perchlorate is not likely to be mutagenic, given its physical and chemical properties (i.e., it is simply an anion). Although perchlorate is an oxidizing agent, it is not expected to produce oxidative DNA damage because of the kinetic considerations discussed in Chapter 2.

5.3.1 In Vitro Assays

Ammonium perchlorate was not found to be mutagenic in the *Salmonella typhimurium* (Ames assay) with and without Arochlor 1254-induced rat liver S9 activation by two separate laboratories (ManTech Environmental Technology, Inc., 1998; NTP, 1999b). In the ManTech study, ammonium perchlorate was dissolved in distilled water and tested at five concentrations (5,000, 2,500, 1,250, 625, and 312.5 µg/plate) in tester strains TA98, TA100, TA1535, and TA1537, without and with Arochlor 1254-induced rat liver S9 using the plate incorporation assay. Although this study was regarded as adequate, the EPA requested that Ames assay be repeated by the National Toxicology Program (NTP) to confirm the negative findings and to include additional tester strains (i.e., TA102 and TA104) which are able to detect a variety of oxidative mutagens. Therefore, NTP evaluated ammonium perchlorate in the Salmonella/Ames assay in tester strains TA98, TA100, TA1535, TA97, TA102, and TA104 (NTP, 1999b). Ammonium perchlorate was dissolved in distilled water and tested using the preincubation procedure at doses of 10,000, 3,333, 1,000, 333, and 100 µg/plate, with and without metabolic activation from Arochlor-induced rat and hamster livers. Ammonium perchlorate was neither toxic nor mutagenic under the conditions of the NTP assay.

The L5178Y/$tk^{+/-}$ mouse lymphoma assay also was used to evaluate the mutagenic and chromosomal breaking potential of ammonium perchlorate in vitro. Ammonium perchlorate was reported to be negative both in the absence and presence of rat Arochlor-induced S9 liver activation (ManTech Environmental Technology, Inc., 1998). Ammonium perchlorate was evaluated at 5.0, 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL without S9 activation, and at 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL with S9 activation. Although a small increase in mutation frequency was found in the absence of S9 activation at 2.5 mg/mL, which appeared to be statistically significant ($p < 0.05$) by the two-tail, Student's t-test, a repeat assay found no increase in

mutation frequency at this concentration compared with controls. Therefore, ammonium perchlorate is considered to be negative in the absence of S9 activation. Confidence in the negative findings without S9 activation is reinforced by the wide range of ammonium perchlorate concentrations evaluated. Although ammonium perchlorate also was reported as negative in the presence of S9 activation, the response of the positive control, 3-methyl cholanthrene (MCA), in the actual experiment was too low (182.6×10^{-6}) to be acceptable. The highest dose of ammonium perchlorate produced a mutation frequency of 194×10^{-6}. The MCA at 2.5 µg/mL should induce a mutation frequency of 300 to 350×10^{-6} or higher. Such a low positive control response weakens the confidence for the negative finding with S9 activation. In addition, the cloning efficiencies for the S9 test appear to be too high (143%), further reducing the confidence in a negative finding. Therefore, only the assays on ammonium perchlorate without S9 are considered unequivocally to be negative. Although perchlorate is not expected to be metabolized to a mutagenic intermediate, these S9 data are not of sufficient quality to support a clear negative-response conclusion.

Because of the problems described above, the sponsor (PSG) had the mouse lymphoma assay repeated. In this recent mouse lymphoma assay, ammonium perchlorate was evaluated at concentrations of 1000, 2000, 3000, 4000, and 5000 ug/ml without and with Arochlor 1254-induced rat liver S9 activation (BioReliance, 1999). No increase in mutant frequencies were found after treatment with perchlorate. The data are judged to be of sufficient quality to determine perchlorate to be nonmutagenic both with and without S9 activation. Although the background mutant frequency was low, particularly in the without S9 experiment, the data set still is considered to be overall very good., as well as internally consistent. The problems that were observed in the data generated by the first laboratory (ManTech Environmental Technology, Inc., 1998) are not present in the data from the BioReliance study.

5.3.2 In Vivo Assays

The potential for ammonium perchlorate to induce micronuclei was evaluated in mice and rats. Ammonium perchlorate was administered by drinking water gavage for 3 consecutive days to Swiss CD-1 mice (5 females and 5 males per dose group) at 1,000, 500, 250, 125, and

62.5 mg/kg-day (ManTech Environmental Technology, Inc., 1998). Twenty-four hours after the last dose, the mice were sacrificed, and the frequency of micronucleated cells were evaluated by counting 1,000 PCEs per animal. The assay was conducted in accordance with existing EPA FIFRA/TSCA testing guidelines. No increase in the frequency of micronuclei were found for any dose group. There is some uncertainty whether a maximum tolerated dose (MTD) was reached in this study. The study authors reported that at 2,000 mg/kg, 4 out of 6 animals died after one dosing of ammonium perchlorate. Typically, the assay is performed at 85% of the MTD, and the 1,000 mg/kg-day represents approximately 50% of the LD_{50}. There was no indication of toxicity to the bone marrow cells because the PCE/NCE ratio was not different from negative controls. Furthermore, the study authors did not report any indication of clinical signs of toxicity in the highest dose group. Despite a rebuttal submitted by Dourson (1998) on behalf of the sponsor (PSG), EPA remained concerned because of the importance of this test in the overall determination of the approach to be taken for the carcinogenicity assessment (i.e., to rule out direct genotoxicity).

The NTP agreed to expedite and repeat this test in response to an EPA request. The assay was performed by ip injection to ensure the greatest delivery to the bone marrow. Male B6C3F1 mice were treated with 125, 250, 500, 1,000, 1,500, and 2,000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Note that this study uses two dose groups higher than those used in the previous study (i.e., 1,500 and 2,000 mg/kg). Furthermore, use of ip injection as the route of administration would result in a direct delivery of the compound to the bone marrow cells versus drinking water gavage. Five mice per group were injected daily for 3 consecutive days and were sacrificed 24 h after the last injection; 2,000 PCEs were scored per animal for micronuclei. All animals in the 1,500- and 2,000-mg/kg groups died after the first ip injection, and 4/5 animals died in the 1,000-mg/kg group after the second ip injection. No increases in percent PCE were observed in any of the remaining test groups (125, 250, and 500 mg/kg). No bone marrow toxicity was seen as indicated by the percent of PCE. These results are interpreted to be consistent with those of the ManTech Environmental Technology, Inc. (1998) study that used gavage drinking water administration, and confirm that perchlorate does not induce micronuclei in rodents.

The 90-day subchronic bioassay using Spraque-Dawley rats also evaluated micronuclei induction (Springborn Laboratories, Inc., 1998). The frequency of micronuclei induction was examined in both the males and females after the 90-day sacrifice in the 10-mg/kg-day dose group of ammonium perchlorate administered by drinking water. Although there was no induction of micronuclei at this dose, 10 mg/kg-day does not appear to reach a MTD because there were no overt signs of toxicity, although the definition of MTD may be somewhat moot, given the changes in thyroid hormone economy and histopathology seen in the thyroids at that dose. There was significant reduction in the PCE/NCE ratio (i.e, an indicator of toxicity to the bone marrow cells).

5.3.3 Summary of Genotoxicity Battery Results

Negative results were reported in all genotoxicity assays conducted on ammonium perchlorate when evaluated by two independent laboratories. Ammonium perchlorate was not mutagenic in the Ames assay (with or without S9 activation). Negative results were also found in the mouse lymphoma gene mutation assay without and with S9 activation. Ammonium perchlorate did not induce chromosomal anomalies when evaluated for micronuclei induction in the bone marrow of mice when administered via drinking water gavage or i.p. injection No increases in micronuclei were found in Spraque-Dawley rats when evaluated from the 90-day study at the highest dose, which produced both thyroid hormone perturbations and follicular cell hyperplasia. It is concluded that ammonium perchlorate does not have the potential to be mutagenic or clastogenic. The in vitro and in vivo studies discussed above provide support for that conclusion. Therefore, mutagenicity is not considered a possible mode of carcinogenic action for this chemical.

February 1, 1999 EPA Assessment Submission

Attachment #2
Analysis of Brain Histopathology at 3 mg/kg-day
Argus (1998a) Neurodevelopmental Study

A. Argus 1/20/98 Data Submission (York, 1998f)
B. EPA analysis (Geller, 1999a)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
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November 20, 1998

Annie Jarabek
USEPA, National Center for
Environmental Assessment
3210 Highway 54, Catawba Bldg.
Research Triangle Park, NC 27709

Telephone: (919) 541-4847
Telefax: (919) 541-1818

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Ms. Jarabek:

Enclosed is a diskette containing the thyroid hormone data for the Fo generation adults and F1 generation pups supplied by AniLytics, as well as a summary table created by Argus to show the mean group values and identify which groups are significantly different than control values. Please note that there is an error in the data supplied by AniLytics. For the F1 generation females, pup number 3668 has been incorrectly identified as being in the 30.0 mg/kg/day dosage group, and should be 3.0 mg/kg/day. The summary table does reflect this correction. AniLytics has been made aware of this incorrect value and will make the necessary changes to their data.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:hmg
Enc.
Copies to: D. Mattie
M. Dourson

Protocol 1416-001: Summary of Thyroid Hormone Data

Fo Generation Rats:

Dosage Group	Dosage Level (mg/kg/day)	TSH (ng/mL)		T3 (ng/dL)		T4 (µg/dL)	
		Male Rats	Female Rats	Male Rats	Female Rats	Male Rats	Female Rats
I	0	1.530	2.054	72.547	57.770	4.641	2.126
II	0.3	1.353	2.213	87.389**	64.789	4.726	2.903**
III	3.0	1.487	1.990	88.452**	56.350	4.744	2.924**
IV	30	3.871**	2.174	78.570	60.373	3.578**	2.421

F1 Generation Pups:

Dosage Group	Dosage Level (mg/kg/day)	TSH (ng/mL)		T3 (ng/dL)		T4 (µg/dL)	
		Male Pups	Female Pups	Male Pups	Female Pups	Male Pups	Female Pups
I	0	1.237	1.120	105.897	105.954	4.403	4.270
II	0.3	.941**	1.188	111.150	109.922	4.615	4.865*
III	3.0	.877**	1.141	109.810	109.293	4.533	4.324
IV	30	1.270	1.301	107.398	97.581*	4.525	3.913

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 27 January 1999

Subject: Analysis of the Brain Morphometry Data from the Neurobehavioral Developmental Study of Ammonium Perchlorate (Argus, 1998a)

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is the statistical analysis of the hormone data from the Argus Neurobehavioral Developmental Study (Argus Protocol #1613-002). Data was received from Argus on November 5, 1998 (York, 1998d) and imported in ASCII form to SAS for further analysis. I have attached a description of how the analyses were done, a description of results, and summary graphs.

Analyses of Brain Morphometry Data from Neurobehavioral Developmental Study (Argus, 1998a)

Summary: A memo from Argus Laboratories (York, 1998d) contains brain morphometry data from the control, 3 mg/kg/day and 10 mg/kg/day dose groups from the Neurobehavioral Developmental Study of ammonium perchlorate in the rat at post-natal day 12 in the F1 generation (Argus, 1998a). This memo adds the morphometric data from the 3 mg/kg/day data to that of the control and high dose (10 mg/kg/day) groups previously reported in Tables 1 and 2 of Appendix P (Argus, 1998a). This data had been requested by the USEPA after initial findings of a morphometric increase in the size of the corpus callosum in the high dose group relative to controls. At the time that the report on Perchlorate Environmental Contamination had been prepared for External Review, only the data from the corpus callosum had been re-analyzed by the USEPA (Crofton, 1998c). The results of analysis of the morphometry data from the other brain regions is reported here..

Data was analyzed using a 2-way analysis of variance, with dose and sex as independent variables. It is desirable in the analysis of developmental data to have litter information; since none was included in Appendix P (Argus, 1998a) or the memo (York, 1998d), it is possible that the effects of sex and litter are confounded.

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen.

The corpus callosum showed an increase in size at the highest dose tested (10 mg/kg /day). The other significant dose effects were driven by effects at the 3.0 mg/kg/day dose group. There was a significant decrease in size in this dose group in hippocampal gyrus and caudate putamen and a significant increase in size in anterior/posterior cerebellum.

Data: All data were supplied in the form of a memo (York, 1998d). Data were keyed in and entered as ASCII files for analyses by SAS.

Data for dependent measures (brain weight, anterior/posterior cerebrum, anterior/posterior cerebellum, frontal cortex, parietal cortex, caudate putamen, corpus callosum, hippocampal gyrus, cerebellum, external germinal layer) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

To correct for multiple comparisons the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.016 (alpha of 0.05 divided by the square root of the number of ANOVAs).

Analyses of Brain Morphometry Data from Neurobehavioral Developmental Study (Argus, 1998a)

Summary: A memo from Argus Laboratories (York, 1998d) contains brain morphometry data from the control, 3 mg/kg/day and 10 mg/kg/day dose groups from the Neurobehavioral Developmental Study of ammonium perchlorate in the rat at post-natal day 12 in the F1 generation (Argus, 1998a). This memo adds the morphometric data from the 3 mg/kg/day data to that of the control and high dose (10 mg/kg/day) groups previously reported in Tables 1 and 2 of Appendix P (Argus, 1998a). This data had been requested by the USEPA after initial findings of a morphometric increase in the size of the corpus callosum in the high dose group relative to controls. At the time that the report on Perchlorate Environmental Contamination had been prepared for External Review, only the data from the corpus callosum had been re-analyzed by the USEPA (Crofton, 1998c). The results of analysis of the morphometry data from the other brain regions is reported here.

Data was analyzed using a 2-way analysis of variance, with dose and sex as independent variables. It is desirable in the analysis of developmental data to have litter information; since none was included in Appendix P (Argus, 1998a) or the memo (York, 1998d), it is possible that the effects of sex and litter are confounded.

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen.

The corpus callosum showed an increase in size at the highest dose tested (10 mg/kg /day). The other significant dose effects were driven by effects at the 3.0 mg/kg/day dose group. There was a significant decrease in size in this dose group in hippocampal gyrus and caudate putamen and a significant increase in size in anterior/posterior cerebellum.

Data: All data were supplied in the form of a memo (York, 1998d). Data were keyed in and entered as ASCII files for analyses by SAS.

Data for dependent measures (brain weight, anterior/posterior cerebrum, anterior/posterior cerebellum, frontal cortex, parietal cortex, caudate putamen, corpus callosum, hippocampal gyrus, cerebellum, external germinal layer) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

To correct for multiple comparisons the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.016 (alpha of 0.05 divided by the square root of the number of ANOVAs).

Data Analysis - Results:

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen (Figure 1). An effect of sex was also found in caudate putamen.

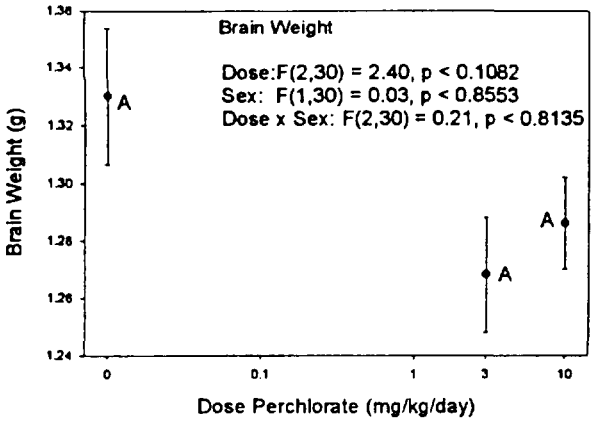
Corpus callosum showed an increase in size in the 10 mg/kg/day dose group, as previously reported in Crofton (1998c).

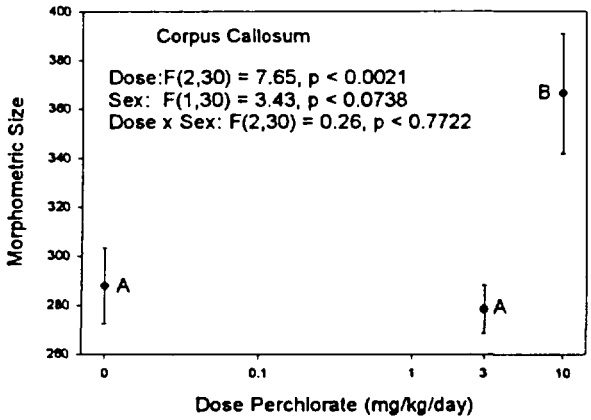
Hippocampal gyrus (12% less than control) and caudate putamen (7.3% less than control) showed a decrease in size at the 3 mg/kg/day dose, with no significant difference between control and high dose, yielding a U-shaped dose response. A/P cerebellum showed a significant increase in size in the 3 mg/kg/day group (13% greater than control), yielding an inverted U-shaped dose response function.

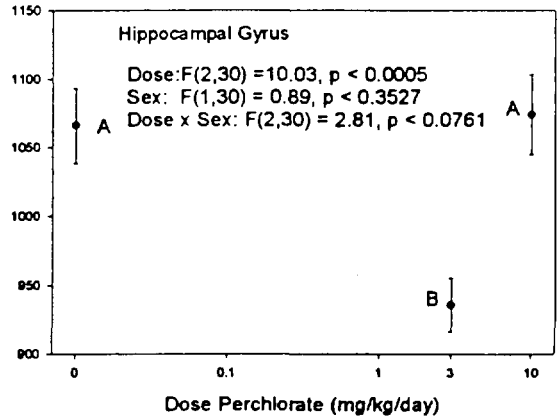
Inhibition of iodide uptake is highly non-linear and saturable, and therefore does not rule out the possibility of a U-shaped dose response. Until the PBPK modeling better characterizes this phenomenon, we are not requesting histopathological evaluation of brain sections at the next lower dose. This is pending commentary with respect to the potential for U-shaped dose response for changes in brain morphology with perchlorate exposure and other recommendations made at the external peer review. We do request, however, that the tissue samples be saved until a final decision is made on this matter.

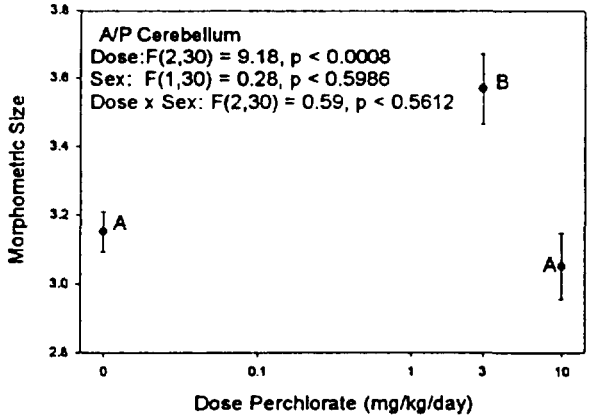
Neurobehavioral Developmental Study of Ammonium Perchlorate in Drinking Water (Argus, 1998a)

F1 Generation, PND12, Male and Female Combined. Brain weight and morphometric size measurements for different brain regions. There is no effect on brain weight. Other plots show regions where significant effect of dose was found in 2-way analysis of variance (independent variables = dose, sex). Within each plot, means with different letters are significantly different ($p < 0.05$).









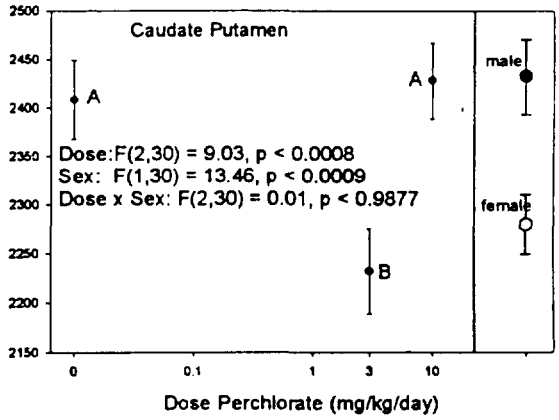


Figure 1

```
NOTE: Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software Release 6.12  TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.
                                                            ?
                                                            ?

WARNING: Your system is scheduled to expire on February 18, 1999, which is 23 days from now. Please contact your installation
         representative to have your system renewed. The SAS system will no longer function on or after that date.
 Welcome to the NHEERL-RTP SAS Information Delivery System.


1          *THIS FILE IS FOUND AT [CRofton.THYROID.perchlorate]perchlorate_dn_pnd5.SAS;
2          *IT ANALYZES THE THYROID HORMONE DATA FROM THE WPAFB 90 DAY PERCHLORATE STUDY;
3
4
5          *INPUT DATA INTO SAS DATASET;
6          DATA RAW; INFILE '[GELLER.BMD]1613-002.Txt';
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
7               INPUT SEX$ DOSE$ RATNO BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX
8                        CAUDPUT CORPCOL HIPPO CEREBLL XGEM;
9
10         * BRAINWT = TOTAL BRAIN WEIGHT;
11         * CEREBRUM = ANTER/POST CEREBRUM;
12         * APCBLM = ANT/POST CEREBELLUM;
13         * FCORTEX = FRONTAL CORTEX;
14         * PCORTEX = PARIETAL CORTEX;
15         * CAUDPUT = CAUDATE PUTAMEN;
16         * CORPCOL = CORPUS CALLOSUM;
17         * HIPPO = HIPPOCAMPAL GYRUS;
18         * CEREBLL = CEREBELLUM;
19         * XGEM = EXT GERM LAYER;
20
21         *ASSIGN TREATMENT VALUES TO DOSE CODES;
22              IF DOSE = '1' THEN TRT = '1--------CONTROL';
23              IF DOSE = '2' THEN TRT = '2--0.1_mg/kg/day';
24              IF DOSE = '3' THEN TRT = '3--1.0_mg/kg/day';
25              IF DOSE = '4' THEN TRT = '4--3.0_mg/kg/day';
26              IF DOSE = '5' THEN TRT = '5-10.0_mg/kg/day';
27

NOTE: The infile '[GELLER.BMD]1613-002.Txt' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]1613-002.TXT

NOTE: 36 records were read from the infile '[GELLER.BMD]1613-002.Txt'.
      The minimum record length was 73.
      The maximum record length was 73.
NOTE: The data set WORK.RAW has 36 observations and 14 variables.

28                 PROC PRINT;
```

```
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
29                    TITLE "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
30
31          *SORT DATA BY TRT -- THEN GET MEANS;
32
33
12                                                    The SAS System                          15:56 Tuesday, January 26, 1999

NOTE: The PROCEDURE PRINT printed page 1.

33            PROC SORT; BY TRT;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
34

NOTE: The data set WORK.RAW has 36 observations and 14 variables.

34            PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY TRT;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
35                    VAR BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
36                           CORPCOL HIPPO CEREBLL XGEM;;
37                    TITLE1 "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
38                    TITLE2 "GROUP MEANS BY TREATMENT";
39
40          *SORT DATA BY TRT AND GENDER -- THEN GET MEANS;
41
42

NOTE: The PROCEDURE MEANS printed page 2.

42            PROC SORT; BY TRT SEX;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
43

NOTE: The data set WORK.RAW has 36 observations and 14 variables.

43            PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY TRT SEX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
44                    VAR BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
45                           CORPCOL HIPPO CEREBLL XGEM;
46                    TITLE1 "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
47                    TITLE2 "GROUP MEANS BY GENDER AND TREATMENT";
48
49          *RUN ONE WAY ANOVAs FOR ALL VARIABLES;
50

NOTE: The PROCEDURE MEANS printed pages 3-4.
```

```
50          PROC SORT; BY TRT SEX;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
51

NOTE: Input data set is already sorted, no sorting done.

51          PROC GLM;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
52                    CLASSES TRT SEX;
53                    MODEL BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
54                           CORPCOL HIPPO CEREBLL XGEM = TRT|SEX;
55                    MEANS TRT/TUKEY LINES;
13                                                  The SAS System                          15:56 Tuesday, January 26, 1999

56                    TITLE1 "ARGUS DEVELOPMENTAL NEURO PND12 CNS MORPHOMETRICS";
57                         TITLE2 "PROC GLM - WITH TUKEYS";
58         ENDSAS;

NOTE: Means from the MEANS statement are not adjusted for other terms in the model.  For adjusted means, use the LSMEANS statement.
NOTE: The PROCEDURE GLM printed pages 5-25.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

OBS	SEX	DOSE	RATNO	BRAINWT	CEREBRUM	APCBLM	FCORTEX	PCORTEX	CAUDPUT	CORPCOL	HIPPO	CEREBLL	XGEM	TRT
1	F	1	2122	1.233	12.6	3.0	1224	1344	2208	192	912	3120	43	1--------CONTROL
2	F	1	2136	1.365	12.8	3.5	1512	1440	2448	259	1056	3696	36	1--------CONTROL
3	F	1	2170	1.342	12.9	3.0	1584	1512	2304	288	1104	3600	41	1--------CONTROL
4	F	1	2172	1.517	13.5	3.0	1632	1536	2496	298	1128	3984	41	1--------CONTROL
5	F	1	2185	1.321	12.5	3.2	1416	1296	2208	269	1152	3552	48	1--------CONTROL
6	F	1	2194	1.280	12.5	2.9	1536	1488	2304	336	960	3552	41	1--------CONTROL
7	F	2	2132	1.259	12.6	4.0	1440	1392	2304	259	984	3360	48	2--0.1_mg/kg/day
8	F	2	2133	1.168	12.3	3.7	1440	1392	2160	269	840	3072	46	2--0.1_mg/kg/day
9	F	2	2145	1.419	13.2	3.3	1560	1656	2256	288	1008	3840	41	2--0.1_mg/kg/day
10	F	2	2151	1.212	12.8	3.5	1488	1416	2016	269	1080	3456	41	2--0.1_mg/kg/day
11	F	2	2165	1.222	12.5	3.3	1488	1488	2064	259	912	3360	41	2--0.1_mg/kg/day
12	F	2	2174	1.347	13.2	4.1	1440	1392	2160	250	960	3696	43	2--0.1_mg/kg/day
13	F	3	2123	1.278	12.4	3.4	1344	1392	2304	307	1080	3024	41	3--1.0_mg/kg/day
14	F	3	2124	1.310	12.9	3.0	1296	1440	2400	336	1032	3552	36	3--1.0_mg/kg/day
15	F	3	2140	1.182	12.6	3.0	1464	1464	2352	355	1056	3264	36	3--1.0_mg/kg/day
16	F	3	2143	1.254	12.9	3.0	2198	1440	2448	346	1008	3168	36	3--1.0_mg/kg/day
17	F	3	2193	1.314	12.6	2.9	1392	1512	2256	355	936	3696	41	3--1.0_mg/kg/day
18	F	3	2198	1.330	13.2	3.3	1632	1608	2352	326	1008	3504	41	3--1.0_mg/kg/day
19	M	1	2002	1.375	13.2	3.4	1440	1416	2592	278	1080	3888	41	1--------CONTROL
20	M	1	2008	1.213	12.7	3.2	1296	1344	2400	240	1056	3648	36	1--------CONTROL
21	M	1	2036	1.357	12.7	3.2	1224	1368	2640	336	1248	3552	36	1--------CONTROL
22	M	1	2062	1.252	12.5	2.9	1368	1368	2352	240	936	3168	41	1--------CONTROL
23	M	1	2067	1.389	13.0	3.4	1368	1392	2544	384	1080	3696	41	1--------CONTROL
24	M	1	2094	1.335	13.2	3.1	1560	1632	2400	336	1080	3216	36	1--------CONTROL
25	M	2	2001	1.335	13.0	3.5	1464	1440	2400	365	984	3456	41	2--0.1_mg/kg/day
26	M	2	2019	1.289	13.0	3.5	1440	1440	2496	307	912	3312	36	2--0.1_mg/kg/day
27	M	2	2026	1.240	13.1	3.1	1392	1368	2304	259	888	3360	34	2--0.1_mg/kg/day
28	M	2	2039	1.250	13.1	3.8	1512	1488	2304	307	912	3312	31	2--0.1_mg/kg/day
29	M	2	2076	1.267	12.6	4.0	1272	1464	2016	240	864	3216	24	2--0.1_mg/kg/day
30	M	2	2097	1.208	12.3	3.0	1464	1464	2304	269	888	3264	43	2--0.1_mg/kg/day
31	M	3	2010	1.356	13.0	3.2	1608	1584	2640	528	1152	3504	36	3--1.0_mg/kg/day
32	M	3	2020	1.194	13.0	3.0	1584	1464	2688	317	984	3168	41	3--1.0_mg/kg/day
33	M	3	2028	1.249	12.7	2.2	1080	1296	2544	557	1200	3120	36	3--1.0_mg/kg/day
34	M	3	2037	1.353	13.0	3.5	1344	1512	2400	307	1032	3792	36	3--1.0_mg/kg/day
35	M	3	2041	1.289	13.0	3.2	1080	1440	2304	298	1104	3216	41	3--1.0_mg/kg/day
36	M	3	2043	1.321	13.0	2.9	1080	1488	2448	365	1296	3744	41	3--1.0_mg/kg/day

GROUP MEANS BY TREATMENT

TRT=1--------CONTROL

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.3315833	0.0237300	1.2130000	1.5170000	0.0822031	0.0067574	6.1733379
CEREBRUM	12	12.8416667	0.0941134	12.5000000	13.5000000	0.3260182	0.1062879	2.5387532
APCBLM	12	3.1500000	0.0583874	2.9000000	3.5000000	0.2022600	0.0409091	6.4209511
FCORTEX	12	1430.00	39.9044313	1224.00	1632.00	138.2330049	19108.36	9.6666437
PCORTEX	12	1428.00	28.1037041	1296.00	1632.00	97.3540866	9477.82	6.8175131
CAUDPUT	12	2408.00	40.8634088	2208.00	2640.00	141.5550006	20037.82	5.8785299
CORPCOL	12	288.0000000	15.4120181	192.0000000	384.0000000	53.3887969	2850.36	18.5377767
HIPPO	12	1066.00	27.3096719	912.0000000	1248.00	94.6034787	8949.82	8.8746228
CEREBLL	12	3556.00	77.8156329	3120.00	3984.00	269.5612597	72663.27	7.5804629
XGEM	12	40.0833333	1.0405297	36.0000000	48.0000000	3.6045006	12.9924242	8.9925170

TRT=2--0.1_mg/kg/day

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.2680000	0.0202286	1.1680000	1.4190000	0.0700740	0.0049104	5.5263397
CEREBRUM	12	12.8083333	0.0972799	12.3000000	13.2000000	0.3369875	0.1135606	2.6310023
APCBLM	12	3.5666667	0.1039619	3.0000000	4.1000000	0.3601347	0.1296970	10.0972333
FCORTEX	12	1450.00	20.3514574	1272.00	1560.00	70.4995164	4970.18	4.8620356
PCORTEX	12	1450.00	22.0000000	1368.00	1656.00	76.2102355	5808.00	5.2558783
CAUDPUT	12	2232.00	43.6181780	2016.00	2496.00	151.0978010	22830.55	6.7696147
CORPCOL	12	278.4166667	9.8868280	240.0000000	365.0000000	34.2489770	1172.99	12.3013386
HIPPO	12	936.0000000	19.8173478	840.0000000	1080.00	68.6493064	4712.73	7.3343276
CEREBLL	12	3392.00	59.4765041	3072.00	3840.00	206.0326541	42449.45	6.0740759
XGEM	12	39.0833333	1.9480306	24.0000000	48.0000000	6.7481760	45.5378788	17.2661219

TRT=3--1.0_mg/kg/day

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.2858333	0.0164353	1.1820000	1.3560000	0.0569335	0.0032414	4.4277517
CEREBRUM	12	12.8583333	0.0668086	12.4000000	13.2000000	0.2314316	0.0535606	1.7998572
APCBLM	12	3.0500000	0.0957427	2.2000000	3.5000000	0.3316625	0.1100000	10.8741796
FCORTEX	12	1425.17	90.8126471	1080.00	2198.00	314.5842374	98963.24	22.0735051
PCORTEX	12	1470.00	23.8403783	1296.00	1608.00	82.5854929	6820.36	5.6180607
CAUDPUT	12	2428.00	38.9498512	2256.00	2688.00	134.9262425	18205.09	5.5570940
CORPCOL	12	366.4166667	24.5956569	298.0000000	557.0000000	85.2018548	7259.36	23.2527236
HIPPO	12	1074.00	29.1141952	936.0000000	1296.00	100.8545307	10171.64	9.3905522
CEREBLL	12	3396.00	77.0643179	3024.00	3792.00	266.9586281	71266.91	7.8609726
XGEM	12	38.5000000	0.7537784	36.0000000	41.0000000	2.6111648	6.8181818	6.7822463

GROUP MEANS BY GENDER AND TREATMENT

TRT=1--------CONTROL SEX=F

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.3430000	0.0397131	1.2330000	1.5170000	0.0972769	0.0094628	7.2432557
CEREBRUM	6	12.8000000	0.1549193	12.5000000	13.5000000	0.3794733	0.1440000	2.9646353
APCBLM	6	3.1000000	0.0894427	2.9000000	3.5000000	0.2190890	0.0480000	7.0673878
FCORTEX	6	1484.00	59.8932383	1224.00	1632.00	146.7078730	21523.20	9.8859753
PCORTEX	6	1436.00	39.3954312	1296.00	1536.00	96.4987047	9312.00	6.7199655
CAUDPUT	6	2328.00	49.1853637	2208.00	2496.00	120.4790438	14515.20	5.1752167
CORPCOL	6	273.6666667	19.6547987	192.0000000	336.0000000	48.1442278	2317.87	17.5922879
HIPPO	6	1052.00	39.3954312	912.0000000	1152.00	96.4987047	9312.00	9.1728807
CEREBLL	6	3584.00	114.0385900	3120.00	3984.00	279.3363564	78028.80	7.7939832
XGEM	6	41.6666667	1.5846486	36.0000000	48.0000000	3.8815804	15.0666667	9.3157930

---------- TRT=1--------CONTROL SEX=M ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.3201667	0.0291278	1.2130000	1.3890000	0.0713482	0.0050906	5.4044848
CEREBRUM	6	12.8833333	0.1194897	12.5000000	13.2000000	0.2926887	0.0856667	2.2718397
APCBLM	6	3.2000000	0.0774597	2.9000000	3.4000000	0.1897367	0.0360000	5.9292706
FCORTEX	6	1376.00	47.4636703	1224.00	1560.00	116.2617736	13516.80	8.4492568
PCORTEX	6	1420.00	43.5614508	1344.00	1632.00	106.7033270	11385.60	7.5143188
CAUDPUT	6	2488.00	48.6621002	2352.00	2640.00	119.1973154	14208.00	4.7908889
CORPCOL	6	302.3333333	24.0134222	240.0000000	384.0000000	58.8206313	3459.87	19.4555561
HIPPO	6	1080.00	40.6349603	936.0000000	1248.00	99.5349185	9907.20	9.2161962
CEREBLL	6	3528.00	115.4330975	3168.00	3888.00	282.7521883	79948.80	8.0145178
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

---------- TRT=2--0.1_mg/kg/day SEX=F ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2711667	0.0384339	1.1680000	1.4190000	0.0941433	0.0088630	7.4060572
CEREBRUM	6	12.7666667	0.1520234	12.3000000	13.2000000	0.3723797	0.1386667	2.9168125
APCBLM	6	3.6500000	0.1408309	3.3000000	4.1000000	0.3449638	0.1190000	9.4510621
FCORTEX	6	1476.00	19.3494186	1440.00	1560.00	47.3962024	2246.40	3.2111248
PCORTEX	6	1456.00	42.7831743	1392.00	1656.00	104.7969465	10982.40	7.1975925
CAUDPUT	6	2160.00	44.6855681	2016.00	2304.00	109.4568408	11980.80	5.0674463
CORPCOL	6	265.6666667	5.3395797	250.0000000	288.0000000	13.0792456	171.0666667	4.9231790
HIPPO	6	964.0000000	33.6095225	840.0000000	1080.00	82.3261805	6777.60	8.5400602
CEREBLL	6	3464.00	111.1395519	3072.00	3840.00	272.2351924	74112.00	7.8589836
XGEM	6	43.3333333	1.2292726	41.0000000	48.0000000	3.0110906	9.0666667	6.9486706

---------- TRT=2--0.1_mg/kg/day SEX=M ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV

BRAINWT	6	1.2648333	0.0178688	1.2080000	1.3350000	0.0437695	0.0019158	3.4604932
CEREBRUM	6	12.8500000	0.1335415	12.3000000	13.1000000	0.3271085	0.1070000	2.5455918
APCBLM	6	3.4833333	0.1579381	3.0000000	4.0000000	0.3868678	0.1496667	11.1062516
FCORTEX	6	1424.00	34.3161769	1272.00	1512.00	84.0571234	7065.60	5.9028879
PCORTEX	6	1444.00	16.8760185	1368.00	1488.00	41.3376342	1708.80	2.8627170
CAUDPUT	6	2304.00	65.5804849	2016.00	2496.00	160.6387251	25804.80	6.9721669
CORPCOL	6	291.1666667	18.3456020	240.0000000	365.0000000	44.9373638	2019.37	15.4335537
HIPPO	6	908.0000000	16.8760185	864.0000000	984.0000000	41.3376342	1708.80	4.5526029
CEREBLL	6	3320.00	33.7520370	3216.00	3456.00	82.6752684	6835.20	2.4902189
XGEM	6	34.8333333	2.8215441	24.0000000	43.0000000	6.9113433	47.7666667	19.8411770

TRT=3--1.0_mg/kg/day SEX=F

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2780000	0.0222231	1.1820000	1.3300000	0.0544353	0.0029632	4.2594118
CEREBRUM	6	12.7666667	0.1173788	12.4000000	13.2000000	0.2875181	0.0826667	2.2521001
APCBLM	6	3.1000000	0.0816497	2.9000000	3.4000000	0.2000000	0.0400000	6.4516129
FCORTEX	6	1554.33	137.3350324	1296.00	2198.00	336.4007531	113165.47	21.6427677
PCORTEX	6	1476.00	30.8285582	1392.00	1608.00	75.5142371	5702.40	5.1161407
CAUDPUT	6	2352.00	27.7128129	2256.00	2448.00	67.8822510	4608.00	2.8861501
CORPCOL	6	337.5000000	7.6365350	307.0000000	355.0000000	18.7056141	349.9000000	5.5424042
HIPPO	6	1020.00	20.3174802	936.0000000	1080.00	49.7674592	2476.80	4.8791627
CEREBLL	6	3368.00	104.7358582	3024.00	3696.00	256.5494104	65817.60	7.6172628
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

TRT=3--1.0_mg/kg/day SEX=M

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2936667	0.0258865	1.1940000	1.3560000	0.0634087	0.0040207	4.9014734
CEREBRUM	6	12.9500000	0.0500000	12.7000000	13.0000000	0.1224745	0.0150000	0.9457489
APCBLM	6	3.0000000	0.1807392	2.2000000	3.5000000	0.4427189	0.1960000	14.7572957
FCORTEX	6	1296.00	103.6918512	1080.00	1608.00	253.9921259	64512.00	19.5981579
PCORTEX	6	1464.00	39.1918359	1296.00	1584.00	96.0000000	9216.00	6.5573770
CAUDPUT	6	2504.00	59.9733274	2304.00	2688.00	146.9040503	21580.80	5.8667752
CORPCOL	6	395.3333333	47.6337882	298.0000000	557.0000000	116.6784756	13613.87	29.5139483
HIPPO	6	1128.00	46.3724056	984.0000000	1296.00	113.5887318	12902.40	10.0699230
CEREBLL	6	3424.00	121.8523697	3120.00	3792.00	298.4761297	89088.00	8.7171767
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

PROC GLM - WITH TUKEYS

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	3	1--------CONTROL 2--0.1_mg/kg/day 3--1.0_mg/kg/day
SEX	2	F M

Number of observations in data set = 36

General Linear Models Procedure

Dependent Variable: BRAINWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.02823647	0.00564729	1.05	0.4079
Error	30	0.16157983	0.00538599		
Corrected Total	35	0.18981631			

R-Square	C.V.	Root MSE	BRAINWT Mean
0.148757	5.666522	0.07338933	1.29513889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	0.02581572	0.01290786	2.40	0.1082
SEX	1	0.00018225	0.00018225	0.03	0.8553
TRT*SEX	2	0.00223850	0.00111925	0.21	0.8135

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.02581572	0.01290786	2.40	0.1082
SEX	1	0.00018225	0.00018225	0.03	0.8553
TRT*SEX	2	0.00223850	0.00111925	0.21	0.8135

General Linear Models Procedure

Dependent Variable: CEREBRUM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.15805556	0.03161111	0.33	0.8902
Error	30	2.86500000	0.09550000		
Corrected Total	35	3.02305556			

R-Square	C.V.	Root MSE	CEREBRUM Mean
0.052283	2.407511	0.30903074	12.83611111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	0.01555556	0.00777778	0.08	0.9220
SEX	1	0.12250000	0.12250000	1.28	0.2664
TRT*SEX	2	0.02000000	0.01000000	0.10	0.9009

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.01555556	0.00777778	0.08	0.9220
SEX	1	0.12250000	0.12250000	1.28	0.2664
TRT*SEX	2	0.02000000	0.01000000	0.10	0.9009

General Linear Models Procedure

Dependent Variable: APCBLM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.94555556	0.38911111	3.97	0.0070
Error	30	2.94333333	0.09811111		
Corrected Total	35	4.88888889			

R-Square	C.V.	Root MSE	APCBLM Mean
0.397955	9.621305	0.31322693	3.25555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	1.80222222	0.90111111	9.18	0.0008
SEX	1	0.02777778	0.02777778	0.28	0.5986
TRT*SEX	2	0.11555556	0.05777778	0.59	0.5612

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	1.80222222	0.90111111	9.18	0.0008
SEX	1	0.02777778	0.02777778	0.28	0.5986
TRT*SEX	2	0.11555556	0.05777778	0.59	0.5612

General Linear Models Procedure

Dependent Variable: FCORTEX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	247472.55555554	49494.51111111	1.34	0.2756
Error	30	1110147.33333334	37004.91111111		
Corrected Total	35	1357619.88888888			

R-Square	C.V.	Root MSE	FCORTEX Mean
0.182284	13.40482	192.36660602	1435.05555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	4160.22222222	2080.11111111	0.06	0.9454
SEX	1	175002.77777778	175002.77777778	4.73	0.0377
TRT*SEX	2	68309.55555556	34154.77777778	0.92	0.4083

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	4160.22222222	2080.11111111	0.06	0.9454
SEX	1	175002.77777778	175002.77777778	4.73	0.0377
TRT*SEX	2	68309.55555556	34154.77777778	0.92	0.4083

General Linear Models Procedure

Dependent Variable: PCORTEX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12224.00000000	2444.80000000	0.30	0.9068
Error	30	241536.00000000	8051.20000000		
Corrected Total	35	253760.00000000			

R-Square	C.V.	Root MSE	PCORTEX Mean
0.048172	6.191017	89.72847931	1449.33333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	10592.00000000	5296.00000000	0.66	0.5253
SEX	1	1600.00000000	1600.00000000	0.20	0.6590
TRT*SEX	2	32.00000000	16.00000000	0.00	0.9980

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	10592.00000000	5296.00000000	0.66	0.5253
SEX	1	1600.00000000	1600.00000000	0.20	0.6590
TRT*SEX	2	32.00000000	16.00000000	0.00	0.9980

General Linear Models Procedure

Dependent Variable: CAUDPUT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	487488.00000000	97497.60000000	6.31	0.0004
Error	30	463488.00000000	15449.60000000		
Corrected Total	35	950976.00000000			

R-Square	C.V.	Root MSE	CAUDPUT Mean
0.512619	5.275739	124.29641990	2356.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	279168.00000000	139584.00000000	9.03	0.0008
SEX	1	207936.00000000	207936.00000000	13.46	0.0009
TRT*SEX	2	384.00000000	192.00000000	0.01	0.9877

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	279168.00000000	139584.00000000	9.03	0.0008
SEX	1	207936.00000000	207936.00000000	13.46	0.0009
TRT*SEX	2	384.00000000	192.00000000	0.01	0.9877

General Linear Models Procedure

Dependent Variable: CORPCOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	70390.22222222	14078.04444444	3.85	0.0082
Error	30	109659.66666667	3655.32222222		
Corrected Total	35	180049.88888889			

R-Square	C.V.	Root MSE	CORPCOL Mean
0.390948	19.44375	60.45926085	310.94444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	55940.05555556	27970.02777778	7.65	0.0021
SEX	1	12544.00000000	12544.00000000	3.43	0.0738
TRT*SEX	2	1906.16666667	953.08333333	0.26	0.7722

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	55940.05555556	27970.02777778	7.65	0.0021
SEX	1	12544.00000000	12544.00000000	3.43	0.0738
TRT*SEX	2	1906.16666667	953.08333333	0.26	0.7722

General Linear Models Procedure

Dependent Variable: HIPPO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	190784.00000000	38156.80000000	5.31	0.0013
Error	30	215424.00000000	7180.80000000		
Corrected Total	35	406208.00000000			

R-Square	C.V.	Root MSE	HIPPO Mean
0.469671	8.264590	84.73960113	1025.33333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	144032.00000000	72016.00000000	10.03	0.0005
SEX	1	6400.00000000	6400.00000000	0.89	0.3527
TRT*SEX	2	40352.00000000	20176.00000000	2.81	0.0761

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	144032.00000000	72016.00000000	10.03	0.0005
SEX	1	6400.00000000	6400.00000000	0.89	0.3527
TRT*SEX	2	40352.00000000	20176.00000000	2.81	0.0761

General Linear Models Procedure

Dependent Variable: CEREBLL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	291072.00000000	58214.40000000	0.89	0.5021
Error	30	1969152.00000000	65638.40000000		
Corrected Total	35	2260224.00000000			

R-Square	C.V.	Root MSE	CEREBLL Mean
0.128780	7.430392	256.19992194	3448.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	210048.00000000	105024.00000000	1.60	0.2186
SEX	1	20736.00000000	20736.00000000	0.32	0.5783
TRT*SEX	2	60288.00000000	30144.00000000	0.46	0.6361

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	210048.00000000	105024.00000000	1.60	0.2186
SEX	1	20736.00000000	20736.00000000	0.32	0.5783
TRT*SEX	2	60288.00000000	30144.00000000	0.46	0.6361

General Linear Models Procedure

Dependent Variable: XGEM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	262.22222222	52.44444444	3.33	0.0164
Error	30	472.00000000	15.73333333		
Corrected Total	35	734.22222222			

R-Square	C.V.	Root MSE	XGEM Mean
0.357143	10.11296	3.96652661	39.22222222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	15.38888889	7.69444444	0.49	0.6180
SEX	1	136.11111111	136.11111111	8.65	0.0062
TRT*SEX	2	110.72222222	55.36111111	3.52	0.0424

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	15.38888889	7.69444444	0.49	0.6180
SEX	1	136.11111111	136.11111111	8.65	0.0062
TRT*SEX	2	110.72222222	55.36111111	3.52	0.0424

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: BRAINWT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.005386
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.0739

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1.33158	12	1--------CONTROL
A			
A	1.28583	12	3--1.0_mg/kg/day
A			
A	1.26800	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CEREBRUM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.0955
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.311

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	12.8583	12	3--1.0_mg/kg/day
A			
A	12.8417	12	1--------CONTROL
A			
A	12.8083	12	2--0.1_mg/kg/day

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: APCBLM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.098111
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.3153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	3.5667	12	2--0.1_mg/kg/day
B	3.1500	12	1--------CONTROL
B			
B	3.0500	12	3--1.0_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: FCORTEX

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 37004.91
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 193.61

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1450.00	12	2--0.1_mg/kg/day
A			
A	1430.00	12	1--------CONTROL
A			
A	1425.17	12	3--1.0_mg/kg/day

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: PCORTEX

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 8051.2
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 90.309

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1470.00	12	3--1.0_mg/kg/day
A			
A	1450.00	12	2--0.1_mg/kg/day
A			
A	1428.00	12	1--------CONTROL

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CAUDPUT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 15449.6
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 125.1

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	2428.00	12	3--1.0_mg/kg/day
A			
A	2408.00	12	1--------CONTROL
B	2232.00	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CORPCOL

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 3655.322
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 60.85

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	366.42	12	3--1.0_mg/kg/day
B	288.00	12	1--------CONTROL
B			
B	278.42	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: HIPPO

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 7180.8
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 85.288

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1074.00	12	3--1.0_mg/kg/day
A			
A	1066.00	12	1--------CONTROL
B	936.00	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CEREBLL

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 65638.4
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 257.86

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	3556.0	12	1--------CONTROL
A			
A	3396.0	12	3--1.0_mg/kg/day
A			
A	3392.0	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: XGEM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 15.73333
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 3.9922

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	40.083	12	1--------CONTROL
A			
A	39.083	12	2--0.1_mg/kg/day
A			
A	38.500	12	3--1.0_mg/kg/day

February 1, 1999 EPA Assessment Submission

Attachment #3
Nonparametric Reanalysis of Thyroid Histopathology in Pups on PND5 from Argus (1998a) Neurodevelopmental Study

A. EPA analysis (Marcus, 1999)

ATTENTION PANEL MEMBER(S):

JOE HASEMAN
SUSAN PORTERFIELD
TOM ZOELLER

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

FROM: Allan H. Marcus, EMAG/NCEA-RTP (MD-52)

TO: Annie Jarabek, HPAG/NCEA-RTP (MD-52)

Attached is a set of statistical analyses of the histology data, provided as severity scores for both histology measures individual animals, that I received from you as a telefax from WPAFB (AFRL/HESD). A copy of these data is appended to the memo. I have corrected some errors in a draft version of 12/29/98, in response to specific comments from Dr. Joseph Haseman regarding the number of animals used in the analyses, identification of sub-groups, and expanding the methods of analysis to exact significance levels appropriate to these small sample sizes.

The raw data in the fax was converted into SYSTAT or StatXact data sets for further analyses. I can also export the data to spreadsheets or to SAS data sets, if needed. They are shown as Tables 1 to 6 in the attached memo. The changes are: (1) Table1 (level 1 at 10 mg/kg-day) frequency = 5; (2) Table 3 (level 1 severity at 0.1 mg/kg-day dose) frequency = 6, (at 10 mg/kg-day) frequency = 7; Table 6 (level 0 at 3 mg/kg-day) frequency = 1, (level 1 at 10 mg/kg-day) frequency = 2; Table 9 (levels "1 + 2" at dose 0) frequency = 6. The reported analyses were done using correct counts.

The exact small-sample Jonckheere-Terpstra test for ordered categories was used in Tables 1-5, and new Tables 1A-6A. These tests were based on assuming ordered categories of both dose and severity, hence are one-tailed tests. Monte Carlo approximation to the P-value was calculated in Table 6. Exact Fisher tests for 2X2 tables using the likelihood ratio criterion were carried out in Tables 7-12.

I understand that these analyses are based on data in the Argus rat developmental neurotoxicology study (Argus, 1998a). The 2x2 contingency table tests of association are straightforward and described in most elementary statistics texts. The logistic regression analyses in this version of SYSTAT used the iteratively reweighted least squares approach to maximum likelihood estimation described on p. 622 of the SYSTAT v. 5.0 manual (1995). These are very simple approaches, easily understood by most non-specialists. Further analyses using categorical regression methods may also be informative.

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The sample sizes are on the small side for testing hypotheses. For that reason, the findings of marginal or statistically significant associations in the contingency table tests at 0.1 and 1 mg/kg-day are worrying, given that the study has small power to detect real effects of only modest magnitude. The logistic regression models are consistent with a steeper dose-response function at low doses than at high doses. The evidence as a whole leans toward a significant response at doses as low as 0.1 to 1 mg/kg-day. A larger study to look at these lower dose ranges would seem to be justified.

Attachment

Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

Allan H. Marcus, Statistician
National Center for Environmental Assessment – RTP

1. DATA STRUCTURE AND PURPOSE OF THE ANALYSES

The purpose of the analyses was to provide an assessment of possible trends in toxicity data provided to me by Annie Jarabek, based on the rat neurodevelopmental study data for pups postnatal on day 5 (PND5), reported in (Argus, 1998a). There were two toxicity endpoints: (1) Follicular epithelial cell hypertrophy (denoted HYPER), and (2) decrease in follicular lumen size (denoted SIZE). Both were coded on a discrete scale of increasing seriousness, as 0, 1, 2 for HYPER and 0, 1, 2, 3 for SIZE. There were separate studies for females and for males, so SEX was also a discrete variable. Each set of experiments was done at 5 dose levels: control (0 mg/kg-day), 0.1, 1, 3, and 10 mg/kg-day. DOSE effects could be evaluated either as an ordered categorical scale or as a numeric scale. Including DOSE as an ordered categorical scale allowed use of contingency table methods, whereas use of DOSE or log(DOSE) as a numeric scale allowed use of logistic regression models. These provide different but complementary information about the relationship, using elementary analytical methods.

2. TESTING ASSOCIATION IN CATEGORICAL RESPONSE DATA

The individual rat data were combined into contingency tables and entered into the SYSTAT (1995) data analysis system. The basic data tables are shown below, along with the results for tests of association with DOSE in a table with r rows and c columns as shown. The first set of tests was done by exact small-sample Jonckheere-Terpstra tests (StatXact, 1998) for association in ordered categories (DOSE, severity) for each sex and for both sexes, for both endpoints. We use the following symbols for significance: * for 0.01<P<0.05, ** for 0.001<P<0.01, *** for P<0.001, and # for 0.05 < P < 0.10. Because of the ordering assumed in both dimensions of the dose-severity relationship, all tests are one-tailed tests.

TABLE 1

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1
1	1	2	3
3	3	2	1
10	0	5	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.0811#
DF=8

TABLE 2

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1
1	2	3	1
3	1	4	1
10	0	2	4

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.0004***
DF=8

TABLE 3

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	6	2
1	3	5	4
3	4	6	2
10	0	7	5

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.0005***, DF=8

TABLE 4
SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE DOSE, mg/kg-day	LEVEL 0	1	2	3
0	2	3	1	0
0.1	1	3	2	0
1	1	4	1	0
3	1	1	2	2
10	0	2	3	1

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.0110*, DF=12

TABLE 5
SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE DOSE, mg/kg-day	LEVEL 0	1	2	3
0	4	1	1	0
0.1	1	3	2	0
1	1	1	4	0
3	0	2	4	0
10	0	0	3	3

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.0001***, DF=12

TABLE 6
SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL DOSE, mg/kg-day	LEVEL 0	1	2	3
0	6	4	2	0
0.1	2	6	4	0
1	2	5	5	0
3	1	3	6	2
10	0	2	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN ALL SEXES: 0.0000***, DF=12

We also repeated these tests for a much more focused assessment of controls vs. dose 0.1 mg/kg, using all levels of severity, but maintaining the ordering of alternatives in the exact small-sample Jonckheere-Terpstra tests. This is shown in Tables 1A-6A.

TABLE 1A

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.4621
DF=2

TABLE 2A

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.0325*
DF=2

TABLE 3A

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	6	2

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.0432*, DF=2

TABLE 4A
SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE DOSE, mg/kg-day	LEVEL 0	1	2
0	2	3	1
0.1	1	3	2

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.3528, DF=2

TABLE 5A
SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	1	3	2

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.1050, DF=2

TABLE 6A
SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL DOSE, mg/kg-day	LEVEL 0	1	2
0	6	4	2
0.1	2	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN BOTH SEXES: 0.0661#, DF=2

Exact Fisher tests were performed on reduced 2 by 2 tables, using DOSE level 0.1 and 1 mg/kg-day vs. controls to see if there was a significant difference at low doses. Tests of the controls against the highest 2 doses were significant and are not shown here. The low-dose tests for HYPER used a combined HYPER score of 1+2 to combine the more serious effects These tables were then combined into single tables for the purpose of providing a concise display of the results. All of the tests are one-tailed likelihood ratio tests, following a natural ordering of alternatives.

TABLE 7
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 0.1	3	3	1	5	4	8
P VALUE	0.5000		0.0400*		0.0498*	

TABLE 8
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 1.0	1	5	2	4	3	9
P VALUE	0.1212		0.1212		0.0196*	

The 2 by 2 tests for SIZE effects required a more detailed level of the aggregated SIZE categories. We show separate results for category 0 vs. 1+2, and categories 0+1 vs. 2. Category 3 had no counts at dose levels 0, 0.1 and 1.

TABLE 9
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE EFFECT AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 0.1	1	5	1	5	2	10
P VALUE	0.1212		0.1212		0.0965#	

TABLE 10
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 0.1	4	2	4	2	8	4
P VALUE	0.1212		0.1212		0.3202	

TABLE 11
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 1	1	5	1	5	2	10
P VALUE	0.1212		0.1212		0.0965#	

TABLE 12
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 1	5	1	2	4	7	5
P VALUE	0.5000		0.1212		0.1854	

3. LOGISTIC REGRESSION ANALYSIS

As a check on the overall relationship, we also carried out logistic regression analyses of response vs. dose and vs. log(dose), for males and females separately and for both sexes combined. The dose for controls was taken as 0, and log(dose) as log(0.01 mg/kg-day). The results are shown in the following tables.

TABLE 13
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.332	0.210	-16.90
MALE	0.614	0.397	-14.78
ALL	0.423*	0.192	-32.06

TABLE 14
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.335	0.313	-12.31
MALE	1.734	1.187	-10.68
ALL	0.614	0.378	-22.30

TABLE 15
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.198#	0.109	-18.34
MALE	0.635#	0.339	-15.15
ALL	0.279***	0.097	-35.66

TABLE 16
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.342*	0.174	-17.08
MALE	0.532**	0.207	-13.95
ALL	0.426***	0.132	-31.49

TABLE 17
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.269	0.205	-12.60
MALE	0.704**	0.284	-10.02
ALL	0.459***	0.166	-22.07

TABLE 18
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.330#	0.179	-18.20
MALE	0.572**	0.208	-15.20
ALL	0.430***	0.132	-34.86

The relationship between non-transformed dose and hypertrophy is statistically significant in both sexes combined, and positive but not significant in both sexes separately. The relationship with the logarithm of dose is significant or very significant in all analyses. This suggests that the risk of a hypertrophic response increases as (roughly) the 0.3 to 0.5 power of dose. Since the dose-response function is nonlinear with a steeper slope near the origin, the possibility of significant responses at low doses is consistent with the contingency table tests.

The regression coefficients of any size > 0 vs. untransformed dose are positive but not significant, whereas after log-transformation, the effects for males and for both sexes are very significant. If the severity cutpoint for SIZE is taken as levels 2+3 vs. levels 0+1, then the relationship with dose is marginally significant in either sex and highly significant when sexes are combined. The effects for males and for both sexes combined are highly significant in the model for log of dose, which also suggests that the SIZE response probability at low doses increases as roughly the 0.3 to 0.5 power of dose.

Additional logistic regression models explored the possibility of a dose-sex interaction, with males having a steeper dose-response curve. No statistically significant gender effect was found, but it is unlikely that these small samples allow sufficient power to detect this effect.

4. SUMMARY

There appears to be strong evidence for a dose-response relationship between perchlorate dose and both endpoints, follicular epithelial cell hypertrophy and decrease in follicular lumen size. Even though the number of rats in each treatment group is smaller than is desirable to have substantial power against real

effects of modest size at the two lowest dose levels, attention should be paid to the simple comparisons in Tables 2A, 3A, 7 and 8, which suggest a significant increase in hypertrophy for males, and for both groups combined at both 0.1 and 1 mg/kg-day (significant). One should note that the differences lie in the expected direction if there is a real dose-response relationship. Although there may be a dose-sex interaction, with males showing stronger effects than females, this was not significant, and combining the sexes gave evidence for an effect on follicular epithelial cell hypertrophy.

Similar analyses did not find strongly significant decreases in follicular lumen cell size at the lowest two levels using the very basic contingency table tests in Tables 9 through12, nor in Tables 4A, 5A, and 6A. However, the logistic regression models suggested that there is a very significant dose response relationship overall, with a strong model-based suggestion of a steeper dose-response relationship for lumen cell size at lower doses.

Taking the small samples sizes and limited power of these data into account, there is an indication of increased effects at levels as low as 0.1 to 1 mg/kg-day, particularly for the follicular epithelial cell hypertrophy in males.

5. REFERENCES

1. Argus, 1998a. A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [report ammendment: July 27, 1998]. Argus Research Laboratories, Inc., Horsham, PA. Argus Protocol #1613-002,

2. Wilkinson, L. SYSTAT: The System for Statistics. SYSTAT Inc., Evanston, Il., 1995.

3. StatXact Program. Cytel Inc., Cambridge, MA. 1998.

Appendix: Data as received by telefax.

February 1, 1999 EPA Assessment Submission

Attachment #4
Hormone Data Analysis for F0 and F1 from Argus (1998b) 2-Generation Reproductive Study

A. EPA analysis (Geller, 1999b)
B. EPA analysis (House, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
JOE HASEMAN
SUSAN PORTERFIELD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: February 1, 1999

Subject: Analysis of the Thyroid Hormone Data from the Rat Two Generation Reproduction Study (Argus, 1998b)

From: for Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is the statistical analysis of the hormone data from the Argus Rat Developmental Neurotoxicology Study (Argus, 1998b). A memo (York, 1999g) from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) provided thyroid hormone and thyrotropin data from the oral (drinking water) two-generation reproductive study of ammonium perhclorate in the rat. Data were supplied on diskette in the form of ASCII text reports, one report for each gender/age group, and imported in ASCII form to SAS for further analysis. I have attached a description of how the analyses were done, a description of results, and summary graphs.

An alternative statistical analysis for the F1 generation, per suggestion by Joseph Haseman, is provided in the memo from Dennis House (1999) using these same data. These analyses have been provided for comparative purposes.

Analyses of Hormone Data from the Argus Oral (Drinking Water) Two-Generation Reproduction Study

Summary: A memo from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) contains thyroid hormone and thyrotrophin data from the Oral (Drinking Water) Two-Generation reproduction Study of ammonium perchlorate in the rat. The following is a statistical analysis of the thyroid and pituitary hormone data (T4, thyroxine; T3, triiodothyronine; TSH, thyroid stimulating hormone) found in that report. At the time of this analysis, data were available from both the F0 generation, females and males sacrificed at 5 and 6 months of age, respectively, and the F1 generation, one male and one female from each litter, sacrificed on postnatal day 21 (PND21). Males were sacrificed after 13 weeks of exposure, i.e., approximately 91 days. Females were sacrificed after 16 weeks, i.e. at weaning, approximately 120 days of exposure.

Data from the F0 generation were re-analyzed to look for dose and gender effects. Data from the F1 generation were re-analyzed using gender as a repeated measure within each litter. Results of these re-analyses are similar to those stated in the memo from Argus RE: Protocol 1416-001 (20 November 1998).

For the F0 generation, a NOEL of 3.0 mg/kg/day was identified from a decrease in T4 and an increase in TSH of male rats. These results are consistent with the known mechanism-of-action (MOA) of perchlorate (inhibition of thyroid hormones). The increased TSH is likely a result of the activation of the pituitary-thyroid feedback mechanism. These data are not consistent with the results of the 90-day drinking water study (Springborn Laboratories, Inc., 1998). In that study, 90 days of exposure resulted in LOELs of 0.01 mg/kg/day for T3 and T4 and a NOEL of 0.05 mg/kg/day for TSH.

For the F1 generation, a LOAEL of 0.3 mg/kg/day was identified for a decrease in TSH level, inconsistent with known MOA of perchlorate. This data is inconsistent with results from the Neurodevelopmental Toxicity Study (Argus, 1998a, Crofton, 1998f). In the Neurodevelopmental study, dose-related decreases of T4 and T3 and dose-related increase of TSH were found. Possible reasons for this disparity are discussed.

Data: All data were supplied in the form of ASCII text reports, one report for each gender/age group. Data were exported as ASCII files for analyses by SAS.

F0 generation: Data for dependent measures (T4, T3 and TSH) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

F1 generation: Data for dependent measures (T4, T3, TSH) were subjected to separate repeated-measures ANOVAs. Treatment (dose) was the independent between-subjects variable. Sex was a within-litter repeated-measures variable. The repeated-measures analysis requires a full set of data for each litter, i.e. 1 male and 1 female. Data was missing from 4 litters (1 male from each of 0, 0.3, and 30 mg/kg/day dose groups and 1 female from 30 mg/kg/day), reducing the sample size in the analysis from 99 to 95. Mean contrasts were performed using Tukey's

Studentized Range (HSD) Test.

To correct for multiple comparisons (i.e., separate analyses for T4 and TSH) the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.029 (alpha of 0.05 divided by the square root of the number of ANOVAs). SAS analysis code and output are attached in Appendix 1.

Data Analysis - Results:

F0 Generation: There were significant dose effects for T4 and TSH, and dose x sex effects for T4 and TSH (Figure 1). Given our assumptions about the mechanism of action (MOA) of perchlorate (i.e., iodide uptake inhibition resulting in reduced levels of T4 and T3, and an increase in TSH), only the effects on T4 and TSH levels for males can be considered biologically significant. NOELs were identified for males only for T4 and TSH at a dose of 3.0 mg/kg/day. There were also significant effects of sex on T4 and T3 levels.

F1 Generation: There were no significant main effects of dose on T4, T3, or TSH. There were significant dose x sex interactions for T4 and TSH (Figure 2). The significant effect of dose on female T4 data is due to an elevated level in the 0.3 mg/kg/day group relative to the high dose group and is not consistent with the MOA of perchlorate. There was a LOEL of 0.3 mg/kg/day for a reduction in TSH level in males; this is not consistent with the known MOA of perchlorate.

These results are different from those in the F1 generation of the Neurodevelopmental Toxicity study (Argus, 1998a, Crofton, 1998f). In PND5 pups exposed through gestation and lactation, there were significant dose-related reductions in T4 and T3, and a significant dose-related increase in TSH. One possible source of this disparity is that the PND21 weanlings tested in the Two-Generation study likely received a reduced dose of the test compound through lactation (Fisher, 1998b) and the slow addition of drinking water to their diets. This may have allowed recovery from the hormone deficits due to gestational effects still visible in the younger pups.

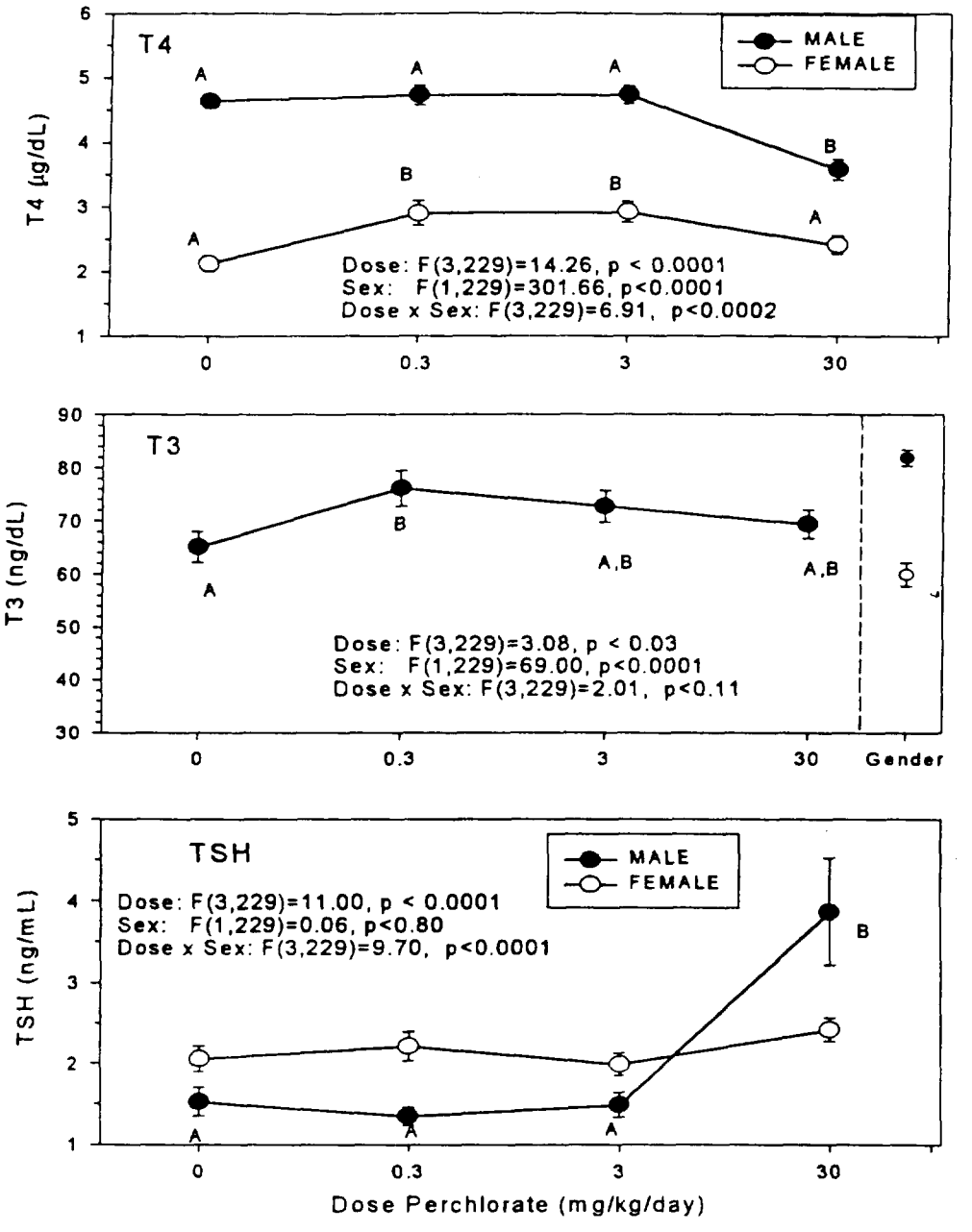
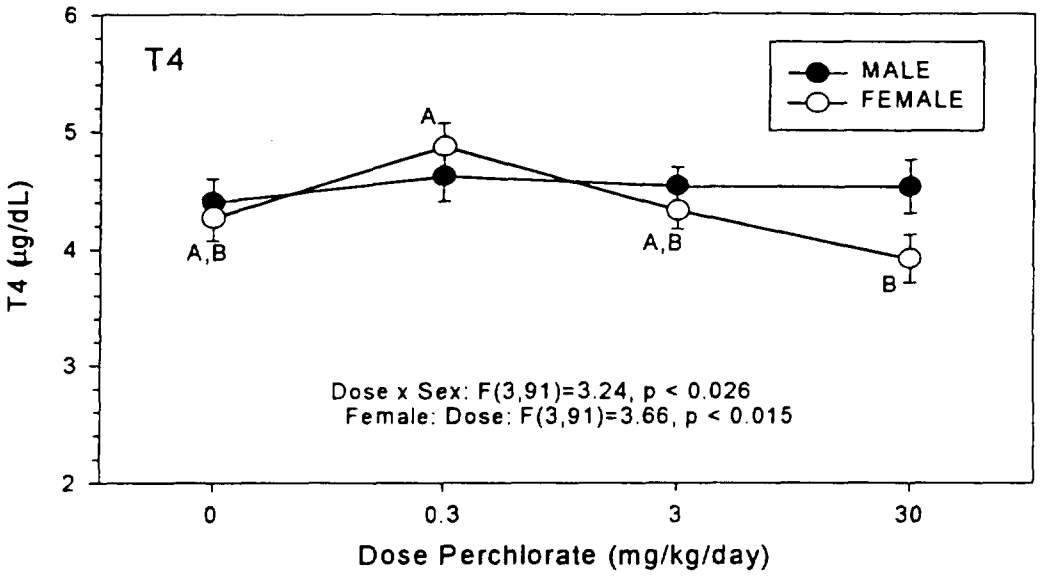


Figure
1. Effects of oral perchlorate exposure on hormone levels in F0 generation. Serum total thyroxine (T4) (top): There were significant dose, sex, and dose x sex effects. Means with different letters (on each function) were significantly different ($p<0.05$). Serum total triiodothyronine (T3)(middle): There was a significant effects of sex and a borderline significant effect of dose. Plot to right of dotted line illustrates sex effect (males>females). Serum thyroid stimulation hormone (TSH) (bottom): There were significant effects of dose and dose x sex. Means with different letters were significantly different ($p<0.05$).



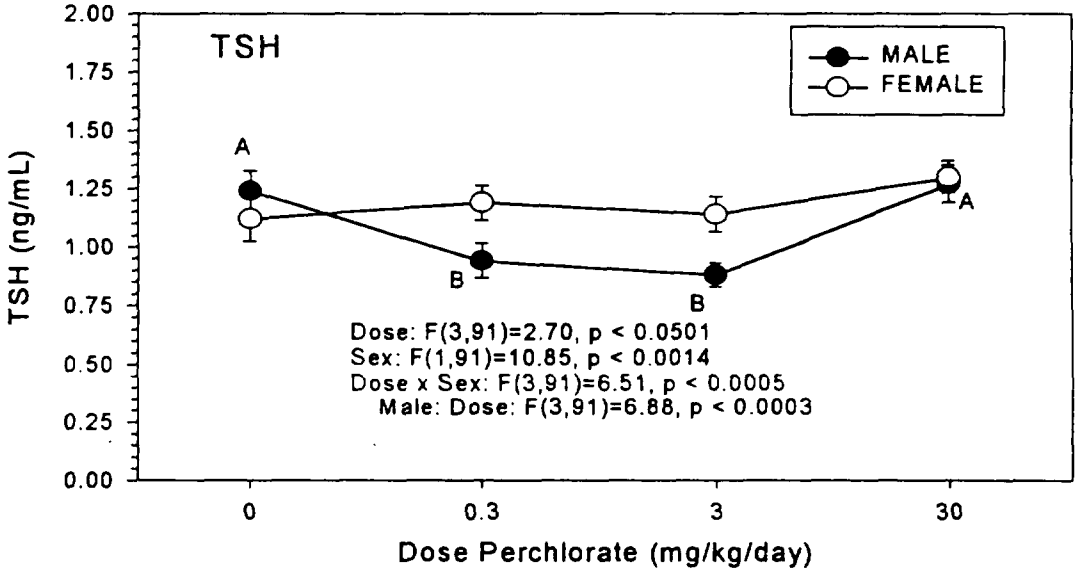


Figure 2. Effects of perchlorate exposure on hormone levels in F1 generation at post-natal day 21. Serum total thyroxine (T4) (top): There was a significant dose x sex effect, with a dose effect in females due to elevated T4 in the 0.3 mg/kg dose group. Means with different letters were significantly different ($p<0.05$). Serum thyroid stimulation hormone (TSH) (bottom): There were significant main effects of sex and dose x sex, with a dose effect in males. Means with different letters were significantly different ($p<0.05$).

APPENDIX 1 - Raw Data and Statistical Analysis

```
NOTE: Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software Release 6.12  TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.


WARNING: Your system is scheduled to expire on February 18, 1999, which is 28 days from now. Please contact your installation
         representative to have your system renewed. The SAS system will no longer function on or after that date.
 Welcome to the NHEERL-RTP SAS Information Delivery System.


1          /* INPUT NEWLY RECEIVED THYROID HORMONE DATA FROM 2 GEN Reproduction STUDY.
2             DATA GENERATED BY ARGUS RESEARCH LABS, RECEIVED JAN 7, 1999 */
3
4          DATA F21; /* Female rats, generation F1,  day 21 */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
5            INFILE '[GELLER.BMD]GEN2F21.DAT';
6            INPUT a $ no id grp sex $ age $ tsh1 t31 t41;
7            DROP a no sex;
8          RUN;

NOTE: The infile '[GELLER.BMD]GEN2F21.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN2F21.DAT

NOTE: 98 records were read from the infile '[GELLER.BMD]GEN2F21.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.F21 has 98 observations and 6 variables.

9
10         DATA M21; /* Male rats, generation F1, day 21 */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
11           INFILE '[GELLER.BMD]GEN2M21.DAT';
12           INPUT a $ no id grp sex $ age $ tsh2 t32 t42;
13           DROP a no sex;
14         RUN;

NOTE: The infile '[GELLER.BMD]GEN2M21.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN2M21.DAT

NOTE: 96 records were read from the infile '[GELLER.BMD]GEN2M21.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.M21 has 96 observations and 6 variables.

15
16         PROC SORT DATA=f21;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
17           BY id;
18         RUN;

NOTE: The data set WORK.F21 has 98 observations and 6 variables.
```

```
19
12                                                    The SAS System                    09:23 Thursday, January 21, 1999

20          PROC SORT DATA=m21;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
21            BY id;
22          RUN;

NOTE: The data set WORK.M21 has 96 observations and 6 variables.

23
24          DATA day21rep;
WARNING: The BASE Product produot with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
25            MERGE f21 m21;
26            BY id;
27          RUN;

NOTE: The data set WORK.DAY21REP has 99 observations and 9 variables.

28
29          /*****************************************************************/
30          /* For F0 generation, rats are not tracked by litter.  Therefore */
31          /* simply concatenate the male and female data sets and analyze */
32          /* with 2 way analysis of variance (grp, sex)                   */
33          /*****************************************************************/
34
35          DATA F5M; /* Female rats, F0, 5 months */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
36            INFILE '[GELLER.BMD]GEN25MF.DAT';
37            INPUT a $ no id grp sex $ age $ tsh t3 t4;
38            DROP a no;
39          RUN;

NOTE: The infile '[GELLER.BMD]GEN25MF.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN25MF.DAT

NOTE: 119 records were read from the infile '[GELLER.BMD]GEN25MF.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.F5M has 119 observations and 7 variables.

40
41          DATA M6M; /* Male rats, F0, 6 months */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
42            INFILE '[GELLER.BMD]GEN26MM.DAT';
43            INPUT a $ no id grp sex $ age $ tsh t3 t4;
44            DROP a no;
45          RUN;

NOTE: The infile '[GELLER.BMD]GEN26MM.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN26MM.DAT

NOTE: 118 records were read from the infile '[GELLER.BMD]GEN26MM.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
```

```
NOTE: The data set WORK.M6M has 118 observations and 7 variables.

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46
47          PROC SORT DATA=f5m;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
48            BY id;
49          RUN;

NOTE: The data set WORK.F5M has 119 observations and 7 variables.

50
51          PROC SORT DATA=m6m;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
52            BY id;
53          RUN;

NOTE: The data set WORK.M6M has 118 observations and 7 variables.

54
55          DATA F0;
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
56            set f5m m6m;
57          RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

58
59
60          /*****************************************************************/
61          /*  Analysis of F1, by dose group with sex as a repeated measure      */
62          /*****************************************************************/
63          PROC SORT DATA=day21rep;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
64            BY grp;
65          RUN;

NOTE: The data set WORK.DAY21REP has 99 observations and 9 variables.

66
67          PROC PRINT;
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
68            TITLE1'Generation F1, DAY 21 data for repeated measures.';
69            TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
70          RUN;

NOTE: The PROCEDURE PRINT printed pages 1-2.

71
72          PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
73            BY grp;
```

```
74          VAR tsh1 t31 t41 tsh2 t32 t42;
75          TITLE1'MEANS of Generation F1, DAY 21 data for repeated measures.';
76          TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
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77        RUN;

NOTE: The PROCEDURE MEANS printed page 3.

78
79        PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
80         CLASS grp;
81         MODEL t41 t42=grp;
82         REPEATED sex 2 /SUMMARY;
83         MEANS grp /TUKEY LINES;
84         TITLE1'Generation F1,DAY 21, T4';
85         TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
86        RUN;

87

NOTE: The PROCEDURE GLM printed pages 4-12.

88        PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
89         CLASS grp;
90         model t31 t32=grp;
91         REPEATED sex 2 /SUMMARY;
92         MEANS grp /TUKEY LINES;
93         TITLE1'Generation F1,DAY 21, T3';
94         TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
95        RUN;

96

NOTE: The PROCEDURE GLM printed pages 13-21.

97        PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
98         CLASS grp;
99         model tsh1 tsh2=grp;
100        REPEATED sex 2 /SUMMARY;
101        MEANS grp /TUKEY LINES;
102        TITLE1'Generation F1,DAY 21, TSH';
103        TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
104       RUN;

105        /*************************************************/
106       /*    ANALYSIS OF F0, BY DOSE GRP AND SEX        */
107      /*************************************************/

NOTE: The PROCEDURE GLM printed pages 22-30.

108       PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
```

```
        representative to have it renewed.
109         BY grp sex;
110       RUN;
```



```
NOTE: The data set WORK.F0 has 237 observations and 7 variables.

111
112       PROC PRINT DATA= F0;
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
        representative to have it renewed.
113         TITLE1'DATA FROM F0 GENERATION';
114       RUN;

NOTE: The PROCEDURE PRINT printed pages 31-35.

115
116       PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
        representative to have it renewed.
117         BY sex;
118       RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

119
120       PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
        representative to have it renewed.
121         BY sex;
122         TITLE'F0 Generation, Means by SEX';
123       RUN;

NOTE: The PROCEDURE MEANS printed page 36.

124
125       PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
        representative to have it renewed.
126         BY grp;
127       RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

128
129       PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
        representative to have it renewed.
130         BY grp;
131         TITLE'F0 Generation, Means by Dose Group';
132       RUN;

NOTE: The PROCEDURE MEANS printed page 37.

133
134       PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
        representative to have it renewed.
```

```
135          BY grp sex;
136        RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.
```



```
137
138        PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
139          BY grp sex;
140          TITLE'F0 Generation, Means by Dose and Sex';
141        RUN;

NOTE: The PROCEDURE MEANS printed pages 38-39.

142
143        PROC GLM DATA=F0;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
144         CLASSES grp sex;
145         model t4 t3 tsh=grp|sex;
146         MEANS grp|sex /TUKEY LINES;
147         TITLE'Generation F0, ADULT';
148        RUN;

NOTE: Means from the MEANS statement are not adjusted for other terms in the model.  For adjusted means, use the LSMEANS statement.
149

NOTE: The PROCEDURE GLM printed pages 40-49.

150        PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
151          BY sex;
152        RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

153
154        PROC GLM DATA=F0;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
155         BY sex;
156         CLASSES grp;
157         MODEL t4 t3 tsh=grp;
158         MEANS grp/TUKEY LINES;
159         TITLE1'Generation F0, ADULT';
160         TITLE2'Analysis by Sex';
161        RUN;

NOTE: Interactivity disabled with BY processing.
162
NOTE: The PROCEDURE GLM printed pages 50-63.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

1 Generation F1, DAY 21 data for repeated measures. 09:23 Thursday, January 21, 1999 1
Suffix=1 for Females; Suffix=2 for Males.

OBS	ID	GRP	AGE	TSH1	T31	T41	TSH2	T32	T42
1	3801	0.0	21D	0.54	118.93	4.57	0.90	120.00	5.66
2	3802	0.0	21D	0.56	127.92	2.80	.	.	.
3	3803	0.0	21D	1.64	93.98	4.37	0.85	99.80	4.97
4	3804	0.0	21D	0.87	112.45	3.55	1.14	104.22	3.83
5	3805	0.0	21D	1.16	114.92	5.82	0.97	112.25	4.76
6	3806	0.0	21D	0.74	95.62	4.24	1.02	98.19	4.09
7	3807	0.0	21D	1.30	107.53	4.34	0.97	104.56	4.46
8	3808	0.0	21D	1.53	100.83	4.66	1.34	109.78	5.27
9	3809	0.0	21D	1.07	107.58	4.42	1.90	86.13	3.07
10	3810	0.0	21D	0.86	102.97	4.53	1.03	99.47	5.02
11	3811	0.0	21D	0.91	122.60	4.05	1.03	110.48	4.54
12	3812	0.0	21D	1.19	104.24	3.55	1.61	99.33	4.41
13	3813	0.0	21D	1.74	103.20	3.18	1.66	116.19	3.59
14	3814	0.0	21D	0.85	109.83	3.14	1.59	118.50	5.95
15	3815	0.0	21D	0.69	88.40	2.48	0.56	103.55	2.67
16	3816	0.0	21D	2.74	85.37	3.32	2.30	96.28	3.17
17	3818	0.0	21D	0.85	104.36	4.89	1.17	116.60	4.52
18	3819	0.0	21D	1.16	101.32	3.59	0.98	94.88	3.10
19	3821	0.0	21D	0.84	79.83	4.37	0.58	111.49	3.81
20	3822	0.0	21D	0.57	90.65	3.09	0.70	97.51	3.49
21	3823	0.0	21D	1.18	107.12	3.08	1.16	105.90	2.55
22	3824	0.0	21D	1.45	117.65	4.68	1.93	108.85	5.97
23	3825	0.0	21D	0.73	104.83	5.96	1.17	115.59	4.89
24	3826	0.0	21D	1.51	105.71	5.29	1.59	85.59	3.77
25	3827	0.0	21D	1.35	115.38	5.59	1.84	114.60	5.76
26	3828	0.0	21D	0.16	137.69	6.65	1.12	125.29	5.41
27	3829	0.0	21D	1.70	115.59	4.80	1.59	106.54	5.60
28	3830	0.0	21D	1.48	90.20	4.54	0.71	97.65	4.56
29	3831	0.3	21D	0.84	118.75	5.65	1.16	140.77	5.55
30	3833	0.3	21D	1.47	122.11	6.87	1.02	102.38	3.45
31	3834	0.3	21D	0.74	105.27	4.72	0.50	98.34	3.77
32	3837	0.3	21D	1.61	109.33	4.62	1.12	109.50	4.00
33	3838	0.3	21D	0.76	116.85	3.92	0.48	104.64	3.91
34	3842	0.3	21D	0.96	116.94	4.97	0.83	116.33	4.59
35	3843	0.3	21D	0.91	123.21	5.54	1.00	126.47	4.72
36	3845	0.3	21D	0.47	95.72	4.43	0.63	77.78	3.68
37	3846	0.3	21D	1.62	99.48	4.52	0.97	100.00	4.52
38	3847	0.3	21D	1.14	139.51	4.28	.	.	.
39	3848	0.3	21D	1.11	99.10	4.77	0.97	83.62	3.07
40	3849	0.3	21D	1.33	125.37	5.77	0.89	117.49	5.07
41	3850	0.3	21D	1.84	85.45	4.11	0.72	91.03	4.41
42	3851	0.3	21D	1.16	105.31	3.56	0.98	116.70	5.70
43	3852	0.3	21D	1.27	106.74	4.42	0.58	109.25	3.97
44	3854	0.3	21D	1.27	116.06	5.47	0.91	128.06	6.23
45	3855	0.3	21D	0.89	119.82	4.15	0.66	135.82	4.82
46	3856	0.3	21D	1.00	114.33	3.58	1.04	119.85	3.95
47	3857	0.3	21D	1.52	93.59	4.84	1.48	115.96	6.37
48	3858	0.3	21D	1.73	104.69	6.25	2.00	117.18	5.60
49	3859	0.3	21D	1.30	111.68	6.70	1.05	126.16	6.00
50	3860	0.3	21D	1.20	88.98	3.89	0.77	96.81	3.53
51	3861	3.0	21D	1.19	94.02	5.40	0.38	96.85	4.73
52	3862	3.0	21D	1.28	100.02	3.30	0.88	91.83	3.51
53	3863	3.0	21D	1.67	104.22	5.34	1.07	115.12	5.69
54	3864	3.0	21D	0.70	81.32	3.63	0.95	86.32	3.81
55	3865	3.0	21D	1.15	88.17	4.35	1.20	86.70	4.04

Suffix=1 for Females; Suffix=2 for Males.

OBS	ID	GRP	AGE	TSH1	T31	T41	TSH2	T32	T42
56	3866	3	21D	1.21	104.71	4.07	0.81	114.95	4.22
57	3867	3	21D	0.61	111.35	4.11	0.67	101.21	3.34
58	3868	3	21D	1.43	109.34	3.28	0.93	85.71	5.11
59	3869	3	21D	0.98	113.15	4.25	0.99	100.07	4.33
60	3872	3	21D	1.48	117.39	5.01	1.34	117.67	4.26
61	3873	3	21D	2.15	118.55	4.18	1.27	114.37	4.86
62	3875	3	21D	1.40	102.32	4.54	0.99	135.10	5.59
63	3876	3	21D	1.62	142.56	4.44	1.38	126.16	4.60
64	3877	3	21D	1.23	125.24	3.94	0.70	127.60	4.77
65	3878	3	21D	0.70	112.91	4.06	0.63	139.04	4.65
66	3879	3	21D	0.90	109.09	3.78	0.60	114.07	3.87
67	3880	3	21D	0.67	89.02	4.96	0.56	101.60	5.67
68	3882	3	21D	0.82	116.22	6.23	0.83	123.71	5.71
69	3883	3	21D	1.01	120.88	3.90	0.80	119.86	4.95
70	3884	3	21D	1.30	131.74	3.78	1.17	120.28	4.03
71	3885	3	21D	0.62	98.85	4.46	0.72	96.82	4.53
72	3887	3	21D	0.86	110.08	4.12	0.88	126.66	4.59
73	3888	3	21D	1.00	108.36	5.20	0.69	102.72	4.37
74	3889	3	21D	1.36	108.76	5.06	0.76	90.42	5.54
75	3890	3	21D	1.18	114.05	2.70	0.72	110.42	2.56
76	3891	30	21D	1.11	101.34	3.64	1.10	103.06	4.09
77	3892	30	21D	1.47	106.42	2.84	.	.	.
78	3893	30	21D	1.01	96.20	4.49	0.74	118.93	5.40
79	3894	30	21D	1.50	110.06	3.96	1.55	107.78	4.94
80	3895	30	21D	2.05	89.95	4.46	1.68	94.67	5.01
81	3897	30	21D	1.32	94.62	3.17	1.20	101.91	4.31
82	3899	30	21D	1.29	94.64	4.82	0.95	91.54	4.67
83	3900	30	21D	1.34	95.71	2.83	1.22	86.49	2.85
84	3901	30	21D	0.60	82.98	2.91	0.74	79.34	4.10
85	3902	30	21D	1.11	95.47	3.52	1.04	94.80	3.59
86	3904	30	21D	1.11	90.80	2.94	1.01	93.58	4.68
87	3905	30	21D	.	.	.	1.71	117.53	2.64
88	3906	30	21D	1.14	87.52	3.42	0.89	100.49	4.94
89	3907	30	21D	1.54	79.28	2.67	1.63	127.13	6.03
90	3910	30	21D	2.14	124.86	4.21	2.43	127.13	6.70
91	3911	30	21D	1.19	90.74	2.88	1.02	104.62	3.62
92	3912	30	21D	1.19	102.83	3.78	1.44	104.71	2.74
93	3913	30	21D	1.70	98.04	4.67	1.62	95.74	5.66
94	3915	30	21D	1.65	106.41	4.65	1.24	118.72	5.75
95	3916	30	21D	1.10	115.86	4.31	1.18	101.66	3.69
96	3917	30	21D	0.93	97.81	5.24	1.28	115.02	5.51
97	3918	30	21D	1.01	93.00	4.54	1.36	142.11	5.34
98	3919	30	21D	1.34	80.96	6.74	0.98	104.32	4.04
99	3920	30	21D	1.09	108.86	3.31	1.20	138.88	3.78

GRP=0

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	28	1.1203571	0.0964152	0.5101814	0.1600000	2.7400000
T31	28	105.9535714	2.4708986	13.0747665	79.8300000	137.6900000
T41	28	4.2696429	0.1926517	1.0194170	2.4800000	6.6500000
TSH2	27	1.2374074	0.0861880	0.4478461	0.5600000	2.3000000
T32	27	105.8970370	1.9197980	9.9755631	85.5900000	125.2900000
T42	27	4.4033333	0.1950272	1.0133911	2.5500000	5.9700000

GRP=0.3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	22	1.1881818	0.0750574	0.3520503	0.4700000	1.8400000
T31	22	109.9222727	2.7857661	13.0664011	85.4500000	139.5100000
T41	22	4.8650000	0.2016799	0.9459626	3.5600000	6.8700000
TSH2	21	0.9409524	0.0745396	0.3415831	0.4800000	2.0000000
T32	21	111.1495238	3.5726718	16.3720391	77.7800000	140.7700000
T42	21	4.6147619	0.2137150	0.9793652	3.0700000	6.3700000

GRP=3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	25	1.1408000	0.0749376	0.3746879	0.6100000	2.1500000
T31	25	109.2928000	2.7136494	13.5682468	81.3200000	142.5600000
T41	25	4.3236000	0.1555141	0.7775704	2.7000000	6.2300000
TSH2	25	0.8768000	0.0508747	0.2543737	0.3800000	1.3800000
T32	25	109.8104000	3.1385677	15.6928385	85.7100000	139.0400000
T42	25	4.5332000	0.1577939	0.7889694	2.5600000	5.7100000

GRP=30

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	23	1.3013043	0.0732916	0.3514943	0.6000000	2.1400000
T31	23	97.5808696	2.3031636	11.0455844	79.2800000	124.8600000
T41	23	3.9130435	0.2049751	0.9830261	2.6700000	6.7400000
TSH2	23	1.2700000	0.0795342	0.3814327	0.7400000	2.4300000
T32	23	107.3982609	3.3487192	16.0598932	79.3400000	142.1100000
T42	23	4.5252174	0.2264661	1.0860934	2.6400000	6.7000000

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: T41

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.51553209	3.17184403	3.66	0.0153
Error	91	78.81858370	0.86613828		
Corrected Total	94	88.33411579			

R-Square	C.V.	Root MSE	T41 Mean
0.107722	21.31723	0.93066551	4.36578947

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	9.51553209	3.17184403	3.66	0.0153

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	9.51553209	3.17184403	3.66	0.0153

1

Generation F1,DAY 21, T4
Suffix=1 for Females; Suffix=2 for Males.

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General Linear Models Procedure

Dependent Variable: T42

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.72784090	0.24261363	0.27	0.8499
Error	91	83.05904963	0.91273681		
Corrected Total	94	83.78689053			

R-Square	C.V.	Root MSE	T42 Mean
0.008687	21.07913	0.95537260	4.53231579

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.72784090	0.24261363	0.27	0.8499

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.72784090	0.24261363	0.27	0.8499

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	T41	T42
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.97207577	2.6141	1	91	0.1094
Pillai's Trace	0.02792423	2.6141	1	91	0.1094
Hotelling-Lawley Trace	0.02872639	2.6141	1	91	0.1094
Roy's Greatest Root	0.02872639	2.6141	1	91	0.1094

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.90340906	3.2432	3	91	0.0256
Pillai's Trace	0.09659094	3.2432	3	91	0.0256
Hotelling-Lawley Trace	0.10691828	3.2432	3	91	0.0256
Roy's Greatest Root	0.10691828	3.2432	3	91	0.0256

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	5.48008956	1.82669652	1.42	0.2430
Error	91	117.32693992	1.28930703		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted G - G	Pr > F H - F
SEX	1	1.27978048	1.27978048	2.61	0.1094	.	.
SEX*GRP	3	4.76328344	1.58776115	3.24	0.0256	.	.
Error(SEX)	91	44.55069341	0.48956806				

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	2.55956097	2.55956097	2.61	0.1094
GRP	3	9.52656687	3.17552229	3.24	0.0256
Error	91	89.10138681	0.97913612		

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Ge[illegible]ion F1,DAY 21, T4 09:23 Thursday, January 21, 1999 11
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T41

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.866138
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.7104
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	4.8929	21	0.3
	A			
B	A	4.3241	27	0
B	A			
B	A	4.3236	25	3
B				
B		3.9618	22	30

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Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T42

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.912737
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.7292
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	4.6148	21	0.3
A			
A	4.6109	22	30
A			
A	4.5332	25	3
A			
A	4.4033	27	0

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

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Generation F1,DAY 21, T3
Suffix=1 for Females; Suffix=2 for Males.

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General Linear Models Procedure

Dependent Variable: T31

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2064.38182225	688.12727408	4.54	0.0052
Error	91	13804.23115248	151.69484783		
Corrected Total	94	15868.61297474			

R-Square	C.V.	Root MSE	T31 Mean
0.130092	11.71489	12.31644623	105.13494737

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	2064.38182225	688.12727408	4.54	0.0052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	2064.38182225	688.12727408	4.54	0.0052

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Generation F1,DAY 21, T3
Suffix=1 for Females; Suffix=2 for Males.

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General Linear Models Procedure

Dependent Variable: T32

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	424.20015417	141.40005139	0.66	0.5773
Error	91	19425.47154057	213.46672023		
Corrected Total	94	19849.67169474			

R-Square	C.V.	Root MSE	T32 Mean
0.021371	13.48716	14.61050034	108.32894737

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	424.20015417	141.40005139	0.66	0.5773

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	424.20015417	141.40005139	0.66	0.5773

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	T31	T32
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.93817904	5.9964	1	91	0.0163
Pillai's Trace	0.06182096	5.9964	1	91	0.0163
Hotelling-Lawley Trace	0.06589462	5.9964	1	91	0.0163
Roy's Greatest Root	0.06589462	5.9964	1	91	0.0163

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.92796295	2.3548	3	91	0.0772
Pillai's Trace	0.07203705	2.3548	3	91	0.0772
Hotelling-Lawley Trace	0.07762923	2.3548	3	91	0.0772
Roy's Greatest Root	0.07762923	2.3548	3	91	0.0772

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1841.56332307	613.85444102	2.24	0.0885
Error	91	24894.97280640	273.57112974		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted G - G	Pr > F H - F
SEX	1	549.21389263	549.21389263	6.00	0.0163	.	.
SEX*GRP	3	647.01865335	215.67288445	2.35	0.0772	.	.
Error(SEX)	91	8334.72988665	91.59043831				

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	1098.42778526	1098.42778526	6.00	0.0163
GRP	3	1294.03730671	431.34576890	2.35	0.0772
Error	91	16669.45977329	183.18087663		

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Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T31

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 151.6948
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 9.4008
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	109.293	25	3
	A			
	A	108.513	21	0.3
	A			
B	A	105.140	27	0
B				
B		97.179	22	30

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Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T32

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 213.4667
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 11.152
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	111.150	21	0.3
A			
A	109.810	25	3
A			
A	106.938	22	30
A			
A	105.897	27	0

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Dependent Variable: TSH1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.36063343	0.12021114	0.71	0.5472
Error	91	15.36005500	0.16879181		
Corrected Total	94	15.72068842			

R-Square	C.V.	Root MSE	TSH1 Mean
0.022940	34.60419	0.41084281	1.18726316

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.36063343	0.12021114	0.71	0.5472

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.36063343	0.12021114	0.71	0.5472

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Generation F1,DAY 21, TSH
Suffix=1 for Females; Suffix=2 for Males.

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General Linear Models Procedure

Dependent Variable: TSH2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.74305548	0.91435183	6.88	0.0003
Error	91	12.09964347	0.13296312		
Corrected Total	94	14.84269895			

R-Square	C.V.	Root MSE	TSH2 Mean
0.184808	33.76635	0.36464108	1.07989474

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	2.74305548	0.91435183	6.88	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	2.74305548	0.91435183	6.88	0.0003

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	TSH1	TSH2
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.89351379	10.8451	1	91	0.0014
Pillai's Trace	0.10648621	10.8451	1	91	0.0014
Hotelling-Lawley Trace	0.11917691	10.8451	1	91	0.0014
Roy's Greatest Root	0.11917691	10.8451	1	91	0.0014

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect.
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.82339898	6.5058	3	91	0.0005
Pillai's Trace	0.17660102	6.5058	3	91	0.0005
Hotelling-Lawley Trace	0.21447806	6.5058	3	91	0.0005
Roy's Greatest Root	0.21447806	6.5058	3	91	0.0005

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1.98018483	0.66006161	2.70	0.0501
Error	91	22.22138149	0.24419101		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted G - G	Pr > F H - F
SEX	1	0.62428642	0.62428642	10.85	0.0014	.	.
SEX*GRP	3	1.12350407	0.37450136	6.51	0.0005	.	.
Error(SEX)	91	5.23831698	0.05756392				

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	1.24857284	1.24857284	10.85	0.0014
GRP	3	2.24700815	0.74900272	6.51	0.0005
Error	91	10.47663396	0.11512785		

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Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH1

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.168792
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.3136
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	1.2936	22	30
A			
A	1.1905	21	0.3
A			
A	1.1411	27	0
A			
A	1.1408	25	3

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Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH2

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.132963
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.2783
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	1.2500	22	30
A			
A	1.2374	27	0
B	0.9410	21	0.3
B			
B	0.8768	25	3

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
1	3801	0	F	5M	0.72	27.62	1.79
2	3802	0	F	5M	3.51	88.20	4.35
3	3803	0	F	5M	0.84	23.88	1.05
4	3804	0	F	5M	1.66	55.70	1.45
5	3805	0	F	5M	1.31	56.03	2.47
6	3806	0	F	5M	2.46	36.57	2.23
7	3807	0	F	5M	1.82	48.65	2.04
8	3808	0	F	5M	1.59	62.75	2.98
9	3809	0	F	5M	1.39	62.30	2.02
10	3810	0	F	5M	2.41	44.70	2.80
11	3811	0	F	5M	1.44	50.48	2.06
12	3812	0	F	5M	1.86	47.12	1.68
13	3813	0	F	5M	2.77	43.52	2.43
14	3814	0	F	5M	1.36	52.48	2.16
15	3815	0	F	5M	1.80	68.54	2.10
16	3816	0	F	5M	3.16	108.80	1.58
17	3817	0	F	5M	1.31	68.48	2.64
18	3818	0	F	5M	1.50	159.25	1.45
19	3819	0	F	5M	2.59	41.89	1.45
20	3820	0	F	5M	1.59	114.68	3.17
21	3821	0	F	5M	2.08	44.07	1.11
22	3822	0	F	5M	5.24	60.97	1.93
23	3823	0	F	5M	1.97	43.95	1.90
24	3824	0	F	5M	1.83	24.39	1.34
25	3825	0	F	5M	2.05	53.42	1.88
26	3826	0	F	5M	2.41	40.63	2.33
27	3827	0	F	5M	2.00	38.53	1.83
28	3828	0	F	5M	2.20	47.03	2.46
29	3829	0	F	5M	2.82	60.18	2.44
30	3830	0	F	5M	1.93	58.28	2.67
31	3601	0	M	6M	1.19	88.35	5.07
32	3602	0	M	6M	0.93	84.17	4.35
33	3603	0	M	6M	0.62	79.49	5.66
34	3604	0	M	6M	0.80	82.88	5.33
35	3605	0	M	6M	2.16	86.13	5.19
36	3606	0	M	6M	1.18	93.42	4.36
37	3607	0	M	6M	1.05	63.72	3.81
38	3608	0	M	6M	1.80	69.21	4.18
39	3609	0	M	6M	4.41	72.29	4.12
40	3610	0	M	6M	1.40	68.40	4.82
41	3611	0	M	6M	0.84	61.11	4.24
42	3612	0	M	6M	1.45	61.69	4.84
43	3613	0	M	6M	0.56	71.49	4.94
44	3614	0	M	6M	1.81	83.01	4.56
45	3615	0	M	6M	1.08	77.97	5.39
46	3616	0	M	6M	3.04	69.67	3.84
47	3617	0	M	6M	1.34	71.30	4.95
48	3618	0	M	6M	2.08	51.76	3.18
49	3619	0	M	6M	0.42	68.45	4.49
50	3620	0	M	6M	0.95	66.34	5.01
51	3621	0	M	6M	2.42	62.40	4.30
52	3622	0	M	6M	1.48	96.45	4.44
53	3623	0	M	6M	3.67	61.89	4.17
54	3624	0	M	6M	0.35	73.45	4.35
55	3625	0	M	6M	1.88	80.55	4.11
56	3627	0	M	6M	1.35	55.93	5.37

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
57	3628	0.0	M	6M	0.90	65.47	5.25
58	3629	0.0	M	6M	0.75	59.35	4.87
59	3630	0.0	M	6M	2.46	77.51	5.40
60	3831	0.3	F	5M	1.46	46.98	1.38
61	3832	0.3	F	5M	2.99	76.19	3.82
62	3833	0.3	F	5M	1.42	118.14	2.13
63	3834	0.3	F	5M	1.40	39.12	3.15
64	3835	0.3	F	5M	1.64	78.71	4.42
65	3836	0.3	F	5M	1.48	102.72	3.65
66	3837	0.3	F	5M	1.30	50.23	3.71
67	3838	0.3	F	5M	2.02	54.58	2.16
68	3839	0.3	F	5M	1.16	82.60	4.07
69	3840	0.3	F	5M	2.40	132.21	5.87
70	3841	0.3	F	5M	1.71	67.83	3.55
71	3842	0.3	F	5M	2.67	50.68	2.93
72	3843	0.3	F	5M	4.82	42.96	2.44
73	3844	0.3	F	5M	1.88	141.56	3.76
74	3845	0.3	F	5M	1.07	43.53	1.14
75	3846	0.3	F	5M	1.26	49.05	2.57
76	3847	0.3	F	5M	1.63	84.55	2.96
77	3848	0.3	F	5M	2.78	54.17	2.11
78	3849	0.3	F	5M	1.13	43.88	2.48
79	3850	0.3	F	5M	2.72	46.08	2.92
80	3851	0.3	F	5M	3.71	45.33	1.66
81	3852	0.3	F	5M	1.76	34.24	1.54
82	3853	0.3	F	5M	2.51	101.45	3.29
83	3854	0.3	F	5M	1.34	42.79	2.46
84	3855	0.3	F	5M	1.62	28.25	1.42
85	3856	0.3	F	5M	3.47	40.06	3.48
86	3857	0.3	F	5M	2.79	46.68	3.64
87	3858	0.3	F	5M	4.32	69.99	2.02
88	3859	0.3	F	5M	3.17	58.12	2.98
89	3860	0.3	F	5M	2.77	70.99	3.39
90	3631	0.3	M	6M	1.83	79.54	4.79
91	3632	0.3	M	6M	0.91	77.36	4.51
92	3633	0.3	M	6M	1.30	131.87	7.01
93	3634	0.3	M	6M	0.81	79.14	4.28
94	3635	0.3	M	6M	2.09	110.78	6.17
95	3636	0.3	M	6M	1.02	79.79	4.55
96	3637	0.3	M	6M	1.69	98.71	4.28
97	3638	0.3	M	6M	0.82	107.52	4.74
98	3639	0.3	M	6M	2.38	82.07	4.28
99	3640	0.3	M	6M	2.14	80.26	3.94
100	3641	0.3	M	6M	1.82	57.22	3.30
101	3642	0.3	M	6M	1.79	87.48	3.75
102	3643	0.3	M	6M	0.65	120.54	4.83
103	3644	0.3	M	6M	1.67	83.31	4.74
104	3645	0.3	M	6M	2.64	91.05	5.01
105	3646	0.3	M	6M	1.08	102.18	5.81
106	3647	0.3	M	6M	1.42	79.21	4.57
107	3648	0.3	M	6M	1.80	72.73	4.18
108	3649	0.3	M	6M	1.26	68.76	4.86
109	3650	0.3	M	6M	0.71	89.35	5.05
110	3651	0.3	M	6M	0.72	78.85	4.25
111	3652	0.3	M	6M	0.61	92.34	5.56
112	3653	0.3	M	6M	2.29	91.63	5.07

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
113	3654	0.3	M	6M	0.31	109.01	5.63
114	3655	0.3	M	6M	0.78	80.77	4.05
115	3656	0.3	M	6M	1.08	70.67	3.80
116	3657	0.3	M	6M	1.43	91.65	5.36
117	3658	0.3	M	6M	1.17	73.19	4.18
118	3659	0.3	M	6M	0.29	74.18	3.57
119	3660	0.3	M	6M	2.07	80.52	5.66
120	3861	3.0	F	5M	1.71	47.40	3.05
121	3862	3.0	F	5M	3.25	65.02	3.67
122	3863	3.0	F	5M	0.87	61.89	2.24
123	3864	3.0	F	5M	0.62	47.74	1.36
124	3865	3.0	F	5M	2.36	42.31	2.07
125	3866	3.0	F	5M	1.46	49.32	2.56
126	3867	3.0	F	5M	2.72	49.26	2.15
127	3868	3.0	F	5M	2.26	48.82	2.20
128	3869	3.0	F	5M	1.09	45.46	2.24
129	3870	3.0	F	5M	1.80	89.35	3.60
130	3871	3.0	F	5M	1.52	85.38	4.03
131	3872	3.0	F	5M	2.11	58.63	2.69
132	3873	3.0	F	5M	2.29	45.69	2.68
133	3874	3.0	F	5M	1.92	90.35	3.97
134	3875	3.0	F	5M	2.16	53.03	3.06
135	3876	3.0	F	5M	2.88	42.69	1.46
136	3877	3.0	F	5M	3.26	52.51	2.74
137	3878	3.0	F	5M	1.50	63.21	4.25
138	3879	3.0	F	5M	1.27	38.87	1.64
139	3880	3.0	F	5M	1.44	53.24	2.68
140	3882	3.0	F	5M	1.41	58.15	2.82
141	3883	3.0	F	5M	3.87	46.05	2.90
142	3884	3.0	F	5M	1.19	75.50	3.59
143	3885	3.0	F	5M	1.44	43.87	3.15
144	3886	3.0	F	5M	2.53	70.42	4.63
145	3887	3.0	F	5M	2.46	47.75	3.83
146	3888	3.0	F	5M	1.38	50.40	2.42
147	3889	3.0	F	5M	2.27	59.71	3.69
148	3890	3.0	F	5M	2.67	52.14	3.43
149	3661	3.0	M	6M	1.29	81.05	5.05
150	3662	3.0	M	6M	2.84	99.22	3.73
151	3663	3.0	M	6M	1.54	92.30	6.09
152	3664	3.0	M	6M	1.36	102.47	4.29
153	3665	3.0	M	6M	1.71	102.42	5.34
154	3666	3.0	M	6M	0.68	67.87	4.79
155	3667	3.0	M	6M	1.39	88.38	4.39
156	3668	3.0	M	6M	0.39	68.59	4.96
157	3669	3.0	M	6M	1.22	75.98	5.99
158	3670	3.0	M	6M	1.91	98.88	4.76
159	3671	3.0	M	6M	3.76	82.85	4.58
160	3672	3.0	M	6M	0.87	90.33	5.27
161	3673	3.0	M	6M	1.40	90.58	4.17
162	3674	3.0	M	6M	1.32	90.37	4.38
163	3675	3.0	M	6M	1.03	84.18	4.57
164	3676	3.0	M	6M	0.90	72.26	4.74
165	3677	3.0	M	6M	0.87	79.72	4.73
166	3678	3.0	M	6M	0.38	69.36	3.34
167	3679	3.0	M	6M	2.47	85.77	3.79
168	3680	3.0	M	6M	1.36	74.30	5.45

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
169	3681	3	M	6M	2.58	92.89	4.40
170	3682	3	M	6M	2.19	98.91	6.02
171	3683	3	M	6M	2.07	111.73	4.99
172	3684	3	M	6M	0.77	70.66	4.27
173	3685	3	M	6M	0.45	166.80	3.47
174	3686	3	M	6M	3.03	81.70	5.52
175	3687	3	M	6M	0.96	81.88	4.39
176	3688	3	M	6M	1.47	94.46	6.49
177	3689	3	M	6M	1.05	79.94	4.74
178	3690	3	M	6M	1.35	77.72	3.62
179	3891	30	F	5M	2.33	37.30	1.90
180	3892	30	F	5M	2.04	56.00	3.92
181	3893	30	F	5M	1.27	45.02	3.02
182	3894	30	F	5M	2.99	43.84	1.83
183	3895	30	F	5M	2.59	40.47	3.06
184	3896	30	F	5M	1.34	68.29	3.24
185	3897	30	F	5M	1.20	55.97	2.39
186	3898	30	F	5M	2.67	108.40	3.11
187	3899	30	F	5M	1.52	60.10	3.29
188	3900	30	F	5M	1.51	42.03	2.28
189	3901	30	F	5M	1.44	38.32	1.83
190	3902	30	F	5M	1.27	46.37	2.25
191	3903	30	F	5M	3.33	89.67	2.39
192	3904	30	F	5M	1.66	54.31	2.59
193	3905	30	F	5M	2.47	91.70	4.62
194	3906	30	F	5M	3.02	47.31	2.46
195	3907	30	F	5M	2.64	64.16	1.88
196	3908	30	F	5M	2.14	107.67	2.78
197	3909	30	F	5M	1.80	100.45	2.54
198	3910	30	F	5M	3.30	49.36	1.58
199	3911	30	F	5M	1.53	79.20	2.98
200	3912	30	F	5M	1.80	88.74	1.59
201	3913	30	F	5M	3.06	41.83	1.57
202	3914	30	F	5M	1.51	77.67	2.47
203	3915	30	F	5M	3.58	50.85	1.08
204	3916	30	F	5M	1.45	47.37	1.53
205	3917	30	F	5M	1.82	47.81	2.06
206	3918	30	F	5M	2.49	42.50	2.93
207	3919	30	F	5M	1.98	39.32	2.28
208	3920	30	F	5M	3.46	49.17	1.19
209	3691	30	M	6M	2.58	64.35	3.66
210	3692	30	M	6M	3.24	89.66	3.66
211	3693	30	M	6M	2.70	91.40	3.10
212	3694	30	M	6M	5.17	95.93	3.94
213	3695	30	M	6M	3.36	85.61	3.95
214	3696	30	M	6M	1.28	83.01	4.05
215	3697	30	M	6M	8.21	100.77	6.08
216	3699	30	M	6M	2.07	60.94	3.23
217	3700	30	M	6M	1.94	59.68	2.63
218	3701	30	M	6M	1.76	81.16	3.52
219	3702	30	M	6M	3.82	92.08	3.39
220	3703	30	M	6M	3.74	98.18	3.63
221	3704	30	M	6M	1.76	80.75	4.37
222	3705	30	M	6M	2.29	68.34	2.62
223	3706	30	M	6M	1.97	74.23	3.00
224	3707	30	M	6M	5.37	61.49	3.27

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
225	3708	30	M	6M	1.13	84.65	2.10
226	3709	30	M	6M	14.15	71.72	1.57
227	3710	30	M	6M	13.31	74.04	2.99
228	3711	30	M	6M	1.14	71.23	4.50
229	3712	30	M	6M	1.93	70.55	4.18
230	3713	30	M	6M	7.40	71.35	4.45
231	3714	30	M	6M	1.05	64.68	3.00
232	3715	30	M	6M	1.79	116.75	4.08
233	3716	30	M	6M	1.97	89.85	4.68
234	3717	30	M	6M	0.94	66.63	3.19
235	3718	30	M	6M	8.27	78.42	3.95
236	3719	30	M	6M	6.87	54.88	3.35
237	3720	30	M	6M	1.04	76.21	3.61

SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	119	3860.33	3.1975080	34.8806948	3801.00	3920.00
GRP	119	8.3697479	1.1609977	12.6649895	0	30.0000000
TSH	119	2.1087395	0.0773778	0.8440927	0.6200000	5.2400000
T3	119	59.8497479	2.2107705	24.1166592	23.8800000	159.2500000
T4	119	2.5910084	0.0827259	0.9024331	1.0500000	5.8700000

SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	118	3660.47	3.2002262	34.7633543	3601.00	3720.00
GRP	118	8.2118644	1.1549327	12.5457806	0	30.0000000
TSH	118	2.0492373	0.1953372	2.1219049	0.2900000	14.1500000
T3	118	81.8444068	1.5246098	16.5615013	51.7600000	166.8000000
T4	118	4.4274576	0.0832387	0.9042040	1.5700000	7.0100000

GRP=0

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3717.02	13.2011036	101.3996007	3601.00	3830.00
TSH	59	1.7964407	0.1230441	0.9451195	0.3500000	5.2400000
T3	59	65.0328814	2.9519008	22.6739804	23.8800000	159.2500000
T4	59	3.3623729	0.1841824	1.4147320	1.0500000	5.6600000

GRP=0.3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	60	3745.50	13.0675667	101.2209364	3631.00	3860.00
TSH	60	1.7830000	0.1202247	0.9312563	0.2900000	4.8200000
T3	60	76.0891667	3.3691531	26.0973476	28.2500000	141.5600000
T4	60	3.8146667	0.1684937	1.3051469	1.1400000	7.0100000

GRP=3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3773.71	13.1659384	101.1294921	3661.00	3890.00
TSH	59	1.7342373	0.1077443	0.8275998	0.3800000	3.8700000
T3	59	72.6733898	2.9975333	23.0244899	38.8700000	166.8000000
T4	59	3.8494915	0.1592210	1.2230000	1.3600000	6.4900000

GRP=30

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3807.32	13.1611298	101.0925559	3691.00	3920.00
TSH	59	3.0077966	0.3421046	2.6277551	0.9400000	14.1500000
T3	59	69.3176271	2.6843263	20.6187013	37.3000000	116.7500000
T4	59	2.9896610	0.1308737	1.0052602	1.0800000	6.0800000

GRP=0 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3815.50	1.6072751	8.8034084	3801.00	3830.00
TSH	30	2.0540000	0.1594023	0.8730825	0.7200000	5.2400000
T3	30	57.7696667	5.1493264	28.2040220	23.8800000	159.2500000
T4	30	2.1263333	0.1236620	0.6773248	1.0500000	4.3500000

GRP=0 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3615.14	1.6209255	8.7289508	3601.00	3630.00
TSH	29	1.5300000	0.1777452	0.9571871	0.3500000	4.4100000
T3	29	72.5465517	2.0850083	11.2281134	51.7600000	96.4500000
T4	29	4.6410345	0.1083501	0.5834830	3.1800000	5.6600000

GRP=0.3 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3845.50	1.6072751	8.8034084	3831.00	3860.00
TSH	30	2.2133333	0.1801745	0.9868561	1.0700000	4.8200000
T3	30	64.7890000	5.3452191	29.2769705	28.2500000	141.5600000
T4	30	2.9033333	0.1896520	1.0387670	1.1400000	5.8700000

GRP=0.3 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3645.50	1.6072751	8.8034084	3631.00	3660.00
TSH	30	1.3526667	0.1165263	0.6382408	0.2900000	2.6400000
T3	30	87.3893333	2.9681270	16.2571009	57.2200000	131.8700000
T4	30	4.7260000	0.1492407	0.8174249	3.3000000	7.0100000

GRP=3 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3875.31	1.6520650	8.8966424	3861.00	3890.00
TSH	29	1.9900000	0.1435424	0.7729997	0.6200000	3.8700000
T3	29	56.3503448	2.5982097	13.9917875	38.8700000	90.3500000
T4	29	2.9241379	0.1562495	0.8414296	1.3600000	4.6300000

GRP=3 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3675.50	1.6072751	8.8034084	3661.00	3690.00
TSH	30	1.4870000	0.1488030	0.8150276	0.3800000	3.7600000
T3	30	88.4523333	3.4021201	18.6341793	67.8700000	166.8000000
T4	30	4.7440000	0.1442050	0.7898433	3.3400000	6.4900000

GRP=30 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3905.50	1.6072751	8.8034084	3891.00	3920.00
TSH	30	2.1736667	0.1357876	0.7437393	1.2000000	3.5800000
T3	30	60.3733333	4.0112100	21.9703018	37.3000000	108.4000000
T4	30	2.4213333	0.1445999	0.7920063	1.0800000	4.6200000

GRP=30 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3705.76	1.6420094	8.8424915	3691.00	3720.00
TSH	29	3.8706897	0.6489744	3.4948339	0.9400000	14.1500000
T3	29	78.5703448	2.6672331	14.3634900	54.8800000	116.7500000
T4	29	3.5775862	0.1596908	0.8599612	1.5700000	6.0800000

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3
SEX	2	F M

Number of observations in data set = 237

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	241.25464107	34.46494872	52.50	0.0001
Error	229	150.32005345	0.65641945		
Corrected Total	236	391.57469451			

R-Square	C.V.	Root MSE	T4 Mean
0.616114	23.11310	0.81019717	3.50535865

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	29.62445542	9.87481847	15.04	0.0001
SEX	1	198.02136032	198.02136032	301.67	0.0001
GRP*SEX	3	13.60882533	4.53627511	6.91	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	28.07875642	9.35958547	14.26	0.0001
SEX	1	198.01581342	198.01581342	301.66	0.0001
GRP*SEX	3	13.60882533	4.53627511	6.91	0.0002

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	34937.85388704	4991.12198386	12.10	0.0001
Error	229	94446.22560494	412.42893277		
Corrected Total	236	129384.07949198			

R-Square	C.V.	Root MSE	T3 Mean
0.270032	28.68383	20.30834638	70.80067511

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	3977.55083365	1325.85027788	3.21	0.0237
SEX	1	28467.84975054	28467.84975054	69.02	0.0001
GRP*SEX	3	2492.45330286	830.81776762	2.01	0.1127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	3811.29675945	1270.43225315	3.08	0.0283
SEX	1	28458.69687677	28458.69687677	69.00	0.0001
GRP*SEX	3	2492.45330286	830.81776762	2.01	0.1127

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	129.23442772	18.46206110	8.77	0.0001
Error	229	481.83968621	2.10410343		
Corrected Total	236	611.07411392			

R-Square	C.V.	Root MSE	TSH Mean
0.211487	69.76784	1.45055280	2.07911392

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	67.87744714	22.62581571	10.75	0.0001
SEX	1	0.15498515	0.15498515	0.07	0.7863
GRP*SEX	3	61.20199542	20.40066514	9.70	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	69.42116553	23.14038851	11.00	0.0001
SEX	1	0.13455387	0.13455387	0.06	0.8006
GRP*SEX	3	61.20199542	20.40066514	9.70	0.0001

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 0.656419
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 0.3852
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.8495	59	3
A			
A	3.8147	60	0.3
B	3.3624	59	0
B			
B	2.9897	59	30

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 412.4289
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 9.6566
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	76.089	60	0.3
	A			
B	A	72.673	59	3
B	A			
B	A	69.318	59	30
B				
B		65.033	59	0

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 2.104103
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 0.6897
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.0078	59	30
B	1.7964	59	0
B			
B	1.7830	60	0.3
B			
B	1.7342	59	3

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 0.656419
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 0.2074
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	4.4275	118	M
B	2.5910	119	F

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 412.4289
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 5.1986
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	81.844	118	M
B	59.850	119	F

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 2.104103
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 0.3713
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	2.1087	119	F
A			
A	2.0492	118	M

Level of GRP	Level of SEX	N	T4 Mean	T4 SD	T3 Mean	T3 SD	TSH Mean	TSH SD
0	F	30	2.12633333	0.67732477	57.7696667	28.2040220	2.05400000	0.87308253
0	M	29	4.64103448	0.58348304	72.5465517	11.2281134	1.53000000	0.95718710
3	F	29	2.92413793	0.84142955	56.3503448	13.9917875	1.99000000	0.77299972
3	M	30	4.74400000	0.78984328	88.4523333	18.6341793	1.48700000	0.81502761
30	F	30	2.42133333	0.79200633	60.3733333	21.9703018	2.17366667	0.74373931
30	M	29	3.57758621	0.85996119	78.5703448	14.3634900	3.87068966	3.49483387
0.3	F	30	2.90333333	1.03876695	64.7890000	29.2769705	2.21333333	0.98685615
0.3	M	30	4.72600000	0.81742489	87.3893333	16.2571009	1.35266667	0.63824076

---------- SEX=F ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in by group = 119

---------- SEX=F ----------

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13.48606554	4.49535518	6.26	0.0006
Error	115	82.61141345	0.71836012		
Corrected Total	118	96.09747899			

R-Square	C.V.	Root MSE	T4 Mean
0.140337	32.71164	0.84756128	2.59100840

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	13.48606554	4.49535518	6.26	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	13.48606554	4.49535518	6.26	0.0006

---------- SEX=F ----------

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1225.04156255	408.34718752	0.70	0.5559
Error	115	67405.32192989	586.13323417		
Corrected Total	118	68630.36349244			

R-Square	C.V.	Root MSE	T3 Mean
0.017850	40.45161	24.21018864	59.84974790

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	1225.04156255	408.34718752	0.70	0.5559

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1225.04156255	408.34718752	0.70	0.5559

---------- SEX=F ----------

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.95342759	0.31780920	0.44	0.7250
Error	115	83.12068333	0.72278855		
Corrected Total	118	84.07411092			

R-Square	C.V.	Root MSE	TSH Mean
0.011340	40.31649	0.85016972	2.10873950

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.95342759	0.31780920	0.44	0.7250

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.95342759	0.31780920	0.44	0.7250

---------- SEX=F ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 0.71836
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.573
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	2.9241	29	3
	A			
	A	2.9033	30	0.3
	A			
B	A	2.4213	30	30
B				
B		2.1263	30	0

---------- SEX=F ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 586.1332
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 16.367
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	64.789	30	0.3
A			
A	60.373	30	30
A			
A	57.770	30	0
A			
A	56.350	29	3

---------- SEX=F ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 0.722789
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.5747
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	2.2133	30	0.3
A			
A	2.1737	30	30
A			
A	2.0540	30	0
A			
A	1.9900	29	3

---------- SEX=M ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in by group = 118

---------- SEX=M ----------

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	27.94879729	9.31626576	15.69	0.0001
Error	114	67.70864000	0.59393544		
Corrected Total	117	95.65743729			

R-Square	C.V.	Root MSE	T4 Mean
0.292176	17.40665	0.77067207	4.42745763

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	27.94879729	9.31626576	15.69	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	27.94879729	9.31626576	15.69	0.0001

---------- SEX=M ----------

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	5050.24523342	1683.41507781	7.10	0.0002
Error	114	27040.90367506	237.20090943		
Corrected Total	117	32091.14890847			

R-Square	C.V.	Root MSE	T3 Mean
0.157372	18.81781	15.40132817	81.84440678

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	5050.24523342	1683.41507781	7.10	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	5050.24523342	1683.41507781	7.10	0.0002

---------- SEX=M ----------

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	128.07122848	42.69040949	12.21	0.0001
Error	114	398.71900287	3.49753511		
Corrected Total	117	526.79023136			

R-Square	C.V.	Root MSE	TSH Mean
0.243116	91.26175	1.87016981	2.04923729

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	128.07122848	42.69040949	12.21	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	128.07122848	42.69040949	12.21	0.0001

---------- SEX=M ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 0.593935
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.5233
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	4.7440	30	3
A			
A	4.7260	30	0.3
A			
A	4.6410	29	0
B	3.5776	29	30

---------- SEX=M ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 237.2009
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 10.457
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	88.452	30	3
	A			
	A	87.389	30	0.3
	A			
B	A	78.570	29	30
B				
B		72.547	29	0

---------- SEX=M ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 3.497535
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 1.2698
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.8707	29	30
B	1.5300	29	0
B			
B	1.4870	30	3
B			
B	1.3527	30	0.3

From: Latendresse, John <JLatendresse@nctr.fda.gov>
To: 'Alan Warren' <awarren@terra1.com>
Date: Monday, January 11, 1999 3:33 PM
Subject: RE: slides

Alan,
I didn't read your message until after I had sent the report out. With few exceptions, I have never been a strong advocate of "blind" histopathology assessment of toxicology studies. Blind reading generally takes much longer, and it can significantly hinder the identification and characterization of lesions induced by exposure to a xenobiotic agent, particularly when they are suttle. With such a study like ammonium perchlorate, I believe that one would get a much more accurate and confident characterization of morphologic alterations by first comparing the high dose and control specimens to establish thresholds for severity scores, for example. Particularly when lesions are suttle, this is an absolutely essential step precluding one's attempt to determine a dose response. To summarize, frankly, in most instances I believe you don't need a blind reading to get a quality, unbiased assessment by the majority of pathologists who characterize morphologic alterations for a living. Often such requests come from scientists who don't understand the process of morphologic assessment. Most pathologists worth their salt actually do some sort of a blind reading anyway, if the study implies a need. For example, after I have carefully compared the morphology of control and high dose specimens, and detect a putative morphologic alteration believed to be due to exposure to a toxicant, I will confirm my observation by examing a pool of unknown specimens. If I can separate the treatment and control specimens based on the morphologic criteria developed during the high dose and control comparison, I proceed with a similar series of exercises in an effort to define a dose response.

Principle In ator: A. Warren, Ph.D.

Table
Ammonium Pe rate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Diplomate,

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
D	0	1	A	thyroid adventicia	ectopic thymus		
D	0	3	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
D	0	4	A	thyroid gland	essentially normal tissue		
D	0	5	A	thyroid gland	essentially normal tissue		
D	0	6	C	thyroid gland	essentially normal tissue		
D	0	2	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
D	30	25	C	thyroid follicle	colloid depletion	2	Follicles are predominantly small to medium with decreased luminal size and colloid.
D	30	26	A	thyroid follicle	colloid depletion	2	
D	30	27	A	thyroid follicle	colloid depletion	2	
D	30	28	A	thyroid follicle	colloid depletion	2	
D	30	29	C	thyroid follicle	colloid depletion	2	
D	30	25	C	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly, diffusely dilated and filled with erythrocytes.
D	30	26	A	capillary	congestion	2	
D	30	27	A	capillary	congestion	2	
D	30	28	A	capillary	congestion	2	
D	30	29	C	capillary	congestion	1	
D	30	25	C	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epithihem is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2. Perinuclear halo often present.
D	30	26	A	epithelium, follicular	hypertrophy	2	
D	30	27	A	epithelium, follicular	hypertrophy	2	
D	30	28	A	epithelium, follicular	hypertrophy	2	
D	30	29	C	epithelium, follicular	hypertrophy	1	
D	30	30	C		NOT EXAMINED		RECUT, NOT ENOUGH TISSUE TO EVALUATE.

Princir' 'nvestigator: A. Warren, Ph.D.

Table 1
Ammoni[illegible]erchlorate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Ph.D.
Diplo ACVP

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
A	0	1	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
A	0	2	A	thyroid gland	essentially normal tissue		
A	0	4	A	thyroid gland	essentially normal tissue		
A	0	5	A	thyroid gland	essentially normal tissue		
A	0	6	C	thyroid gland	essentially normal tissue		
A	0	3	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
A	30	28	C	thyroid follicle	colloid depletion	2	
A	30	29	A	thyroid follicle	colloid depletion	2	
A	30	30	A	thyroid follicle	colloid depletion	2	
A	30	25	A	thyroid follicle	colloid depletion	2	
A	30	26	A	thyroid follicle	colloid depletion	2	
A	30	27	A	thyroid follicle	colloid depletion	2	
A	30	25	A	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly dilated and filled with erythrocytes.
A	30	26	A	capillary	congestion	2	
A	30	27	A	capillary	congestion	2	
A	30	28	C	capillary	congestion	2	
A	30	29	A	capillary	congestion	2	
A	30	30	A	capillary	congestion	2	
A	30	25	A	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epiththem is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2.
A	30	26	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	epithelium, follicular	hypertrophy	3	
A	30	28	C	epithelium, follicular	hypertrophy	2	
A	30	29	A	epithelium, follicular	hypertrophy	2	
A	30	30	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	thyroid gland	thyroglossal duct cyst	2	

Table 3. Study A
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day)	
		0	30
Thyroid follicle	Colloid depletion	0/6 (0)	6/6 (100) [2]*
Capillary	Congestion	0/6 (0)	6/6 (100) [2]
Epithelium, follicular	Hypertrophy	0/6 (0)	6/6 (100) [2]

- [Mean severity]

Table 4. Study D
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day)	
		0	30
Thyroid follicle	Colloid depletion	0/5 (0)	5/5 (100) [2]*
Capillary	Congestion	0/6 (0)	5/5 (100) [1.8]
Epithelium, follicular	Hypertrophy	0/6 (0)	5/5 (100) [1.8]

- [Mean severity]

Inhibition of iodide uptake by the thyroid follicular epithelium has been reported as the mechanism of action of ammonium perchlorate in the rat thyroid gland. Iodination of tyrosine residues of thyroglobulin is one of the essential steps in the production of T3 and T4 (thyroxin). Decreased synthesis of T4 and T3 results in lowered serum concentration that triggers the synthesis and release of TSH from the anterior pituitary gland. TSH receptor activation of cyclic AMP intracellular signaling culminates in hypertrophy of the follicular epithelium. Epithelial hypertrophy, colloid depletion, and the appearance of increased blood flow to the thyroid gland observed in the mice in these studies are consistent with persistent TSH stimulation secondary to deficient production of T3 and/or thyroxin. These observations support a hypothesis of a similar mechanism of action of ammonium perchlorate in the thyroid gland of the mouse that has been shown in the rat.

John R. Latendresse
Diplomate, College of Veterinary Pathologists

John R. Latendresse, D.V.M., Ph.D.
Diplomate of the American College of Veterinary Pathologists

Phone 870-543-7404
E-mail jlatendresse@nctr.fda.gov

Interim Pathology Report
Histopathologic Effects of Ammonium Perchlorate in Thyroid Gland of Mice

Methods

Eight to nine week old male B6C3F1 mice were administered ammonium perchlorate in drinking water for 90 days at 0, 0.1, 1.0, 3.0, and 30 mg/kg/day in two different studies (Studies A and D). For inclusion in this report, only the control and high dose groups from each study were examined. Three histologic sections (A, B, and C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for a detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used.

Results and Discussion

Morphologies by anatomical site and individual animal are given in the Histopathology Databases (Tables 1 and 2). Thyroid glands from control mice were essentially normal. The follicles were variably sized with complements of relatively large, medium and small colloid-filled lumens. The height of the follicular epithelium was mostly low to medium cuboidal, and the nuclear to cytoplasmic ratio was usually one or less. The cytoplasm of the follicular cells often contained abundant small vacuoles.

The incidence of lesions induced by treatment with ammonium perchlorate is given in the tables 3 and 4. In the 30 mg/kg/day group, although a few peripheral follicles were large with abundant colloid in their lumens, the majority of the follicles tended to be smaller on the average with less colloid compared to controls. Both the inter- and intrafollicular capillaries were mildly congested diffusely, distinguishing them from those of the control thyroid glands. The mildly hypertrophied follicular epithelium was characteristically high cuboidal to low columnar. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2. The follicular cells often contained clear perinuclear halos, but the distinct pattern of vacuolization observed in the control group was absent.

TERRA, Inc.
Toxicology, Ecology, Research, and Risk Assessment

January 13, 1999

Annie Jarabek
NCEA National Center for Environmental Assessment
3210 Highway 54 Catawba Bldg.
RTP Durham, NC 27709-

RE:
Our Case File: MUSC-6872

Dear Ms. Jarabek:

Dave Mattie asked that I send you a copy of the interim pathology report prepared in relation to the perchlorate research effort ongoing at the Medical University of South Carolina. Although my involvement in the research project has been minimal since submitting the grant proposal, as a consultant I have had to stay informed on the issue. I congratulate you and your colleagues for your success in tackling a complex subject in such a systematic and expeditious fashion. I will forward the pathology analysis of the remaining dose groups to you in the near future. Please feel free to call me with your questions or concerns.

Best Regards,

Alan Warren
TERRA, INC.

Mahan Station • 1709 Mahan Drive, Suite E • Tallahassee, Florida 32308 • Phone (850) 309-1330 • Fax (850) 309-1336

severity range was not provided. The majority of follicles tended to be smaller (a few exceptions on the periphery) with less colloid. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2.0.

These lesions in mice are consistent with those seen in the other species tested and with the proposed mode-of-action for the assessment model. Quantitative interspecies comparison is precluded at this time due to the lack of completed histopathology at the other doses. The Caldwell et al. (1995) study in rats is the only one that tested as high as approximately 22 mg/kg-day, but the difference in severity ratings and lack of statistics for both reports prevents further analysis. In the rabbit developmental study, histopathology was observed at the 30 mg/kg-day dose and this was not the lowest observed effect level. The best data for comparison may be the pending histopathology in the adults of the 2-generation reproductive study in rats, since there was a 30 mg/kg-day testing dose.

In conclusion, this preliminary analysis suggests that the mode-of-action is similar in mice, rabbits and rats. Quantitative interspecies comparison awaits dose-response data in the mice (i.e., histopathology for the remaining dose groups) and possibly a systematic pathology working group (PWG) evaluation of all the histopathology data once they are available.

Attachment

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Review of Interim Pathology Report in Mice from 90-day Immunotoxicity Studies

FROM: Annie M. Jarabek
National Center for Environmental Assessment
RTP (MD-52)

TO: EPA Perchlorate Health Assessment Team

I have reviewed the interim histopathology report received on January 13, 1999 for the mice from the immunotoxicity studies ongoing at the Medical Center of South Carolina (Warren, 1999). These are reported for two 90-day experiments ("A" and "D") and only for the control and high-dose groups. Thus, this analysis is preliminary and limited but nevertheless worthwhile to include at this time since it may add some perspective on interspecies sensitivity. The report is attached.

Three histologic sections (A,B,C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of the thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used. The study pathologist did not read the slides blind, but rather as he notes in the E-mail attached to the report, read the control and high dose specimens to detect a putative morphologic alteration and to characterize the full range of the alterations. Although I understand these points, no mention of a second pathologist to provide QA (per typical NTP SOP) on the study was mentioned. I expect the issue that we have already raised regarding the lack of QA or blind assessment will be resolved in the disposition of the decision regarding a potential pathology working group (PWG) of all the thyroid histopathology, so that I will not belabor the point herein.

In both 90-day experiments ("A" and "D"), the incidence of lesions induced by treatment were 0 in the control and 100% in the 30 mg/kg-day group. Lesions consistent with our proposed mode-of-action were observed, including: colloid depletion, congestion, hypertrophy. Mean values for these lesions are given but the

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February 1, 1999 EPA Assessment Submission

Attachment #6
Sheep Red Blood Cell (SRBC) Assay in 90-day Studies

A. Keil 1/23/99 Data Submission
B. EPA analysis (Smialowicz, 1999)

ATTENTION PANEL MEMBER(S):

KIMBER WHITE

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 4): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 4			30.0 MG/KG/DAY		
7291	6	1	7306	6	4
7292	4a	3	7307	6	8b
7293	3	1	7308	5	4
7294	6	4	7309	6	4
7295	4	1	7310	5	2
7296	6	2	7311	5	2
7297	6	4	7312	3	3
7298	6	2	7313	4	1
7299	4a	2	7314	6	1
7300	4	2	7315	5	1
7301	5	6	7316	5	3
7302	5	3	7317	5	2
7303	4	3	7318	5	2
7304	3a	3	7319	4	2
7305	5	2	7320	7	1

a Six or more consecutive days of diestrus were observed
c. Dam 7307 was cohabited with a second male rat; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE #44 (PAGE 3): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 3			3.0 MG/KG/DAY		
7261	5	1	7276	5	2
7262	6	4	7277	5	1
7263	5	7	7278	5	2
7264	5	4	7279	5	1
7265	4	2	7280	5	6
7266	6	1	7281	4	6
7267	4	2	7282	5	3
7268	5	1	7283	5	3
7269	3	2	7284	5	2
7270	5	3	7285	5	2
7271	5	3	7286	6	4
7272	6	4	7287	5	2
7273	3	5	7288	5	3
7274	5	3	7289	5	3
7275	5	1	7290	5	3

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 2): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 2			0.3 MG/KG/DAY		
7231	3a	6	7246	5	1
7232	5	6	7247	5	2
7233	6	4	7248	5	2
7234	5	2	7249	5	1
7235	5	2	7250	3a	3
7236	5	3	7251	4	3
7237	3	1	7252	5	4
7238	5	1	7253b		
7239	5	3	7254	5	1
7240	5	1	7255	3a	7
7241	5	2	7256	5	3
7242	6	4	7257	5	2
7243	5	14c	7258	5	4
7244	5	3	7259	5	4
7245	5	3	7260	5	2

a. Six or more consecutive days of diestrus were observed.
b. Rat 7253 was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).
c. Mating not confirmed.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 1): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 1			0 (CARRIER) MG/KG/DAY		
7201	5	3	7216	5	1
7202	4a	1	7217	5	1
7203	6	4	7218	5	2
7204	5	1	7219	5	1
7205	3a	3	7220	4	2
7206	6	4	7221	4	1
7207	5	5	7222	5	2
7208	5	9	7223	5	1
7209	4	2	7224	5	3
7210	4a	3	7225	6	1
7211	3	1	7226	6	1
7212	5	1	7227	5	3
7213	5	3	7228	6	4
7214	6	1	7229	5	3
7215	6	5	7230	6	14b

a. Six or more consecutive days of diestrus were observed.
b. Mating not confirmed.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E22 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS IN COHABITATION	N	30	29a	30	30
DAYS IN COHABITATION b	MEAN±S.D	2.9 ± 2.8	3.2 ± 2.6	2.9 ± 1.6	2.4 ± 1.2 [29]c
RATS THAT MATED	N(%)	29(96.7)	28(96.6)	30(100.0)	30(100.0)
FERTILITY INDEX d	N/N (%)	21/29 (72.4)	27/28** (96.4)	28/30** (93.3)	27/30** (90.0)
RATS WITH CONFIRMED MATING DATES	N	29	28	30	29c
RATS MATING c,e					
DAYS 1- 7	N(%)	28(96.6)	28(100.0)	30(100.0)	29(96.7)
DAYS 8-14	N(%)	1(3.4)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	21/30 (70.0)	27/29** (93.1)	28/30** (93.3)	27/30** (90.0)

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Excludes values for dam 7307, which was cohabited with a second male rat.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E22 (PAGE 1): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
PRECOHABITATION ESTROUS CYCLING					
RATS EVALUATED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	29a	30	30
ESTROUS STAGES/ 14 DAYS	MEAN±S.D	5.0 ± 0.8	4.8 ± 0.8	4.9 ± 0.7	4.9 ± 1.0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	3	3	0	3
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0

a. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 4): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 4						30.0 MG/KG/DAY							
7091	190	0	0	0	0	8	2	0	0	0	0	0	5.0
7092	174	0	0	0	0	22	3	0	0	0	0	1	13.0
7093	182	0	0	0	0	17	1	0	0	0	0	0	9.0
7094	194	0	0	0	0	6	0	0	0	0	0	0	3.0
7095	189	0	0	0	0	7	4	0	0	0	0	0	5.5
7096	190	0	0	1	0	4	4	0	0	0	0	1	5.0
7097	184	0	0	0	0	11	3	0	0	0	0	2	8.0
7098	193	0	0	0	1	6	0	0	0	0	0	0	3.5
7099	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7100	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7101	188	2	0	0	0	0	6	4	0	0	0	0	6.0
7102	196	0	0	0	0	1	3	0	0	0	0	0	2.0
7103	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7104	181	0	0	0	0	13	6	0	0	0	0	0	9.5
7105	183	0	0	0	0	11	5	0	0	0	0	1	8.5
7106	194	0	0	0	0	2	3	0	0	0	0	1	3.0
7107	190	0	0	0	0	7	2	0	0	0	0	1	5.0
7108	188	0	0	0	0	5	6	0	0	0	0	1	6.0
7109	181	0	0	0	0	10	5	0	0	0	0	1	8.1
7110	188	1	0	0	0	8	3	0	0	0	0	0	6.0
7111	FOUND DEAD ON DAY 131 POSTWEANING												
7112	192	0	0	0	0	6	2	0	0	0	0	0	4.0
7113	FOUND DEAD ON DAY 95 POSTWEANING												
7114	193	0	0	0	0	5	1	0	0	0	0	1	3.5
7115	FOUND DEAD ON DAY 82 POSTWEANING												
7116	188	0	0	0	0	8	3	0	0	0	0	1	6.0
7117	195	0	0	0	0	5	0	0	0	0	0	0	2.5
7118	191	0	0	0	0	8	0	1	0	0	0	0	4.5
7119	181	0	0	0	0	17	2	0	0	0	0	0	9.5
7120	181	0	1	0	0	12	4	0	0	1	0	1	9.5

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 3): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 3						3.0 MG/KG/DAY							
7061	194	0	0	0	0	4	2	0	0	0	0	0	3.0
7062	190	1	0	0	0	2	3	0	1	0	0	3	5.0
7063	185	0	0	0	0	10	5	0	0	0	0	0	7.5
7064	188	0	0	0	0	8	3	0	0	0	0	1	6.0
7065	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7066	193	0	0	0	0	4	2	1	0	0	0	0	3.5
7067	195	0	0	0	0	3	2	0	0	0	0	0	2.5
7068	191	0	0	0	0	5	4	0	0	0	0	0	4.5
7069	193	0	0	0	0	6	1	0	0	0	0	0	3.5
7070a	11	0	0	0	0	2	8	0	0	0	0	0	47.6
7071	191	0	0	0	0	6	2	0	0	1	0	0	4.5
7072	186	1	0	0	0	11	2	0	0	0	0	0	7.0
7073	195	0	0	0	0	4	1	0	0	0	0	0	2.5
7074	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7075	197	0	0	0	0	2	1	0	0	0	0	0	1.5
7076	191	0	0	0	0	7	7	0	0	0	0	0	4.5
7077	173	0	0	0	0	20	7	0	0	0	0	0	13.5
7078	193	0	0	0	0	7	0	0	0	0	0	0	3.5
7079	188	0	0	0	0	10	2	0	0	0	0	0	6.0
7080	188	0	0	0	0	9	2	1	0	0	0	0	6.0
7081	185	0	0	0	0	11	3	0	0	0	0	1	7.5
7082	196	0	0	0	0	2	2	0	0	0	0	0	2.0
7083	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7084	195	0	0	0	0	3	2	0	0	0	0	0	2.5
7085	190	0	0	0	0	8	1	1	0	0	0	0	5.0
7086	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7087	190	0	0	0	0	5	3	0	0	0	0	2	5.0
7088	185	0	0	0	0	14	1	0	0	0	0	0	7.5
7089	184	0	0	0	0	10	4	1	0	0	0	1	8.0
7090	195	0	0	0	0	3	2	0	0	0	0	0	2.5

a. Rat 7070 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 2): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCES-SIVE HOOK	AMOR-PHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	BROKEN FLAGEL-LUM	PERCENT ABNORMAL
DOSAGE GROUP 2						0.3 MG/KG/DAY							
7031	190	0	0	0	0	8	1	0	0	0	0	1	5.0
7032	183	0	0	0	0	14	2	0	0	0	0	1	8.5
7033	194	0	0	0	0	5	1	0	0	0	0	0	3.0
7034	191	1	0	0	0	3	5	0	0	0	0	0	4.5
7035	193	0	0	1	0	4	1	0	0	0	0	1	3.5
7036	190	0	0	0	0	6	3	0	1	0	0	0	5.0
7037	181	0	0	0	0	9	7	1	0	0	0	2	9.5
7038	192	0	0	0	0	5	3	0	0	0	0	0	4.0
7039	187	0	0	0	0	8	3	0	0	0	0	2	6.5
7040	189	0	0	0	0	9	1	0	1	0	0	0	5.5
7041	185	0	0	0	0	9	3	0	0	0	0	3	7.5
7042	196	0	0	0	0	3	1	0	0	0	0	0	2.0
7043	189	0	0	0	0	4	7	0	0	0	0	0	5.5
7044	190	0	0	0	0	8	1	0	0	0	0	1	5.0
7045	195	0	0	0	0	4	0	1	0	0	0	0	2.5
7046	186	2	0	0	0	7	4	0	0	0	2	1	7.0
7047	186	2	0	1	0	7	2	0	0	0	0	0	6.0
7048	182	0	0	0	0	13	5	0	0	0	0	0	9.0
7049	188	0	0	0	0	10	2	0	0	0	0	0	6.0
7050	189	1	0	0	0	8	2	0	0	0	0	0	5.5
7051	194	0	0	0	0	4	2	0	0	0	0	0	3.0
7052	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7053	182	0	0	0	0	18	0	0	0	0	0	0	9.0
7054	193	0	0	0	0	5	1	0	0	0	0	1	3.5
7055	189	0	0	0	0	9	1	0	0	0	0	1	5.5
7056	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7057	174	0	0	0	0	18	7	0	0	0	0	1	13.0
7058	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7059	185	0	0	0	0	13	2	0	0	0	0	0	7.5
7060	195	0	0	0	0	3	1	0	0	0	0	1	2.5

PROTOCOL 1436-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCES-SIVE HOOK	AMOR-PHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	BROKEN FLAGEL-LUM	PERCENT ABNORMAL
DOSAGE GROUP 1						0 (CARRIER) MG/KG/DAY							
7001	196	0	0	0	0	3	2	0	0	0	0	1	3.0
7002	168	2	0	0	0	25	3	0	0	0	0	2	16.0
7003	187	0	0	0	0	8	3	0	0	0	0	2	6.5
7004a	9	0	0	0	0	1	8	0	0	0	0	0	50.0
7005	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7006	195	0	0	0	0	4	1	0	0	0	0	0	2.5
7007	192	0	0	0	0	4	3	0	0	0	0	1	4.0
7008	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7009	181	1	0	0	0	10	7	0	0	0	0	1	9.5
7010	193	0	0	0	0	7	0	0	0	0	0	0	3.5
7011	177	0	0	0	0	20	3	0	0	0	0	0	11.5
7012	188	0	0	0	0	9	3	0	0	0	0	0	6.0
7013	195	0	0	0	0	3	2	0	0	0	0	0	2.5
5450	188	1	0	0	0	9	1	0	1	0	0	0	6.0
7015	192	1	0	0	0	4	3	0	0	0	0	0	4.0
7016	195	0	0	0	0	1	2	1	0	0	0	1	2.5
7017	187	1	0	0	0	8	3	0	0	0	0	1	6.5
7018	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7019	186	1	0	0	0	7	3	0	0	0	0	3	7.0
7020	192	0	0	0	0	4	3	0	0	0	0	1	4.0
7021	190	0	0	0	0	9	1	0	0	0	0	0	5.0
7022	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7023	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7024	184	0	0	0	0	14	2	0	0	0	0	0	8.0
7025	189	0	0	0	0	9	2	0	0	0	0	0	5.5
7026	191	0	0	0	0	9	0	0	0	0	0	0	4.5
7027	184	0	0	0	0	12	4	0	0	0	0	0	8.0
7028	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7029	190	0	0	0	0	9	1	0	0	0	0	0	5.0
7030	197	0	0	0	0	9	3	0	0	0	0	1	6.2

a. Rat 7004 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 4): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 4 30.0 MG/KG/DAY

RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7091	355	70	153	508	124	7.2	1087.0	25	1.4	91.4
7092	245	76	76	321	295	17.1	2056.3	33	1.9	95.2
7093	540	72	210	750	190	11.0	1557.0	51	3.7	194.7
7094	296	84	58	354	271	15.7	1810.9	28	1.6	84.1
7095	425	78	120	545	190	11.0	1548.2	27	1.6	89.2
7096	249	79	65	314	224	13.0	1670.0	40	2.3	134.0
7097	471	82	102	573	213	12.3	1760.4	22	1.3	84.2
7098	647	81	154	801	120	6.9	1084.8	18	1.0	65.9
7099	467	89	56	523	135	7.8	1259.7	25	1.4	82.5
7100	333	86	56	389	245	14.2	2109.3	16	0.9	59.0
7101	397	85	69	466	215	12.4	1671.9	18	1.0	62.5
7102	249	80	62	311	273	15.8	2134.4	18	1.0	54.8
7103	431	78	125	556	228	13.2	1653.0	14	0.8	39.0
7104	638	88	83	721	132	7.6	1051.9	15	0.9	53.6
7105	562	83	114	676	103	6.0	721.4	18	1.0	41.9
7106	495	89	69	564	80	4.6	628.9	31	1.8	100.9
7107	212	75	70	282	136	7.9	936.7	4	0.2	11.9
7108	437	78	126	563	98	5.7	869.6	38	2.2	128.5
7109	444	68	207	651	140	8.1	1121.8	38	2.2	124.8
7110	835	85	144	980	283	16.4	1944.5	43	2.5	120.0
7111	FOUND DEAD ON DAY 131 POSTWEANING									
7112	605	81	146	751	159	9.2	1054.9	37	2.1	129.7
7113	FOUND DEAD ON DAY 95 POSTWEANING									
7114	537	75	182	719	152	8.8	1289.4	48	2.8	181.0
7115	FOUND DEAD ON DAY 82 POSTWEANING									
7116	467	84	89	556	139	8.0	1104.6	26	1.5	94.2
7117	293	75	100	393	195	11.3	1598.0	42	2.4	131.8
7118	552	87	83	635	225	13.0	1575.9	40	2.3	107.3
7119	508	87	79	587	114	6.6	774.1	44	2.5	127.7
7120	433	82	93	526	154	8.9	976.9	39	2.3	115.8

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6}), multiplying by 2 (dilution factor) and multiplying by 10^6 to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

PROTOCOL 1415-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D25 (PAGE 3): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 3 — 3.0 MG/KG/DAY

RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7061	336	74	117	453	190	11.0	1531.0	32	1.9	94.9
7062	257	74	92	349	159	9.2	1390.0	26	1.5	83.3
7063	212	64	117	329	142	8.2	1229.8	18	1.0	65.8
7064	169	68	80	249	196	11.3	1723.3	43	2.5	141.8
7065	246	77	75	321	202	11.7	1240.6	38	2.2	105.9
7066	311	74	108	419	177	10.2	1296.2	24	1.4	90.3
7067	278	64	153	427	306	17.7	2405.2	24	1.4	77.6
7068	215	77	66	281	262	15.2	2468.7	27	1.6	100.8
7069	622	80	160	782	195	11.3	1532.8	34	2.0	118.2
7070d	0	0	0	0	6	0.3	92.8	0	0.0	0.0
7071	331	76	103	434	168	9.7	1717.2	24	1.4	83.3
7072	162	72	64	226	286	16.5	2224.0	38	2.2	114.5
7073	201	64	113	314	89	5.1	770.8	33	1.9	111.8
7074	558	82	125	683	168	9.7	1327.8	31	1.8	93.4
7075	834	91	83	917	138	8.0	1078.9	14	0.8	42.8
7076	274	87	42	316	164	9.5	1379.1	37	2.1	115.1
7077	338	80	86	424	144	8.3	980.1	45	2.6	148.1
7078	699	92	58	757	127	7.3	1043.7	48	2.8	139.1
7079	312	81	71	383	146	8.4	1056.3	32	1.9	120.6
7080	398	71	163	561	169	9.8	1800.8	47	2.7	149.2
7081	446	77	130	576	157	9.1	1170.5	28	1.6	96.9
7082	461	85	84	545	147	10.6	1436.6	27	1.6	95.6
7083	285	71	115	400	196	11.3	1633.9	42	2.4	160.3
7084	335	79	90	425	151	8.7	1456.0	29	1.7	103.0
7085	638	73	231	869	130	7.5	924.0	34	2.0	108.5
7086	408	81	95	503	190	11.0	1380.9	39	2.3	128.3
7087	346	78	100	446	239	13.8	1942.0	57	3.3	176.4
7088	425	68	200	625	155	9.0	1088.3	31	1.8	96.3
7089	704	79	185	889	315	18.2	2139.0	28	1.6	97.5
7090	742	77	217	959	150	8.7	957.9	40	2.3	109.3

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-4}), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Rat 7070 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 2): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 2 0.3 MG/KG/DAY

RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7031	162	72	62	224	176	10.2	1414.2	15	0.9	45.6
7032	455	81	105	560	171	9.9	1242.8	38	2.2	110.4
7033	192	71	79	271	199	11.5	1560.0	23	1.3	80.1
7034	475	71	194	669	131	7.6	1013.2	27	1.6	86.1
7035	326	78	92	418	226	13.1	1594.5	34	2.0	101.7
7036	314	80	79	393	224	13.0	1810.0	52	3.0	159.2
7037	311	78	87	398	286	16.5	2154.5	40	2.3	120.8
7038	537	77	161	698	256	14.8	2204.0	29	1.7	99.3
7039	277	68	129	406	329	19.0	2524.4	33	1.9	121.4
7040	438	80	108	546	216	12.5	1899.2	39	2.3	120.4
7041	212	70	89	301	199	11.5	1683.2	15	0.9	52.8
7042	436	90	48	484	292	16.9	2513.9	8	0.5	32.3
7043	423	78	117	540	265	15.3	2100.2	41	2.4	121.5
7044	523	92	48	571	205	11.9	1382.3	39	2.3	137.2
7045	620	82	133	753	179	10.4	1341.4	17	1.0	58.5
7046	236	63	136	372	293	17.0	2278.4	21	1.2	67.4
7047	339	85	58	397	325	18.8	2254.5	15	0.9	45.9
7048	222	79	59	281	84	4.9	843.7	39	2.3	144.7
7049	568	83	114	682	113	6.5	1089.6	44	2.5	180.0
7050	175	60	115	290	254	18.4	2005.3	61	3.5	189.2
7051	592	77	174	766	126	7.3	1088.0	38	2.2	130.1
7052	785	84	147	932	176	10.2	1308.8	39	2.3	128.8
7053	316	74	110	426	292	16.9	1968.9	54	3.1	182.5
7054	167	61	107	274	257	14.9	1891.7	53	3.1	152.7
7055	400	68	192	592	91	5.3	849.2	44	2.5	147.8
7056	517	80	131	648	121	7.0	1035.6	43	2.5	167.9
7057	419	85	72	491	100	5.8	747.5	49	2.8	162.5
7058	687	88	98	785	201	11.6	1498.5	25	1.4	73.6
7059	542	81	129	671	127	7.3	959.2	32	1.9	98.9
7060	345	70	146	491	112	6.5	892.5	61	3.5	192.0

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6}), multiplying by 2 (dilution factor) and multiplying by 10^{-4} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 1): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 1 — 0 (CARRIER) MG/KG/DAY

RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7001	192	74	69	261	163	9.4	1399.1	43	2.5	153.5
7002	438	76	142	580	96	5.6	826.5	48	2.8	182.1
7003	588	90	64	652	182	10.5	1339.6	26	1.5	90.3
7004d	0	0	6	6	2	0.1	41.3	16	0.9	279.7
7005	360	76	113	473	237	13.7	1722.5	34	2.0	120.9
7006	178	56	142	320	146	8.4	1422.0	26	1.5	100.5
7007	281	75	92	373	247	14.3	2205.2	33	1.9	107.4
7008	447	85	77	524	254	14.7	1800.9	27	1.6	97.2
7009	211	74	73	284	243	14.1	2466.4	33	1.9	112.8
7010	669	83	133	802	183	10.6	1470.5	29	1.7	97.4
7011	235	81	54	289	165	9.5	1391.5	29	1.7	107.8
7012	306	70	145	451	230	13.5	2330.1	35	2.0	110.2
7013	657	85	116	773	341	19.7	2644.5	4	0.2	12.6
5650	711	80	180	891	169	9.8	1433.6	33	1.9	117.1
7015	559	85	100	659	157	9.1	1316.4	21	1.2	77.0
7016	274	69	122	396	310	17.9	2591.7	26	1.5	95.2
7017	654	83	131	785	195	11.3	1500.2	37	2.1	135.5
7018	351	84	66	417	178	10.3	1418.5	33	1.9	122.7
7019	332	76	107	439	225	13.0	2140.1	27	1.6	115.0
7020	326	69	149	475	144	8.3	1033.5	59	3.4	177.4
7021	626	86	98	724	155	9.0	1042.7	20	1.2	69.7
7022	456	74	161	617	107	6.2	834.3	44	2.5	159.3
7023	464	87	71	535	190	11.0	1473.5	46	2.7	142.0
7024	625	79	166	791	158	9.1	1151.3	78	4.5	207.4
7025	347	73	128	475	125	7.2	1092.4	35	2.0	130.6
7026	289	78	82	371	169	9.8	1384.9	26	1.5	89.9
7027	490	85	85	575	248	14.3	1897.9	44	2.5	142.5
7028	452	73	169	621	187	10.8	1494.3	66	3.8	209.9
7029	309	62	193	502	101	5.8	936.4	33	1.9	120.2
7030	329	70	138	467	111	6.4	991.0	67	3.9	212.9

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.9×10^{-4}), multiplying by 2 (dilution factor) and multiplying by 10^{-4} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Rat 7004 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D16 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1		2		3		4	
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)		0.3		3.0		30.0	
RATS EXAMINED	N	30		30		30		27a	
INCLUDED IN ANALYSES	N	29b		30		29b		27	
NORMAL	MEAN±S.D.	189.5±	6.3	188.8±	4.9	190.1±	4.9	188.1±	5.4
PERCENT ABNORMAL	MEAN±S.D.	5.4±	3.1	5.6±	2.5	4.9±	2.4	5.9±	2.7
NO HOOK	MEAN±S.D.	0.2±	0.5	0.2±	0.6	0.1±	0.2	0.1±	0.4
EXCESSIVE HOOK	MEAN±S.D.	0.0±	0.0	0.0±	0.0	0.0±	0.0	0.0±	0.2
AMORPHOUS	MEAN±S.D.	0.0±	0.0	0.1±	0.2	0.0±	0.0	0.0±	0.2
PIN HEAD	MEAN±S.D.	0.0±	0.0	0.0±	0.0	0.0±	0.0	0.0±	0.2
DETACHED HEAD	MEAN±S.D.	7.9±	5.1	7.7±	4.1	6.9±	4.0	8.1±	5.0
NO HEAD	MEAN±S.D.	2.2±	1.5	2.6±	2.0	2.4±	1.4	2.8±	1.9
BANANA	MEAN±S.D.	0.0±	0.2	0.1±	0.2	0.1±	0.4	0.2±	0.8
COILED FLAGELLUM	MEAN±S.D.	0.0±	0.2	0.1±	0.2	0.0±	0.2	0.0±	0.0
BENT FLAGELLUM	MEAN±S.D.	0.0±	0.0	0.0±	0.0	0.0±	0.2	0.0±	0.2
BENT FLAGELLUM TIP	MEAN±S.D.	0.0±	0.0	0.0±	0.0	0.0±	0.0	0.0±	0.0
BROKEN FLAGELLUM	MEAN±S.D.	0.5±	0.8	0.5±	0.8	0.3±	0.7	0.4±	0.6

a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal epididymides and testes.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D15 (PAGE 1): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS EXAMINED	N	30	30	30	27a
INCLUDED IN ANALYSES	N	29b	30	29b	27
NUMBER MOTILE	MEAN±S.D.	420.6 ± 158.4	400.4 ± 163.2	397.9 ± 186.0	449.0 ± 145.0
MOTILE PERCENT	MEAN±S.D.	77.2 ± 7.8	76.9 ± 8.1	76.4 ± 7.2	80.6 ± 5.7
STATIC COUNT (NONMOTILE)	MEAN±S.D.	116.1 ± 38.6	110.6 ± 39.7	114.6 ± 48.3	107.1 ± 45.4
TOTAL COUNT	MEAN±S.D.	536.6 ± 171.6	511.0 ± 181.3	512.5 ± 212.9	556.1 ± 170.6
SPERM COUNT	MEAN±S.D.	186.9 ± 59.7	200.9 ± 73.1	181.3 ± 53.9	179.0 ± 61.8
SPERM CONCENTRATION	MEAN±S.D.	10.9 ± 3.4	11.8 ± [illegible]	10.6 ± 3.1	10.4 ± 3.6
SPERM DENSITY	MEAN±S.D.	1543.6 ± 520.8	1571.6 ± 536.1	1461.2 ± 438.8	1372.6 ± 464.6
SPERMATID COUNT	MEAN±S.D.	36.8 ± 15.6	35.6 ± 14.0	33.4 ± 9.4	29.6 ± 12.1
SPERMATID CONCENTRATION	MEAN±S.D.	2.1 ± 0.9	2.1 ± 0.8	1.9 ± 0.5	1.7 ± 0.8
SPERMATID DENSITY	MEAN±S.D.	125.0 ± 44.4	117.2 ± 46.2	109.3 ± 28.6	97.6 ± 41.2

a. Excludes values for rats that were found dead.

b. Excludes values for rats that had abnormal epididymides and testes.

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

January 15, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached is a copy of the audited individual and summary tables with the F1 generation sperm and estrous cycling data you requested. Please remember, these data could still change based on the final audit of the other study data.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

January 28, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Assimilation of F1 mating, estrous cyclicity and sperm measure results with P results

FROM: Eric D. Clegg, Ph.D. E. D. Clegg
National Center for Environmental Assessment (8623D)
Washington, DC

TO: Annie Jarabek
National Center for Environmental Assessment (MD-52)
Research Triangle Park, NC

I have reviewed the result tables on the F1 mating, estrous cyclicity and sperm measure results provided by Ray York of Argus Laboratories on January 15, 1999. The only statistically different result in the new data are in the fertility results where the control mating and pregnancy rates were significantly lower than the dosed groups. The values for the dosed groups were uniformly high. There was nothing remarkable in the results for the other parameters. The results with the P generation in mating and estrous cycle monitoring hinted at effects at 0.3 mg/kg, but those were not replicated with the F1 generation. Thyroid and ovarian weight data are not available yet for the F1. Thus, to this point, the F1 data are not supporting the existence of U-shaped dose-responses.

February 1, 1999 EPA Assessment Submission

Attachment #5
Analysis of Reproductive Parameters from the F1 Mating in Argus (1998b) 2-Generation Reproductive Study

A. Argus 1/15/99 Data Submission (York, 1999a)
B. EPA analysis (Clegg, 1999)

ATTENTION PANEL MEMBER(S):

ROCHELLE TYL

Figure 1
TSH by dose and gender

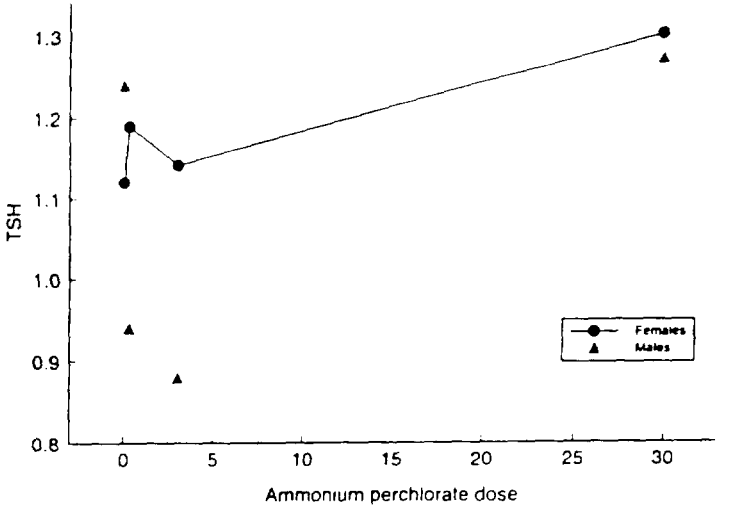


Figure 2
T3 by dose and gender

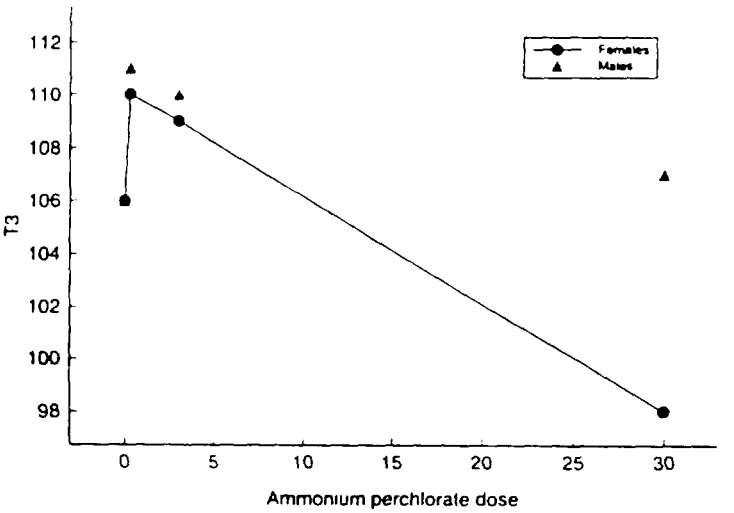


Figure 3
T4 by dose and gender

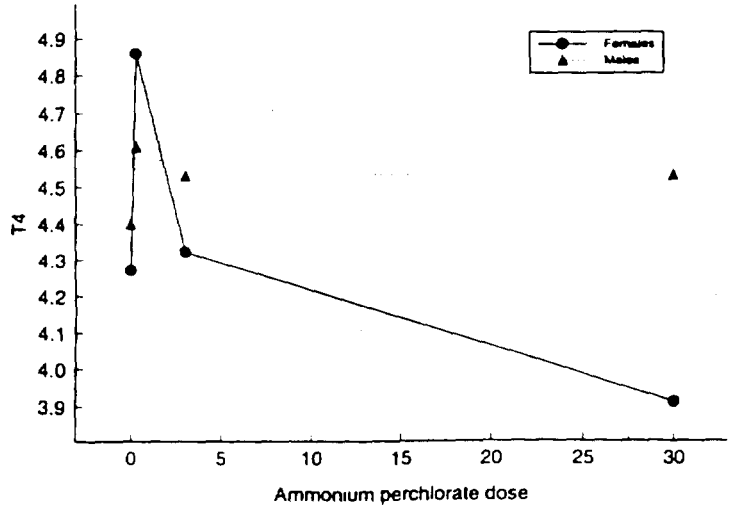


Table 1

Means and S.E.s of each variable by gender and ammonium perchlorate dose

Gender	Ammonium perchlorate dose	TSH n	TSH Mean	TSH S.E.	T3 n	T3 Mean	T3 S.E.	T4 n	T4 Mean	T4 S.E.
Female	0	28	1.12	0.10	28	106	2.5	28	4.27	0.19
	.3	22	1.19	0.08	22	110	2.8	22	4.86	0.20
	3	25	1.14	0.07	25	109	2.7	25	4.32	0.16
	30	23	1.30	0.07	23	98	2.3	23	3.91	0.20
Male	0	27	1.24	0.09	27	106	1.9	27	4.40	0.20
	.3	21	0.94	0.07	21	111	3.6	21	4.61	0.21
	3	25	0.88	0.05	25	110	3.1	25	4.53	0.16
	30	23	1.27	0.08	23	107	3.3	23	4.53	0.23

Table 2

Multivariate analysis of variance of ammonium perchlorate data. Results for Wilks' Lambda statistic.

Source	D.F.	F	P
Dose	9, 226	2.29	.018
Gender	3, 89	5.69	.001
Dose x Gender	9, 217	3.67	<.001

Table 3

Analysis of variance of each variable in ammonium perchlorate experiment

Source	D.F.	Mean Square	F	P	Adjusted P
TSH					
Dose	3	.7510	3.13	.029	.087
Error 1	95	.2398			
Gender	1	.6243	10.85	.001	.004
Dose x Gender	3	.3745	6.51	<.001	.002
Error 2	91	.0576			
T3					
Dose	3	605.0	2.17	.097	.291
Error 1	95	279.0			
Gender	1	549.2	6.00	.016	.049
Dose x Gender	3	215.7	2.35	.077	.232
Error 2	91	91.6			
T4					
Dose	3	2.517	1.92	.132	.396
Error 1	95	1.314			
Gender	1	1.280	2.61	.109	.328
Dose x Gender	3	1.588	3.24	.026	.077
Error 2	91	.490			

The attached Table 1 gives the sample size, mean, and S.E. for each gender, dose, and variable combination. The means for the three variables are plotted in Figures 1 through 3. The multivariate analysis of variance results for the two main effects and the interaction effect are given in Table 2. All three effects are statistically significant ($p<.05$). The next step in the analysis is to do a univariate analysis of variance on each variable in order to understand the meaning of the significant multivariate effects. These latter analyses are for a spit-plot design. Since we are doing three analyses on one experiment, the Bonferroni adjustment to the p-values is made and are given under "Adjusted P" in each table. These adjusted p-values will be used to make conclusions from the experiment.

In the analysis of TSH, the dose by gender interaction is significant ($p=.002$) so a separate analysis of the dose effect only was done on each gender. The dose effect was not significant ($p=.420$) for females, but was significant ($p<.001$) for males. The conclusion for females is that there are no significant differences in TSH by dose. For males, Tukey's multiple comparison procedure was done on the dose means to determine which were different from each other. The conclusion for males is that dose 3 was significantly lower than doses 0 and 30 and no other differences are significant ($p<.05$).

The gender effect is the only significant one for T3 ($p=.049$). The mean of 108.4 (S.E.=1.5) for males is larger than the mean of 105.7 (S.E.=1.4) for females. The conclusion is that there are no dose effects on T3, but there is a small but significant gender effect.

No effect or interaction is significant for T4. The conclusion is that neither dose nor gender had a significant effect on T4.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

DATE: February 1, 1999

SUBJECT: Statistical analysis of ammonium perchlocate experiment

FROM: Dennis E. House Dennis E. House
NHEERL/BRSS/MD-55

TO: Andrew Geller
NHEERL/NTD/MD-74B

"Attached is the statistical analysis of the hormone data from the Argus Rat Developmental Neurotoxicology Study (Argus, 1998b). A memo from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) contains thyroid hormone and thyrotrophin data from the Oral (Drinking Water) Two-Generation reproduction Study of ammonium perchlorate in the rat. Data were supplied on diskette in the form of ASCII text reports, one report for each gender/age group, and imported in ASCII form to SAS for further analysis.

The following is a statistical analysis of the thyroid and pituitary hormone data (T4, thyroxine; T3, triiodothyronine; TSH, thyroid stimulating hormone) found in that report. At the time of this analysis, data were available from both the F0 generation, females and males sacrificed at 5 and 6 months of age, respectively, and the F1 generation, one male and one female from each litter, sacrificed on postnatal day 21 (PND21). Males were sacrificed after 13 weeks of exposure, i.e., approximately 91 days. Females were sacrificed after 16 weeks, i.e. at weaning, approximately 120 days of exposure."

This report gives the results of some statistical analyses of the ammonium perchlocate experiment. The design of the experiment was to randomly assign rat parent pairs to one of four ammonium perchlocate dose groups. The doses were 0, .3, 3, and 30 (units unknown). Both parents were dosed 10 weeks before mating. Dosing of females continued through weaning or about age 21 days. One male and one female pup from each litter were sacrificed at age 21 days and TSH, T3, and T4 measurements were made.

The design of this experiment is a split-plot. The main plot treatment is the perchlocate dose which was applied to litters (since treatments were applied to the parents-mainly the mother) and the subplot "treatment" is gender. These designs are characterized by different mean square errors for evaluating different effects or classification variables in the experiment. Since three variables are measured on each pup, the proper analysis is a multivariate analysis of variance for a split-plot experiment. Essentially this is an analysis of the vector of three measurements from each pup.

February 1, 1999 EPA Assessment Submission

Attachment #7
Interim Thyroid Histopathology in Mice
(Control and High Dose) from
Keil et al. (1998) Immunotoxicity Studies

A. Warren 1/13/99 Data Submission
B. EPA analysis (Jarabek, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
SUSAN PORTERFIELD

One-way Analysis of Variance 90d IgM

Analysis of Variance

Source	DF	SS	MS	F	P
treatmen	4	0.02701	0.00675	3.13	0.022
Error	54	0.11640	0.00216		
Total	58	0.14341			

Level	N	Mean	StDev
0.0	12	0.20442	0.05200
0.1	11	0.16827	0.04506
1.0	12	0.15342	0.02899
3.0	12	0.16608	0.02613
30.0	12	0.20508	0.06714

```
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
                                   --------+---------+---------+--------
                                                    (--------*--------)
                                        (--------*--------)
                                   (--------*--------)
                                       (--------*--------)
                                                    (--------*--------)
                                   --------+---------+---------+--------
                                         0.150     0.180     0.210
```

Pooled StDev = 0.04643

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00668

Critical value = 3.99

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.01853 0.09082			
1.0	-0.00248 0.10448	-0.03982 0.06953		
3.0	-0.01514 0.09181	-0.05249 0.05687	-0.06614 0.04081	
30.0	-0.05414 0.05281	-0.09149 0.01787	-0.10514 0.00181	-0.09248 0.01448

Descriptive Statistics 90d IgM

Variable	N	N*	Mean	Median	TrMean	StDev
c	12	0	0.2044	0.2080	0.2014	0.0520
0.1	11	1	0.1683	0.1560	0.1649	0.0451
1	12	0	0.15342	0.15050	0.15030	0.02899
3	12	0	0.16608	0.16350	0.16580	0.02613
30	12	0	0.2051	0.2085	0.2083	0.0671

Variable	SE Mean	Minimum	Maximum	Q1	Q3
c	0.0150	0.1400	0.2990	0.1535	0.2415
0.1	0.0136	0.1020	0.2650	0.1450	0.1880
1	0.00837	0.12400	0.21400	0.12625	0.17100
3	0.00754	0.11700	0.21800	0.14825	0.18075
30	0.0194	0.0890	0.2890	0.1410	0.2682

Statistics

The calculated O.D. 50 for each of the treatment groups was compared to controls ($p<0.05$). A total of 59 serum samples from independently challenged mice were analyzed for both IgG and IgM.

One-way Analysis of Variance 90d IgG

Analysis of Variance

Source	DF	SS	MS	F	P
C4	4	0.1163	0.0291	0.92	0.458
Error	54	1.7020	0.0315		
Total	58	1.8183			

Level	N	Mean	StDev
0.0	12	0.3270	0.1413
0.1	11	0.3008	0.1827
1.0	12	0.2374	0.1499
3.0	12	0.2880	0.1522
30.0	12	0.3708	0.2424

```
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
                                   -------+---------+---------+---------
                                          (----------*---------)
                                       (----------*----------)
                                   (----------*---------)
                                       (---------*---------)
                                               (---------*---------)
                                   -------+---------+---------+---------
                                        0.20      0.30      0.40
```

Pooled StDev = 0.1775

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00668

Critical value = 3.99

Intervals for (column level mean) - (row level mean)

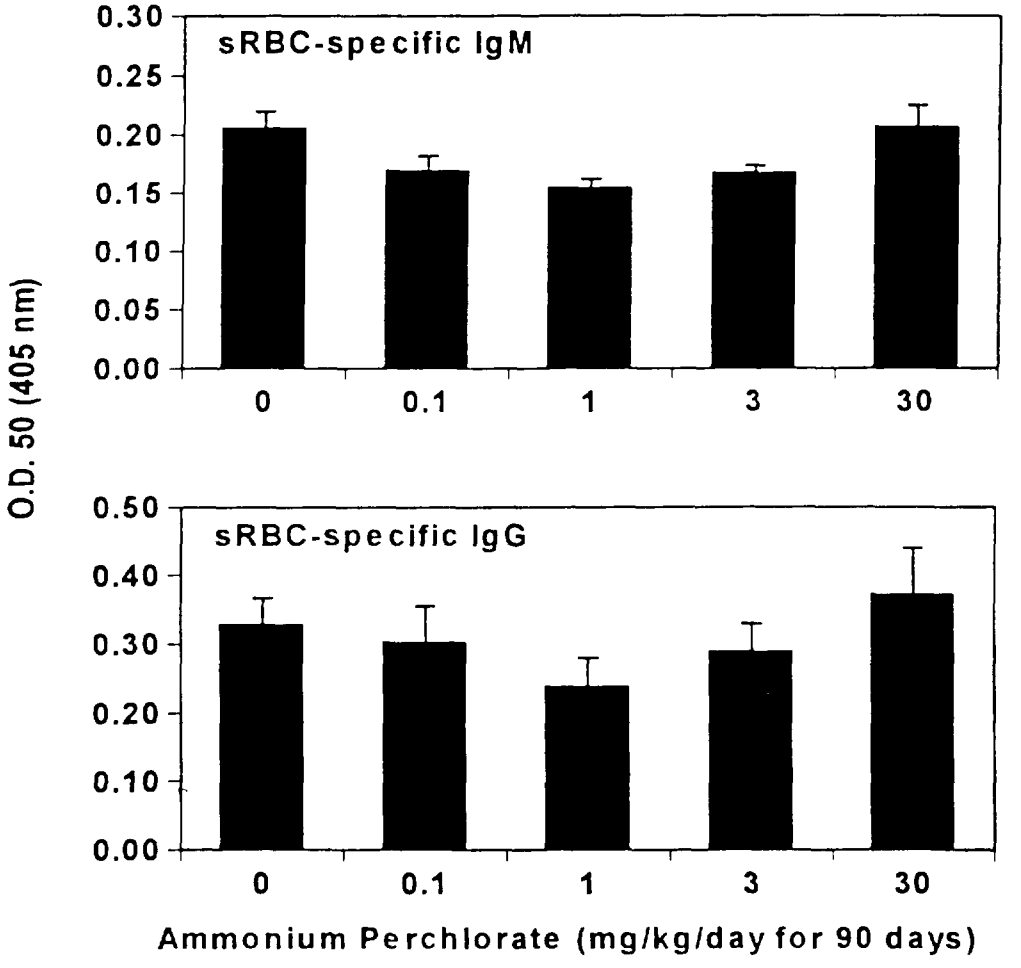
	0.0	0.1	1.0	3.0
0.1	-0.1829 0.2353			
1.0	-0.1149 0.2941	-0.1457 0.2725		
3.0	-0.1655 0.2435	-0.1963 0.2219	-0.2551 0.1539	
30.0	-0.2483 0.1607	-0.2791 0.1391	-0.3379 0.0711	-0.2873 0.1217

Descriptive Statistics 90d IgG

Variable	N	N*	Mean	Median	TrMean	StDev
C6	12	0	0.3270	0.2940	0.3222	0.1413
C7	11	1	0.3008	0.2350	0.2908	0.1827
C8	12	0	0.2374	0.2050	0.2210	0.1499
C9	12	0	0.2880	0.2555	0.2728	0.1522
C10	12	0	0.3708	0.2925	0.3431	0.2424

Variable	SE Mean	Minimum	Maximum	Q1	Q3
C6	0.0408	0.1500	0.5520	0.2002	0.4737
C7	0.0551	0.0470	0.6450	0.1450	0.4330
C8	0.0433	0.0430	0.5960	0.1685	0.3143
C9	0.0439	0.1260	0.6020	0.1563	0.4192
C10	0.0700	0.1480	0.8710	0.1670	0.5853

Serum IgM or IgG Levels after sRBC Challenge During a 90-Day Exposure to Ammonium Perchlorate



Adult B6C3F1 female mice were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. On day 75, animals were immunized by i.v. tail injection with sRBC (1×10^8 cells). Following the immunization, animals were bled on day 79 (4 days post challenge) and day 90 (15 days post challenge) to obtain serum for detection of specific IgM or IgG respectively. Detection of specific IgM or IgG was performed using an ELISA based on a protocol provided by L. Temple at the Medical College of Virginia. The O.D. 50 was determined for both IgM and IgG. Each of the above graphs represent the means and standard errors of a total of 59 mice. No significant differences were observed in any of the treatment groups as compared to controls using analysis of variance and Tukey's pairwise comparisons ($p<0.05$).

SRBC Specific Serum IgM or IgG Determination after Exposure to Ammonium Perchlorate for 90 Days

Submitted by Deborah Keil, PhD
Medical University of South Carolina
January 23, 1999

Animals and Ammonium Perchlorate Exposure: B6C3F1 female mice aged 8-10 weeks were exposed to ammonium perchlorate (AP) (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. A total of 60 mice with 12 animals per treatment group were used to determine specific IgM and IgG levels after immunization with sRBC. Animals were housed in an AAALAC accredited facility and provided water (with and without AP) and mouse chow ad libidum.

Immunization: Mice were immunized with sheep red blood cells (sRBC) ($1x10^8$ total cells) by intravenous tail injection on day 75. Serum was collected on day 79 (4 days post challenge) and day 90 (15 days post challenge) to determine specific IgM or IgG sRBC antibody levels, respectively. A semi-quantitative ELISA detected levels of specific IgM or IgG sRBC antibody in serially diluted serum (1:20, 1:40, 1:80, 1:160, 1:320). A SOP based on a protocol provided by L. Temple of the Medical College of Virginia was used.

Optimization of the ELISA: Optimization of the ELISA was performed prior to testing the serum samples to establish the appropriate titer of sRBC membrane coating antigen (1μg/ml) and the secondary antibody dilution (1: 5,000 for IgM and 1:7,500 for IgG). In addition, pooled serum samples from controls were used in the optimization. Controls for non-specific binding were included and were less than 0.070 O.D. (405 nm) in both the optimization and testing ELISAs.

Data Analysis:
nalysis of sRBC specific IgG serum titers were analyzed as described in a SOP provided by Louise Temple of the Medical College of Virginia. The average absorbance unit values of the replicates for each dilution of the test serum were calculated. Background in the ELISA was subtracted from these values. Five consecutive average absorbance values versus log base 2 of the dilution of the serum were plotted. The best-fit linear line was calculated in an Excel spreadsheet by determining the value for the slope and intercept. Log base 2 of the titer was considered the independent variable and O.D. was considered the dependent variable. In this experiment, the absorbance at the mid-point of the 5 serial dilutions was 1:80 (log base 2 (80) = 6.3219). Using the equation for the best-fit line, the O.D. 50 (absorbance at mid-point 1:80) was calculated for each animal.

Results:
No significant differences were observed in any of the AP treatment groups as compared to controls for specific IgM or IgG levels after immunization with sRBC. This was determined by using the calculated O.D.50 for each sample and performing an analysis of variance with Tukey's pairwise comparisons ($p<0.05$). Refer to graphs and statistical analysis that have been included in this report.

Limitations: A time course to determine the peak levels of IgM or IgG after sRBC immunization in B6C3F1 female mice was not performed in this study. However, bleeding times (day 4 for IgM and day 15 for IgG) have been previously used and reported in the literature (Holsapple, et al, 1984).
In addition, these data may be analyzed by additional methods to include expression of the "serum titer to achieve 0.5 O.D." At this time, the data manipulation involved to determine the "serum titer to achieve 0.5 O.D." has been laborious and time-consuming, particularly when specialized software (i.e., Softmax) not available to produce specialized graphs and corresponding equations for each of the 120 samples. Consequently, I have submitted the calculated O.D. 50 as described by a SOP provided by L. Temple.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Experimental Toxicology Division
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

DATE: January 28, 1999

FROM: Ralph J. Smialowicz (MD-92) RJ. Smialowicz

TO: Annie Jarabek (MD-52)
National Center for Environmental Assessment

SUBJECT: Review of 90-Day Ammonium Perchlorate Exposure on the Antibody Response to SRBC in Mice

As indicated in the external review draft of the NCEA document entitled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information*, an evaluation of the potential effects of ammonium perchlorate on humoral immunity was not performed as part of the original immunotoxicity testing protocol. This raised concern that a significant component of the immune system was not assessed in perchlorate-exposed animals. Consequently, the sponsor and contract laboratory agreed to perform 14-day and 90-day studies in which the antibody response to sheep red blood cells (SRBC) would be determined.

Results of a 90-day study were received on January 23,1999. In this study, B6C3F1 female mice, 12 mice per group, were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. Mice where immunized intravenously with SRBC on day 75. Serum was collected on day 79 (4 days post-immunization) and on day 90 (15 days post-immunization), and the SRBC-specific IgM and IgG antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA) "based on a protocol provided by L. Temple of the Medical College of Virginia". Analysis of the ELISA data, which was expressed as the O.D. 50, indicated that neither the IgM nor IgG titers were affected by ammonium perchlorate exposure. In the report, the contract laboratory indicated limitations which were the following: 1) a kinetic study to determine the day of peak levels of IgM and IgG was not performed; and 2) since specialized software (e.g., Softmax®) was not available, serum antibody titers were calculated as the O.D. 50 or midpoint "as described by a SOP provided by L. Temple", rather than the conventional "titer to achieve 0.5 O.D.".

The results of a 14-day exposure study on SRBC-specific antibody responses in mice is expected on February 3, 1999. In addition, because of concern expressed in the external review draft about the infectivity data (i.e., *L. monocytogenes* challenge model) additional studies are currently in progress. The expected due date for the report of these data is June 1, 1999.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 8, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

Dr. Susan Goldhaber
Scientific Coordinator for Perchlorate Peer Review
Research Triangle Institute
3040 Cornwallis Road
P.O. Box 12194
Research Triangle Park, NC 27711

Dear Dr. Goldhaber:

Per our February 1, 1999 submission to Dr. Klaassen via your contract, enclosed please find the second set of analyses we promised (see Table 1). This is another set of new analyses based on data that were not provided in sufficient time to include in the December 31, 1998 external review draft of the document *Perchlorate Environmental Contamination: Toxicology Review and Risk Characterization Based on Emerging Information.* These data represent important information that is being made available as part of completing the original set of studies in the testing strategy. As for the previous data submitted, we will present brief summaries of these data at the peer review meeting. All of these fall into the latter two categories that we described (Category 2 or Category 3) as follows.

1.	*Completed EPA analysis:*	EPA has finalized its analyses utilizing final audited data from a particular study.
2.	*Preliminary EPA analysis:*	EPA has either analyzed audited data for individual parameters but the final report audit is not completed, or the analyses EPA performed may not be complete.
3.	*Pending data:*	These are studies that are in the pipeline. Due dates and thoughts on how these data inform the current effort will be presented.

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I will be staying at the contract hotel for the meeting. Should there be any questions on this submission, do not hesitate to contact me there. The NCEA risk assessment team is looking forward to a stimulating and valuable peer review of these data and their anticipated interpretation/integration into the assessment effort. We will be seeing you shortly at the review.

Sincerely,

Annie M. Jarabek
EPA Perchlorate Assessment Team Leader and
Interagency Perchlorate Steering Committee
(IPSC) Executive Committee (NCEA)

Enclosures

cc: **w/o enclosures**

W. Farland, NCEA
Lt. Col. Dan Rogers, IPSC Executive Committee (USAF)
Peter Grevatt, IPSC Executive Committee (OSWER)
Kevin Mayer, IPSC Executive Committee (Region 9)
Mike Osinski, IPSC Executive Committee (OW)

Table 1. Data Analyses in February 8, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Different BMD analysis for neurodevelopmental study as suggested by Joe Haseman.	Preliminary — Reanalysis of BMD for neurodevelopmental study (Geller, 1999b) using data as litter-by-litter rather than only those for which hormone and histopathology were performed (Table 6B-7 of document).	Joe Haseman Rochelle Tyl
2. Occupational cross-sectional study of workers exposed via inhalation and an epidemiological study	Preliminary — Manuscripts submitted as accepted on 1/22/99. EPA analysis not complete.	Susan Porterfield Tom Zoeller Charles Emerson
3. Body weight and organ weight audited data and unaudited histopathology from F1 generation in 2-generation reproductive study (Argus, 1998b)	Preliminary — Body weight and organ weight data audited, histopathology is unaudited and final report has not been audited or released. No EPA statistical analysis performed.	Rochelle Tyl Susan Porterfield Tom Zoeller
4. Sheep red blood cell (SRBC) from 14-day experiment (repeat) in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
5. Correlations between percent of iodide uptake inhibition and hormone perturbations using single dose and repeated 14-day dose PK studies	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen

February 8, 1999 EPA Assessment Submission

Attachment #1
Calculations of Quantal Benchmark Doses on Data from Neurodevelopmental Toxicity Study (Argus 1998; York, 1998c) with Full Data

A. EPA Analysis Geller (1999c)

ATTENTION PANEL MEMBER(S):

JOE HASEMAN

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 8 February 1999

Subject: Calculations of Quantal Benchmark Doses on Data from Neurobehavioral Developmental Toxicity Study (Argus, 1998a; York, 1998c) with full data set.

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Memo contains benchmark dose calculations on standard histopathology data set, using all data. Data are considered litter-by-litter; where there was more than one pup considered from a particular litter, the mean severity rating was used, yielding an n = 46 litters. Tables and calculations are for comparison with benchmark calculations for this study initially presented in Table 6B-7 of Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information. Table 6B-7 was calculated with a subset of 36 litters, derived from those animals from whom both histopathology and hormone levels were available.

The inclusion of the additional litters did not significantly affect the benchmark calculations. The benchmark dose and benchmark dose lower confidence limits remained virtually identical to those previously presented. As before, the quantal linear, Weibull, and gamma function models all produced the same fit.

BMDs from Developmental Neurotoxicity Study, PND5, Incidence of Follicular Hyperplasia/Hypertrophy Data. BMDs reflect 10% extra risk with 46 litters.

Function	p of fit, df	BMD	BMDL	LOAEL	BMD: LOAEL	BMDL: LOAEL
Gamma	0.36, 3	0.237	0.101	0.1	2.37	1.01
Logistic	0.36, 3	0.306	0.298	0.1	3.06	2.98
Probit	0.36, 3	0.339	0.303	0.1	3.39	3.03
Quantal Linear	0.36, 3	0.236	0.101	0.1	2.36	1.01
Quantal Quadratic	0.33, 3	0.908	0.528	0.1	9.08	5.28
Weibull	0.36, 3	0.236	0.101	0.1	2.36	1.01

==========

Gamma Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:32:39 1999

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BMDS MODEL RUN

The form of the probability function is:

P[response]=
background+(1-background)*CumGamma[slope*dose,power],
where CumGamma(.) is the cummulative Gamma distribution function

Dependent variable = fh
Independent variable = dose
Power parameter is restricted as power>=1

Total number of observations = 5
Total number of records with missing values = 0

Maximum number of iterations = 250
Relative Function Convergence = 2.22045e-016
Parameter Convergence = 1.49012e-008

Default Initial Parameter Values

Background =	0.375
Slope =	0.482731
Power =	1.2

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.581016	0.121886
Slope	0.444908	0.997695
Power	1	1.90119

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	0.3616	0.5064
Slope	0.3616	1	0.9572
Power	0.5064	0.9572	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.109	3.37943	3	0.184572
Reduced model	-25.3035	8.38885	2	0.015079

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5810	4.648	3	8
0.1000	0.5992	5.992	8	10
1.0000	0.7315	7.315	7	10
3.0000	0.8897	7.118	7	8
10.0000	0.9951	9.951	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3632

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.236814

BMDL = 0.1013

==========

Logistic Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:33:19 1999

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BMDS MODEL RUN

The form of the probability function is:

P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = fh
Independent variable = dose
Slope parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
intercept = 0.469692
slope = 0.419527

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	0.365989	0.494797
slope	0.56306	0.232602

Asymptotic Correlation Matrix of Parameter Estimates

	intercept	slope
intercept	1	-0.3873
slope	-0.3873	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.0899	3.34115	3	0.341957
Reduced model	-25.3035	8.42713	1	0.0036966

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5905	4.724	3	8
0.1000	0.6040	6.040	8	10
1.0000	0.7169	7.169	7	10
3.0000	0.8865	7.092	7	8
10.0000	0.9975	9.975	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3631

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.306206

BMDL = 0.2978

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==========

Multistage Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:33:41 1999

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BMDS MODEL RUN

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = fh
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

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==========

Probit Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:34:00 1999

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BMDS MODEL RUN

The form of the probability function is:

P[response] = CumNorm(Intercept+Slope*Dose),

where CumNorm( ) is the cumulative normal distribution function

Dependent variable = fh
Independent variable = dose
Slope parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Intercept = 0.334036
Slope = 0.227262

Parameter Estimates

Variable	Estimate	Std. Err.
Intercept	0.242784	0.341629
Slope	0.312349	0.218901

Asymptotic Correlation Matrix of Parameter Estimates

	Intercept	Slope
Intercept	1	-0.6042
Slope	-0.6042	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.0682	3.29771	3	0.347962
Reduced model	-25.3035	8.47057	1	0.0036094

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5959	4.767	3	8
0.1000	0.6080	6.080	8	10
1.0000	0.7106	7.106	7	10
3.0000	0.8810	7.048	7	8
10.0000	0.9996	9.996	10	10

Chi-square = 3.18 DF = 3 P-value = 0.3646

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.338921

BMDL = 0.3028

==========

Quantal Linear Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:34:48 1999

==========

BMDS MODEL RUN

The form of the probability function is:

P[response] = background +
(1-background)*[1-EXP(-slope*dose)]

Dependent variable = fh
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.706952

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.581016	0.143536
Slope	0.444908	0.290282

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope
Background	1	-0.4765
Slope	-0.4765	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.109	3.37943	3	0.33674
Reduced model	-25.3035	8.38885	1	0.0037753

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5810	4.648	3	8
0.1000	0.5992	5.992	8	10
1.0000	0.7315	7.315	7	10
3.0000	0.8897	7.118	7	8
10.0000	0.9951	9.951	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3632

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.236814

BMDL = 0.1013

==========

Quantal Quadratic Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:35:03 1999

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BMDS MODEL RUN

The form of the probability function is:

P[response] = background +
(1-background)*[1-EXP(-slope*dose^2)]

Dependent variable = fh
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.0906102

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.624661	0.123807
Slope	0.127549	0.122104

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope
Background	1	-0.4048
Slope	-0.4048	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.1773	3.51586	3	0.318711
Reduced model	-25.3035	8.25242	1	0.0040698

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.6247	4.997	3	8
0.1000	0.6251	6.251	8	10
1.0000	0.6696	6.696	7	10
3.0000	0.8809	7.047	7	8
10.0000	1.0000	10.000	10	10

Chi-square = 3.48 DF = 3 P-value = 0.3239

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.908867

BMDL = 0.5280

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Weibull Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:35:19 1999

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BMDS MODEL RUN

The form of the probability function is:

P[response] = background +
(1-background)*[1-EXP(-slope*dose^power)]

Dependent variable = fh
Independent variable = dose
Power parameter is restricted as power>=1

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.482731
Power = 1.2

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.581016	0.236161
Slope	0.444908	1.17357
Power	1	1.72216

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	-0.8411	0.7941
Slope	-0.8411	1	-0.9689
Power	0.7941	-0.9689	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.109	3.37943	3	0.184572
Reduced model	-25.3035	8.38885	2	0.015079

Goodness of Fit

Dose	Est._Prob	Expected	Observed	Size
0.0000	0.5810	4.648	3	8
0.1000	0.5992	5.992	8	10
1.0000	0.7315	7.315	7	10
3.0000	0.8897	7.118	7	8
10.0000	0.9951	9.951	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3632

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.236814

BMDL = 0.1013

Consultants in Epidemiology and Occupational Health, Inc.

2428 Wisconsin Avenue, NW / Washington, DC 20007 / 202-333-2364 / Fax 202-333-2239
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January 21, 1999

Annie M. Jarabek
National Center for Environmental Assessment (NCEA)
Environmental Protection Agency (MD-52)
3210 Highway 54 Room 320
Research Triangle Park, NC 27709

Dear Annie,

On September 1, 1998, you received from us a peer-reviewed report of our perchlorate worker occupational health study entitled "Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational health Study". You had asked that we notify when it had been submitted for publication. We did, and we sent you a copy of the submitted version. You asked that we inform you if and when the paper is accepted by the journal.

This is to notify you that the editor of the Journal of Occupational and Environmental Medicine (JOEM), Paul W. Brandt-Rauf, MD, ScD, DrPH, has informed me that the paper has been accepted for publication in the JOEM. Attached is the as-accepted version of that paper. We hope that it will be found useful to your process to assess the risks to human health from exposure to perchlorate. Please let us know if there are any questions that either you or your associates have for us.

Cordially,

Steven H. Lamm, MD DTPH

Sent by Federal Express

Cc: Linda Ferguson - AMPAC

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

Running title: Thyroid Function in Perchlorate Workers (39 spaces)

By
Steven H. Lamm, MD, Lewis E. Braverman, MD, Feng Xiao Li, MD, DrPH,
Kent Richman, PhD, Sam Pino, BSc, and Gregory Howearth, BSc

From Consultants in Epidemiology and Occupational Health, Inc. (CEOH, Inc.), Washington, DC (Dr. Lamm, Dr. Li), Department of Medicine, Brigham and Women's Hospital, Boston, MA (Dr. Braverman, Mr. Pino), and American Pacific Corporation, Cedar City, UT (Dr. Richman, Mr. Howearth).

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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.

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 highly 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 or below daily for 1 year with excellent results and no serious side effects.[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.

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, and THBR being defined as values at or above the normal lower limit for the laboratory and with normal values of TSH and anti-TPO being defined as values at or below the normal upper limit for the laboratory. There were no significant differences across the exposure groups, between the two plants, or in comparison to the laboratory range of normal. 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. 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 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. 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 developing 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 anti-TPO antibodies, and a clinical thyroid examination to their assessment of thyroid function outcome measures. 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 Grave's disease patients being treated therapeutically with exposures of 1,000 mg per day or greater, occasionally at 600 mg per day, and only once in a patient at 450 mg per day (who had previously shown a toxic reaction at 800 mg per day).[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 Grave's disease patients may occur 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.

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 health status 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 assessment of human risk from environmental exposure to perchlorate. The results of the present study do not support the hypothesis that chronic exposure to perchlorates at the levels encountered in this study has an 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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This paper is dedicated to Dr. Irving R. Tabershaw who taught me (SHL) the role of occupational medicine in assessing the effect of chemical exposures on human health and whose footsteps I have tried to follow for over twenty years.

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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.

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.

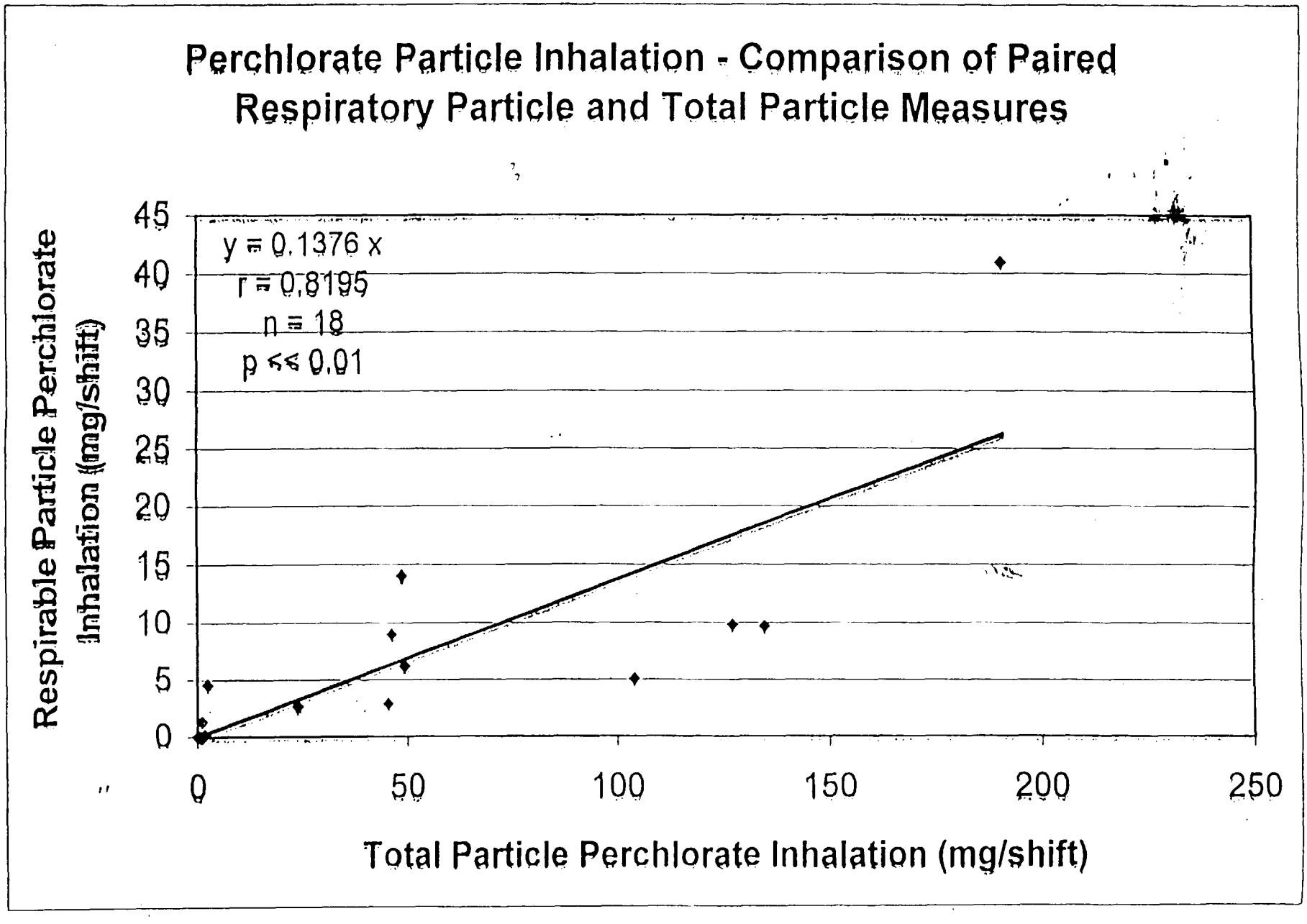
Table 4. Descriptive statistics of blood cell counts by plants and exposure groups

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

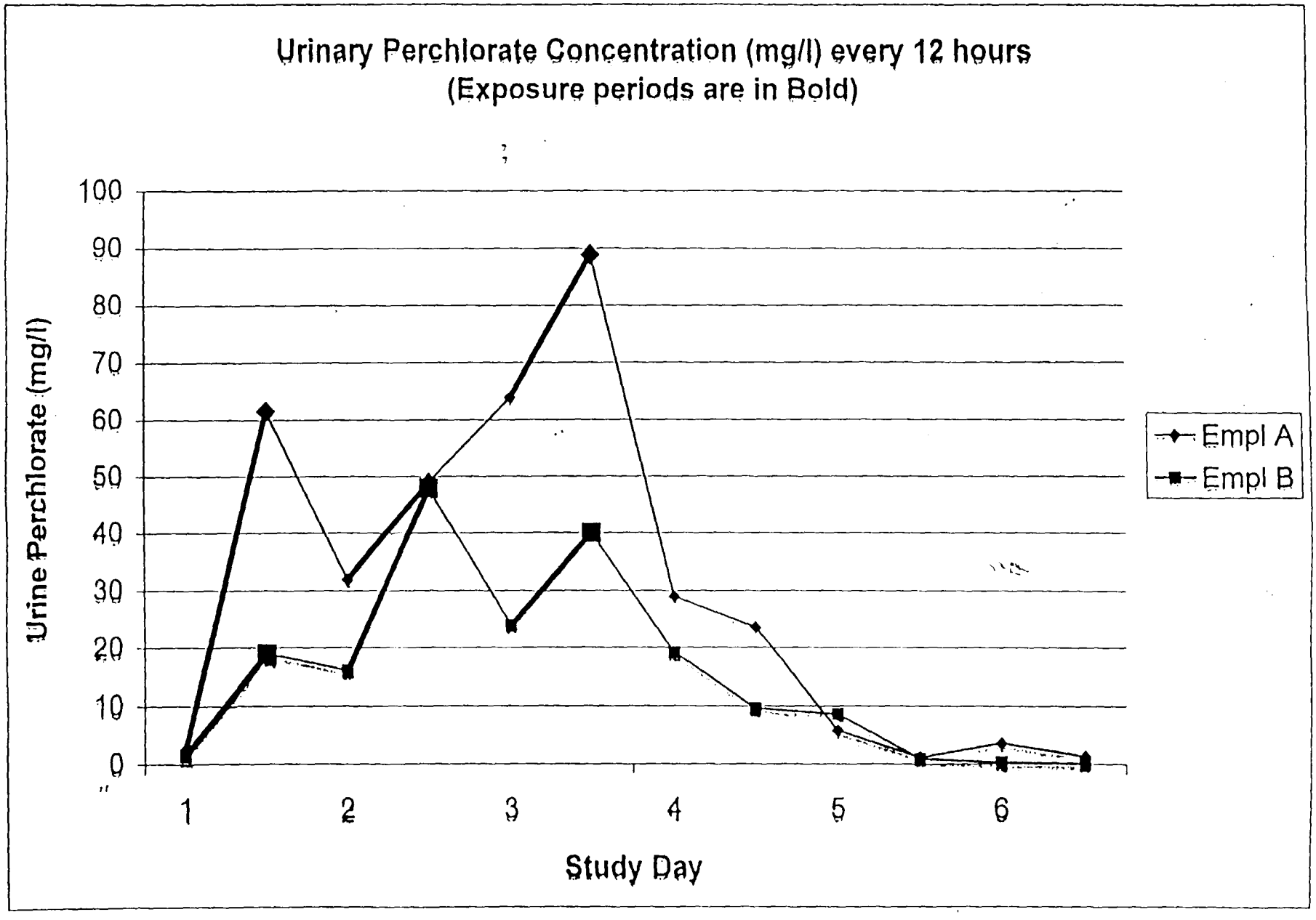
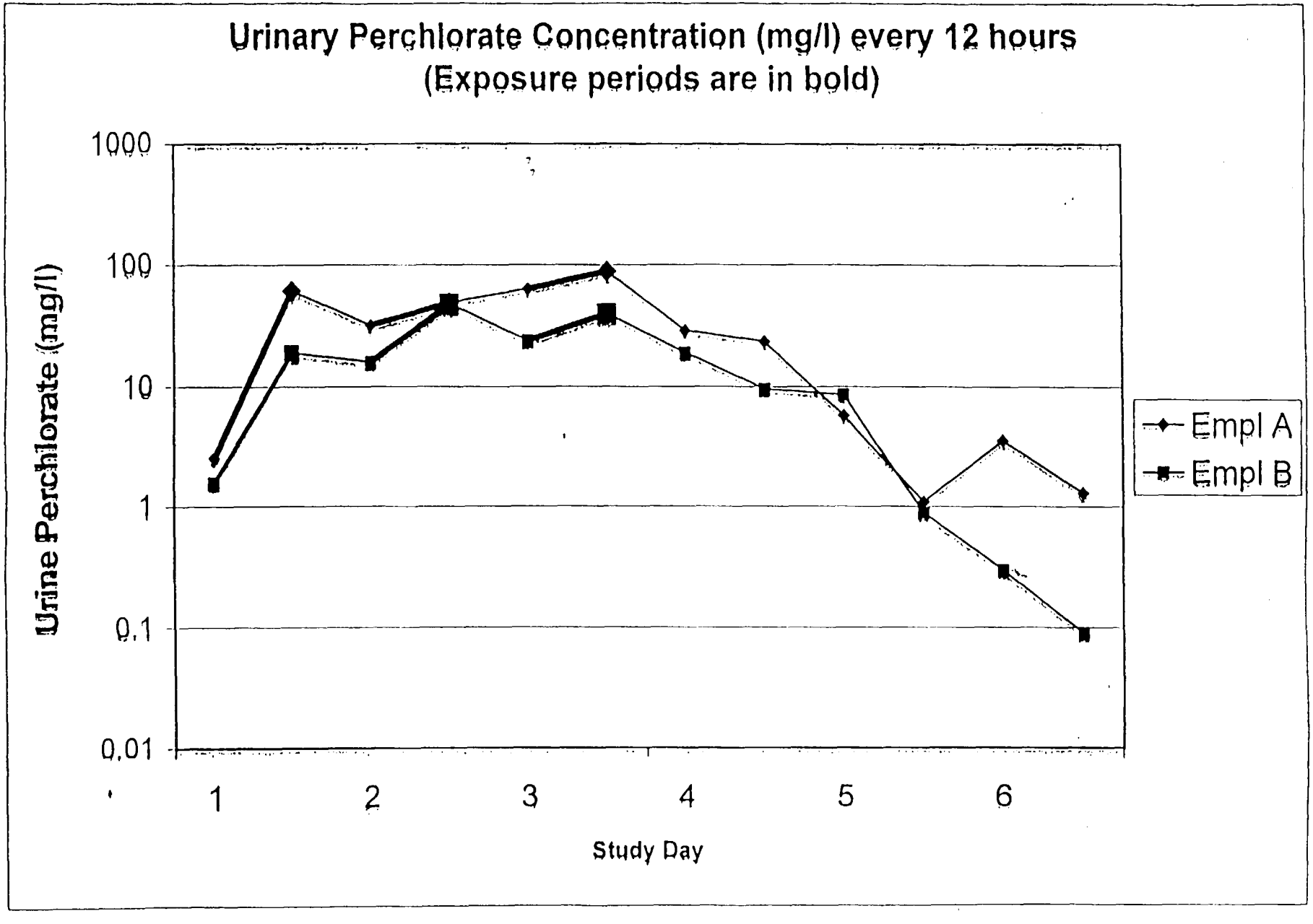
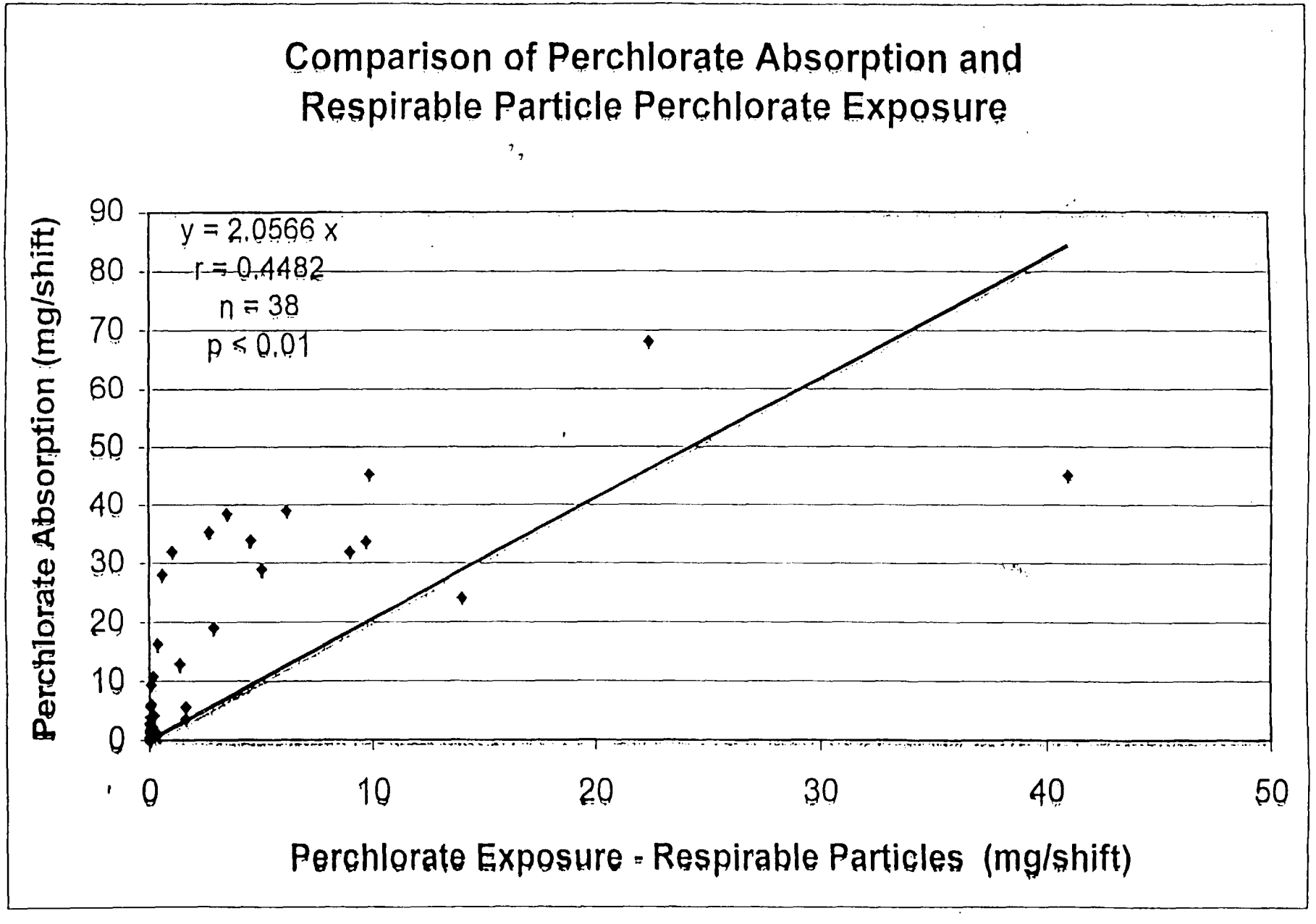
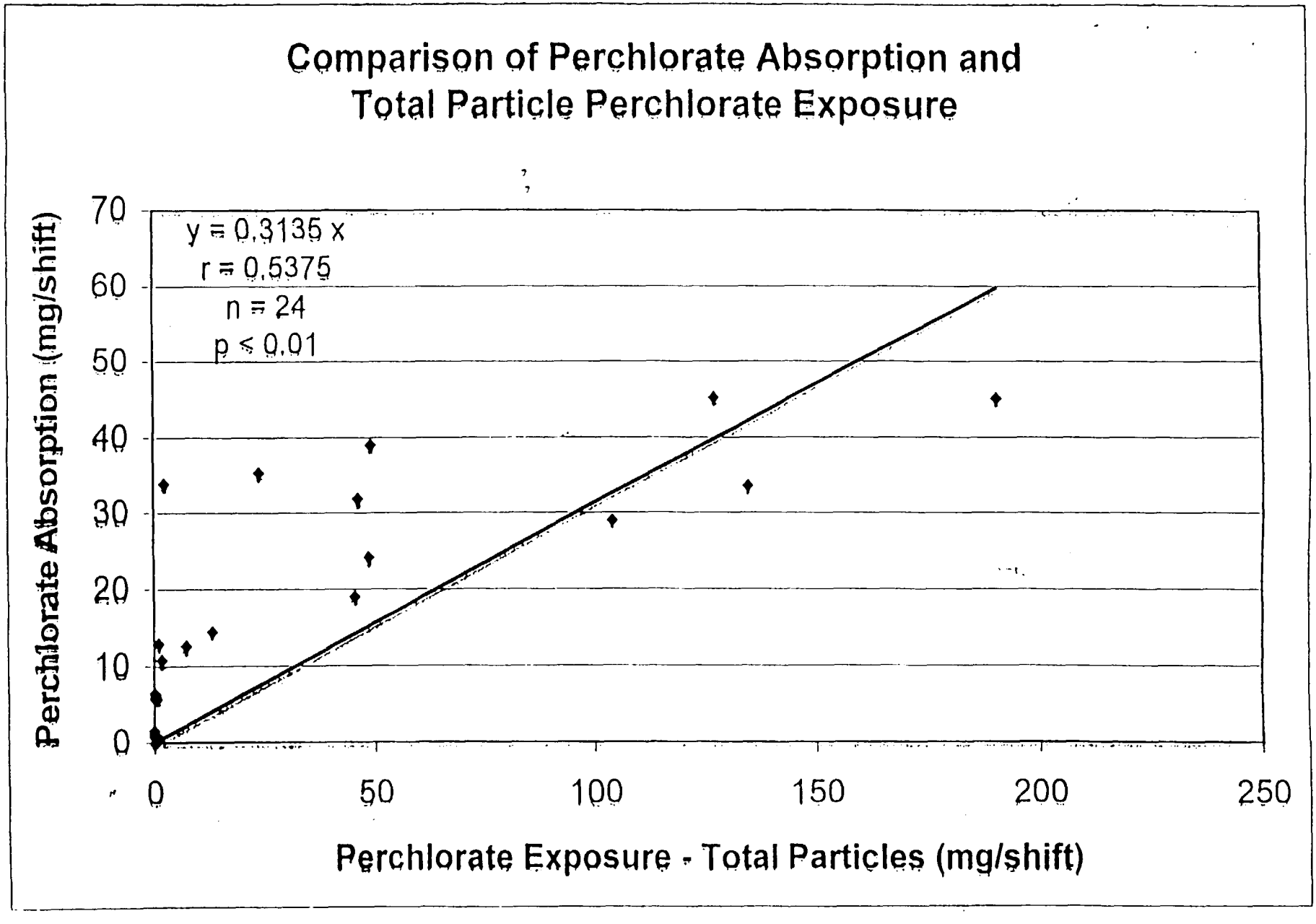

Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in Bold)
Urine Perchlorate (mg/l)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Study Day
Empl A
Empl B

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


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)


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)

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

Running Title: Perchlorate and Congenital Hypothyroidism

Word Count: 777

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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.

References

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[6]Delange F, Heidemann P, Bourdoux P, Larsson A, Vigneri R, Klett M, Beckers C, and Stubbe P.: Regional variations of iodine nutrition and thyroid function during the neonatal period in Europe. Biol. Neonate, 1986; 49: 322-330.

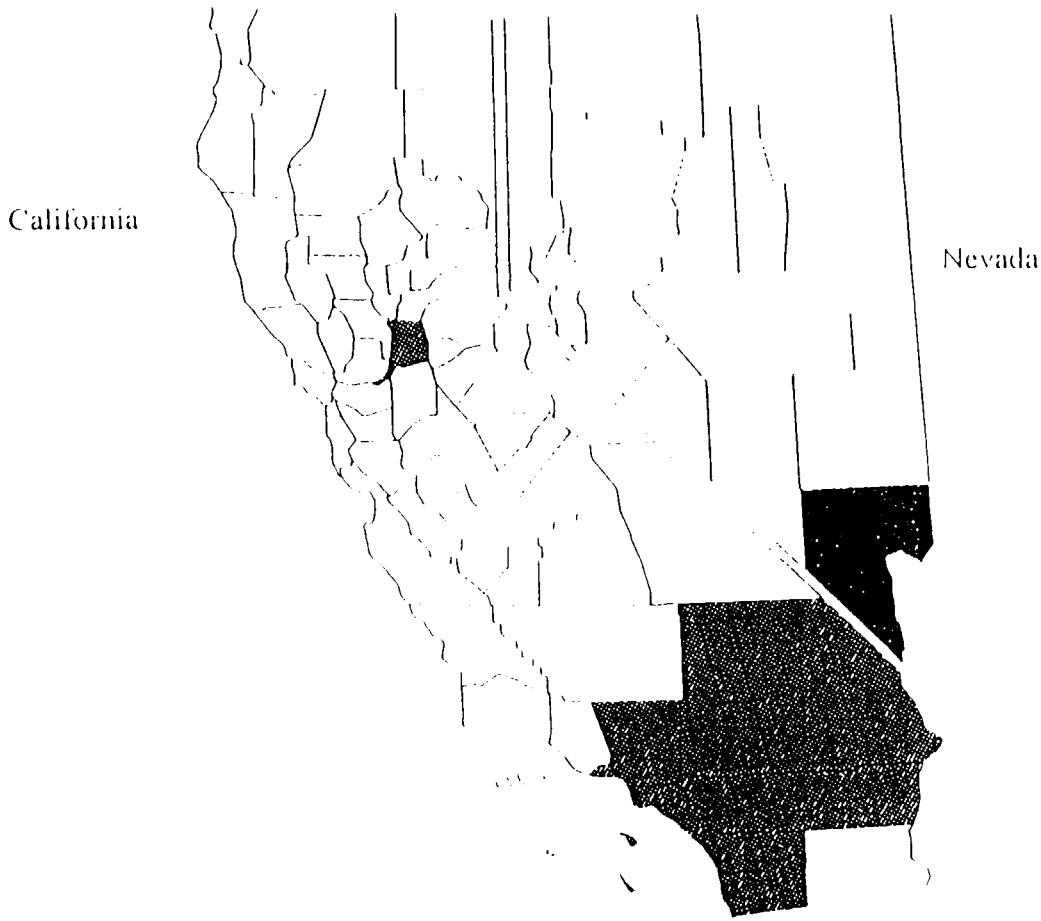
Table 1

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



February 8, 1999 EPA Assessment Submission

Attachment #3
Review of Unadudited Terminal Body Weights, Organ Weights, and Ratios; T3, T4, TSH data; and Histopathology in the F1 Generation Rats of the 2-Generation Reproductive Study

A. Body weights, Organ weights, Ratios (York ,1999a)
B. Histopathology and Hormone Data (York, 1999a)
C. Preliminary EPA analysis (Jarabek and Clegg, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
ROCHELLE TYL
SUSAN PORTERFIELD
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PRIMEDICA

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February 1, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached is a copy of the summary tables with the F1 generation male and female rats terminal body weights and absolute organ weights and ratios of these organ weights to terminal body weights and to brain weights you requested. Please remember these tables are unaudited and could still change based on the final audit of the study.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Valerie A. Sharpe

for Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D12 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION MALE PUPS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
TERMINAL BODY WEIGHT	MEAN±S.D.	617.2 ± 76.1	664.6 ± 58.2*	652.6 ± 74.4	638.0 ± 48.8
EPIDIDYMIS LEFT	MEAN±S.D.	0.7617 ±0.0676 [29]b	0.7900 ±0.0695	0.7939 ±0.0651 [29]b	0.7975 ±0.0522
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.3535 ±0.0370 [29]b	0.3702 ±0.0390	0.3654 ±0.0459 [29]b	0.3774 ±0.0403
TESTIS LEFT	MEAN±S.D.	1.8489 ±0.1672 [29]b	1.9460 ±0.1718*	1.9329 ±0.1653 [29]b	1.9670 ±0.1676*
LEFT TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	1.6764 ±0.1636 [29]b	1.7619 ±0.1632	1.7683 ±0.1604 [29]b	1.7813 ±0.1781
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	1.7615 ±0.2457	1.8277 ±0.3127 [28]b	1.7521 ±0.3505	1.7811 ±0.3458
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.7135 ±0.1138	0.7485 ±0.1260	0.7540 ±0.1335	0.7724 ±0.1139
EPIDIDYMIS RIGHT	MEAN±S.D.	0.7669 ±0.0466 [29]b	0.8033 ±0.0596*	0.8070 ±0.0643* [29]b	0.7947 ±0.0487
TESTIS RIGHT	MEAN±S.D.	1.8548 ±0.1572 [29]b	1.9637 ±0.1673	1.9418 ±0.1636 [29]b	1.9518 ±0.1784
PROSTATE	MEAN±S.D.	1.1603 ±0.1966	1.1375 ±0.2382	1.1584 ±0.2321 [29]b	1.2163 ±0.2826
PITUITARY	MEAN±S.D.	0.014 ± 0.002	0.016 ± 0.003**	0.015 ± 0.003	0.016 ± 0.002*
BRAIN	MEAN±S.D.	2.38 ± 0.10	2.44 ± 0.15	2.46 ± 0.11*	2.39 ± 0.10

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 8, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Review of Unaudited Terminal Body Weights, Organ Weights, and Ratios; T3, T4, TSH data, and Histopathology in the F1 Generation Rats of the 2-Generation Reproductive Study

FROM: Annie M. Jarabek
NCEA-RTP (MD-52)

for Eric Clegg
NCEA-WA (8623)

TO: Perchlorate Risk Assessment Team

We have reviewed the February 1, 1999 submissions (York, 1999b,c) from Primedica / Argus Research Laboratories. York (1999b) provided unaudited terminal body weights, absolute organ weights, and ratios of these organ weights to terminal body weights and to brain weights. York (1999c) provided the hormone and histopathology data. These data could still change based on the final report audit.

Thyroid weights, thyroid to body weight ratio, and thyroid to brain weight were statistically significantly increased in F1 male pups at 3.0 and 30.0 mg/kg-day and in females at 0.3, 3.0 and 30 mg/kg-day. There was also an effect on the pituitary weight and pituitary to brain weight ratio that was significant at 0.3 and 30.0, but not at 3.0 mg/kg-day, although these dose group values were increased over those of controls.

The only treatment-related effect observed was primarily hypertrophy and hyperplasia of the thyroid follicular epithelium. In thyroids with moderate or marked hyperplasia/hypertrophy in the P1 rats, there was a decrease or complete absence of visible colloid. The P1 rats were affected at all dose groups, with incidence and severity increasing in a dose-related manner. In the F1 pups, these changes were noted at the mid and high dose groups. The statistical analyses were run separately for the males versus females and EPA would like to look at the combined data.

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There was no apparent dose trend in any of the hormone measures (T3, T4, TSH) as provided, but again EPA would like to run additional alternative analyses using the combined data.

These unaudited data suggest at this time a corroboration of the thyroid effects seen in pups of the neurodevelopmental study. More rigorous analysis to look at the severity scoring and alternative analyses of the combined data are recommended. The incidence of histopathology suggests support of an effect at 0.3 mg/kg-day seen in the organ weight data. The hormone data may not sufficiently analyzed at this time but do not appear to correspond with the histopathology and weight data. The results of the inter-laboratory validation study for the hormone analyses may be informative to interpreting this discrepancy.

Attachments

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D12 (PAGE 2): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION MALE PUPS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
LIVER	MEAN±S.D.	24.72 ± 4.03	27.30 ± 3.54**	26.18 ± 3.26	25.28 ± 3.17 [26]b
KIDNEY PAIRED	MEAN±S.D.	4.84 ± 0.59	5.23 ± 0.45*	5.18 ± 0.64*	5.11 ± 0.49
ADRENAL PAIRED	MEAN±S.D.	0.069 ± 0.011	0.071 ± 0.011	0.073 ± 0.013	0.071 ± 0.010
SPLEEN	MEAN±S.D.	1.01 ± 0.16	1.08 ± 0.22	1.12 ± 0.18*	1.08 ± 0.12*
THYROID	MEAN±S.D.	0.036 ± 0.005	0.041 ± 0.009	0.044 ± 0.005**	0.063 ± 0.012**
HEART	MEAN±S.D.	1.91 ± 0.21	2.01 ± 0.27	2.04 ± 0.22	[26]c 1.96 ± 0.19

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Excludes value for rat 7095, value was not recorded.
c. Excludes values for rats that had organs damaged (weight affected).
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D13 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
TERMINAL BODY WEIGHT	MEAN±S.D.	617.2 ± 76.1	664.6 ± 58.2*	652.6 ± 74.4	638.0 ± 48.8
EPIDIDYMIS LEFT	MEAN±S.D.	0.124 ± 0.014 [29]b	0.118 ± 0.017	0.122 ± 0.017 [29]b	0.125 ± 0.010
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.057 ± 0.008 [29]b	0.056 ± 0.009	0.057 ± 0.009 [29]b	0.059 ± 0.007
TESTIS LEFT	MEAN±S.D.	0.302 ± 0.033 [29]b	0.294 ± 0.038	0.298 ± 0.039 [29]b	0.310 ± 0.028
LEFT TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	0.273 ± 0.032 [29]b	0.265 ± 0.034	0.272 ± 0.038 [29]b	0.280 ± 0.028
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.288 ± 0.052	0.275 ± 0.058 [28]b	0.273 ± 0.069	0.281 ± 0.056
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.116 ± 0.025	0.112 ± 0.021	0.117 ± 0.025	0.121 ± 0.020
EPIDIDYMIS RIGHT	MEAN±S.D.	0.126 ± 0.016 [29]b	0.121 ± 0.016	0.123 ± 0.017 [29]b	0.125 ± 0.011
TESTIS RIGHT	MEAN±S.D.	0.303 ± 0.037 [29]b	0.297 ± 0.038	0.300 ± 0.038 [29]b	0.307 ± 0.032
PROSTATE	MEAN±S.D.	0.189 ± 0.032	0.171 ± 0.033	0.179 ± 0.042 [29]b	0.190 ± 0.040
PITUITARY c	MEAN±S.D.	2.267 ± 0.336	2.437 ± 0.394	2.353 ± 0.517	2.470 ± 0.368
BRAIN	MEAN±S.D.	0.390 ± 0.048	0.369 ± 0.030	0.379 ± 0.037	0.376 ± 0.028

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).

c. Value was multiplied by 1000.

* Significantly different from the carrier group value ($p \leq 0.05$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D13 (PAGE 2): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
LIVER	MEAN±S.D.	3.996 ± 0.330	4.113 ± 0.436	4.016 ± 0.268	3.951 ± 0.368 [26]b
KIDNEY PAIRED	MEAN±S.D.	0.786 ± 0.065	0.789 ± 0.075	0.795 ± 0.066	0.800 ± 0.056
ADRENAL PAIRED	MEAN±S.D.	0.010 ± 0.000	0.010 ± 0.000	0.011 ± 0.002	0.010 ± 0.002
SPLEEN	MEAN±S.D.	0.165 ± 0.021	0.163 ± 0.033	0.172 ± 0.024	0.170 ± 0.017
THYROID c	MEAN±S.D.	5.935 ± 0.840	6.116 ± 1.092	6.705 ± 0.600**	9.888 ± 1.823** [26]d
HEART	MEAN±S.D.	0.311 ± 0.036	0.303 ± 0.037	0.316 ± 0.035	0.306 ± 0.025

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes value for rat 7095, value was not recorded.

c. Value was multiplied by 1000.

d. Excludes values for rats that had organs damaged (weight affected).

** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D14 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO BRAIN WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
BRAIN WEIGHT	MEAN±S.D.	2.38 ± 0.10	2.44 ± 0.15	2.46 ± 0.11*	2.39 ± 0.10
EPIDIDYMIS LEFT	MEAN±S.D.	32.02 ± 2.72 [29]b	32.50 ± 3.23	32.30 ± 3.01 [29]b	33.45 ± 2.41
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	14.86 ± 1.53 [29]b	15.22 ± 1.70	14.86 ± 1.93 [29]b	15.82 ± 1.62
TESTIS LEFT	MEAN±S.D.	77.68 ± 6.11 [29]b	79.95 ± 6.80	78.62 ± 7.18 [29]b	82.44 ± 6.76
LEFT TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	70.43 ± 6.09 [29]b	72.40 ± 6.71	71.93 ± 6.92 [29]b	74.63 ± 6.89
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	74.09 ± 10.98	75.29 ± 13.09 [28]b	71.44 ± 15.18	74.45 ± 13.08
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	30.01 ± 4.97	30.71 ± 4.76	30.75 ± 5.94	32.33 ± 4.46
EPIDIDYMIS RIGHT	MEAN±S.D.	32.25 ± 1.92 [29]b	33.06 ± 2.95	32.85 ± 3.06 [29]b	33.33 ± 2.28
TESTIS RIGHT	MEAN±S.D.	77.93 ± 5.61 [29]b	80.68 ± 6.67	78.96 ± 6.92 [29]b	81.80 ± 7.17
PROSTATE	MEAN±S.D.	48.72 ± 8.11	46.78 ± 10.00	47.08 ± 9.43 [29]b	50.89 ± 11.26
PITUITARY	MEAN±S.D.	0.58 ± 0.10	0.66 ± 0.12*	0.62 ± 0.12	0.66 ± 0.09*
LIVER	MEAN±S.D.	1037.23 ±158.37	1120.77 ±135.98	1063.74 ±116.27	1062.09 ±122.13 [26]c

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/BRAIN WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).

c. Excludes value for rat 7095, value was not recorded.

* Significantly different from the carrier group value ($p \leq 0.05$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D14 (PAGE 2): RATIOS (%) OF BRAIN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
KIDNEY PAIRED	MEAN±S.D.	203.09 ± 22.84	214.72 ± 17.71	210.10 ± 21.08	214.30 ± 19.96
ADRENAL PAIRED	MEAN±S.D.	2.91 ± 0.46	2.93 ± 0.44	2.98 ± 0.54	3.00 ± 0.51
SPLEEN	MEAN±S.D.	42.56 ± 6.02	44.51 ± 9.10	45.78 ± 7.49	45.40 ± 4.73
THYROID	MEAN±S.D.	1.52 ± 0.21	1.68 ± 0.37	1.77 ± 0.17**	2.64 ± 0.52**
HEART	MEAN±S.D.	80.26 ± 9.44	82.59 ± 11.57	83.18 ± 8.57	[26]b 81.86 ± 6.64

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/BRAIN WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes values for rats that had organs damaged (weight affected).

** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E27 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
PREGNANT	N	21	27	28	27
INCLUDED IN ANALYSES	N	20a	27b	28	25c,d
TERMINAL BODY WEIGHT	MEAN±S.D.	354.0 ± 18.6	361.0 ± 22.6	368.0 ± 21.4	367.2 ± 21.0
PITUITARY	MEAN±S.D.	0.016 ± 0.003	0.016 ± 0.003	0.016 ± 0.004	0.016 ± 0.004
BRAIN	MEAN±S.D.	2.20 ± 0.11	2.23 ± 0.10	2.23 ± 0.08	2.19 ± 0.11
LIVER	MEAN±S.D.	19.92 ± 2.10	20.27 ± 1.73	19.67 ± 1.39	19.11 ± 1.76
KIDNEY PAIRED	MEAN±S.D.	3.31 ± 0.27	3.32 ± 0.26	3.38 ± 0.25	3.26 ± 0.32
ADRENAL PAIRED	MEAN±S.D.	0.112 ± 0.013	0.107 ± 0.013	0.112 ± 0.011	0.103 ± 0.014*
SPLEEN	MEAN±S.D.	0.74 ± 0.12	0.76 ± 0.12	0.77 ± 0.10	0.77 ± 0.09
OVARY PAIRED	MEAN±S.D.	0.126 ± 0.027	0.126 ± 0.027	0.130 ± 0.029	0.131 ± 0.029
THYROID	MEAN±S.D.	0.022 ± 0.003	0.025 ± 0.004*	0.028 ± 0.004** [27]	0.033 ± 0.005**
HEART	MEAN±S.D.	1.37 ± 0.13	1.38 ± 0.10	1.39 ± 0.12	1.39 ± 0.12

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for dam 7212, which did not deliver a litter; only one early resorption was present *in utero* on day 25 of gestation.

b. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning.

c. Excludes values for dam 7293, which did not deliver a litter; only three early resorptions was present *in utero* on day 25 of gestation.

d. Excludes values for dam 7295, which was found dead on day 22 of gestation.

* Significantly different from the carrier group value ($p \leq 0.05$).

** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E28 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
PREGNANT	N	21	27	28	27
INCLUDED IN ANALYSES	N	20a	27b	28	25c,d
TERMINAL BODY WEIGHT	MEAN±S.D.	354.0 ± 18.6	361.0 ± 22.6	368.0 ± 21.4	367.2 ± 21.0
PITUITARY e	MEAN±S.D.	4.469 ± 0.688	4.559 ± 0.752	4.315 ± 1.098	4.462 ± 0.997
BRAIN	MEAN±S.D.	0.622 ± 0.038	0.621 ± 0.048	0.607 ± 0.034	0.599 ± 0.042
LIVER	MEAN±S.D.	5.632 ± 0.575	5.652 ± 0.339	5.350 ± 0.339	5.206 ± 0.385*
KIDNEY PAIRED	MEAN±S.D.	0.934 ± 0.074	0.923 ± 0.073	0.918 ± 0.059	0.887 ± 0.080
ADRENAL PAIRED	MEAN±S.D.	0.030 ± 0.004	0.029 ± 0.005	0.031 ± 0.004	0.028 ± 0.004
SPLEEN	MEAN±S.D.	0.208 ± 0.034	0.211 ± 0.035	0.210 ± 0.029	0.208 ± 0.027
OVARY PAIRED	MEAN±S.D.	0.036 ± 0.008	0.034 ± 0.008	0.035 ± 0.008	0.035 ± 0.008
THYROID e	MEAN±S.D.	6.279 ± 0.917	7.032 ± 1.224*	7.707 ± 1.172** [27]	9.018 ± 1.333**
HEART	MEAN±S.D.	0.388 ± 0.034	0.382 ± 0.029	0.377 ± 0.026	0.377 ± 0.028

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for dam 7212, which did not deliver a litter; only one early resorption was present in utero on day 25 of gestation.

b. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning.

c. Excludes values for dam 7293, which did not deliver a litter; only three early resorptions was present in utero on day 25 of gestation.

d. Excludes values for dam 7295, which was found dead on day 22 of gestation.

e. Value was multiplied by 1000.

* Significantly different from the carrier group value ($p \leq 0.05$).

** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E29 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO BRAIN WEIGHT - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
PREGNANT	N	21	27	28	27
INCLUDED IN ANALYSES	N	20a	27b	28	25c,d
BRAIN WEIGHT	MEAN±S.D.	2.20 ± 0.11	2.23 ± 0.10	2.23 ± 0.08	2.19 ± 0.11
PITUITARY	MEAN±S.D.	0.72 ± 0.12	0.73 ± 0.13	0.71 ± 0.18	0.74 ± 0.15
LIVER	MEAN±S.D.	908.83 ±100.57	909.06 ± 86.73	883.81 ± 65.55	873.83 ± 95.77
KIDNEY PAIRED	MEAN±S.D.	150.81 ± 11.38	148.76 ± 13.01	151.69 ± 10.18	148.88 ± 16.50
ADRENAL PAIRED	MEAN±S.D.	5.12 ± 0.59	4.78 ± 0.59	5.03 ± 0.48	4.70 ± 0.70
SPLEEN	MEAN±S.D.	33.77 ± 6.24	34.00 ± 5.60	34.75 ± 5.04	34.95 ± 4.00
OVARY PAIRED	MEAN±S.D.	5.75 ± 1.23	5.66 ± 1.30	5.84 ± 1.38	6.00 ± 1.35
THYROID	MEAN±S.D.	1.01 ± 0.14	1.13 ± 0.18*	1.28 ± 0.19** [27]	1.51 ± 0.23**
HEART	MEAN±S.D.	62.39 ± 6.23	61.60 ± 3.89	62.24 ± 4.75	63.32 ± 5.47

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for dam 7212, which did not deliver a litter; only one early resorption was present *in utero* on day 25 of gestation.

b. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning.

c. Excludes values for dam 7293, which did not deliver a litter; only three early resorptions was present *in utero* on day 25 of gestation.

d. Excludes values for dam 7295, which was found dead on day 22 of gestation.

e. Value was multiplied by 1000.

* Significantly different from the carrier group value ($p \leq 0.05$).

** Significantly different from the carrier group value ($p \leq 0.01$).

PRIMEDICA

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February 01, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached are unaudited copies of summary tables with the P1 and F1 generation male and female rats histopatholgy and Ray Brown's draft report. The hormone data sent to us from AniLytics has also been statistically analyzed by us put into a table for you. I also have enclosed another copy of the organ weigh and organ weight ratios. Please remember these tables are unaudited and could still change based on the final audit of the study.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Ray

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

P1 Female-adult

		TSH	T3	T4
Group 1	Mean	2.054	57.77	2.126
	S.D.	0.873	28.204	0.677
Group 2	Mean	2.213	64.789	2.903**
	S.D.	0.987	29.277	1.039
Group 3	Mean	1.99	56.35	2.924**
	S.D.	0.773	13.992	0.841
Group 4	Mean	2.174	60.373	2.421
	S.D.	0.744	21.97	0.792

P1 Male-adult

		TSH	T3	T4
Group 1	Mean	1.53	72.546	4.641
	S.D.	0.957	11.228	0.583
Group 2	Mean	1.353	87.389**	4.726
	S.D.	0.638	16.257	0.817
Group 3	Mean	1.487	88.452**	4.744
	S.D.	0.815	18.634	0.79
Group 4	Mean	3.871**	78.57	3.578**
	S.D.	3.495	14.363	0.86

F1 Female-pup

Group 1	Mean	1.12	105.954	4.27
	S.D.	0.51	13.075	1.019
Group 2	Mean	1.188	109.922	4.865*
	S.D.	0.352	13.066	0.946
Group 3	Mean	1.141	109.293	4.324
	S.D.	0.375	13.568	0.778
Group 4	Mean	1.301	97.581*	3.913
	S.D.	0.351	11.046	0.983

F1 Male-pup

Group 1	Mean	1.237	105.897	4.403
	S.D.	0.448	9.976	1.013
Group 2	Mean	0.941**	111.15	4.615
	S.D.	0.342	16.372	0.979
Group 3	Mean	0.877**	109.81	4.533
	S.D.	0.254	15.693	0.789
Group 4	Mean	1.27	107.398	4.525
	S.D.	0.381	16.06	1.086

1416-001

F1 Female-adult

		TSH	T3	T4
Group 1	Mean	1.625	61.47	2.219
	S.D.	1.006	25.235	1.025
Group 2	Mean	1.223	51.191	2.026
	S.D.	0.663	21.939	0.836
Group 3	Mean	1.647	53.41	2.273
	S.D.	0.882	19.582	1.053
Group 4	Mean	2.12*	56.856	2.131
	S.D.	0.692	18.888	0.863

F1 Male-adult

		TSH	T3	T4
Group 1	Mean	2.507	82.5	3.784
	S.D.	1.01	8.677	0.547
Group 2	Mean	2.164	81.27	4.214*
	S.D.	1.044	15.435	0.862
Group 3	Mean	2.303	83.232	4.204*
	S.D.	1.729	16.496	0.865
Group 4	Mean	5.176**	82.969	2.777**
	S.D.	2.523	13.378	0.716

F2 Female-pup

Group 1	Mean	0.938	108.391	3.402
	S.D.	0.284	21.091	0.724
Group 2	Mean	0.911	107.395	3.336
	S.D.	0.29	13.002	0.787
Group 3	Mean	0.959	107.856	4.151**
	S.D.	0.222	20.657	1.046
Group 4	Mean	0.966	98.846	3.834
	S.D.	0.218	23.988	0.832

F2 Male-pup

Group 1	Mean	0.818	106.346	3.231
	S.D.	0.193	18.27	0.839
Group 2	Mean	0.876	108.035	3.307
	S.D.	0.255	14.633	0.859
Group 3	Mean	0.949	119.532*	3.794
	S.D.	0.277	20.083	0.881
Group 4	Mean	0.959	107.094	3.422
	S.D.	0.214	21.357	0.804

Histopathology - P1 Generation Male Rats

Hyperplasia/hypertrophy, follicular epithelium (N=30):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	0	2	1	0
-mild	1	5	5	0
-moderate	1	2	11	1
-marked	0	0	8	29
Total	2	9	25**	30**

** Significantly different from control $p \leq 0.01$

Histopathology - P1 Generation Female Rats

Hyperplasia/hypertrophy, follicular epithelium (N=22 to 28):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	2	4	4	0
-mild	1	3	8	0
-moderate	1	3	8	2
-marked	0	0	0	22
Total	4	10	20**	24**

** Significantly different from control $p \leq 0.01$

Histopathology - F1 Generation Male Rats

Hyperplasia/hypertrophy, follicular epithelium (N=30):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	0	0	3	0
-mild	3	4	5	0
-moderate	2	4	5	3
-marked	0	0	6	23
Total	5	8	19**	26**

** Significantly different from control $p<0.01$

Histopathology - F1 Generation Female Rats

Hyperplasia/hypertrophy, follicular epithelium (N=20 to 28):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	4	5	6	1
-mild	2	1	6	2
-moderate	0	0	1	10
-marked	0	0	0	11
Total	6	6	13	24**

** Significantly different from control $p<0.01$

RESEARCH
PATHOLOGY
SERVICES, INC.

January 25, 1999

TO: Raymond G. York, Ph.D., D.A.B.T.
Argus Research Laboratories, Inc.
905 Sheehy Drive
Horsham, PA 19044

FROM: W. Ray Brown, D.V.M., Ph.D. WRB 1-25-99
Veterinary Pathologist

SUBJECT: Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats
Protocol 1416-001 - Interim Histopathology Report
Preliminary Histopathology Data - P1 and F1 Generation Adult Rats

Method:

Microscopic examination was made of the specified tissues from parental male and parental female Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats in four treatment groups used in a two-generation reproduction study of ammonium perchlorate. A brief outline of the study design is shown below.

Group	Number of Parental Rats/Group P1	F1	Target Dosage (mg/kg/day)	Concentration (µg/mL)[a]
1	30M,28F	30M,20F	0 (Carrier)[b]	0
2	30M,22F	30M,27F	0.3	2.3
3	30M,26F	30M,28F	3.0	22.8
4	30M,24F	30M,25F	30.0	227.4

[a]Based on average water consumption of 33 mL/animal/day (132 mL/kg/day) for a 250g rat. These concentrations were adjusted weekly based on actual body weight and water consumption levels recorded the previous week. The test article was considered 100% pure for the purpose of dosage calculations.
[b]Reverse osmosis membrane processed deionized water (R. O. deionized water).

P1 generation rats were given continual access to the test substance in the drinking water beginning at least 70 days before cohabitation (approximately six weeks of age) and continuing through the day before sacrifice. Rats of the F1 generation were given the same test substance concentrations as their respective

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P1 generation sires and dams once daily beginning at weaning and continued through the day before sacrifice. Rats of this generation may have been exposed *in utero* during gestation and via maternal milk and water during the postpartum period.

Results:

The type and incidence by degree of severity of histomorphologic changes in the P1 and F1 generation parental male and female rats are presented in Tables 1 and 2, respectively.

The only tissue in which a compound-related change occurred was the thyroid gland and the change occurred in male and female rats of the P1 and F1 generations. The treatment-related change in the thyroid was primarily hypertrophy and hyperplasia of the thyroid follicular epithelium. In many of the affected thyroids, there were increased numbers of small follicles (hyperplasia) and these follicles had enlarged (hypertrophied) follicular epithelial cells. In the thyroids with a moderate or marked hyperplasia/hypertrophy, there was a decrease or complete absence of visible colloid in the affected follicles. The incidence by degree of severity of the thyroid changes in the P1 generation male and female rats is presented in the following text table.

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Rats/Group:	30	30	30	30	28	22	26	24
THYROID:								
-hyperplasia/hypertrophy, follicular epithelium								
minimal	0	2	1	0	2	4	4	0
mild	1	5	5	0	1	3	8	0
moderate	1	2	11	1	1	3	8	2
marked	0	0	8	29	0	0	0	22
Total Incidence	2	9	25	30	4	10	20	24

The same information is presented in the following text table for the F1 generation male and female rats.

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Rats/Group:	30	30	30	30	20	27	28	25
THYROID:								
-hyperplasia/hypertrophy, follicular epithelium								
minimal	0	0	3	0	4	5	6	1
mild	3	4	5	0	2	1	6	2
moderate	2	4	5	3	0	0	1	10
marked	0	0	6	23	0	0	0	11
Total Incidence	5	8	19	26	6	6	13	24

The degree of the thyroid change ranged from minimal to marked and generally occurred in a dose-related fashion. For the P1 generation, the incidence and severity of the thyroid hypertrophy and hyperplasia was considered to be increased in all compound-treated groups, while in the F1 generation, the treatment-related effect (increased incidence and severity of thyroid hyperplasia and hypertrophy) was judged to have occurred in the mid and high dosage groups. The incidence in the low-dose F1 generation rats was considered to be comparable to the controls. There were a few control male and female rats in both generations with a similar type of histomorphologic change in the thyroid, but with a lower incidence and severity as compared to the affected compound-treated P1 and F1 generation male and female rats listed above.

There were no other tissues with compound-related changes in any of the P1 or F1 generation parental rats given 30 mg/kg/day of the test article for the respective treatment periods. The other changes that were observed were typical of those that occur spontaneously in male and female rats used in reproductive studies and their type or incidence was not considered to have been influenced by compound administration. These changes are also listed and summarized in the attached Tables 1 and 2.

Summary:

Microscopic examination was made of the specified tissues from parental male and female rats of the P1 and F1 generations of an oral (drinking water) two generation reproduction study of ammonium perchlorate. The dosages used in the study were 0 (carrier), 0.3, 3.0 or 30.0 mg/kg/day.

Treatment-related microscopic changes were observed in the thyroid gland of the parental male and female rats of the P1 generation and F1 generation rats given the test article.

The treatment-related change consisted of an increased incidence and severity of thyroid follicular epithelial hyperplasia and hypertrophy in male and female rats of all compound-treatment groups in the P1 generation and in the mid and high dosage groups of the F1 generation. There was a low incidence of a similar type thyroid change in a few control animals, but the incidence and severity of the above-mentioned groups was considered to have been increased in a dose-related manner and to be compound-related.

All other microscopic changes observed in the other organs and tissues specified for examination from the P1 and F1 generation parental male and female rats given 30 mg/kg/day of ammonium perchlorate were considered to have occurred spontaneously and to be unrelated to treatment.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
ADRENAL GLANDS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	24	0	0	19	20	0	0	18
-hypertrophy, cortical, focal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[1]
minimal	0	0	0	1	1	0	0	1
-vacuolation, cortical, focal	[6]	[0]	[0]	[11]	[8]	[0]	[0]	[5]
minimal	4	0	0	7	8	0	0	5
mild	2	0	0	4	0	0	0	0
AORTA:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
BONE (FEMUR):								
NO. EXAMINED	29	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	23
-cyst(s), epiphysis	0	0	0	0	0	0	0	1
-fibrosis, epiphysis, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
BONE MARROW (FEMUR):								
NO. EXAMINED	29	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	24
-lymphoma, malignant	0	0	0	1	0	0	0	0
BRAIN:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
CECUM:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	28	0	0	30	24	0	0	23
-edema, submucosa	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
-hyperplasia, lymphoid	[2]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
mild	2	0	0	0	0	0	0	0
-inflammation, chronic, mucosa	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
-parasite(s), lumen	0	0	0	0	2	0	0	1
CERVIX:								
NO. EXAMINED					28	0	0	23
NO. NORMAL					28	0	0	23

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
COLON:								
NO. EXAMINED	30	0	0	30	28	0	0	23
NO. NORMAL	29	0	0	30	27	0	0	18
-parasite(s), lumen	1	0	0	0	1	0	0	5
DUODENUM:								
NO. EXAMINED	29	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	30	28	0	0	24
EAR(S):								
NO. EXAMINED	0	0	0	0	4	2	3	0
NO. NORMAL	0	0	0	0	1	0	0	0
-chondritis, auricular	[0]	[0]	[0]	[0]	[2]	[2]	[3]	[0]
mild	0	0	0	0	0	0	1	0
moderate	0	0	0	0	2	0	0	0
marked	0	0	0	0	0	2	2	0
-dermatitis, chronic	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
EPIDIDYMIDES:								
NO. EXAMINED	30	1	1	30				
NO. NORMAL	17	0	0	19				
-hypospermia	[0]	[0]	[1]	[0]				
marked	0	0	1	0				
-infiltration, mononuclear-cell, focal	[13]	[0]	[1]	[11]				
minimal	13	0	1	11				
-necrotic germ cells	0	1	0	0				
ESOPHAGUS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
EYES:								
NO. EXAMINED	30	1	1	30	28	0	0	24
NO. NORMAL	30	0	0	28	28	0	0	24
-atrophy, retinal, focal	[0]	[1]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	1	0	2	0	0	0	0
-keratitis, chronic	[0]	[0]	[1]	[0]	[0]	[0]	[0]	[0]
mild	0	0	1	0	0	0	0	0
-mineralization, cornea, focal	[0]	[0]	[1]	[0]	[0]	[0]	[0]	[0]
minimal	0	0	1	0	0	0	0	0
HEART:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	21	0	0	15	28	0	0	24

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
HEART (Continued):								
-fibrosis, myocardial, focal	[7]	[0]	[0]	[10]	[0]	[0]	[0]	[0]
minimal	7	0	0	8	0	0	0	0
mild	0	0	0	2	0	0	0	0
-hemosiderosis	[4]	[0]	[0]	[6]	[0]	[0]	[0]	[0]
minimal	4	0	0	6	0	0	0	0
-inflammation, chronic, multifocal	[3]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	3	0	0	5	0	0	0	0
ILEUM:								
NO. EXAMINED	30	0	0	28	27	0	0	24
NO. NORMAL	29	0	0	28	27	0	0	24
-Advanced autolysis precludes evaluation	1	0	0	0	0	0	0	0
JEJUNUM:								
NO. EXAMINED	30	0	0	29	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	24
-Advanced autolysis precludes evaluation	1	0	0	0	0	0	0	0
KIDNEYS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	14	0	0	17	23	0	0	16
-cyst(s), medulla	2	0	0	0	0	0	0	0
-degeneration/basophilia, cortical tubules, focal	[7]	[0]	[0]	[7]	[0]	[0]	[0]	[0]
minimal	6	0	0	6	0	0	0	0
mild	1	0	0	1	0	0	0	0
-dilatation, cortical tubules	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	0	0	0	0	0	0	0	1
-dilatation, pelvis	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
mild	0	0	0	1	0	0	0	0
-fibrosis, focal	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	0	0	0	0	0	0	0	1
-glomerulonephritis, chronic	[1]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	1	0	0	3	0	0	0	0
-hyaline droplets, cortical tubules	[2]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
mild	2	0	0	1	0	0	0	0
moderate	0	0	0	1	0	0	0	0
-infiltration, mononuclear-cell, focal/multifocal	[10]	[0]	[0]	[5]	[2]	[0]	[0]	[1]
minimal	10	0	0	5	2	0	0	1

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
KIDNEYS (Continued):								
-mineralization, multifocal	[0]	[0]	[0]	[0]	[2]	[0]	[0]	[6]
minimal	0	0	0	0	2	0	0	6
-mineralization, pelvis	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
-vacuolation, cortical tubules	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
mild	0	0	0	0	0	0	0	1
LIVER:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	18	0	0	19	24	0	0	19
-cellular alteration, clear-cell, focal	[1]	[0]	[0]	[1]	[0]	[0]	[0]	[1]
minimal	1	0	0	1	0	0	0	1
-hematopoiesis, extramedullary	[1]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	2	0	0	0	0
-inflammation, chronic, focal/multifocal	[9]	[0]	[0]	[9]	[4]	[0]	[0]	[3]
minimal	9	0	0	9	4	0	0	3
-necrosis, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[2]
minimal	1	0	0	0	0	0	0	2
-vacuolation, hepatocellular, periportal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
LUNG:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	19	0	0	17	17	0	0	12
-alveolitis, acute, focal/multifocal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-hypertrophy, arterial	[1]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
mild	0	0	0	1	0	0	0	0
moderate	1	0	0	0	0	0	0	0
-infiltration, eosinophilic, perivascular and peribronchial	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[2]
minimal	0	0	0	0	0	0	0	2
-inflammation, interstitial, focal	[1]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	1	0	0	1	1	0	0	0
-macrophages, alveoli, focal	[9]	[0]	[0]	[11]	[9]	[0]	[0]	[11]
minimal	9	0	0	9	8	0	0	10
mild	0	0	0	2	1	0	0	1

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
LUNG (Continued):								
-proliferation, lymphoid, peribronchial/perivascular	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
LYMPH NODE, MANDIBULAR:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	18	0	0	18	11	0	0	8
-hyperplasia, lymphocytic/plasmacytic	[12]	[0]	[0]	[12]	[17]	[0]	[0]	[16]
minimal	5	0	0	5	5	0	0	5
mild	2	0	0	5	3	0	0	6
moderate	5	0	0	2	9	0	0	5
-lymphadenopathy, cystic	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
LYMPH NODE, MESENTERIC:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	27	0	0	30	23	0	0	22
-congestion/erythrophagocytosis	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
-edema	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
moderate	0	0	0	0	1	0	0	0
-hemorrhage	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
mild	1	0	0	0	0	0	0	0
-histiocytosis	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[2]
mild	0	0	0	0	3	0	0	1
moderate	0	0	0	0	0	0	0	1
-hyperplasia, lymphoid	[1]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	1	0	0	0	1	0	0	0
LYMPHORETICULAR SYSTEM:								
-lymphoma, malignant	0	0	0	1	0	0	0	0
MAMMARY GLAND:								
NO. EXAMINED	19	0	0	22	28	1	0	24
NO. NORMAL	19	0	0	22	26	0	0	23
-adenocarcinoma	0	0	0	0	0	1/2	0	0
-cystic gland/duct	0	0	0	0	1	0	0	1

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
MAMMARY GLAND (Continued):								
-inflammation, subacute	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
MUSCLE, SKELETAL:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	24
-inflammation, chronic, focal	[1]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
mild	1	0	0	0	0	0	0	0
NERVE, SCIATIC:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
OVARIES:								
NO. EXAMINED					28	0	0	24
NO. NORMAL					28	0	0	24
PALATE:								
NO. EXAMINED	0	0	0	1	0	0	0	0
NO. NORMAL	0	0	0	0	0	0	0	0
-abscess	0	0	0	1	0	0	0	0
-fracture	0	0	0	1	0	0	0	0
PANCREAS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	17	0	0	19	28	0	0	24
-atrophy, acinar, focal	[3]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	2	0	0	4	0	0	0	0
moderate	1	0	0	1	0	0	0	0
-fibrosis, islet, focal	[8]	[0]	[0]	[8]	[0]	[0]	[0]	[0]
minimal	3	0	0	2	0	0	0	0
mild	2	0	0	4	0	0	0	0
moderate	3	0	0	2	0	0	0	0
-hemorrhage	[3]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	2	0	0	0	0	0	0	0
mild	1	0	0	0	0	0	0	0
-hypertrophy, acinar-cell, focal	[2]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	2	0	0	0	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
PANCREAS (Continued):								
-inflammation, chronic, focal/multifocal	[5]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	3	0	0	2	0	0	0	0
mild	1	0	0	0	0	0	0	0
moderate	1	0	0	1	0	0	0	0
-microgranuloma(s)	1	0	0	0	0	0	0	0
PARATHYROID:								
NO. EXAMINED	27	0	0	25	24	0	0	23
NO. NORMAL	26	0	0	25	24	0	0	23
-ectopic thymic tissue	1	0	0	0	0	0	0	0
PITUITARY:								
NO. EXAMINED	30	0	0	30	28	0	0	23
NO. NORMAL	9	0	0	7	28	0	0	23
-cyst(s), pars distalis	1	0	0	0	0	0	0	0
-hypertrophy/vacuolation, pars distalis	[21]	[0]	[0]	[23]	[0]	[0]	[0]	[0]
minimal	12	0	0	15	0	0	0	0
mild	6	0	0	6	0	0	0	0
moderate	3	0	0	2	0	0	0	0
PROSTATE:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	21	0	0	23				
-prostatitis, interstitial, chronic	[7]	[0]	[0]	[7]				
minimal	5	0	0	6				
mild	2	0	0	1				
-prostatitis, suppurative	[2]	[0]	[0]	[0]				
minimal	1	0	0	0				
mild	1	0	0	0				
RECTUM:								
NO. EXAMINED	29	0	0	29	28	0	0	24
NO. NORMAL	29	0	0	28	25	0	0	23
-parasite(s), lumen	0	0	0	1	3	0	0	1
SALIVARY GLAND:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
SEMINAL VESICLES:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	30	0	0	30				
SKIN (ROUTINE SECTION):								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	20	0	0	18

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
SKIN (ROUTINE SECTION) (Continued):								
-dermatitis, chronic	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[2]
minimal	0	0	0	0	1	0	0	1
mild	0	0	0	0	2	0	0	0
moderate	0	0	0	0	0	0	0	1
-hyperplasia/hyperkeratosis, epidermis	[0]	[0]	[0]	[0]	[8]	[0]	[0]	[4]
mild	0	0	0	0	4	0	0	2
moderate	0	0	0	0	4	0	0	2
-necrosis, epidermis, focal	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
mild	0	0	0	0	0	0	0	1
SKIN (GROSS LESION):								
NO. EXAMINED	1	0	0	0	0	0	0	0
NO. NORMAL	0	0	0	0	0	0	0	0
-fibroma	1	0	0	0	0	0	0	0
SPINAL CORD, CERVICAL:								
NO. EXAMINED	30	0	0	30	28	0	0	21
NO. NORMAL	30	0	0	30	28	0	0	21
SPINAL CORD, LUMBAR:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	29	28	0	0	24
-cyst(s), epidermal	0	0	0	1	0	0	0	0
SPINAL CORD, THORACIC:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
SPLEEN:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	21	0	0	18	27	0	0	24
-cyst(s)	0	0	0	0	1	0	0	0
-hematopoiesis, extramedullary	[9]	[0]	[0]	[10]	[0]	[0]	[0]	[0]
minimal	6	0	0	7	0	0	0	0
mild	3	0	0	3	0	0	0	0
-hemosiderosis	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
mild	0	0	0	2	0	0	0	0
-lymphoma, malignant	0	0	0	1	0	0	0	0
STOMACH:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	22	0	0	26	25	0	0	21
-cystic gland(s), glandular mucosa	0	0	0	0	1	0	0	0

[ ] = *Total incidence of specified lesion, all grades.*

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
STOMACH (Continued):								
-dilatation, mucosal glands	[7]	[0]	[0]	[3]	[2]	[0]	[0]	[3]
minimal	4	0	0	2	2	0	0	3
mild	2	0	0	1	0	0	0	0
moderate	1	0	0	0	0	0	0	0
-infiltration, mixed inflammatory cell, submucosa	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-infiltration, polymorphonuclear, submucosa	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
TESTIS:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	30	0	0	30				
THYMUS:								
NO. EXAMINED	30	0	0	30	28	0	0	23
NO. NORMAL	27	0	0	28	23	0	0	19
-atrophy	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[1]
moderate	0	0	0	0	1	0	0	1
-congestion	[3]	[0]	[0]	[2]	[0]	[0]	[0]	[2]
minimal	1	0	0	1	0	0	0	2
mild	2	0	0	1	0	0	0	0
-cyst(s)	0	0	0	0	4	0	0	1
THYROID:								
NO. EXAMINED	30	30	30	30	28	21	26	24
NO. NORMAL	19	18	3	0	20	5	4	0
-Advanced autolysis precludes evaluation	1	0	0	0	0	0	0	0
-adenoma, follicular	1	0	0	0	0	0	0	0
-follicle(s), cystic	2	0	0	0	1	0	1	0
-hyperplasia, C-cell, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-hyperplasia/hypertrophy, follicular epithelium	[2]	[9]	[25]	[30]	[4]	[10]	[20]	[24]
minimal	0	2	1	0	2	4	4	0
mild	1	5	5	0	1	3	8	0
moderate	1	2	11	1	1	3	8	2
marked	0	0	8	29	0	0	0	22
-inflammation, chronic, focal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
-ultimobranchial body/cyst	6	6	4	1	4	8	8	3

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
TRACHEA:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	24	0	0	23	26	0	0	22
-cystic tracheal glands	0	0	0	1	0	0	0	0
-tracheitis, chronic, focal	[6]	[0]	[0]	[6]	[2]	[0]	[0]	[2]
minimal	6	0	0	6	2	0	0	2
URINARY BLADDER:								
NO. EXAMINED	29	0	0	28	27	0	0	24
NO. NORMAL	28	0	0	28	27	0	0	24
-infiltration, mononuclear-cell, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
UTERUS:								
NO. EXAMINED					28	0	0	24
NO. NORMAL					7	0	0	8
-distention, lumen					[3]	[0]	[0]	[0]
minimal					2	0	0	0
mild					1	0	0	0
-macrophages, pigmented					[21]	[0]	[0]	[16]
minimal					3	0	0	1
mild					5	0	0	2
moderate					12	0	0	13
marked					1	0	0	0
VAGINA:								
NO. EXAMINED					28	0	0	24
NO. NORMAL					28	0	0	24
ZYMBAL'S GLAND:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	28	0	0	28	28	0	0	24
-cyst(s)	2	0	0	2	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
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TABLE 2

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
ABDOMINAL CAVITY:								
NO. EXAMINED	0	0	0	0	0	0	0	1
NO. NORMAL	0	0	0	0	0	0	0	1
ADRENAL GLANDS:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	24	0	0	23	14	0	0	15
-hypertrophy, cortical, focal	[2]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	2	0	0	0	0	0	0	0
-hypertrophy, zona glomerulosa	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
mild	0	0	0	0	0	0	0	1
-vacuolation, cortical, focal	[4]	[0]	[0]	[7]	[6]	[0]	[0]	[9]
minimal	3	0	0	1	6	0	0	8
mild	0	0	0	4	0	0	0	1
moderate	1	0	0	2	0	0	0	0
AORTA:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
BONE (FEMUR):								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	30	20	0	0	25
-cyst(s), epiphysis	1	0	0	0	0	0	0	0
BONE MARROW (FEMUR):								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
BRAIN:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
CECUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	27	19	0	0	25
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-hyperplasia, lymphoid	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	0	0	2	0	0	0	0
-inflammation, chronic, mucosa	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
mild	1	0	0	0	0	0	0	0
-parasite(s), lumen	0	0	0	0	1	0	0	0
CERVIX:								
NO. EXAMINED					19	0	0	25
NO. NORMAL					19	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
COLON:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	19	0	0	23
-parasite(s), lumen	0	0	0	0	1	0	0	2
DUODENUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
EAR(S):								
NO. EXAMINED	0	1	0	0	2	0	1	3
NO. NORMAL	0	0	0	0	0	0	0	0
-chondritis, auricular	[0]	[1]	[0]	[0]	[2]	[0]	[1]	[3]
mild	0	1	0	0	0	0	0	0
moderate	0	0	0	0	0	0	1	1
marked	0	0	0	0	2	0	0	2
EPIDIDYMIDES:								
NO. EXAMINED	30	0	1	30				
NO. NORMAL	21	0	0	26				
-hypospermia	[1]	[0]	[1]	[0]				
marked	1	0	1	0				
-infiltration, mononuclear-cell, focal	[8]	[0]	[0]	[4]				
minimal	8	0	0	4				
ESOPHAGUS:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	30	0	0	30	19	0	0	24
-dilatation	0	0	0	0	0	0	0	1
EYES:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	28	19	0	0	25
-atrophy, retinal, diffuse	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
moderate	0	0	0	0	1	0	0	0
marked	0	0	0	1	0	0	0	0
-keratitis, chronic, focal	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
-mineralization, cornea, focal	[1]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	2	0	0	0	0
HEART:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	19	0	0	18	20	0	0	23

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
HEART (Continued):								
-fibrosis, myocardial, focal	[6]	[0]	[0]	[6]	[0]	[0]	[0]	[1]
minimal	6	0	0	3	0	0	0	1
mild	0	0	0	3	0	0	0	0
-hemosiderosis	[5]	[0]	[0]	[6]	[0]	[0]	[0]	[0]
minimal	5	0	0	5	0	0	0	0
mild	0	0	0	1	0	0	0	0
-inflammation, chronic, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
-inflammation, chronic, multifocal	[6]	[0]	[0]	[6]	[0]	[0]	[0]	[1]
minimal	6	0	0	5	0	0	0	1
mild	0	0	0	1	0	0	0	0
ILEUM:								
NO. EXAMINED	30	0	0	29	20	0	0	25
NO. NORMAL	30	0	0	29	20	0	0	25
JEJUNUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	29	20	0	0	25
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
KIDNEYS:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	12	0	0	14	19	0	0	20
-cast(s), granular	0	0	0	1	0	0	0	0
-cyst(s), cortex	1	0	0	2	0	0	0	1
-cyst(s), medulla	1	0	0	0	1	0	0	0
-degeneration/basophilia, cortical tubules, focal	[3]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	3	0	0	5	0	0	0	0
-dilatation, cortical tubules	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	0	0	2	0	0	0	0
-dilatation, medullary tubules	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-dilatation, pelvis	[2]	[0]	[0]	[5]	[1]	[0]	[0]	[0]
mild	2	0	0	5	1	0	0	0
-dilatation, tubules, papilla	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
KIDNEYS (Continued):								
-glomerulonephritis, chronic	[2]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	1	0	0	0	0
mild	1	0	0	1	0	0	0	0
-hyaline droplets, cortical tubules	[9]	[0]	[0]	[7]	[0]	[0]	[0]	[0]
minimal	3	0	0	0	0	0	0	0
mild	5	0	0	3	0	0	0	0
moderate	1	0	0	4	0	0	0	0
-infiltration, mononuclear-cell, focal/multifocal	[2]	[0]	[0]	[5]	[0]	[0]	[0]	[1]
minimal	2	0	0	5	0	0	0	1
-mineralization, multifocal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	1	0	0	0	0	0	0	1
-mineralization, pelvis	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[2]
minimal	0	0	0	0	0	0	0	2
LIVER:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	13	0	0	16	13	0	0	22
-hematopoiesis, extramedullary	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
-inflammation, chronic, focal/multifocal	[17]	[0]	[0]	[13]	[6]	[0]	[0]	[3]
minimal	17	0	0	12	6	0	0	3
mild	0	0	0	1	0	0	0	0
LUNG:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	18	0	0	23	5	0	0	13
-infiltration, eosinophilic, perivascular and peribronchial	[0]	[0]	[0]	[0]	[2]	[0]	[0]	[1]
minimal	0	0	0	0	2	0	0	1
-inflammation, interstitial, acute, focal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[3]
minimal	0	0	0	1	1	0	0	3
-inflammation, interstitial, chronic, focal	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	0	0	0	0	0	0	0	1
-macrophages, alveoli, focal	[10]	[0]	[0]	[6]	[12]	[0]	[0]	[9]
minimal	8	0	0	5	11	0	0	8
mild	2	0	0	1	1	0	0	1
-proliferation, lymphoid, peribronchial/perivascular	[3]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	3	0	0	2	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
LYMPH NODE, MANDIBULAR:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	16	0	0	16	9	0	0	10
-hyperplasia, lymphocytic/plasmacytic	[14]	[0]	[0]	[14]	[11]	[0]	[0]	[15]
minimal	7	0	0	6	3	0	0	8
mild	3	0	0	3	2	0	0	5
moderate	4	0	0	4	6	0	0	2
marked	0	0	0	1	0	0	0	0
LYMPH NODE, MESENTERIC:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	28	14	0	0	18
-congestion	[0]	[0]	[0]	[0]	[2]	[0]	[0]	[0]
minimal	0	0	0	0	2	0	0	0
-histiocytosis	[0]	[0]	[0]	[2]	[4]	[0]	[0]	[7]
minimal	0	0	0	1	2	0	0	3
mild	0	0	0	1	2	0	0	4
-hyperplasia, lymphoid	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
MAMMARY GLAND:								
NO. EXAMINED	22	0	0	24	20	0	0	24
NO. NORMAL	22	0	0	24	20	0	0	23
-cystic gland/duct	0	0	0	0	0	0	0	1
MUSCLE, SKELETAL:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	30	20	0	0	25
-inflammation, chronic, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
NERVE, SCIATIC:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
OVARIES:								
NO. EXAMINED					20	0	0	25
NO. NORMAL					19	0	0	25
-cyst(s)					1	0	0	0
PANCREAS:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	16	0	0	18	20	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
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TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
PANCREAS (Continued):								
-atrophy, acinar, focal	[2]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	1	0	0	0	0
mild	1	0	0	1	0	0	0	0
-fibrosis, islet, focal	[9]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	5	0	0	4	0	0	0	0
mild	4	0	0	1	0	0	0	0
-hemorrhage	[3]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	2	0	0	2	0	0	0	0
mild	1	0	0	0	0	0	0	0
-hypertrophy, acinar-cell, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-inflammation, acute, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-inflammation, chronic, focal/multifocal	[5]	[0]	[0]	[6]	[0]	[0]	[0]	[0]
minimal	5	0	0	4	0	0	0	0
mild	0	0	0	2	0	0	0	0
-inflammation, subacute, focal	[3]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	3	0	0	0	0	0	0	0
PARATHYROID:								
NO. EXAMINED	25	0	0	27	19	0	0	25
NO. NORMAL	25	0	0	27	19	0	0	25
PITUITARY:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	17	0	0	20	20	0	0	25
-cyst(s), pars distalis	1	0	0	0	0	0	0	0
-hyperplasia, pars distalis, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-hypertrophy/vacuolation, pars distalis	[13]	[0]	[0]	[9]	[0]	[0]	[0]	[0]
minimal	10	0	0	4	0	0	0	0
mild	1	0	0	4	0	0	0	0
moderate	2	0	0	1	0	0	0	0
PROSTATE:								
NO. EXAMINED	30	0	1	30				
NO. NORMAL	21	0	0	24				

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
PROSTATE (Continued):								
-prostatitis, interstitial, chronic	[9]	[0]	[0]	[6]				
minimal	5	0	0	5				
mild	4	0	0	0				
moderate	0	0	0	1				
-prostatitis, suppurative	[0]	[0]	[1]	[0]				
marked	0	0	1	0				
RECTUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	16	0	0	25
-parasite(s), lumen	0	0	0	0	4	0	0	0
SALIVARY GLAND:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
SEMINAL VESICLES:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	30	0	0	30				
SKIN (ROUTINE SECTION):								
NO. EXAMINED	29	0	0	29	20	0	0	24
NO. NORMAL	28	0	0	29	14	0	0	16
-dermatitis, chronic, focal	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
mild	0	0	0	0	1	0	0	0
moderate	0	0	0	0	1	0	0	0
-hyperplasia, epidermis	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
-hyperplasia/hyperkeratosis, epidermis	[0]	[0]	[0]	[0]	[4]	[0]	[0]	[8]
minimal	0	0	0	0	3	0	0	2
mild	0	0	0	0	1	0	0	5
moderate	0	0	0	0	0	0	0	1
-necrosis, focal	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
moderate	0	0	0	0	1	0	0	0
SKIN (GROSS LESION):								
NO. EXAMINED	0	0	1	0	0	0	1	0
NO. NORMAL	0	0	0	0	0	0	0	0
-dermatitis, chronic, focal	[0]	[0]	[1]	[0]	[0]	[0]	[1]	[0]
minimal	0	0	0	0	0	0	1	0
moderate	0	0	1	0	0	0	0	0
SPINAL CORD, CERVICAL:								
NO. EXAMINED	29	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	30	20	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
SPINAL CORD, LUMBAR:								
NO. EXAMINED	30	0	0	29	20	0	0	25
NO. NORMAL	30	0	0	29	20	0	0	25
SPINAL CORD, THORACIC:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
SPLEEN:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	22	0	0	20	16	0	0	23
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-hematopoiesis, extramedullary	[1]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	1	0	0	3	0	0	0	0
-hemosiderosis	[8]	[0]	[0]	[6]	[4]	[0]	[0]	[2]
minimal	2	0	0	2	1	0	0	2
mild	5	0	0	3	3	0	0	0
moderate	1	0	0	1	0	0	0	0
STOMACH:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	24	0	0	24	17	0	0	18
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-cyst(s), squamous	1	0	0	0	0	0	0	0
-dilatation, mucosal glands	[5]	[0]	[0]	[5]	[3]	[0]	[0]	[7]
minimal	3	0	0	3	1	0	0	7
mild	2	0	0	2	2	0	0	0
TESTIS:								
NO. EXAMINED	30	0	1	30				
NO. NORMAL	29	0	0	30				
-atrophy, diffuse	[1]	[0]	[1]	[0]				
marked	1	0	1	0				
THYMUS:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	24	0	0	21	14	0	0	20
-atrophy	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[2]
minimal	0	0	0	0	0	0	0	1
mild	0	0	0	0	3	0	0	1
-congestion	[6]	[0]	[0]	[9]	[0]	[0]	[0]	[1]
minimal	4	0	0	4	0	0	0	1
mild	1	0	0	4	0	0	0	0
moderate	1	0	0	1	0	0	0	0
-cyst(s)	0	0	0	0	2	0	0	2

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
THYROID:								
NO. EXAMINED	30	30	30	30	20	27	27	25
NO. NORMAL	21	14	7	0	5	16	8	0
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-adenoma, follicular	0	0	0	1	0	0	0	0
-hyperplasia, follicular, focal/nodular	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
marked	0	0	0	1	0	0	0	0
-hyperplasia/hypertrophy, follicular epithelium	[5]	[8]	[19]	[26]	[6]	[6]	[13]	[24]
minimal	0	0	3	0	4	5	6	1
mild	3	4	5	0	2	1	6	2
moderate	2	4	5	3	0	0	1	10
marked	0	0	6	23	0	0	0	11
-ultimobranchial body/cyst	7	8	6	6	13	9	15	8
TRACHEA:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	27	0	0	28	18	0	0	24
-tracheitis, chronic, focal	[3]	[0]	[0]	[2]	[1]	[0]	[0]	[1]
minimal	3	0	0	2	1	0	0	1
URINARY BLADDER:								
NO. EXAMINED	30	0	0	29	20	0	0	25
NO. NORMAL	30	0	0	27	20	0	0	25
-infiltration, mononuclear-cell, focal	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	0	0	2	0	0	0	0
UTERUS:								
NO. EXAMINED					20	0	0	25
NO. NORMAL					1	0	0	2
-deciduoma					1	0	0	0
-distention, lumen					[3]	[0]	[0]	[3]
minimal					0	0	0	1
mild					2	0	0	1
moderate					1	0	0	1
-macrophages, pigmented					[17]	[0]	[0]	[22]
minimal					1	0	0	3
mild					7	0	0	8
moderate					9	0	0	11
VAGINA:								
NO. EXAMINED					19	0	0	25
NO. NORMAL					19	0	0	25
ZYMBAL'S GLAND:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	29	20	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
ZYMBAL'S GLAND (Continued):								
-cyst(s)	1	0	0	1	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

February 8, 1999 EPA Assessment Submission

Attachment #4
Review of 14-Day Exposure on Antibody Response to SRBC in Mice

A. SRBC Specific Serum IgM Antibody Response (Keil,1999b)
B. Preliminary EPA analysis (Smialowicz, 1999b)

ATTENTION PANEL MEMBER(S):

KIMBER WHITE

Immunotoxicity Studies at Medical University of South Carolina

(14-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"C, G, I, J, T, K"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured. Supplementary experiments were needed to acquire additional serum samples for hormone analysis or to repeat the NK assay.
"U, V"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and were challenged with listeria to assess **delayed type hypersensitivity**.
"H, F, M"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
SRBC Experiments	B6C3F1 female mice (two experiments of 30 mice each) were exposed to **90 days of AP** (0, 0.1, 3.0, or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 75, bled on day 79 to determine specific IgM antibody levels, and bled on day 90 to determine specific IgG antibody levels.

Immunotoxicity Studies at Medical University of South Carolina (90-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"A, D, N"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of immune, hematological, or thyroid parameters were measured. In experiments "A" and "D", thyroid histopathology was performed. Experiment "N" included a variety of other parameters: macrophage phagocytosis and nitrite production, NKh, assay organ weights and cellularities, flow cytometry, and serum for hormone analysis.
"B, E"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured.
"P"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
"Q"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with **B16F10 melanomas** on day 76.
"L"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with ***Listeria monocytogenes*** on day 86.
SRBC Experiments	B6C3F1 Female mice (1 experiment of 30 mice) were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0 or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 9 and bled on day 14 to determine specific IgM antibody levels.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Experimental Toxicology Division
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

DATE: February 5, 1999

FROM: Ralph J. Smialowicz (MD-92) RJ. Smialowicz

TO: Annie Jarabek (MD-52)
National Center for Environmental Assessment

SUBJECT: Review of 14-Day Ammonium Perchlorate Exposure on the Antibody Response to SRBC in Mice

Results of a 14-day study were received on February 4, 1999 for review. In this study, B6C3F1 female mice, 6 mice per group, were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 14 days. Mice where immunized intravenously with SRBC on day 9. Serum was collected on day 14 (5 days post-immunization)and the SRBC-specific IgM antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA). The ELISA data were analyzed "as described by L. Temple of the Medical College of Virginia", and expressed as the O.D. 50. Analysis of the ELISA data indicated that the IgM titers were not affected in mice exposed to ammonium perchlorate for 14 days compared to controls.

SRBC Specific Serum IgM Determination after Exposure to Ammonium Perchlorate for 14 Days

Submitted by Deborah Keil, PhD
Medical University of South Carolina
February 4, 1999

Animals and Ammonium Perchlorate Exposure: B6C3F1 female mice aged 8-10 weeks were exposed to ammonium perchlorate (AP) (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 14 days. A total of 30 mice with 6 animals per treatment group were used to determine specific IgM levels after immunization with sRBC. Animals were housed in an AAALAC accredited facility and provided water (with and without AP) and mouse chow ad libidum.

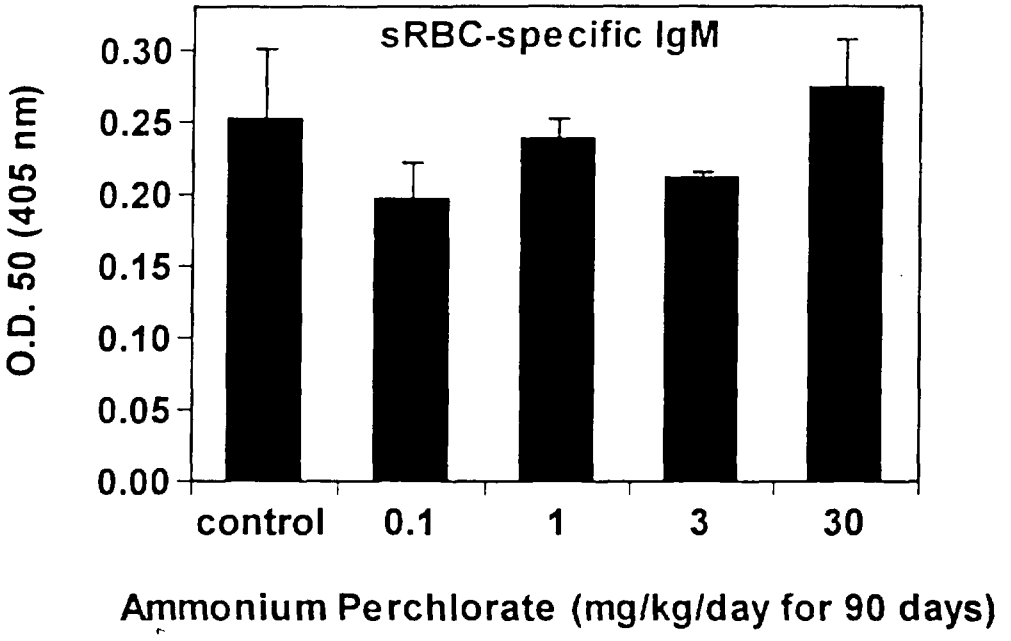
Immunization: Mice were immunized with sheep red blood cells (sRBC) ($1x10^8$ total cells) by intravenous tail injection on day 9. Serum was collected on day 14 (5 days post challenge) to determine specific IgM antibody levels, respectively. A semi-quantitative ELISA detected levels of specific IgM sRBC antibody in serially diluted serum (1:10, 1:20, 1:40, 1:80, 1:160).

Optimization of the ELISA: Optimization of the ELISA was performed prior to testing the serum samples to establish the appropriate titer of sRBC membrane coating antigen (1μg/ml) and the secondary antibody dilution (1:5,000 for IgM). In addition, pooled serum samples from controls were used in the optimization. Controls for non-specific binding were included and were approximately 0.070 O.D. (405 nm) in both the optimization and testing ELISAs.

Data Analysis:
Analysis of sRBC specific IgG serum titers were analyzed as described by L. Temple of the Medical College of Virginia. The average absorbance unit values of the replicates for each dilution of the test serum were calculated. Background in the ELISA was subtracted from these values. Five consecutive average absorbance values versus log base 2 of the dilution of the serum were plotted. The best-fit linear line was calculated in an Excel spreadsheet by determining the value for the slope and intercept. Log base 2 of the titer was considered the independent variable and O.D. was considered the dependent variable. In this experiment, the absorbance at the mid-point of the 5 serial dilutions was 1:40. Using the equation for the best-fit line, the O.D. 50 (absorbance at mid-point 1:40) was calculated for each animal.

Results:
No significant differences in specific sRBC IgM antibody were observed in any of the AP treatment groups as compared to controls. This is consistent with results obtained after 90-day exposure to AP. The results were expressed as the O.D.50 and an analysis of variance was performed using Tukey's pairwise comparisons ($p<0.05$). Refer to graphs and statistical analysis that have been included in this report.

Serum IgM Levels after sRBC Challenge During a 14-Day Exposure to Ammonium Perchlorate



Adult B6C3F1 female mice were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 14 days. On day 9 (5 days before serum was collected), animals were immunized by i.v. tail injection with sRBC (1×10^8 cells). Detection of specific IgM was performed using an ELISA based on a protocol by L. Temple at the Medical College of Virginia. The O.D. 50 was determined for IgM. The above graph represents the means and standard errors of 6 animals per group for a total of 30 mice. No significant differences were observed in any of the treatment groups as compared to controls using analysis of variance and Tukey's pairwise comparisons ($p<0.05$).

Statistics

The calculated O.D. 50 for each of the treatment groups was compared to controls ($p<0.05$). A total of 30 serum samples from independently challenged mice (6 mice per group) were analyzed for both IgM levels.

Descriptive Statistics

Variable	N	Mean	Median	TrMean	StDev	SE Mean
control	6	0.2512	0.2275	0.2512	0.1216	0.0496
0.1	6	0.1958	0.1965	0.1958	0.0624	0.0255
1.0	6	0.2373	0.2285	0.2373	0.0359	0.0147
3.0	6	0.21067	0.21250	0.21067	0.01073	0.00438
30	6	0.2730	0.2850	0.2730	0.0832	0.0340

Variable	Minimum	Maximum	Q1	Q3
control	0.1250	0.4790	0.1707	0.3162
0.1	0.1250	0.2860	0.1302	0.2508
1.0	0.1980	0.2840	0.2063	0.2780
3.0	0.19800	0.22400	0.19875	0.21950
30	0.1760	0.3620	0.1827	0.3493

One-way Analysis of Variance

Analysis of Variance for IgM 14d

Source	DF	SS	MS	F	P
treatmen	4	0.02296	0.00574	1.06	0.395
Error	25	0.13499	0.00540		
Total	29	0.15795			

```
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
 0.0        6   0.25117   0.12159            (---------*---------)
 0.1        6   0.19583   0.06237  (----------*---------)
 1.0        6   0.23733   0.03590         (----------*---------)
 3.0        6   0.21067   0.01073     (---------*---------)
30.0        6   0.27300   0.08318                (----------*---------)
                                   --------+---------+---------+--------
Pooled StDev =  0.07348                  0.180     0.240     0.300
```

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.00706

Critical value = 4.15

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.06916 0.17983			
1.0	-0.11066 0.13833	-0.16599 0.08299		
3.0	-0.08399 0.16499	-0.13933 0.10966	-0.09783 0.15116	
30.0	-0.14633 0.10266	-0.20166 0.04733	-0.16016 0.08883	-0.18683 0.06216

February 8, 1999 EPA Assessment Submission

Attachment #5
Correlations of Hormone and Histopathology Data from Caldwell et al. (1995), Neurodevelopmental Study (Argus, 1998a), and Subchronic Study (Springborn, 1998) with Percent Iodide Inhibition (Meyer, 1998; Channel, 1999)

A. Iodide Inhibition 14-day study (Channel, 1999)
B. Preliminary EPA analysis (Geller, 1999d)

ATTENTION PANEL MEMBER(S):

MEL ANDERSEN

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 8 February 1999

Subject: Correlations of Hormone and Histopathology Data from Caldwell et. al. (1995), Neurobehavioral Developmental (Argus, 1998a), and Subchronic (Springborn, 1998) Studies with Percent Iodide Uptake Inhibition (Meyer, 1998).

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Correlations and linear regressions were run, as requested, to relate estimated administered dose or iodide uptake inhibition associated with a particular dose of ammonium perchlorate to thyroxine (T4), 3,5,3'-triiodothyronine (T3), and thyrotrophin (TSH) in the Caldwell 14-day Study, the Neurobehavioral Developmental Study, and the Subchronic Study. These were run to begin to evaluate the efficacy of using the inhibition of iodide uptake by the thyroid as an internal dosimeter rather than using estimated administered dose.

The results of two studies were considered to establish the relationship of administered dose of ammonium perchlorate and iodide uptake inhibition. Meyer (1998) measured iodide uptake inhibition 9 hours after the acute i.v. administration of a single dose of ammonium perchlorate was used in these calculations. This study showed a dose-related decrease in iodide uptake by the thyroid. Channel (1999) measured iodide uptake inhibition in adult male animals that had been dosed for 14 days with ammonium perchlorate in the drinking water. This study did not show a systematic change in iodide uptake with administered dose of perchlorate.

Caldwell 14 Day study: Note that these correlations are run using only 4 of 9 doses (0, 0.1, 1.0, 3.0 mg/kg/day) used in the study, since not all study doses were included in Meyer (1998) and Channel (1999).

Correlations with Meyer (1998): Percentage of iodide uptake inhibition correlated more highly with T4 and T3 levels than did estimated administered dose. The correlation of TSH with administered dose or iodide uptake inhibition was approximately the same (Table 1A).

Correlations with Channel (1999): Correlations with the non-monotonic uptake data (Table 1B) were much worse than those with administered dose or with uptake as established in the Meyer (1998) study.

Neurobehavioral Developmental study: Note that these correlations are run using only 4 of 5 doses (0. 0.1, 1.0, 3.0 mg/kg/day) used in the study, since not all study doses were included in Meyer (1998) and Channel (1999).

Correlations with Meyer (1998), Post-natal day 5 (PND5) pups: Correlations of T3 and T4 with administered dose or iodide uptake inhibition were significant. The correlation of TSH with iodide uptake inhibition was marginally significant. Correlations of administered dose or iodide uptake inhibition with T4 and TSH were approximately equal. T3 correlated more highly with administered dose than iodide uptake inhibition (Table 2A).

Correlations with Channel (1999): Correlations of hormone levels with iodide uptake as estimated by the results reported in Channel (1999) were worse than those with administered dose or the iodide uptake numbers of Meyer (1998).

Subchronic Study: Note that these correlations are run using only 3 of 6 doses (0, 0.01, 1.0 mg/kg/day) used in the study, since not all study doses were included in Meyer (1998) and Channel (1999).

Correlations with Meyer (1998):

14 day timepoint: Correlations of T3 and TSH with administered dose or iodide uptake inhibition were significant. Correlations with T4 were not. Correlations of T4, T3 and TSH were

marginally higher with iodide uptake inhibition than with administered dose (Table 3).

90 day timepoint: Correlations of T4, T3 and TSH were significant with both administered dose and iodide uptake inhibition. Correlations were marginally higher with iodide uptake inhibition than with administered dose (Table 4).

Correlations with Channel (1999):

At both 14 and 90 day timepoints, the correlations with the estimates of iodide uptake from the 14 day drinking water administration of perchlorate were worse than from the other measures of dosimetry discussed in this memo and were of opposite sign.

Discussion: The correlations run in this memo were done with subsets of the data from each of the relevant studies. These subsets were composed of animals from the dose groups in the studies that were identical to those considered in the iodide uptake studies. For future consideration, it may prove useful to fit the monotonic data of Meyer (1998) with an analytic function that would allow for the interpolation of iodide uptake values at all study doses. The data included in Channel (1999) are more problematic. Previous data on absorption, distribution, metabolism, and elimination do not suggest a U-shaped relationship between perchlorate dose and iodide uptake by the thyroid. Further work is necessary to elaborate differences between injected and oral dosing.

Table 3 – Subchronic Study – Correlations of hormone levels from 14 day time point with estimated administered dose of ammonium perchlorate and with % iodide uptake inhibition.

A. Uptake inhibiton derived from 9 hour timepoint of Meyer (1998).

	T4	T3	TSH
Administered Dose	-0.11 0.44	-0.49 0.0002	0.44 0.001
% Iodide Uptake Inhibition	-0.14 0.334	-0.52 0.0001	0.45 0.0007

B. Uptake inhibition derived from 2 hour timepoint of Channel (1999).

	T4	T3	TSH
Administered Dose	-0.11 0.44	-0.49 0.0002	0.44 0.001
% Iodide Uptake Inhibition	0.04 0.77	0.38 0.005	-0.38 0.005

Table 4 – Subchronic Study – Correlations of hormone levels from 90 day time point with estimated administered dose of ammonium perchlorate and with % iodide uptake inhibition

A. Uptake inhibition derived from 9 hour timepoint of Meyer (1998).

	T4	T3	TSH
Administered Dose	-0.62 0.0001	-0.72 0.0001	0.47 0.0002
% Iodide Uptake Inhibition	-0.69 0.0001	-0.77 0.0001	0.48 0.0001

B. Uptake inhibition derived from 2 hour timepoint of Channel (1999).

	T4	T3	TSH
Administered Dose	-0.62 0.0001	-0.72 0.0001	0.47 0.0002
% Iodide Uptake Inhibition	0.42 0.001	0.53 0.001	-0.398 0.002

1 T4, T3, TSH, and Follicle Hyperplasia and follicular lumen 1
area data from Caldwell 14-day perchlorate study with PK internal dose approximation from Meyer, 1998
14:54 Thursday, February 4, 1999

OBS	ID	DOSE	SEX	FH	FL	T4	T3	TSH	DCORR	INTDOSE
1	68	0.00	F	1	1	4.49	124.33	10.92	0.0	0
2	71	0.00	F	1	1	4.50	138.36	10.71	0.0	0
3	86	0.00	F	0	1	5.36	127.89	12.01	0.0	0
4	90	0.00	F	0	1	5.36	133.14	11.40	0.0	0
5	95	0.00	F	0	0	4.94	134.17	10.96	0.0	0
6	100	0.00	F	0	0	5.33	113.17	11.49	0.0	0
7	51	1.25	F	0	0	4.41	93.91	11.96	0.1	29
8	55	1.25	F	.	.	4.37	75.70	11.71	0.1	29
9	58	1.25	F	0	1	4.47	84.17	14.74	0.1	29
10	67	1.25	F	1	0	4.37	85.04	13.42	0.1	29
11	73	1.25	F	0	1	4.13	86.66	13.68	0.1	29
12	84	1.25	F	0	0	4.69	82.11	12.81	0.1	29
13	60	12.50	F	1	1	3.75	94.00	15.45	1.0	55
14	66	12.50	F	1	1	3.84	79.42	12.54	1.0	55
15	70	12.50	F	0	1	4.11	76.16	16.42	1.0	55
16	77	12.50	F	0	0	3.97	77.21	15.93	1.0	55
17	92	12.50	F	1	1	4.11	78.67	16.43	1.0	55
18	99	12.50	F	1	1	3.92	78.61	15.37	1.0	55
19	59	25.00	F	2	1	3.75	73.91	17.17	3.0	82
20	64	25.00	F	1	1	3.96	85.00	17.21	3.0	82
21	76	25.00	F	1	2	3.80	73.47	17.33	3.0	82
22	80	25.00	F	1	1	3.75	74.16	17.55	3.0	82
23	87	25.00	F	2	2	4.11	88.61	16.77	3.0	82
24	94	25.00	F	1	1	3.76	80.65	18.29	3.0	82
25	11	0.00	M	0	1	5.19	139.85	14.24	0.0	0
26	15	0.00	M	1	1	5.12	141.54	13.76	0.0	0
27	24	0.00	M	0	0	5.16	136.13	14.68	0.0	0
28	39	0.00	M	0	0	4.98	110.68	13.88	0.0	0
29	44	0.00	M	0	1	5.44	129.32	16.69	0.0	0
30	47	0.00	M	1	0	4.85	139.67	13.59	0.0	0
31	1	1.25	M	1	0	4.86	132.68	14.87	0.1	29
32	12	1.25	M	0	1	4.79	127.29	15.03	0.1	29
33	14	1.25	M	1	0	4.83	137.89	12.32	0.1	29
34	19	1.25	M	1	1	4.73	126.69	15.85	0.1	29
35	25	1.25	M	1	1	4.70	106.41	16.70	0.1	29
36	27	1.25	M	1	1	4.91	113.15	15.35	0.1	29
37	17	12.50	M	1	2	4.35	99.33	22.88	1.0	55
38	20	12.50	M	1	1	4.34	96.77	21.74	1.0	55
39	26	12.50	M	0	1	3.92	85.19	18.79	1.0	55
40	38	12.50	M	1	0	4.53	98.02	18.45	1.0	55
41	41	12.50	M	2	2	4.29	81.90	19.92	1.0	55
42	45	12.50	M	1	1	4.49	81.55	19.72	1.0	55
43	4	25.00	M	1	2	4.06	85.83	32.66	3.0	82
44	7	25.00	M	2	1	4.26	70.56	28.76	3.0	82
45	16	25.00	M	2	2	4.16	77.17	30.41	3.0	82

	0.0001 47	0.0001 47	0.0001 47	0.0010 47	0.0180 47	0.0011 47	0.0 47	0.0006 47
FL	0.53089 0.0001 47	0.51594 0.0002 47	0.49650 0.0004 47	-0.38990 0.0067 47	-0.34003 0.0194 47	0.48478 0.0006 47	0.48237 0.0006 47	1.00000 0.0 47

Correlation Analysis

Spearman Correlation Coefficients / Prob > |R| under Ho: Rho=0 / Number of Observations

	DOSE	DCORR	INTDOSE	T4	T3	TSH	FH	FL
DOSE	1.00000 0.0 48	1.00000 0.0001 48	1.00000 0.0001 48	-0.83826 0.0001 48	-0.81379 0.0001 48	0.81243 0.0001 48	0.59016 0.0001 47	0.49760 0.0004 47
DCORR	1.00000 0.0001 48	1.00000 0.0 48	1.00000 0.0001 48	-0.83826 0.0001 48	-0.81379 0.0001 48	0.81243 0.0001 48	0.59016 0.0001 47	0.49760 0.0004 47
INTDOSE	1.00000 0.0001 48	1.00000 0.0001 48	1.00000 0.0 48	-0.83826 0.0001 48	-0.81379 0.0001 48	0.81243 0.0001 48	0.59016 0.0001 47	0.49760 0.0004 47
T4	-0.83826 0.0001 48	-0.83826 0.0001 48	-0.83826 0.0001 48	1.00000 0.0 48	0.77215 0.0001 48	-0.50928 0.0002 48	-0.43423 0.0023 47	-0.41697 0.0035 47
T3	-0.81379 0.0001 48	-0.81379 0.0001 48	-0.81379 0.0001 48	0.77215 0.0001 48	1.00000 0.0 48	-0.56564 0.0001 48	-0.35229 0.0152 47	-0.33194 0.0226 47
TSH	0.81243 0.0001 48	0.81243 0.0001 48	0.81243 0.0001 48	-0.50928 0.0002 48	-0.56564 0.0001 48	1.00000 0.0 48	0.50107 0.0003 47	0.54035 0.0001 47
FH	0.59016 0.0001 47	0.59016 0.0001 47	0.59016 0.0001 47	-0.43423 0.0023 47	-0.35229 0.0152 47	0.50107 0.0003 47	1.00000 0.0 47	0.45934 0.0012 47
FL	0.49760 0.0004 47	0.49760 0.0004 47	0.49760 0.0004 47	-0.41697 0.0035 47	-0.33194 0.0226 47	0.54035 0.0001 47	0.45934 0.0012 47	1.00000 0.0 47

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	780.35450	780.35450	46.011	0.0001
Error	45	763.20148	16.96003		
C Total	46	1543.55598			

Root MSE	4.11826	R-square	0.5056
Dep Mean	17.24298	Adj R-sq	0.4946
C.V.	23.88367		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	11.696723	1.01459484	11.528	0.0001
INTDOSE	1	0.132794	0.01957694	6.783	0.0001

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6.40591	6.40591	23.983	0.0001
Error	45	12.01962	0.26710		
C Total	46	18.42553			

Root MSE	0.51682	R-square	0.3477
Dep Mean	0.76596	Adj R-sq	0.3332
C.V.	67.47370		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.263448	0.12732641	2.069	0.0443
INTDOSE	1	0.012032	0.00245680	4.897	0.0001

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.4900	5.0114	0.067	4.8756	5.1471	-0.5214
2	4.5000	5.0114	0.067	4.8756	5.1471	-0.5114
3	5.3600	5.0114	0.067	4.8756	5.1471	0.3486
4	5.3600	5.0114	0.067	4.8756	5.1471	0.3486
5	4.9400	5.0114	0.067	4.8756	5.1471	-0.0714
6	5.3300	5.0114	0.067	4.8756	5.1471	0.3186
7	4.4100	4.6268	0.043	4.5397	4.7138	-0.2168
8	4.3700	4.6268	0.043	4.5397	4.7138	-0.2568
9	4.4700	4.6268	0.043	4.5397	4.7138	-0.1568
10	4.3700	4.6268	0.043	4.5397	4.7138	-0.2568
11	4.1300	4.6268	0.043	4.5397	4.7138	-0.4968
12	4.6900	4.6268	0.043	4.5397	4.7138	0.0632
13	3.7500	4.2819	0.043	4.1944	4.3694	-0.5319
14	3.8400	4.2819	0.043	4.1944	4.3694	-0.4419
15	4.1100	4.2819	0.043	4.1944	4.3694	-0.1719
16	3.9700	4.2819	0.043	4.1944	4.3694	-0.3119
17	4.1100	4.2819	0.043	4.1944	4.3694	-0.1719
18	3.9200	4.2819	0.043	4.1944	4.3694	-0.3619
19	3.7500	3.9238	0.066	3.7913	4.0564	-0.1738
20	3.9600	3.9238	0.066	3.7913	4.0564	0.0362
21	3.8000	3.9238	0.066	3.7913	4.0564	-0.1238
22	3.7500	3.9238	0.066	3.7913	4.0564	-0.1738
23	4.1100	3.9238	0.066	3.7913	4.0564	0.1862
24	3.7600	3.9238	0.066	3.7913	4.0564	-0.1638
25	5.1900	5.0114	0.067	4.8756	5.1471	0.1786
26	5.1200	5.0114	0.067	4.8756	5.1471	0.1086
27	5.1600	5.0114	0.067	4.8756	5.1471	0.1486
28	4.9800	5.0114	0.067	4.8756	5.1471	-0.0314
29	5.4400	5.0114	0.067	4.8756	5.1471	0.4286
30	4.8500	5.0114	0.067	4.8756	5.1471	-0.1614
31	4.8600	4.6268	0.043	4.5397	4.7138	0.2332
32	4.7900	4.6268	0.043	4.5397	4.7138	0.1632
33	4.8300	4.6268	0.043	4.5397	4.7138	0.2032
34	4.7300	4.6268	0.043	4.5397	4.7138	0.1032
35	4.7000	4.6268	0.043	4.5397	4.7138	0.0732
36	4.9100	4.6268	0.043	4.5397	4.7138	0.2832
37	4.3500	4.2819	0.043	4.1944	4.3694	0.0681
38	4.3400	4.2819	0.043	4.1944	4.3694	0.0581
39	3.9200	4.2819	0.043	4.1944	4.3694	-0.3619
40	4.5300	4.2819	0.043	4.1944	4.3694	0.2481
41	4.2900	4.2819	0.043	4.1944	4.3694	0.00808
42	4.4900	4.2819	0.043	4.1944	4.3694	0.2081
43	4.0600	3.9238	0.066	3.7913	4.0564	0.1362

34	126.7	108.5	2.083	104.3	112.7	18.1718
35	106.4	108.5	2.083	104.3	112.7	-2.1082
36	113.2	108.5	2.083	104.3	112.7	4.6318
37	99.3300	91.1422	2.094	86.9243	95.3601	8.1878
38	96.7700	91.1422	2.094	86.9243	95.3601	5.6278
39	85.1900	91.1422	2.094	86.9243	95.3601	-5.9522
40	98.0200	91.1422	2.094	86.9243	95.3601	6.8778
41	81.9000	91.1422	2.094	86.9243	95.3601	-9.2422
42	81.5500	91.1422	2.094	86.9243	95.3601	-9.5922
43	85.8300	73.0979	3.171	66.7111	79.4846	12.7321
44	70.5600	73.0979	3.171	66.7111	79.4846	-2.5379
45	77.1700	73.0979	3.171	66.7111	79.4846	4.0721
46	76.2200	73.0979	3.171	66.7111	79.4846	3.1221
47	68.5500	73.0979	3.171	66.7111	79.4846	-4.5479
48	74.1600	73.0979	3.171	66.7111	79.4846	1.0621

Sum of Residuals	0
Sum of Squared Residuals	7820.8130
Predicted Resid SS (Press)	8377.3462

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 9

14:54 Thursday, February 4, 1999

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	10.9200	11.6967	1.015	9.6532	13.7402	-0.7767
2	10.7100	11.6967	1.015	9.6532	13.7402	-0.9867
3	12.0100	11.6967	1.015	9.6532	13.7402	0.3133
4	11.4000	11.6967	1.015	9.6532	13.7402	-0.2967
5	10.9600	11.6967	1.015	9.6532	13.7402	-0.7367
6	11.4900	11.6967	1.015	9.6532	13.7402	-0.2067
7	11.9600	15.5477	0.651	14.2373	16.8582	-3.5877
8	11.7100	15.5477	0.651	14.2373	16.8582	-3.8377
9	14.7400	15.5477	0.651	14.2373	16.8582	-0.8077
10	13.4200	15.5477	0.651	14.2373	16.8582	-2.1277
11	13.6800	15.5477	0.651	14.2373	16.8582	-1.8677
12	12.8100	15.5477	0.651	14.2373	16.8582	-2.7377
13	15.4500	19.0004	0.654	17.6828	20.3180	-3.5504
14	12.5400	19.0004	0.654	17.6828	20.3180	-6.4604
15	16.4200	19.0004	0.654	17.6828	20.3180	-2.5804
16	15.9300	19.0004	0.654	17.6828	20.3180	-3.0704
17	16.4300	19.0004	0.654	17.6828	20.3180	-2.5704
18	15.3700	19.0004	0.654	17.6828	20.3180	-3.6304
19	17.1700	22.5858	0.991	20.5907	24.5809	-5.4158
20	17.2100	22.5858	0.991	20.5907	24.5809	-5.3758
21	17.3300	22.5858	0.991	20.5907	24.5809	-5.2558
22	17.5500	22.5858	0.991	20.5907	24.5809	-5.0358
23	16.7700	22.5858	0.991	20.5907	24.5809	-5.8158

14	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
15	0	0.9252	0.082	0.7598	1.0905	-0.9252
16	0	0.9252	0.082	0.7598	1.0905	-0.9252
17	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
18	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
19	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
20	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
21	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
22	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
23	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
24	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
25	0	0.2634	0.127	0.00700	0.5199	-0.2634
26	1.0000	0.2634	0.127	0.00700	0.5199	0.7366
27	0	0.2634	0.127	0.00700	0.5199	-0.2634
28	0	0.2634	0.127	0.00700	0.5199	-0.2634
29	0	0.2634	0.127	0.00700	0.5199	-0.2634
30	1.0000	0.2634	0.127	0.00700	0.5199	0.7366
31	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
32	0	0.6124	0.082	0.4479	0.7768	-0.6124
33	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
34	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
35	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
36	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
37	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
38	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
39	0	0.9252	0.082	0.7598	1.0905	-0.9252
40	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
41	2.0000	0.9252	0.082	0.7598	1.0905	1.0748
42	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
43	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
44	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
45	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
46	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
47	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
48	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500

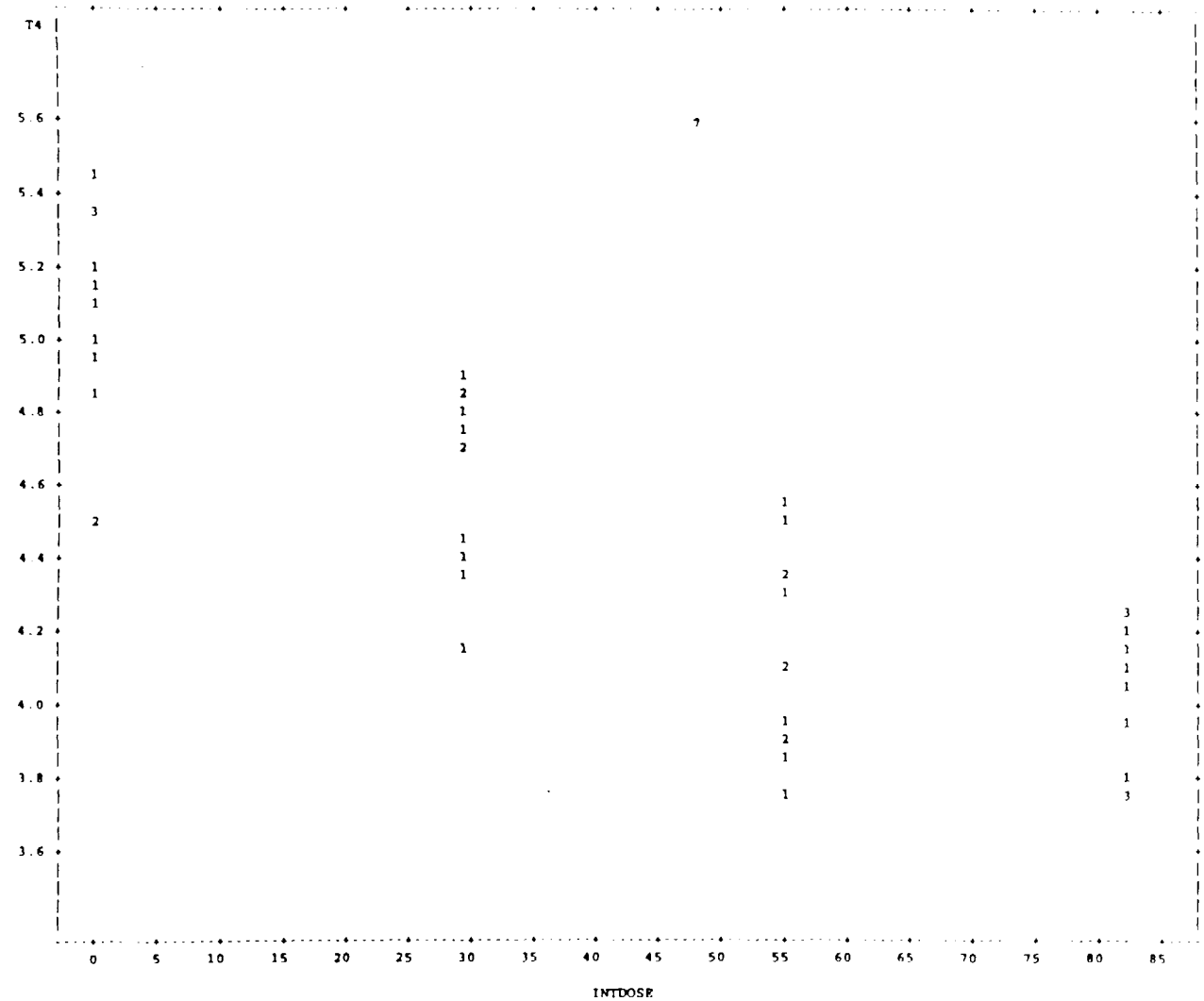
Sum of Residuals	0
Sum of Squared Residuals	12.0196
Predicted Resid SS (Press)	13.0729

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 11

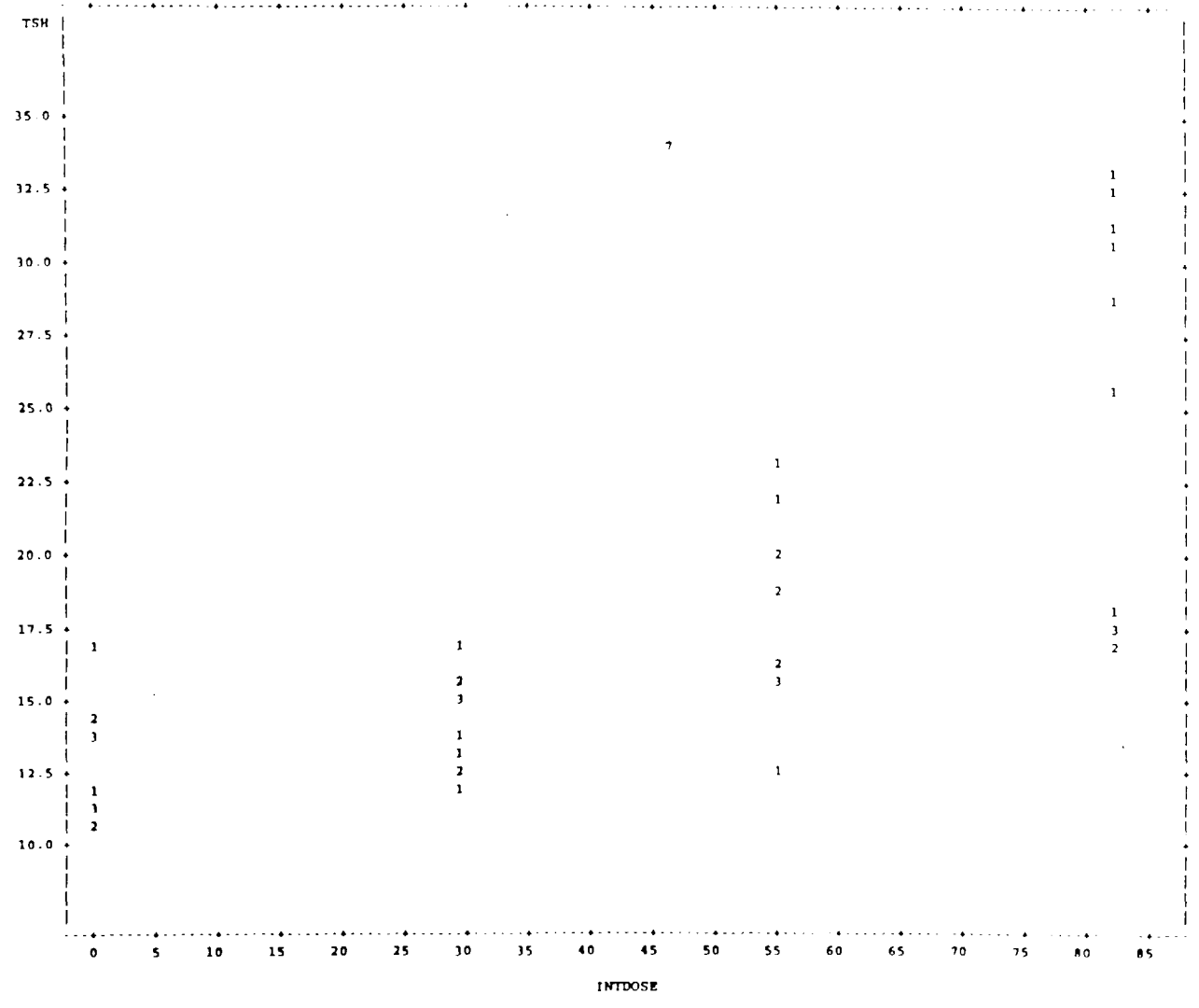
14:54 Thursday, February 4, 1999

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.4631	0.132	0.1966	0.7297	0.5369
2	1.0000	0.4631	0.132	0.1966	0.7297	0.5369
3	1.0000	0.4631	0.132	0.1966	0.7297	0.5369

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 14:54 Thursday, February 4, 1999 12



1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 14



Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	835.91606	835.91606	53.157	0.0001
Error	45	707.63992	15.72533		
C Total	46	1543.55598			

Root MSE	3.96552	R-square	0.5416
Dep Mean	17.24298	Adj R-sq	0.5314
C.V.	22.99787		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	13.601987	0.76417937	17.799	0.0001
DCORR	1	3.485267	0.47802945	7.291	0.0001

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6.40318	6.40318	23.967	0.0001
Error	45	12.02235	0.26716		
C Total	46	18.42553			

Root MSE	0.51688	R-square	0.3475
Dep Mean	0.76596	Adj R-sq	0.3330
C.V.	67.48135		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.447291	0.09960561	4.491	0.0001
DCORR	1	0.305037	0.06230790	4.896	0.0001

21	3.8000	3.9079	0.099	3.7089	4.1069	-0.1079
22	3.7500	3.9079	0.099	3.7089	4.1069	-0.1579
23	4.1100	3.9079	0.099	3.7089	4.1069	0.2021
24	3.7600	3.9079	0.099	3.7089	4.1069	-0.1479
25	5.1900	4.7511	0.069	4.6127	4.8894	0.4389
26	5.1200	4.7511	0.069	4.6127	4.8894	0.3689
27	5.1600	4.7511	0.069	4.6127	4.8894	0.4089
28	4.9800	4.7511	0.069	4.6127	4.8894	0.2289
29	5.4400	4.7511	0.069	4.6127	4.8894	0.6889
30	4.8500	4.7511	0.069	4.6127	4.8894	0.0989
31	4.8600	4.7230	0.066	4.5901	4.8558	0.1370
32	4.7900	4.7230	0.066	4.5901	4.8558	0.0670
33	4.8300	4.7230	0.066	4.5901	4.8558	0.1070
34	4.7300	4.7230	0.066	4.5901	4.8558	0.00704
35	4.7000	4.7230	0.066	4.5901	4.8558	-0.0230
36	4.9100	4.7230	0.066	4.5901	4.8558	0.1870
37	4.3500	4.4700	0.052	4.3652	4.5748	-0.1200
38	4.3400	4.4700	0.052	4.3652	4.5748	0.1300
39	3.9200	4.4700	0.052	4.3652	4.5748	-0.5500
40	4.5300	4.4700	0.052	4.3652	4.5748	0.0600
41	4.2900	4.4700	0.052	4.3652	4.5748	-0.1800
42	4.4900	4.4700	0.052	4.3652	4.5748	0.0200
43	4.0600	3.9079	0.099	3.7089	4.1069	0.1521
44	4.2600	3.9079	0.099	3.7089	4.1069	0.3521
45	4.1600	3.9079	0.099	3.7089	4.1069	0.2521
46	4.2500	3.9079	0.099	3.7089	4.1069	0.3421
47	4.2300	3.9079	0.099	3.7089	4.1069	0.3221
48	4.1800	3.9079	0.099	3.7089	4.1069	0.2721

Sum of Residuals	0
Sum of Squared Residuals	5.7157
Predicted Resid SS (Press)	6.1478

1 CALDWELL STUDY, CORRELATIONS WITH Administered DOSE 14:54 Thursday, February 4, 1999 19

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	124.3	114.9	3.354	108.1	121.6	9.4656
2	138.4	114.9	3.354	108.1	121.6	23.4956
3	127.9	114.9	3.354	108.1	121.6	13.0256
4	133.1	114.9	3.354	108.1	121.6	18.2756
5	134.2	114.9	3.354	108.1	121.6	19.3056
6	113.2	114.9	3.354	108.1	121.6	-1.6944
7	93.9100	113.4	3.220	107.0	119.9	-19.5302
8	75.7000	113.4	3.220	107.0	119.9	-37.7402
9	84.1700	113.4	3.220	107.0	119.9	-29.2702
10	85.0400	113.4	3.220	107.0	119.9	-28.4002
11	86.6600	113.4	3.220	107.0	119.9	-26.7802

3	12.0100	13.6020	0.764	12.0629	15.1411	-1.5920
4	11.4000	13.6020	0.764	12.0629	15.1411	-2.2020
5	10.9600	13.6020	0.764	12.0629	15.1411	-2.6420
6	11.4900	13.6020	0.764	12.0629	15.1411	-2.1120
7	11.9600	13.9505	0.734	12.4725	15.4285	1.9905
8	11.7100	13.9505	0.734	12.4725	15.4285	-2.2405
9	14.7400	13.9505	0.734	12.4725	15.4285	0.7895
10	13.4200	13.9505	0.734	12.4725	15.4285	-0.5305
11	13.6800	13.9505	0.734	12.4725	15.4285	-0.2705
12	12.8100	13.9505	0.734	12.4725	15.4285	-1.1405
13	15.4500	17.0873	0.579	15.9214	18.2531	-1.6373
14	12.5400	17.0873	0.579	15.9214	18.2531	4.5473
15	16.4200	17.0873	0.579	15.9214	18.2531	-0.6673
16	15.9300	17.0873	0.579	15.9214	18.2531	1.1573
17	16.4300	17.0873	0.579	15.9214	18.2531	-0.6573
18	15.3700	17.0873	0.579	15.9214	18.2531	-1.7173
19	17.1700	24.0578	1.099	21.8439	26.2717	-6.8878
20	17.2100	24.0578	1.099	21.8439	26.2717	-6.8478
21	17.3300	24.0578	1.099	21.8439	26.2717	-6.7278
22	17.5500	24.0578	1.099	21.8439	26.2717	-6.5078
23	16.7700	24.0578	1.099	21.8439	26.2717	-7.2878
24	18.2900	24.0578	1.099	21.8439	26.2717	-5.7678
25	14.2400	13.6020	0.764	12.0629	15.1411	0.6380
26	13.7600	13.6020	0.764	12.0629	15.1411	0.1580
27	14.6800	13.6020	0.764	12.0629	15.1411	1.0780
28	13.8800	13.6020	0.764	12.0629	15.1411	0.2780
29	16.6900	13.6020	0.764	12.0629	15.1411	3.0880
30	13.5900	13.6020	0.764	12.0629	15.1411	0.0120
31	14.8700	13.9505	0.734	12.4725	15.4285	0.9195
32	15.0300	13.9505	0.734	12.4725	15.4285	1.0795
33	12.3200	13.9505	0.734	12.4725	15.4285	1.6305
34	15.8500	13.9505	0.734	12.4725	15.4285	1.8995
35	16.7000	13.9505	0.734	12.4725	15.4285	2.7495
36	15.3500	13.9505	0.734	12.4725	15.4285	1.3995
37	22.8800	17.0873	0.579	15.9214	18.2531	5.7927
38	21.7400	17.0873	0.579	15.9214	18.2531	4.6527
39	18.7900	17.0873	0.579	15.9214	18.2531	1.7027
40	18.4500	17.0873	0.579	15.9214	18.2531	1.3627
41	19.9200	17.0873	0.579	15.9214	18.2531	2.8327
42	19.7200	17.0873	0.579	15.9214	18.2531	2.6327
43	32.6600	24.0578	1.099	21.8439	26.2717	8.6022
44	28.7600	24.0578	1.099	21.8439	26.2717	4.7022
45	30.4100	24.0578	1.099	21.8439	26.2717	6.3522
46	31.1800	24.0578	1.099	21.8439	26.2717	7.1222
47	25.5700	24.0578	1.099	21.8439	26.2717	1.5122
48	32.8200	24.0578	1.099	21.8439	26.2717	8.7622

Sum of Residuals	0
Sum of Squared Residuals	707.6399

43	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
44	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
45	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
46	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
47	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
48	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624

Sum of Residuals	0
Sum of Squared Residuals	12.0223
Predicted Resid SS (Press)	13.0737

1 CALDWELL STUDY, CORRELATIONS WITH Administered DOSE 14:54 Thursday, February 4, 1999 22

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
2	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
3	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
4	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
5	0	0.6026	0.102	0.3969	0.8084	-0.6026
6	0	0.6026	0.102	0.3969	0.8084	-0.6026
7	0	0.6284	0.098	0.4308	0.8260	-0.6284
8	.	0.6284	0.098	0.4308	0.8260	.
9	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
10	0	0.6284	0.098	0.4308	0.8260	-0.6284
11	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
12	0	0.6284	0.098	0.4308	0.8260	-0.6284
13	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
14	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
15	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
16	0	0.8608	0.077	0.7050	1.0167	-0.8608
17	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
18	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
19	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
20	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
21	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
22	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
23	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
24	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
25	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
26	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
27	0	0.6026	0.102	0.3969	0.8084	-0.6026
28	0	0.6026	0.102	0.3969	0.8084	-0.6026
29	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
30	0	0.6026	0.102	0.3969	0.8084	-0.6026
31	0	0.6284	0.098	0.4308	0.8260	-0.6284
32	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
33	0	0.6284	0.098	0.4308	0.8260	-0.6284

1 T4, T3, TSH, and Follicle Hyperplasia and follicular lumen 09:46 Monday, February 8, 1999 1
area data from Caldwell 14-day perchlorate study with PK internal dose approximation from Channel, 1999

OBS	ID	DOSE	SEX	FH	FL	T4	T3	TSH	DCORR	INTDOSE
1	68	0.00	F	1	1	4.49	124.33	10.92	0.0	0.0
2	71	0.00	F	1	1	4.50	138.36	10.71	0.0	0.0
3	86	0.00	F	0	1	5.36	127.89	12.01	0.0	0.0
4	90	0.00	F	0	1	5.36	133.14	11.40	0.0	0.0
5	95	0.00	F	0	0	4.94	134.17	10.96	0.0	0.0
6	100	0.00	F	0	0	5.33	113.17	11.49	0.0	0.0
7	51	1.25	F	0	0	4.41	93.91	11.96	0.1	-17.5
8	55	1.25	F	.	.	4.37	75.70	11.71	0.1	-17.5
9	58	1.25	F	0	1	4.47	84.17	14.74	0.1	-17.5
10	67	1.25	F	1	0	4.37	85.04	13.42	0.1	-17.5
11	73	1.25	F	0	1	4.13	86.66	13.68	0.1	-17.5
12	84	1.25	F	0	0	4.69	82.11	12.81	0.1	-17.5
13	60	12.50	F	1	1	3.75	94.00	15.45	1.0	-9.0
14	66	12.50	F	1	1	3.84	79.42	12.54	1.0	-9.0
15	70	12.50	F	0	1	4.11	76.16	16.42	1.0	-9.0
16	77	12.50	F	0	0	3.97	77.21	15.93	1.0	-9.0
17	92	12.50	F	1	1	4.11	78.67	16.43	1.0	-9.0
18	99	12.50	F	1	1	3.92	78.61	15.37	1.0	-9.0
19	59	25.00	F	2	1	3.75	73.91	17.17	3.0	3.0
20	64	25.00	F	1	1	3.96	85.00	17.21	3.0	3.0
21	76	25.00	F	1	2	3.80	73.47	17.33	3.0	3.0
22	80	25.00	F	1	1	3.75	74.16	17.55	3.0	3.0
23	87	25.00	F	2	2	4.11	88.61	16.77	3.0	3.0
24	94	25.00	F	1	1	3.76	80.65	18.29	3.0	3.0
25	11	0.00	M	0	1	5.19	139.85	14.24	0.0	0.0
26	15	0.00	M	1	1	5.12	141.54	13.76	0.0	0.0
27	24	0.00	M	0	0	5.16	136.13	14.68	0.0	0.0
28	39	0.00	M	0	0	4.98	110.68	13.88	0.0	0.0
29	44	0.00	M	0	1	5.44	129.32	16.69	0.0	0.0
30	47	0.00	M	1	0	4.85	139.67	13.59	0.0	0.0
31	1	1.25	M	1	0	4.86	132.68	14.87	0.1	-17.5
32	12	1.25	M	0	1	4.79	127.29	15.03	0.1	-17.5
33	14	1.25	M	1	0	4.83	137.89	12.32	0.1	-17.5
34	19	1.25	M	1	1	4.73	126.69	15.85	0.1	-17.5
35	25	1.25	M	1	1	4.70	106.41	16.70	0.1	-17.5
36	27	1.25	M	1	1	4.91	113.15	15.35	0.1	-17.5
37	17	12.50	M	1	2	4.35	99.33	22.88	1.0	-9.0
38	20	12.50	M	1	1	4.34	96.77	21.74	1.0	-9.0
39	26	12.50	M	0	1	3.92	85.19	18.79	1.0	-9.0
40	38	12.50	M	1	0	4.53	98.02	18.45	1.0	-9.0
41	41	12.50	M	2	2	4.29	81.90	19.92	1.0	-9.0
42	45	12.50	M	1	1	4.49	81.55	19.72	1.0	-9.0
43	4	25.00	M	1	2	4.06	85.83	32.66	3.0	3.0
44	7	25.00	M	2	1	4.26	70.56	28.76	3.0	3.0

TSH	0.74258	0.73973	0.36989	-0.38004	-0.50939	1.00000	0.46247	0.48478
	0.0001	0.0001	0.0097	0.0077	0.0002	0.0	0.0011	0.0006
	48	48	48	48	48	48	47	47
FH	0.59512	0.58951	0.23805	-0.46378	-0.34372	0.46247	1.00000	0.48237
	0.0001	0.0001	0.1071	0.0010	0.0180	0.0011	0.0	0.0006
	47	47	47	47	47	47	47	47
FL	0.53089	0.51594	0.29103	-0.38990	-0.34003	0.48478	0.48237	1.00000
	0.0001	0.0002	0.0472	0.0067	0.0194	0.0006	0.0006	0.0
	47	47	47	47	47	47	47	47

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE from Channel 1999 09:46 Monday, February 8, 1999 3

Correlation Analysis

Spearman Correlation Coefficients / Prob > |R| under Ho: Rho=0 / Number of Observations

	DOSE	DCORR	INTDOSE	T4	T3	TSH	FH	FL
DOSE	1.00000	1.00000	0.40000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0	0.0001	0.0048	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
DCORR	1.00000	1.00000	0.40000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0001	0.0	0.0048	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
INTDOSE	0.40000	0.40000	1.00000	-0.25363	-0.25288	0.39680	0.31695	0.35173
	0.0048	0.0048	0.0	0.0820	0.0829	0.0052	0.0300	0.0153
	48	48	48	48	48	48	47	47
T4	-0.83826	-0.83826	-0.25363	1.00000	0.77215	-0.50928	-0.43423	-0.41697
	0.0001	0.0001	0.0820	0.0	0.0001	0.0002	0.0023	0.0035
	48	48	48	48	48	48	47	47
T3	-0.81379	-0.81379	-0.25288	0.77215	1.00000	-0.56564	-0.35229	-0.33194
	0.0001	0.0001	0.0829	0.0001	0.0	0.0001	0.0152	0.0226
	48	48	48	48	48	48	47	47
TSH	0.81243	0.81243	0.39680	-0.50928	-0.56564	1.00000	0.50107	0.54035
	0.0001	0.0001	0.0052	0.0002	0.0001	0.0	0.0003	0.0001
	48	48	48	48	48	48	47	47
FH	0.59016	0.59016	0.31695	-0.43423	-0.35229	0.50107	1.00000	0.45934
	0.0001	0.0001	0.0300	0.0023	0.0152	0.0003	0.0	0.0012
	47	47	47	47	47	47	47	47
FL	0.49760	0.49760	0.35173	-0.41697	-0.33194	0.54035	0.45934	1.00000
	0.0004	0.0004	0.0153	0.0035	0.0226	0.0001	0.0012	0.0

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	191.23320	191.23320	6.363	0.0153
Error	45	1352.32278	30.05162		
C Total	46	1543.55598			

Root MSE	5.48194	R-square	0.1239
Dep Mean	17.24298	Adj R-sq	0.1044
C.V.	31.79228		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	18.673342	0.98025930	19.049	0.0001
INTDOSE	1	0.254167	0.10075600	2.523	0.0153

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	1.04414	1.04414	2.703	0.1071
Error	45	17.38140	0.38625		
C Total	46	18.42553			

Root MSE	0.62149	R-square	0.0567
Dep Mean	0.76596	Adj R-sq	0.0357
C.V.	81.13933		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.871650	0.11113302	7.843	0.0001
INTDOSE	1	0.018781	0.01142281	1.644	0.1071

20	3.9600	4.4185	0.107	4.2031	4.6340	-0.4585
21	3.8000	4.4185	0.107	4.2031	4.6340	-0.6185
22	3.7500	4.4185	0.107	4.2031	4.6340	-0.6685
23	4.1100	4.4185	0.107	4.2031	4.6340	-0.3085
24	3.7600	4.4185	0.107	4.2031	4.6340	-0.6585
25	5.1900	4.4321	0.089	4.2533	4.6109	0.7579
26	5.1200	4.4321	0.089	4.2533	4.6109	0.6879
27	5.1600	4.4321	0.089	4.2533	4.6109	0.7279
28	4.9800	4.4321	0.089	4.2533	4.6109	0.5479
29	5.4400	4.4321	0.089	4.2533	4.6109	1.0079
30	4.8500	4.4321	0.089	4.2533	4.6109	0.4179
31	4.8600	4.5110	0.130	4.2485	4.7734	0.3490
32	4.7900	4.5110	0.130	4.2485	4.7734	0.2790
33	4.8300	4.5110	0.130	4.2485	4.7734	0.3190
34	4.7300	4.5110	0.130	4.2485	4.7734	0.2190
35	4.7000	4.5110	0.130	4.2485	4.7734	0.1890
36	4.9100	4.5110	0.130	4.2485	4.7734	0.3990
37	4.3500	4.4727	0.079	4.3142	4.6311	-0.1227
38	4.3400	4.4727	0.079	4.3142	4.6311	-0.1327
39	3.9200	4.4727	0.079	4.3142	4.6311	-0.5527
40	4.5300	4.4727	0.079	4.3142	4.6311	0.0573
41	4.2900	4.4727	0.079	4.3142	4.6311	-0.1827
42	4.4900	4.4727	0.079	4.3142	4.6311	0.0173
43	4.0600	4.4185	0.107	4.2031	4.6340	-0.3585
44	4.2600	4.4185	0.107	4.2031	4.6340	-0.1585
45	4.1600	4.4185	0.107	4.2031	4.6340	-0.2585
46	4.2500	4.4185	0.107	4.2031	4.6340	-0.1685
47	4.2300	4.4185	0.107	4.2031	4.6340	-0.1885
48	4.1800	4.4185	0.107	4.2031	4.6340	-0.2385

Sum of Residuals	0
Sum of Squared Residuals	11.0917
Predicted Resid SS (Press)	11.9466

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 09:46 Monday, February 8, 1999 8

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	124.3	98.8647	4.418	89.9666	107.8	25.4653
2	138.4	98.8647	4.418	89.9666	107.8	39.4953
3	127.9	98.8647	4.418	89.9666	107.8	29.0253
4	133.1	98.8647	4.418	89.9666	107.8	34.2753
5	134.2	98.8647	4.418	89.9666	107.8	35.3053
6	113.2	98.8647	4.418	89.9666	107.8	14.3053
7	93.9100	102.4	6.485	89.2926	115.4	-8.4434
8	75.7000	102.4	6.485	89.2926	115.4	-26.6534
9	84.1700	102.4	6.485	89.2926	115.4	-18.1834
10	85.0400	102.4	6.485	89.2926	115.4	-17.3134

2	10.7100	18.6733	0.980	16.6990	20.6477	-7.9633
3	12.0100	18.6733	0.980	16.6990	20.6477	-6.6633
4	11.4000	18.6733	0.980	16.6990	20.6477	-7.2733
5	10.9600	18.6733	0.980	16.6990	20.6477	-7.7133
6	11.4900	18.6733	0.980	16.6990	20.6477	-7.1833
7	11.9600	14.2254	1.439	11.3274	17.1234	-2.2654
8	11.7100	14.2254	1.439	11.3274	17.1234	2.5154
9	14.7400	14.2254	1.439	11.3274	17.1234	0.5146
10	13.4200	14.2254	1.439	11.3274	17.1234	-0.8054
11	13.6800	14.2254	1.439	11.3274	17.1234	-0.5454
12	12.8100	14.2254	1.439	11.3274	17.1234	-1.4154
13	15.4500	16.3858	0.869	14.6359	18.1357	-0.9358
14	12.5400	16.3858	0.869	14.6359	18.1357	-3.8458
15	16.4200	16.3858	0.869	14.6359	18.1357	0.0342
16	15.9300	16.3858	0.869	14.6359	18.1357	-0.4558
17	16.4300	16.3858	0.869	14.6359	18.1357	0.0442
18	15.3700	16.3858	0.869	14.6359	18.1357	-1.0158
19	17.1700	19.4358	1.181	17.0569	21.8148	-2.2658
20	17.2100	19.4358	1.181	17.0569	21.8148	-2.2258
21	17.3300	19.4358	1.181	17.0569	21.8148	-2.1058
22	17.5500	19.4358	1.181	17.0569	21.8148	-1.8858
23	16.7700	19.4358	1.181	17.0569	21.8148	-2.6658
24	18.2900	19.4358	1.181	17.0569	21.8148	-1.1458
25	14.2400	18.6733	0.980	16.6990	20.6477	-4.4333
26	13.7600	18.6733	0.980	16.6990	20.6477	-4.9133
27	14.6800	18.6733	0.980	16.6990	20.6477	-3.9933
28	13.8800	18.6733	0.980	16.6990	20.6477	-4.7933
29	16.6900	18.6733	0.980	16.6990	20.6477	-1.9833
30	13.5900	18.6733	0.980	16.6990	20.6477	-5.0833
31	14.8700	14.2254	1.439	11.3274	17.1234	0.6446
32	15.0300	14.2254	1.439	11.3274	17.1234	0.8046
33	12.3200	14.2254	1.439	11.3274	17.1234	-1.9054
34	15.8500	14.2254	1.439	11.3274	17.1234	1.6246
35	16.7000	14.2254	1.439	11.3274	17.1234	2.4746
36	15.3500	14.2254	1.439	11.3274	17.1234	1.1246
37	22.8800	16.3858	0.869	14.6359	18.1357	6.4942
38	21.7400	16.3858	0.869	14.6359	18.1357	5.3542
39	18.7900	16.3858	0.869	14.6359	18.1357	2.4042
40	18.4500	16.3858	0.869	14.6359	18.1357	2.0642
41	19.9200	16.3858	0.869	14.6359	18.1357	3.5342
42	19.7200	16.3858	0.869	14.6359	18.1357	3.3342
43	32.6600	19.4358	1.181	17.0569	21.8148	13.2242
44	28.7600	19.4358	1.181	17.0569	21.8148	9.3242
45	30.4100	19.4358	1.181	17.0569	21.8148	10.9742
46	31.1800	19.4358	1.181	17.0569	21.8148	11.7442
47	25.5700	19.4358	1.181	17.0569	21.8148	6.1342
48	32.8200	19.4358	1.181	17.0569	21.8148	13.3842

Sum of Residuals 0

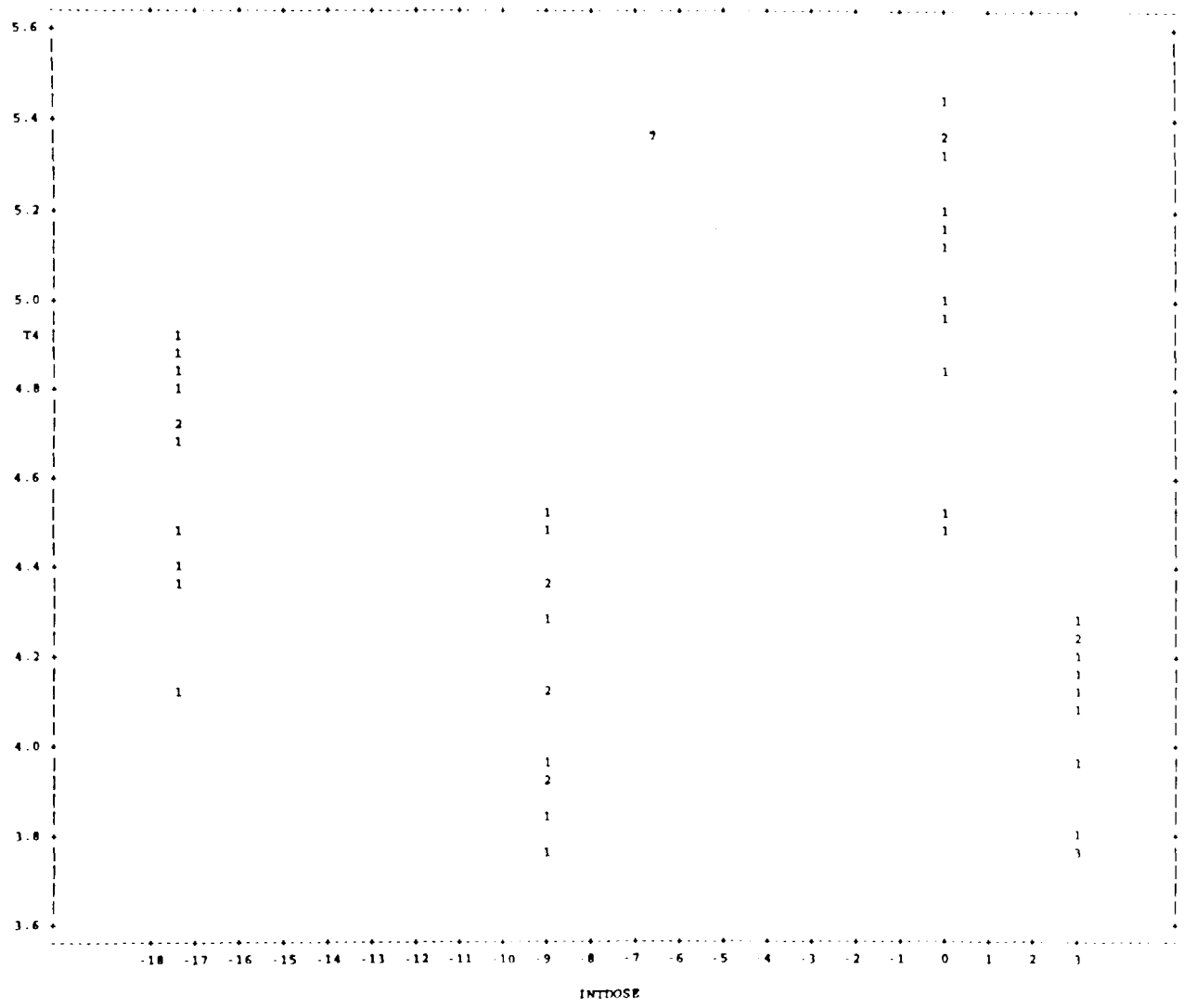
42	1.0000	0.7026	0.098	0.5042	0.9010	0.2974
43	1.0000	0.9280	0.134	0.6583	1.1977	0.0720
44	2.0000	0.9280	0.134	0.6583	1.1977	1.0720
45	2.0000	0.9280	0.134	0.6583	1.1977	1.0720
46	1.0000	0.9280	0.134	0.6583	1.1977	0.0720
47	1.0000	0.9280	0.134	0.6583	1.1977	0.0720
48	1.0000	0.9280	0.134	0.6583	1.1977	0.0720

Sum of Residuals	0
Sum of Squared Residuals	17.3814
Predicted Resid SS (Press)	18.8772

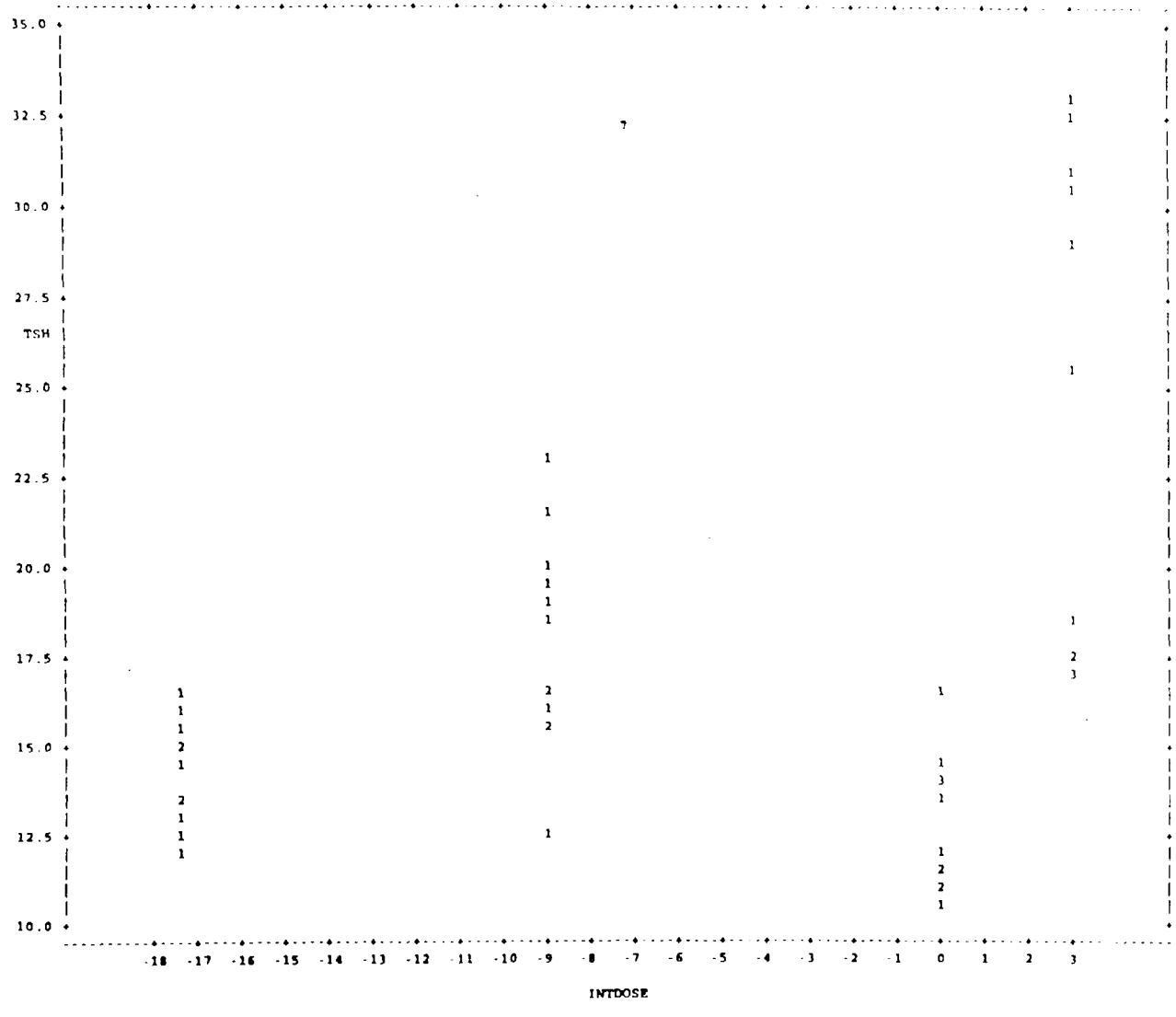
1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 09:46 Monday, February 8, 1999 11

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
2	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
3	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
4	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
5	0	0.9973	0.106	0.7841	1.2105	-0.9973
6	0	0.9973	0.106	0.7841	1.2105	-0.9973
7	0	0.6087	0.155	0.2957	0.9217	-0.6087
8	.	0.6087	0.155	0.2957	0.9217	.
9	1.0000	0.6087	0.155	0.2957	0.9217	0.3913
10	0	0.6087	0.155	0.2957	0.9217	-0.6087
11	1.0000	0.6087	0.155	0.2957	0.9217	0.3913
12	0	0.6087	0.155	0.2957	0.9217	-0.6087
13	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
14	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
15	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
16	0	0.7975	0.094	0.6085	0.9864	-0.7975
17	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
18	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
19	1.0000	1.0639	0.128	0.8070	1.3209	0.0639
20	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
21	2.0000	1.0639	0.128	0.8070	1.3209	0.9361
22	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
23	2.0000	1.0639	0.128	0.8070	1.3209	0.9361
24	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
25	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
26	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
27	0	0.9973	0.106	0.7841	1.2105	-0.9973
28	0	0.9973	0.106	0.7841	1.2105	-0.9973
29	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
30	0	0.9973	0.106	0.7841	1.2105	-0.9973
31	0	0.6087	0.155	0.2957	0.9217	-0.6087
32	1.0000	0.6087	0.155	0.2957	0.9217	0.3913

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 09:46 Monday, February 8, 1999 12



1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 09:46 Monday, February 8, 1999 14



Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	835.91606	835.91606	53.157	0.0001
Error	45	707.63992	15.72533		
C Total	46	1543.55598			

Root MSE	3.96552	R-square	0.5416
Dep Mean	17.24298	Adj R-sq	0.5314
C.V.	22.99787		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	13.601987	0.76417937	17.799	0.0001
DCORR	1	3.485267	0.47802945	7.291	0.0001

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6.40318	6.40318	23.967	0.0001
Error	45	12.02235	0.26716		
C Total	46	18.42553			

Root MSE	0.51688	R-square	0.3475
Dep Mean	0.76596	Adj R-sq	0.3330
C.V.	67.48135		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.447291	0.09960561	4.491	0.0001
DCORR	1	0.305037	0.06230790	4.896	0.0001

21	3.8000	3.9079	0.099	3.7089	4.1069	-0.1079
22	3.7500	3.9079	0.099	3.7089	4.1069	-0.1579
23	4.1100	3.9079	0.099	3.7089	4.1069	0.2021
24	3.7600	3.9079	0.099	3.7089	4.1069	-0.1479
25	5.1900	4.7511	0.069	4.6127	4.8894	0.4389
26	5.1200	4.7511	0.069	4.6127	4.8894	0.3689
27	5.1600	4.7511	0.069	4.6127	4.8894	0.4089
28	4.9800	4.7511	0.069	4.6127	4.8894	0.2289
29	5.4400	4.7511	0.069	4.6127	4.8894	0.6889
30	4.8500	4.7511	0.069	4.6127	4.8894	0.0989
31	4.8600	4.7230	0.066	4.5901	4.8558	0.1370
32	4.7900	4.7230	0.066	4.5901	4.8558	0.0670
33	4.8300	4.7230	0.066	4.5901	4.8558	0.1070
34	4.7300	4.7230	0.066	4.5901	4.8558	0.00704
35	4.7000	4.7230	0.066	4.5901	4.8558	-0.0230
36	4.9100	4.7230	0.066	4.5901	4.8558	0.1870
37	4.3500	4.4700	0.052	4.3652	4.5748	-0.1200
38	4.3400	4.4700	0.052	4.3652	4.5748	-0.1300
39	3.9200	4.4700	0.052	4.3652	4.5748	-0.5500
40	4.5300	4.4700	0.052	4.3652	4.5748	0.0600
41	4.2900	4.4700	0.052	4.3652	4.5748	-0.1800
42	4.4900	4.4700	0.052	4.3652	4.5748	0.0200
43	4.0600	3.9079	0.099	3.7089	4.1069	0.1521
44	4.2600	3.9079	0.099	3.7089	4.1069	0.3521
45	4.1600	3.9079	0.099	3.7089	4.1069	0.2521
46	4.2500	3.9079	0.099	3.7089	4.1069	0.3421
47	4.2300	3.9079	0.099	3.7089	4.1069	0.3221
48	4.1800	3.9079	0.099	3.7089	4.1069	0.2721

Sum of Residuals	0
Sum of Squared Residuals	5.7157
Predicted Resid SS (Press)	6.1478

1 CALDWELL STUDY, CORRELATIONS WITH Administered DOSE 09:46 Monday, February 8, 1999 19

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	124.3	114.9	3.354	108.1	121.6	9.4656
2	138.4	114.9	3.354	108.1	121.6	23.4956
3	127.9	114.9	3.354	108.1	121.6	13.0256
4	133.1	114.9	3.354	108.1	121.6	18.2756
5	134.2	114.9	3.354	108.1	121.6	19.3056
6	113.2	114.9	3.354	108.1	121.6	-1.6944
7	93.9100	113.4	3.220	107.0	119.9	-19.5302
8	75.7000	113.4	3.220	107.0	119.9	-37.7402
9	84.1700	113.4	3.220	107.0	119.9	-29.2702
10	85.0400	113.4	3.220	107.0	119.9	-28.4002
11	86.6600	113.4	3.220	107.0	119.9	-26.7802

3	12.0100	13.6020	0.764	12.0629	15.1411	-1.5920
4	11.4000	13.6020	0.764	12.0629	15.1411	-2.2020
5	10.9600	13.6020	0.764	12.0629	15.1411	-2.6420
6	11.4900	13.6020	0.764	12.0629	15.1411	-2.1120
7	11.9600	13.9505	0.734	12.4725	15.4285	-1.9905
8	11.7100	13.9505	0.734	12.4725	15.4285	-2.2405
9	14.7400	13.9505	0.734	12.4725	15.4285	0.7895
10	13.4200	13.9505	0.734	12.4725	15.4285	0.5305
11	13.6800	13.9505	0.734	12.4725	15.4285	-0.2705
12	12.8100	13.9505	0.734	12.4725	15.4285	-1.1405
13	15.4500	17.0873	0.579	15.9214	18.2531	-1.6373
14	12.5400	17.0873	0.579	15.9214	18.2531	-4.5473
15	16.4200	17.0873	0.579	15.9214	18.2531	-0.6673
16	15.9300	17.0873	0.579	15.9214	18.2531	-1.1573
17	16.4300	17.0873	0.579	15.9214	18.2531	-0.6573
18	15.3700	17.0873	0.579	15.9214	18.2531	-1.7173
19	17.1700	24.0578	1.099	21.8439	26.2717	-6.8878
20	17.2100	24.0578	1.099	21.8439	26.2717	-6.8478
21	17.3300	24.0578	1.099	21.8439	26.2717	-6.7278
22	17.5500	24.0578	1.099	21.8439	26.2717	-6.5078
23	16.7700	24.0578	1.099	21.8439	26.2717	-7.2878
24	18.2900	24.0578	1.099	21.8439	26.2717	-5.7678
25	14.2400	13.6020	0.764	12.0629	15.1411	0.6380
26	13.7600	13.6020	0.764	12.0629	15.1411	0.1580
27	14.6800	13.6020	0.764	12.0629	15.1411	1.0780
28	13.8800	13.6020	0.764	12.0629	15.1411	0.2780
29	16.6900	13.6020	0.764	12.0629	15.1411	3.0880
30	13.5900	13.6020	0.764	12.0629	15.1411	-0.0120
31	14.8700	13.9505	0.734	12.4725	15.4285	0.9195
32	15.0300	13.9505	0.734	12.4725	15.4285	1.0795
33	12.3200	13.9505	0.734	12.4725	15.4285	-1.6305
34	15.8500	13.9505	0.734	12.4725	15.4285	1.8995
35	16.7000	13.9505	0.734	12.4725	15.4285	2.7495
36	15.3500	13.9505	0.734	12.4725	15.4285	1.3995
37	22.8800	17.0873	0.579	15.9214	18.2531	5.7927
38	21.7400	17.0873	0.579	15.9214	18.2531	4.6527
39	18.7900	17.0873	0.579	15.9214	18.2531	1.7027
40	18.4500	17.0873	0.579	15.9214	18.2531	1.3627
41	19.9200	17.0873	0.579	15.9214	18.2531	2.8327
42	19.7200	17.0873	0.579	15.9214	18.2531	2.6327
43	32.6600	24.0578	1.099	21.8439	26.2717	8.6022
44	28.7600	24.0578	1.099	21.8439	26.2717	4.7022
45	30.4100	24.0578	1.099	21.8439	26.2717	6.3522
46	31.1800	24.0578	1.099	21.8439	26.2717	7.1222
47	25.5700	24.0578	1.099	21.8439	26.2717	1.5122
48	32.8200	24.0578	1.099	21.8439	26.2717	8.7622

Sum of Residuals	0
Sum of Squared Residuals	707.6399

43	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
44	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
45	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
46	1.0000	1.3624	0.143	1.0738	1.6510	0.3624
47	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
48	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624

Sum of Residuals	0
Sum of Squared Residuals	12.0223
Predicted Resid SS (Press)	13.0737

1 CALDWELL STUDY, CORRELATIONS WITH Administered DOSE 09:46 Monday, February 8, 1999 22

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
2	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
3	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
4	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
5	0	0.6026	0.102	0.3969	0.8084	-0.6026
6	0	0.6026	0.102	0.3969	0.8084	-0.6026
7	0	0.6284	0.098	0.4308	0.8260	-0.6284
8	.	0.6284	0.098	0.4308	0.8260	.
9	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
10	0	0.6284	0.098	0.4308	0.8260	-0.6284
11	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
12	0	0.6284	0.098	0.4308	0.8260	-0.6284
13	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
14	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
15	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
16	0	0.8608	0.077	0.7050	1.0167	-0.8608
17	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
18	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
19	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
20	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
21	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
22	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
23	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
24	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
25	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
26	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
27	0	0.6026	0.102	0.3969	0.8084	-0.6026
28	0	0.6026	0.102	0.3969	0.8084	-0.6026
29	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
30	0	0.6026	0.102	0.3969	0.8084	-0.6026
31	0	0.6284	0.098	0.4308	0.8260	-0.6284
32	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
33	0	0.6284	0.098	0.4308	0.8260	-0.6284

1 T4, T3, TSH data from Subchronic Perchlorate study with int dose from Meyer, 1998 15:56 Thursday, February 4, 1999 1

OBS	SEX	TMPT	DOSE	AGE	T4	T3	TSH	CODE	FOLL	INTDOSE	DCORR
1	M	15-18	1	16	5.10	200.14	13.08	n	0	0	0.00
2	M	15-18	1	15	5.64	208.45	13.72	n	0	0	0.00
3	M	15-18	1	17	6.64	186.23	14.20	n	0	0	0.00
4	M	15-18	1	17	5.88	234.21	16.38	n	0	0	0.00
5	M	15-18	1	16	4.84	205.08	13.21	ab	1	0	0.00
6	M	15-18	1	15	5.42	178.53	15.52	n	0	0	0.00
7	M	15-18	1	18	5.29	170.16	17.45	n	0	0	0.00
8	M	15-18	1	17	5.19	187.11	15.30	n	0	0	0.00
9	M	15-18	2	18	5.10	173.23	15.22	n	0	11	0.01
10	M	15-18	2	17	4.23	170.49	12.76	n	0	11	0.01
11	M	15-18	2	17	5.21	154.81	17.63	ab	0	11	0.01
12	M	15-18	2	17	6.03	136.30	18.41	n	0	11	0.01
13	M	15-18	2	18	5.70	183.30	16.49	n	0	11	0.01
14	M	15-18	5	15	5.58	122.39	19.93	n	0	55	1.00
15	M	15-18	5	18	4.84	130.71	20.27	n	0	55	1.00
16	M	15-18	5	15	5.17	139.56	17.39	n	0	55	1.00
17	M	15-18	5	17	4.69	137.44	17.03	ab	0	55	1.00
18	M	15-18	5	18	4.92	133.18	20.87	n	0	55	1.00
19	M	15-18	5	16	6.06	101.61	18.72	n	0	55	1.00
20	M	15-18	5	15	4.81	125.91	21.75	n	0	55	1.00
21	M	15-18	5	17	4.86	109.93	16.11	n	0	55	1.00
22	M	15-18	5	16	5.24	124.57	17.48	n	0	55	1.00
23	M	15-18	5	17	5.74	112.27	18.45	n	0	55	1.00
24	F	15-18	1	16	4.89	133.28	9.89	n	0	0	0.00
25	F	15-18	1	15	4.23	146.98	9.49	n	0	0	0.00
26	F	15-18	1	15	4.45	148.52	9.00	n	0	0	0.00
27	F	15-18	1	16	4.33	113.01	10.51	n	0	0	0.00
28	F	15-18	1	17	4.87	141.79	10.86	n	0	0	0.00
29	F	15-18	1	16	3.47	115.12	11.30	n	0	0	0.00
30	F	15-18	1	17	4.81	125.46	10.27	n	0	0	0.00
31	F	15-18	1	18	4.36	155.28	11.15	n	0	0	0.00
32	F	15-18	1	18	4.75	117.27	10.57	n	0	0	0.00
33	F	15-18	1	18	4.72	133.24	11.36	n	0	0	0.00
34	F	15-18	2	18	4.48	144.32	11.86	n	0	11	0.01
35	F	15-18	2	15	4.39	134.14	11.83	n	0	11	0.01
36	F	15-18	2	15	4.59	134.16	10.33	n	0	11	0.01
37	F	15-18	2	16	4.58	134.89	10.03	ab	0	11	0.01
38	F	15-18	2	16	4.74	142.55	13.47	n	0	11	0.01
39	F	15-18	2	16	4.60	113.00	10.78	n	0	11	0.01
40	F	15-18	2	18	3.24	147.24	13.71	n	0	11	0.01
41	F	15-18	2	17	4.44	128.84	11.46	n	0	11	0.01
42	F	15-18	2	18	3.83	145.91	11.36	n	0	11	0.01
43	F	15-18	2	17	4.34	115.29	12.35	n	0	11	0.01
44	F	15-18	5	16	4.02	139.95	13.07	n	0	55	1.00
45	F	15-18	5	18	3.45	122.78	12.33	n	0	55	1.00

91	F	92-95	1	95	4.75	157.34	15.31	n	0	0	0.00
92	F	92-95	1	95	4.25	154.03	19.14	n	0	0	0.00
93	F	92-95	1	95	4.72	148.72	15.41	n	0	0	0.00
94	F	92-95	2	92	3.61	161.10	17.85	n	0	11	0.01
95	F	92-95	2	92	3.73	133.70	15.76	n	0	11	0.01
96	F	92-95	2	93	3.60	153.42	17.47	n	0	11	0.01
97	F	92-95	2	93	3.36	140.29	14.80	n	0	11	0.01
98	F	92-95	2	93	3.18	122.90	19.76	n	0	11	0.01
99	F	92-95	2	94	3.30	126.23	19.48	n	0	11	0.01
100	F	92-95	2	94	3.10	136.10	15.89	n	0	11	0.01
101	F	92-95	2	95	3.46	158.85	16.64	n	0	11	0.01
102	F	92-95	2	95	4.29	134.94	14.45	n	0	11	0.01
103	F	92-95	2	95	3.58	163.78	15.73	ab	0	11	0.01
104	F	92-95	5	92	3.02	122.86	17.75	n	0	55	1.00
105	F	92-95	5	92	3.10	116.09	18.81	n	0	55	1.00
106	F	92-95	5	93	3.41	116.59	16.26	n	0	55	1.00
107	F	92-95	5	93	2.90	107.73	19.20	n	0	55	1.00
108	F	92-95	5	93	3.78	135.30	18.76	n	0	55	1.00
109	F	92-95	5	94	3.43	117.29	15.69	n	0	55	1.00
110	F	92-95	5	95	3.18	137.19	18.27	n	0	55	1.00
111	F	92-95	5	95	2.79	124.03	14.97	n	0	55	1.00
112	F	92-95	5	95	3.04	125.61	18.93	n	0	55	1.00

1 T4, T3, TSH data from Dev NT, PND 5Perchlorate study including approx of internal dose from Meyer, 1998

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15:56 Thursday, February 4, 1999

OBS	DOSE	LITTER	TSH	T4	T3	INTDOSE	DCORR
1	1	19508	4.15	3.17	.	0	0.0
2	1	19520	4.23	2.67	88.8	0	0.0
3	1	19523	4.36	3.49	91.1	0	0.0
4	1	19526	4.06	.	97.9	0	0.0
5	1	19530	4.12	3.37	84.0	0	0.0
6	1	19549	4.68	3.67	82.1	0	0.0
7	1	19556	4.19	3.83	84.1	0	0.0
8	1	19558	4.94	3.29	86.0	0	0.0
9	1	19571	4.09	.	86.5	0	0.0
10	1	19580	4.15	3.07	.	0	0.0
11	1	19582	4.30	2.90	.	0	0.0
12	1	19585	4.72	3.52	.	0	0.0
13	1	19608	4.34	3.81	.	0	0.0
14	1	19610	4.94	3.85	.	0	0.0
15	1	19611	5.46	3.54	.	0	0.0
16	1	19614	5.53	3.85	81.5	0	0.0
17	1	19621	4.45	3.11	97.7	0	0.0
18	2	19511	4.60	3.37	94.1	29	0.1
19	2	19514	3.87	3.04	.	29	0.1
20	2	19527	5.01	3.67	77.8	29	0.1
21	2	19533	3.88	2.87	79.5	29	0.1

66 4 19618 4.89 . . 82 3

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE from Meyer, 1998 5

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Correlation Analysis

5 'VAR' Variables: DCORR INTDOSE T4 T3 TSH

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DCORR	66	1.066667	1.215583	70.400000	0	3.000000
INTDOSE	66	42.272727	31.031272	2790.000000	0	82.000000
T4	50	3.158000	0.408841	157.900000	2.280000	3.850000
T3	42	71.304762	21.611717	2994.800000	34.500000	97.900000
TSH	66	4.647576	0.472255	306.740000	3.760000	5.630000

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / Number of Observations

	DCORR	INTDOSE	T4	T3	TSH
DCORR	1.00000 0.0 66	0.91220 0.0001 66	-0.67382 0.0001 50	-0.94857 0.0001 42	0.21926 0.0769 66
INTDOSE	0.91220 0.0001 66	1.00000 0.0 66	-0.63297 0.0001 50	-0.83589 0.0001 42	0.24164 0.0506 66
T4	-0.67382 0.0001 50	-0.63297 0.0001 50	1.00000 0.0 50	0.60491 0.0001 39	0.01721 0.9056 50
T3	-0.94857 0.0001 42	-0.83589 0.0001 42	0.60491 0.0001 39	1.00000 0.0 42	-0.28791 0.0645 42
TSH	0.21926 0.0769 66	0.24164 0.0506 66	0.01721 0.9056 50	-0.28791 0.0645 42	1.00000 0.0 66

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 6

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Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.56696	0.56696	2.568	0.1175
Error	37	8.16763	0.22075		
C Total	38	8.73459			

Root MSE	0.46984	R-square	0.0649
Dep Mean	4.68718	Adj R-sq	0.0396
C.V.	10.02388		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.506929	0.13531605	33.307	0.0001
INTDOSE	1	0.004035	0.00251806	1.603	0.1175

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 8

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Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	3.1700	3.5209	0.092	3.3341	3.7078	-0.3509
2	2.6700	3.5209	0.092	3.3341	3.7078	-0.8509
3	3.4900	3.5209	0.092	3.3341	3.7078	-0.0309
4	.	3.5209	0.092	3.3341	3.7078	.
5	3.3700	3.5209	0.092	3.3341	3.7078	-0.1509
6	3.6700	3.5209	0.092	3.3341	3.7078	0.1491
7	3.8300	3.5209	0.092	3.3341	3.7078	0.3091
8	3.2900	3.5209	0.092	3.3341	3.7078	-0.2309
9	.	3.5209	0.092	3.3341	3.7078	.
10	3.0700	3.5209	0.092	3.3341	3.7078	-0.4509
11	2.9000	3.5209	0.092	3.3341	3.7078	-0.6209
12	3.5200	3.5209	0.092	3.3341	3.7078	-0.00094
13	3.8100	3.5209	0.092	3.3341	3.7078	0.2891
14	3.8500	3.5209	0.092	3.3341	3.7078	0.3291
15	3.5400	3.5209	0.092	3.3341	3.7078	0.0191
16	3.8500	3.5209	0.092	3.3341	3.7078	0.3291
17	3.1100	3.5209	0.092	3.3341	3.7078	0.4109
18	3.3700	3.2528	0.058	3.1355	3.3701	0.1172
19	3.0400	3.2528	0.058	3.1355	3.3701	-0.2128
20	3.6700	3.2528	0.058	3.1355	3.3701	0.4172
21	2.8700	3.2528	0.058	3.1355	3.3701	-0.3828

65	2.6400	2.7628	0.082	2.5965	2.9290	-0.1228
66	.	2.7628	0.082	2.5965	2.9290	.

Sum of Residuals	0
Sum of Squared Residuals	3.7944
Predicted Resid SS (Press)	4.2470

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	.	96.9481	3.553	89.7482	104.1	.
2	88.8000	96.9481	3.553	89.7482	104.1	-8.1481
3	91.1000	96.9481	3.553	89.7482	104.1	-5.8481
4	97.9000	96.9481	3.553	89.7482	104.1	0.9519
5	84.0000	96.9481	3.553	89.7482	104.1	-12.9481
6	82.1000	96.9481	3.553	89.7482	104.1	-14.8481
7	84.1000	96.9481	3.553	89.7482	104.1	-12.8481
8	86.0000	96.9481	3.553	89.7482	104.1	-10.9481
9	86.5000	96.9481	3.553	89.7482	104.1	-10.4481
10	.	96.9481	3.553	89.7482	104.1	.
11	.	96.9481	3.553	89.7482	104.1	.
12	.	96.9481	3.553	89.7482	104.1	.
13	.	96.9481	3.553	89.7482	104.1	.
14	.	96.9481	3.553	89.7482	104.1	.
15	.	96.9481	3.553	89.7482	104.1	.
16	81.5000	96.9481	3.553	89.7482	104.1	-15.4481
17	97.7000	96.9481	3.553	89.7482	104.1	0.7519
18	94.1000	80.0679	2.231	75.5479	84.5879	14.0321
19	.	80.0679	2.231	75.5479	84.5879	.
20	77.8000	80.0679	2.231	75.5479	84.5879	2.2679
21	79.5000	80.0679	2.231	75.5479	84.5879	-0.5679
22	92.3000	80.0679	2.231	75.5479	84.5879	12.2321
23	.	80.0679	2.231	75.5479	84.5879	.
24	77.9000	80.0679	2.231	75.5479	84.5879	-2.1679
25	.	80.0679	2.231	75.5479	84.5879	.
26	90.9000	80.0679	2.231	75.5479	84.5879	10.8321
27	83.3000	80.0679	2.231	75.5479	84.5879	3.2321
28	74.5000	80.0679	2.231	75.5479	84.5879	-5.5679
29	94.9000	80.0679	2.231	75.5479	84.5879	14.8321
30	.	80.0679	2.231	75.5479	84.5879	.
31	86.1000	80.0679	2.231	75.5479	84.5879	6.0321
32	74.3000	64.9339	2.090	60.6983	69.1696	9.3661
33	89.5000	64.9339	2.090	60.6983	69.1696	24.5661

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

7	4.1900	4.5069	0.135	4.2328	4.7811	-0.3169
8	4.9400	4.5069	0.135	4.2328	4.7811	0.4331
9	4.0900	4.5069	0.135	4.2328	4.7811	-0.4169
10	4.1500	4.5069	0.135	4.2328	4.7811	-0.3569
11	4.3000	4.5069	0.135	4.2328	4.7811	-0.2069
12	4.7200	4.5069	0.135	4.2328	4.7811	0.2131

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 11

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Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
13	4.3400	4.5069	0.135	4.2328	4.7811	-0.1669
14	4.9400	4.5069	0.135	4.2328	4.7811	0.4331
15	5.4600	4.5069	0.135	4.2328	4.7811	0.9531
16	5.5300	4.5069	0.135	4.2328	4.7811	1.0231
17	4.4500	4.5069	0.135	4.2328	4.7811	-0.0569
18	4.6000	4.6240	0.085	4.4518	4.7961	-0.0240
19	3.8700	4.6240	0.085	4.4518	4.7961	-0.7540
20	5.0100	4.6240	0.085	4.4518	4.7961	0.3860
21	3.8800	4.6240	0.085	4.4518	4.7961	-0.7440
22	4.1900	4.6240	0.085	4.4518	4.7961	-0.4340
23	4.6200	4.6240	0.085	4.4518	4.7961	-0.00396
24	4.9400	4.6240	0.085	4.4518	4.7961	0.3160
25	4.7100	4.6240	0.085	4.4518	4.7961	0.0860
26	4.2000	4.6240	0.085	4.4518	4.7961	-0.4240
27	4.0900	4.6240	0.085	4.4518	4.7961	-0.5340
28	4.3000	4.6240	0.085	4.4518	4.7961	-0.3240
29	4.7600	4.6240	0.085	4.4518	4.7961	0.1360
30	4.8900	4.6240	0.085	4.4518	4.7961	0.2660
31	5.2500	4.6240	0.085	4.4518	4.7961	0.6260
32	4.5600	4.7289	0.080	4.5676	4.8902	-0.1689
33	4.7400	4.7289	0.080	4.5676	4.8902	0.0111
34	4.0400	4.7289	0.080	4.5676	4.8902	-0.6889
35	5.1300	4.7289	0.080	4.5676	4.8902	0.4011
36	4.4000	4.7289	0.080	4.5676	4.8902	-0.3289
37	4.5100	4.7289	0.080	4.5676	4.8902	-0.2189
38	4.3900	4.7289	0.080	4.5676	4.8902	-0.3389
39	4.3800	4.7289	0.080	4.5676	4.8902	-0.3489
40	4.8900	4.7289	0.080	4.5676	4.8902	0.1611
41	4.2200	4.7289	0.080	4.5676	4.8902	-0.5089
42	5.6100	4.7289	0.080	4.5676	4.8902	0.8811
43	4.9800	4.7289	0.080	4.5676	4.8902	0.2511
44	5.6300	4.7289	0.080	4.5676	4.8902	0.9011
45	4.7800	4.7289	0.080	4.5676	4.8902	0.0511
46	5.2800	4.7289	0.080	4.5676	4.8902	0.5511
47	4.0300	4.7289	0.080	4.5676	4.8902	-0.6989
48	4.8800	4.7289	0.080	4.5676	4.8902	0.1511
49	5.1100	4.7289	0.080	4.5676	4.8902	0.3811

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 13

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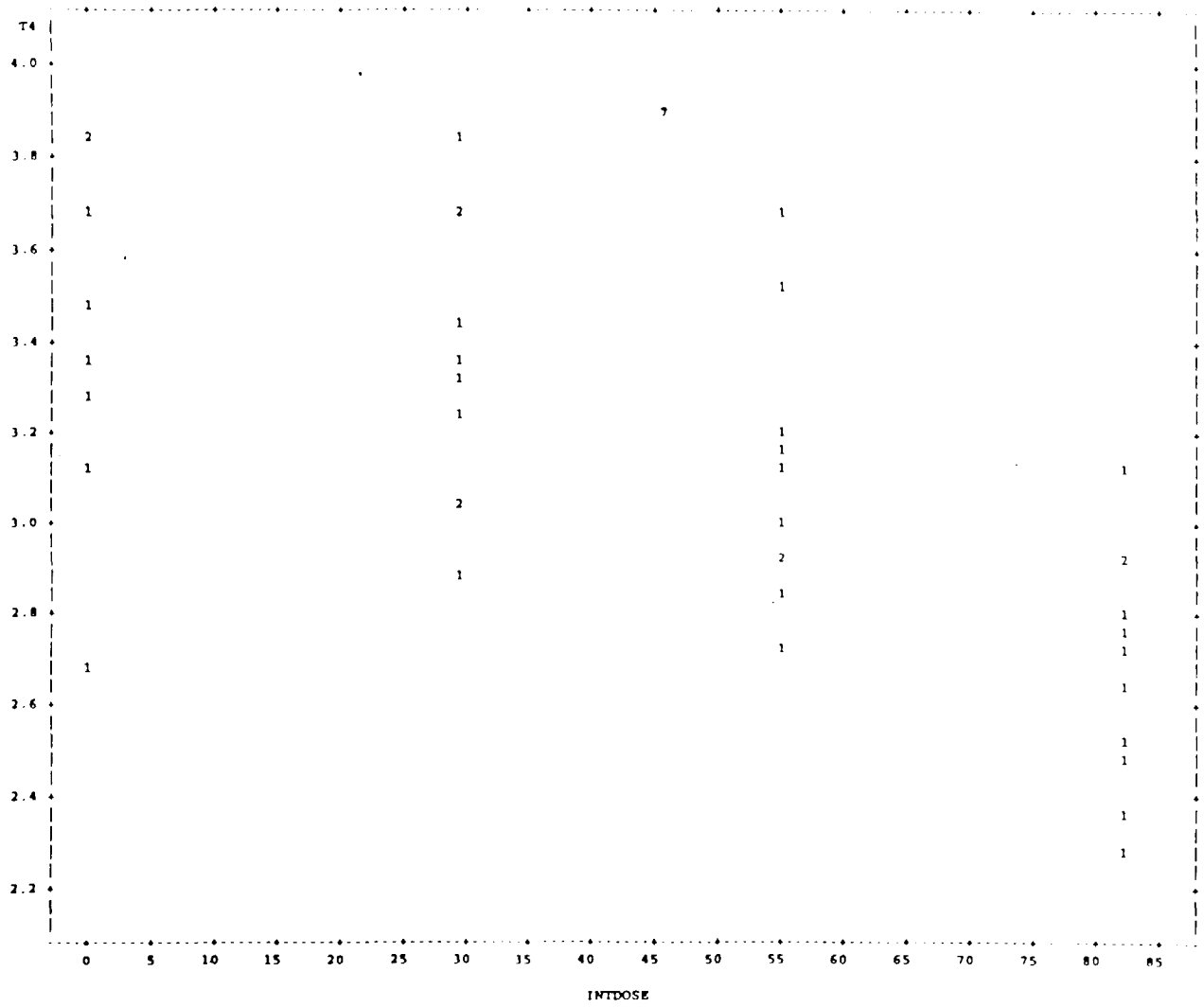

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 15

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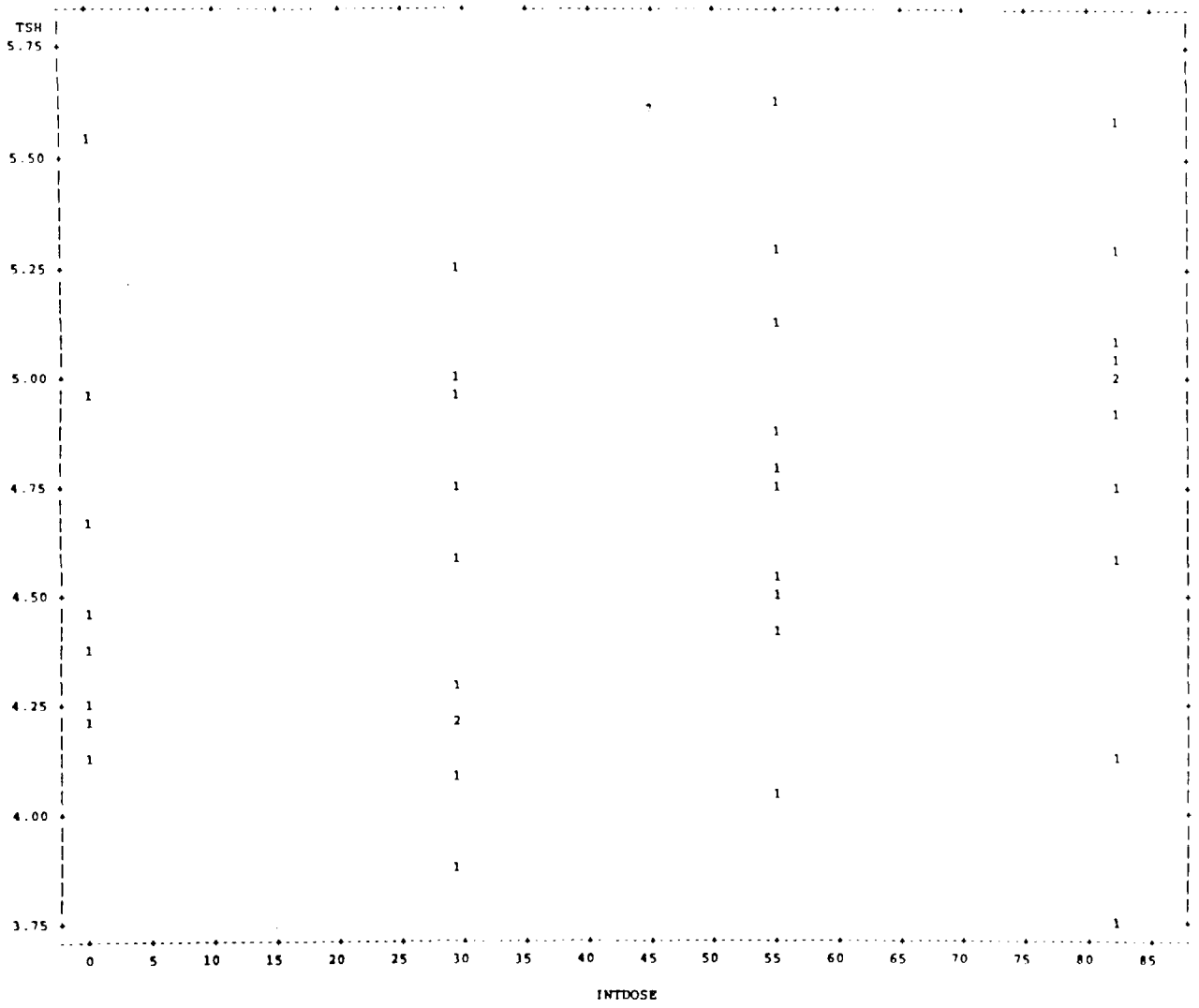

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH EXTERNAL DOSE 17
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Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.53614	0.53614	2.420	0.1283
Error	37	8.19845	0.22158		
C Total	38	8.73459			

Root MSE	0.47072	R-square	0.0614
Dep Mean	4.68718	Adj R-sq	0.0360
C.V.	10.04277		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.580047	0.10210248	44.857	0.0001
DCORR	1	0.094958	0.06104586	1.556	0.1283

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH EXTERNAL DOSE 18
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Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	3.1700	3.3731	0.067	3.2382	3.5080	-0.2031
2	2.6700	3.3731	0.067	3.2382	3.5080	-0.7031
3	3.4900	3.3731	0.067	3.2382	3.5080	0.1169
4	.	3.3731	0.067	3.2382	3.5080	.
5	3.3700	3.3731	0.067	3.2382	3.5080	-0.00309
6	3.6700	3.3731	0.067	3.2382	3.5080	0.2969
7	3.8300	3.3731	0.067	3.2382	3.5080	0.4569
8	3.2900	3.3731	0.067	3.2382	3.5080	-0.0831
9	.	3.3731	0.067	3.2382	3.5080	.
10	3.0700	3.3731	0.067	3.2382	3.5080	-0.3031
11	2.9000	3.3731	0.067	3.2382	3.5080	-0.4731
12	3.5200	3.3731	0.067	3.2382	3.5080	0.1469
13	3.8100	3.3731	0.067	3.2382	3.5080	0.4369
14	3.8500	3.3731	0.067	3.2382	3.5080	0.4769
15	3.5400	3.3731	0.067	3.2382	3.5080	0.1669
16	3.8500	3.3731	0.067	3.2382	3.5080	0.4769

60	2.7600	2.6680	0.089	2.4872	2.8489	0.0920
61	2.9000	2.6680	0.089	2.4872	2.8489	0.2320
62	.	2.6680	0.089	2.4872	2.8489	.
63	.	2.6680	0.089	2.4872	2.8489	.
64	.	2.6680	0.089	2.4872	2.8489	.
65	2.6400	2.6680	0.089	2.4872	2.8489	-0.0280
66	.	2.6680	0.089	2.4872	2.8489	.

Sum of Residuals	0
Sum of Squared Residuals	3.4866
Predicted Resid SS (Press)	3.8525

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	.	89.2214	1.526	86.1300	92.3128	.
2	88.8000	89.2214	1.526	86.1300	92.3128	-0.4214
3	91.1000	89.2214	1.526	86.1300	92.3128	1.8786
4	97.9000	89.2214	1.526	86.1300	92.3128	8.6786
5	84.0000	89.2214	1.526	86.1300	92.3128	-5.2214
6	82.1000	89.2214	1.526	86.1300	92.3128	-7.1214
7	84.1000	89.2214	1.526	86.1300	92.3128	-5.1214
8	86.0000	89.2214	1.526	86.1300	92.3128	-3.2214
9	86.5000	89.2214	1.526	86.1300	92.3128	-2.7214
10	.	89.2214	1.526	86.1300	92.3128	.
11	.	89.2214	1.526	86.1300	92.3128	.
12	.	89.2214	1.526	86.1300	92.3128	.
13	.	89.2214	1.526	86.1300	92.3128	.
14	.	89.2214	1.526	86.1300	92.3128	.
15	.	89.2214	1.526	86.1300	92.3128	.
16	81.5000	89.2214	1.526	86.1300	92.3128	-7.7214
17	97.7000	89.2214	1.526	86.1300	92.3128	8.4786
18	94.1000	87.6018	1.466	84.6319	90.5717	6.4982
19	.	87.6018	1.466	84.6319	90.5717	.
20	77.8000	87.6018	1.466	84.6319	90.5717	-9.8018
21	79.5000	87.6018	1.466	84.6319	90.5717	-8.1018
22	92.3000	87.6018	1.466	84.6319	90.5717	4.6982
23	.	87.6018	1.466	84.6319	90.5717	.
24	77.9000	87.6018	1.466	84.6319	90.5717	-9.7018
25	.	87.6018	1.466	84.6319	90.5717	.
26	90.9000	87.6018	1.466	84.6319	90.5717	3.2982
27	83.3000	87.6018	1.466	84.6319	90.5717	-4.3018
28	74.5000	87.6018	1.466	84.6319	90.5717	-13.1018
29	94.9000	87.6018	1.466	84.6319	90.5717	7.2982
30	.	87.6018	1.466	84.6319	90.5717	.
31	86.1000	87.6018	1.466	84.6319	90.5717	-1.5018
32	74.3000	73.0252	1.132	70.7307	75.3196	1.2748
33	89.5000	73.0252	1.132	70.7307	75.3196	16.4748

2	4.2300	4.5800	0.102	4.3732	4.7869	-0.3500
3	4.3600	4.5800	0.102	4.3732	4.7869	-0.2200
4	4.0600	4.5800	0.102	4.3732	4.7869	-0.5200
5	4.1200	4.5800	0.102	4.3732	4.7869	-0.4600
6	4.6800	4.5800	0.102	4.3732	4.7869	0.1000
7	4.1900	4.5800	0.102	4.3732	4.7869	-0.3900
8	4.9400	4.5800	0.102	4.3732	4.7869	0.3600
9	4.0900	4.5800	0.102	4.3732	4.7869	-0.4900
10	4.1500	4.5800	0.102	4.3732	4.7869	-0.4300
11	4.3000	4.5800	0.102	4.3732	4.7869	-0.2800
12	4.7200	4.5800	0.102	4.3732	4.7869	0.1400

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH EXTERNAL DOSE 21

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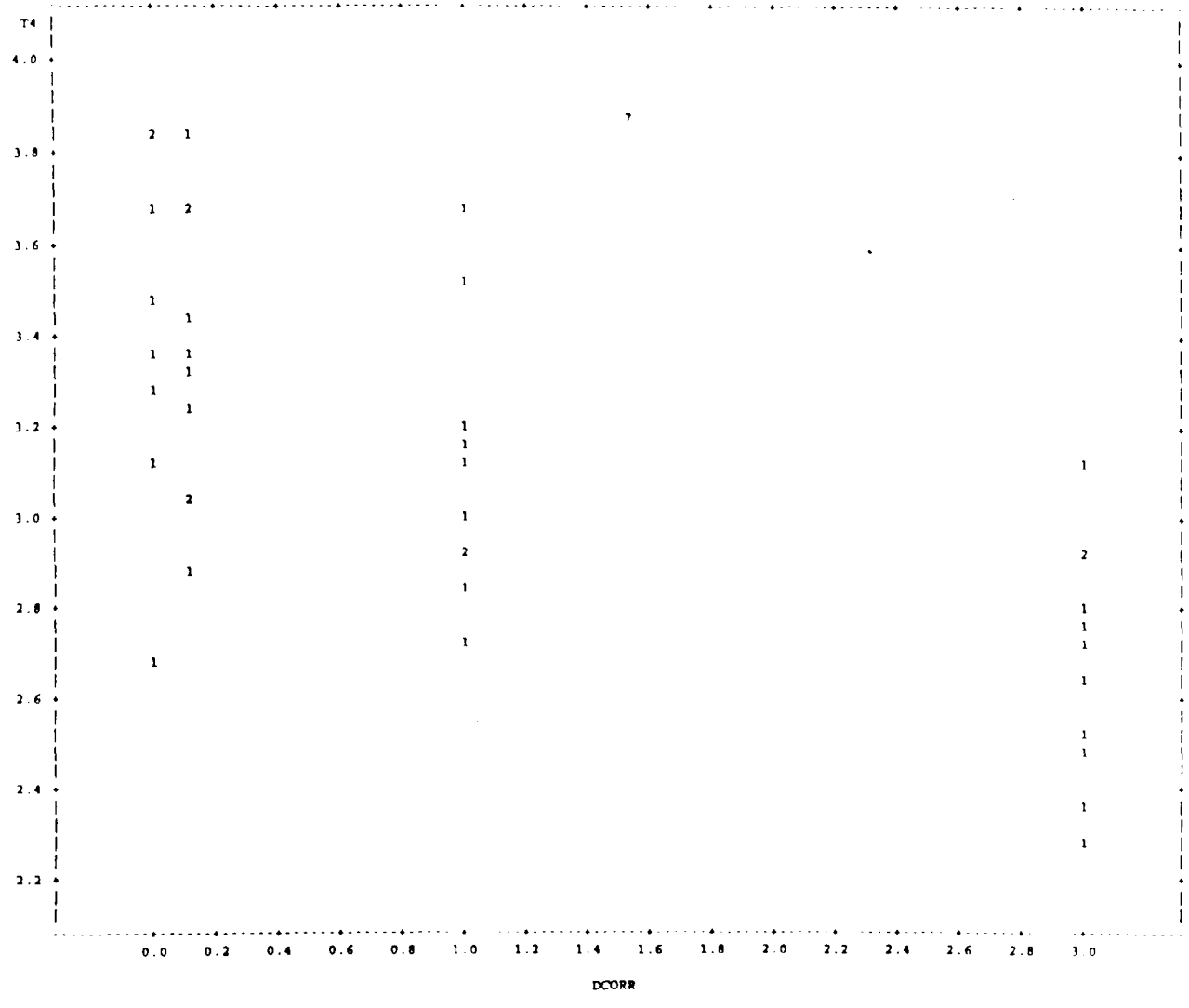
Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
13	4.3400	4.5800	0.102	4.3732	4.7869	-0.2400
14	4.9400	4.5800	0.102	4.3732	4.7869	0.3600
15	5.4600	4.5800	0.102	4.3732	4.7869	0.8800
16	5.5300	4.5800	0.102	4.3732	4.7869	0.9500
17	4.4500	4.5800	0.102	4.3732	4.7869	-0.1300
18	4.6000	4.5895	0.098	4.3908	4.7883	0.0105
19	3.8700	4.5895	0.098	4.3908	4.7883	-0.7195
20	5.0100	4.5895	0.098	4.3908	4.7883	0.4205
21	3.8800	4.5895	0.098	4.3908	4.7883	-0.7095
22	4.1900	4.5895	0.098	4.3908	4.7883	-0.3995
23	4.6200	4.5895	0.098	4.3908	4.7883	0.0305
24	4.9400	4.5895	0.098	4.3908	4.7883	0.3505
25	4.7100	4.5895	0.098	4.3908	4.7883	0.1205
26	4.2000	4.5895	0.098	4.3908	4.7883	-0.3895
27	4.0900	4.5895	0.098	4.3908	4.7883	-0.4995
28	4.3000	4.5895	0.098	4.3908	4.7883	-0.2895
29	4.7600	4.5895	0.098	4.3908	4.7883	0.1705
30	4.8900	4.5895	0.098	4.3908	4.7883	0.3005
31	5.2500	4.5895	0.098	4.3908	4.7883	0.6605
32	4.5600	4.6750	0.076	4.5215	4.8286	-0.1150
33	4.7400	4.6750	0.076	4.5215	4.8286	0.0650
34	4.0400	4.6750	0.076	4.5215	4.8286	-0.6350
35	5.1300	4.6750	0.076	4.5215	4.8286	0.4550
36	4.4000	4.6750	0.076	4.5215	4.8286	-0.2750
37	4.5100	4.6750	0.076	4.5215	4.8286	-0.1650
38	4.3900	4.6750	0.076	4.5215	4.8286	-0.2850
39	4.3800	4.6750	0.076	4.5215	4.8286	-0.2950
40	4.8900	4.6750	0.076	4.5215	4.8286	0.2150
41	4.2200	4.6750	0.076	4.5215	4.8286	-0.4550
42	5.6100	4.6750	0.076	4.5215	4.8286	0.9350
43	4.9800	4.6750	0.076	4.5215	4.8286	0.3050
44	5.6300	4.6750	0.076	4.5215	4.8286	0.9550


T4
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0
DCORR

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH EXTERNAL DOSE 25

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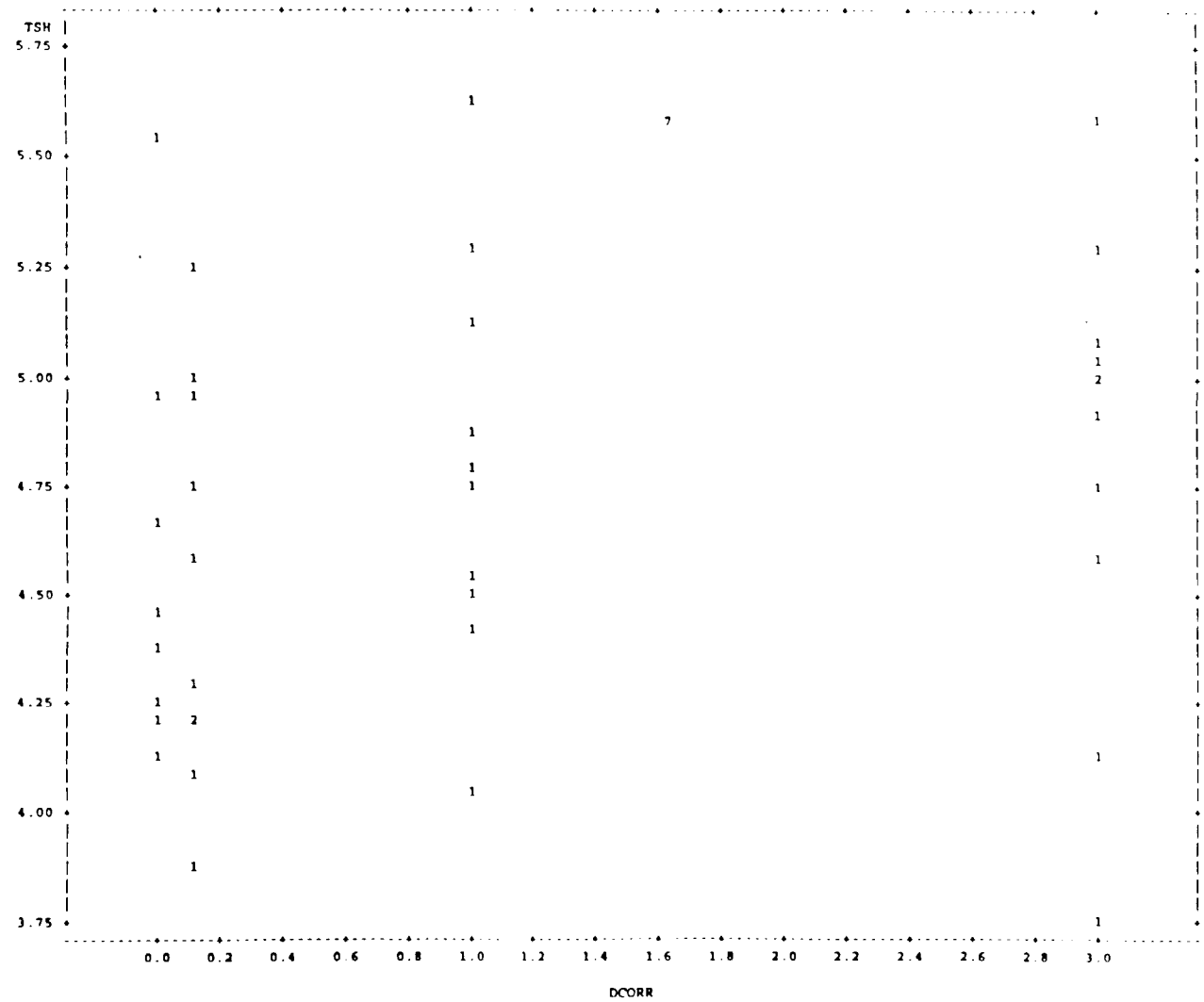

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DCORR	59	0.325424	0.468929	19.200000	0	1.000000
INTDOSE	59	21.440678	23.770651	1265.000000	0	55.000000
T4	59	4.018644	0.774731	237.100000	2.790000	6.350000
T3	59	149.588475	26.736364	8825.720000	103.330000	215.330000
TSH	59	17.151525	1.802690	1011.940000	13.650000	21.520000

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 59

	DCORR	INTDOSE	T4	T3	TSH
DCORR	1.00000 0.0	0.98303 0.0001	-0.61935 0.0001	-0.71706 0.0001	0.47101 0.0002
INTDOSE	0.98303 0.0001	1.00000 0.0	-0.68767 0.0001	-0.77399 0.0001	0.48249 0.0001
T4	-0.61935 0.0001	-0.68767 0.0001	1.00000 0.0	0.69366 0.0001	-0.31694 0.0145
T3	-0.71706 0.0001	-0.77399 0.0001	0.69366 0.0001	1.00000 0.0	-0.45130 0.0003
TSH	0.47101 0.0002	0.48249 0.0001	-0.31694 0.0145	-0.45130 0.0003	1.00000 0.0

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 28

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---------- TMPT=15-18 ----------

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.46317	0.46317	0.951	0.3340
Error	51	24.83270	0.48692		
C Total	52	25.29587			

Root MSE	0.69779	R-square	0.0183
Dep Mean	4.74208	Adj R-sq	-0.0009

Root MSE	2.95180	R-square	0.2018
Dep Mean	13.86491	Adj R-sq	0.1861
C.V.	21.28975		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	12.453554	0.56473417	22.052	0.0001
INTDOSE	1	0.059132	0.01646970	3.590	0.0007

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 30

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---------- TMPT=15-18 ----------

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	5.1000	4.8327	0.134	4.5647	5.1007	0.2673
2	5.6400	4.8327	0.134	4.5647	5.1007	0.8073
3	6.6400	4.8327	0.134	4.5647	5.1007	1.8073
4	5.8800	4.8327	0.134	4.5647	5.1007	1.0473
5	4.8400	4.8327	0.134	4.5647	5.1007	0.00729
6	5.4200	4.8327	0.134	4.5647	5.1007	0.5873
7	5.2900	4.8327	0.134	4.5647	5.1007	0.4573
8	5.1900	4.8327	0.134	4.5647	5.1007	0.3573
9	5.1000	4.7909	0.108	4.5738	5.0081	0.3091
10	4.2300	4.7909	0.108	4.5738	5.0081	-0.5609
11	5.2100	4.7909	0.108	4.5738	5.0081	0.4191
12	6.0300	4.7909	0.108	4.5738	5.0081	1.2391
13	5.7000	4.7909	0.108	4.5738	5.0081	0.9091
14	5.5800	4.6239	0.155	4.3136	4.9341	0.9561
15	4.8400	4.6239	0.155	4.3136	4.9341	0.2161
16	5.1700	4.6239	0.155	4.3136	4.9341	0.5461
17	4.6900	4.6239	0.155	4.3136	4.9341	0.0661
18	4.9200	4.6239	0.155	4.3136	4.9341	0.2961
19	6.0600	4.6239	0.155	4.3136	4.9341	1.4361
20	4.8100	4.6239	0.155	4.3136	4.9341	0.1861
21	4.8600	4.6239	0.155	4.3136	4.9341	0.2361
22	5.2400	4.6239	0.155	4.3136	4.9341	0.6161
23	5.7400	4.6239	0.155	4.3136	4.9341	1.1161
24	4.8900	4.8327	0.134	4.5647	5.1007	0.0573
25	4.2300	4.8327	0.134	4.5647	5.1007	-0.6027
26	4.4500	4.8327	0.134	4.5647	5.1007	-0.3827
27	4.3300	4.8327	0.134	4.5647	5.1007	-0.5027
28	4.8700	4.8327	0.134	4.5647	5.1007	0.0373
29	3.4700	4.8327	0.134	4.5647	5.1007	-1.3627

9	173.2	150.2	3.865	142.5	158.0	23.0172
10	170.5	150.2	3.865	142.5	158.0	20.2772
11	154.8	150.2	3.865	142.5	158.0	4.5972
12	136.3	150.2	3.865	142.5	158.0	-13.9128
13	183.3	150.2	3.865	142.5	158.0	33.0872
14	122.4	123.5	5.522	112.4	134.6	-1.0998
15	130.7	123.5	5.522	112.4	134.6	7.2202
16	139.6	123.5	5.522	112.4	134.6	16.0702
17	137.4	123.5	5.522	112.4	134.6	13.9502
18	133.2	123.5	5.522	112.4	134.6	9.6902
19	101.6	123.5	5.522	112.4	134.6	-21.8798
20	125.9	123.5	5.522	112.4	134.6	2.4202
21	109.9	123.5	5.522	112.4	134.6	-13.5598
22	124.6	123.5	5.522	112.4	134.6	1.0802
23	112.3	123.5	5.522	112.4	134.6	-11.2198
24	133.3	156.9	4.770	147.3	166.5	-23.6135
25	147.0	156.9	4.770	147.3	166.5	-9.9135
26	148.5	156.9	4.770	147.3	166.5	-8.3735
27	113.0	156.9	4.770	147.3	166.5	-43.8835
28	141.8	156.9	4.770	147.3	166.5	-15.1035
29	115.1	156.9	4.770	147.3	166.5	-41.7735
30	125.5	156.9	4.770	147.3	166.5	-31.4335
31	155.3	156.9	4.770	147.3	166.5	-1.6135
32	117.3	156.9	4.770	147.3	166.5	-39.6235
33	133.2	156.9	4.770	147.3	166.5	-23.6535
34	144.3	150.2	3.865	142.5	158.0	-5.8928
35	134.1	150.2	3.865	142.5	158.0	-16.0728
36	134.2	150.2	3.865	142.5	158.0	-16.0528
37	134.9	150.2	3.865	142.5	158.0	-15.3228
38	142.6	150.2	3.865	142.5	158.0	-7.6628
39	113.0	150.2	3.865	142.5	158.0	-37.2128
40	147.2	150.2	3.865	142.5	158.0	-2.9728
41	128.8	150.2	3.865	142.5	158.0	-21.3728
42	145.9	150.2	3.865	142.5	158.0	-4.3028

---------- TMPT=15-18 ----------

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
43	115.3	150.2	3.865	142.5	158.0	-34.9228
44	140.0	123.5	5.522	112.4	134.6	16.4602
45	122.8	123.5	5.522	112.4	134.6	-0.7098
46	109.9	123.5	5.522	112.4	134.6	-13.5598
47	121.8	123.5	5.522	112.4	134.6	-1.6898
48	117.3	123.5	5.522	112.4	134.6	-6.1998
49	127.9	123.5	5.522	112.4	134.6	4.3902

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
33	11.3600	12.4536	0.565	11.3198	13.5873	-1.0936
34	11.8600	13.1040	0.458	12.1855	14.0225	-1.2440
35	11.8300	13.1040	0.458	12.1855	14.0225	-1.2740
36	10.3300	13.1040	0.458	12.1855	14.0225	-2.7740
37	10.0300	13.1040	0.458	12.1855	14.0225	-3.0740
38	13.4700	13.1040	0.458	12.1855	14.0225	0.3660
39	10.7800	13.1040	0.458	12.1855	14.0225	-2.3240
40	13.7100	13.1040	0.458	12.1855	14.0225	0.6060
41	11.4600	13.1040	0.458	12.1855	14.0225	-1.6440
42	11.3600	13.1040	0.458	12.1855	14.0225	-1.7440
43	12.3500	13.1040	0.458	12.1855	14.0225	-0.7540
44	13.0700	15.7058	0.654	14.3935	17.0181	-2.6358
45	12.3300	15.7058	0.654	14.3935	17.0181	-3.3758
46	10.1300	15.7058	0.654	14.3935	17.0181	-5.5758
47	12.0200	15.7058	0.654	14.3935	17.0181	-3.6858
48	13.4800	15.7058	0.654	14.3935	17.0181	-2.2258
49	11.7000	15.7058	0.654	14.3935	17.0181	-4.0058
50	12.4800	15.7058	0.654	14.3935	17.0181	-3.2258
51	13.1400	15.7058	0.654	14.3935	17.0181	-2.5658
52	14.9100	15.7058	0.654	14.3935	17.0181	-0.7958
53	12.6300	15.7058	0.654	14.3935	17.0181	-3.0758

Sum of Residuals	0
Sum of Squared Residuals	444.3705
Predicted Resid SS (Press)	482.8323

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 35

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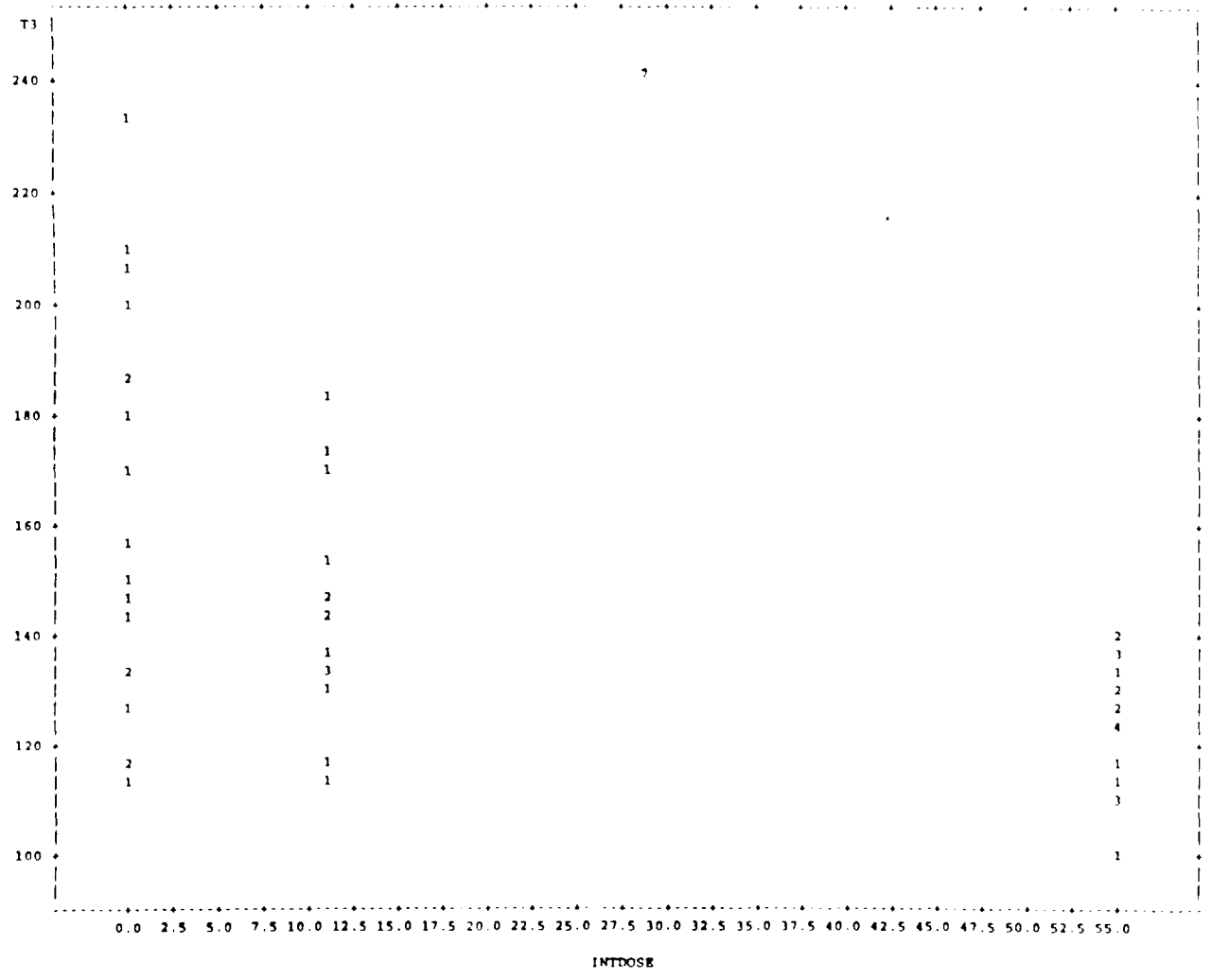
TMPT-15.18



---------- TMPT=92-95 ----------

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	16.46231	16.46231	51.137	0.0001
Error	57	18.34978	0.32193		
C Total	58	34.81209			

Root MSE	0.56739	R-square	0.4729
Dep Mean	4.01864	Adj R-sq	0.4636
C.V.	14.11882		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.499183	0.09986014	45.055	0.0001
INTDOSE	1	-0.022413	0.00313417	-7.151	0.0001

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	24837.40430	24837.40430	85.167	0.0001
Error	57	16622.91907	291.63016		
C Total	58	41460.32336			

Root MSE	17.07718	R-square	0.5991
Dep Mean	149.58847	Adj R-sq	0.5920
C.V.	11.41611		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|

11	3.2200	4.2526	0.081	4.0909	4.4144	-1.0326
12	4.2200	4.2526	0.081	4.0909	4.4144	-0.0326
13	4.7000	4.2526	0.081	4.0909	4.4144	0.4474
14	4.2300	4.2526	0.081	4.0909	4.4144	-0.0226
15	4.1200	4.2526	0.081	4.0909	4.4144	-0.1326
16	4.7600	4.2526	0.081	4.0909	4.4144	0.5074
17	4.3800	4.2526	0.081	4.0909	4.4144	0.1274
18	4.4900	4.2526	0.081	4.0909	4.4144	0.2374
19	4.6500	4.2526	0.081	4.0909	4.4144	0.3974
20	4.7900	4.2526	0.081	4.0909	4.4144	0.5374
21	3.8700	3.2665	0.129	3.0091	3.5239	0.6035
22	3.1400	3.2665	0.129	3.0091	3.5239	-0.1265
23	3.8400	3.2665	0.129	3.0091	3.5239	0.5735
24	3.3200	3.2665	0.129	3.0091	3.5239	0.0535
25	3.5900	3.2665	0.129	3.0091	3.5239	0.3235
26	2.7900	3.2665	0.129	3.0091	3.5239	-0.4765
27	3.2000	3.2665	0.129	3.0091	3.5239	-0.0665
28	3.6100	3.2665	0.129	3.0091	3.5239	0.3435
29	3.8200	3.2665	0.129	3.0091	3.5239	0.5535
30	3.4900	3.2665	0.129	3.0091	3.5239	0.2235
31	3.7300	4.4992	0.100	4.2992	4.6991	-0.7692
32	4.3900	4.4992	0.100	4.2992	4.6991	-0.1092
33	4.1100	4.4992	0.100	4.2992	4.6991	-0.3892
34	4.3800	4.4992	0.100	4.2992	4.6991	-0.1192
35	4.7000	4.4992	0.100	4.2992	4.6991	0.2008
36	4.1000	4.4992	0.100	4.2992	4.6991	-0.3992
37	5.2800	4.4992	0.100	4.2992	4.6991	0.7808
38	4.7500	4.4992	0.100	4.2992	4.6991	0.2508
39	4.2500	4.4992	0.100	4.2992	4.6991	-0.2492
40	4.7200	4.4992	0.100	4.2992	4.6991	0.2208
41	3.6100	4.2526	0.081	4.0909	4.4144	-0.6426
42	3.7300	4.2526	0.081	4.0909	4.4144	-0.5226
43	3.6000	4.2526	0.081	4.0909	4.4144	-0.6526
44	3.3600	4.2526	0.081	4.0909	4.4144	-0.8926
45	3.1800	4.2526	0.081	4.0909	4.4144	-1.0726
46	3.3000	4.2526	0.081	4.0909	4.4144	0.9526
47	3.1000	4.2526	0.081	4.0909	4.4144	-1.1526
48	3.4600	4.2526	0.081	4.0909	4.4144	-0.7926
49	4.2900	4.2526	0.081	4.0909	4.4144	0.0374
50	3.5800	4.2526	0.081	4.0909	4.4144	-0.6726
51	3.0200	3.2665	0.129	3.0091	3.5239	-0.2465
52	3.1000	3.2665	0.129	3.0091	3.5239	-0.1665

TMPT=92-95

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

33	156.3	168.3	3.006	162.2	174.3	-11.9538
34	184.1	168.3	3.006	162.2	174.3	15.7962
35	162.7	168.3	3.006	162.2	174.3	-5.5538
36	174.6	168.3	3.006	162.2	174.3	6.3062

---------- TMPT=92-95 ----------

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
37	178.4	168.3	3.006	162.2	174.3	10.1262
38	157.3	168.3	3.006	162.2	174.3	-10.9138
39	154.0	168.3	3.006	162.2	174.3	-14.2238
40	148.7	168.3	3.006	162.2	174.3	-19.5338
41	161.1	158.7	2.432	153.8	163.5	2.4223
42	133.7	158.7	2.432	153.8	163.5	-24.9777
43	153.4	158.7	2.432	153.8	163.5	-5.2577
44	140.3	158.7	2.432	153.8	163.5	-18.3877
45	122.9	158.7	2.432	153.8	163.5	-35.7777
46	126.2	158.7	2.432	153.8	163.5	-32.4477
47	136.1	158.7	2.432	153.8	163.5	-22.5777
48	158.9	158.7	2.432	153.8	163.5	0.1723
49	134.9	158.7	2.432	153.8	163.5	-23.7377
50	163.8	158.7	2.432	153.8	163.5	5.1023
51	122.9	120.4	3.868	112.6	128.1	2.4869
52	116.1	120.4	3.868	112.6	128.1	-4.2831
53	116.6	120.4	3.868	112.6	128.1	-3.7831
54	107.7	120.4	3.868	112.6	128.1	-12.6431
55	135.3	120.4	3.868	112.6	128.1	14.9269
56	117.3	120.4	3.868	112.6	128.1	-3.0831
57	137.2	120.4	3.868	112.6	128.1	16.8169
58	124.0	120.4	3.868	112.6	128.1	3.6569
59	125.6	120.4	3.868	112.6	128.1	5.2369

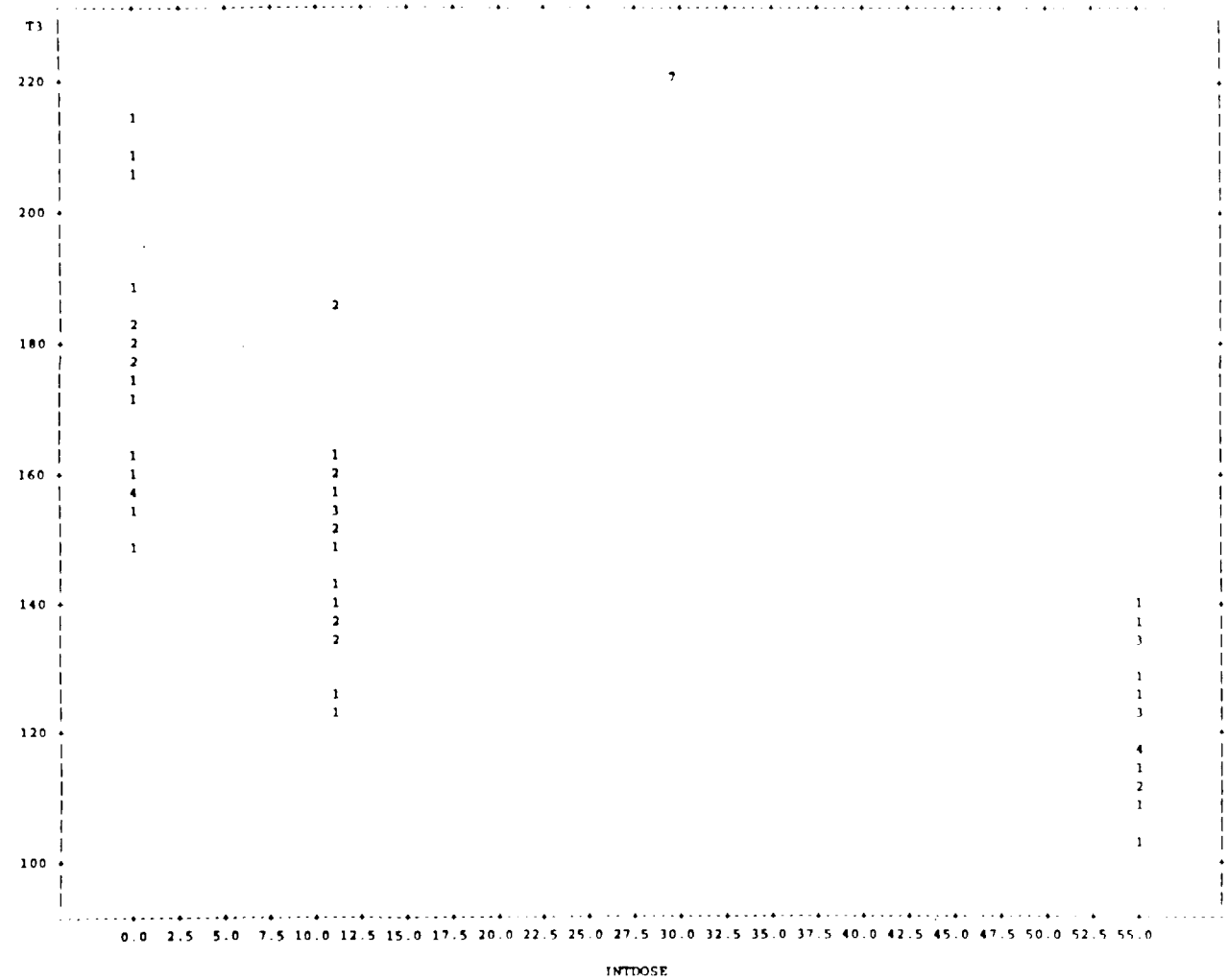
Sum of Residuals	0
Sum of Squared Residuals	16622.9191
Predicted Resid SS (Press)	17636.1185

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	15.7100	16.3670	0.280	15.8057	16.9284	-0.6570
2	14.3800	16.3670	0.280	15.8057	16.9284	-1.9870
3	15.1800	16.3670	0.280	15.8057	16.9284	-1.1870
4	17.7200	16.3670	0.280	15.8057	16.9284	1.3530
5	17.5000	16.3670	0.280	15.8057	16.9284	1.1330

47	15.8900	16.7695	0.227	16.3153	17.2237	-0.8795
48	16.6400	16.7695	0.227	16.3153	17.2237	-0.1295
49	14.4500	16.7695	0.227	16.3153	17.2237	-2.3195
50	15.7300	16.7695	0.227	16.3153	17.2237	-1.0395
51	17.7500	18.3795	0.361	17.6570	19.1020	-0.6295
52	18.8100	18.3795	0.361	17.6570	19.1020	0.4305
53	16.2600	18.3795	0.361	17.6570	19.1020	-2.1195
54	19.2000	18.3795	0.361	17.6570	19.1020	0.8205
55	18.7600	18.3795	0.361	17.6570	19.1020	0.3805
56	15.6900	18.3795	0.361	17.6570	19.1020	-2.6895
57	18.2700	18.3795	0.361	17.6570	19.1020	-0.1095
58	14.9700	18.3795	0.361	17.6570	19.1020	-3.4095
59	18.9300	18.3795	0.361	17.6570	19.1020	0.5505

Sum of Residuals	0
Sum of Squared Residuals	144.6044
Predicted Resid SS (Press)	154.8007

TMPT=92 95


T3
220
200
180
160
140
120
100
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.5 35.0 37.5 40.0 42.5 45.0 47.5 50.0 52.5 55.0
INTDOSE

---------- TMPT=15-18 ----------

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.29494	0.29494	0.602	0.4415
Error	51	25.00093	0.49021		
C Total	52	25.29587			

Root MSE	0.70015	R-square	0.0117
Dep Mean	4.74208	Adj R-sq	-0.0077
C.V.	14.76470		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.800851	0.12243807	39.210	0.0001
DCORR	1	-0.154596	0.19930727	-0.776	0.4415

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	10442.75272	10442.75272	16.083	0.0002
Error	51	33114.80266	649.30986		
C Total	52	43557.55538			

Root MSE	25.48156	R-square	0.2397
Dep Mean	142.39755	Adj R-sq	0.2248
C.V.	17.89466		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|

11	5.2100	4.7993	0.121	4.5560	5.0427	0.4107
12	6.0300	4.7993	0.121	4.5560	5.0427	1.2307
13	5.7000	4.7993	0.121	4.5560	5.0427	0.9007
14	5.5800	4.6463	0.157	4.3320	4.9606	0.9337
15	4.8400	4.6463	0.157	4.3320	4.9606	0.1937
16	5.1700	4.6463	0.157	4.3320	4.9606	0.5237
17	4.6900	4.6463	0.157	4.3320	4.9606	0.0437
18	4.9200	4.6463	0.157	4.3320	4.9606	0.2737
19	6.0600	4.6463	0.157	4.3320	4.9606	1.4137
20	4.8100	4.6463	0.157	4.3320	4.9606	0.1637
21	4.8600	4.6463	0.157	4.3320	4.9606	0.2137
22	5.2400	4.6463	0.157	4.3320	4.9606	0.5937
23	5.7400	4.6463	0.157	4.3320	4.9606	1.0937
24	4.8900	4.8009	0.122	4.5550	5.0467	0.0891
25	4.2300	4.8009	0.122	4.5550	5.0467	-0.5709
26	4.4500	4.8009	0.122	4.5550	5.0467	-0.3509
27	4.3300	4.8009	0.122	4.5550	5.0467	-0.4709
28	4.8700	4.8009	0.122	4.5550	5.0467	0.0691
29	3.4700	4.8009	0.122	4.5550	5.0467	-1.3309
30	4.8100	4.8009	0.122	4.5550	5.0467	0.00915
31	4.3600	4.8009	0.122	4.5550	5.0467	-0.4409
32	4.7500	4.8009	0.122	4.5550	5.0467	-0.0509
33	4.7200	4.8009	0.122	4.5550	5.0467	-0.0809
34	4.4800	4.7993	0.121	4.5560	5.0427	-0.3193
35	4.3900	4.7993	0.121	4.5560	5.0427	-0.4093
36	4.5900	4.7993	0.121	4.5560	5.0427	-0.2093
37	4.5800	4.7993	0.121	4.5560	5.0427	-0.2193
38	4.7400	4.7993	0.121	4.5560	5.0427	-0.0593
39	4.6000	4.7993	0.121	4.5560	5.0427	-0.1993
40	3.2400	4.7993	0.121	4.5560	5.0427	-1.5593
41	4.4400	4.7993	0.121	4.5560	5.0427	-0.3593
42	3.8300	4.7993	0.121	4.5560	5.0427	-0.9693
43	4.3400	4.7993	0.121	4.5560	5.0427	-0.4593
44	4.0200	4.6463	0.157	4.3320	4.9606	-0.6263
45	3.4500	4.6463	0.157	4.3320	4.9606	-1.1963
46	4.3000	4.6463	0.157	4.3320	4.9606	-0.3463
47	4.4600	4.6463	0.157	4.3320	4.9606	-0.1863
48	3.6700	4.6463	0.157	4.3320	4.9606	-0.9763
49	3.8100	4.6463	0.157	4.3320	4.9606	-0.8363
50	4.6000	4.6463	0.157	4.3320	4.9606	-0.0463
51	4.6100	4.6463	0.157	4.3320	4.9606	-0.0363
52	4.0500	4.6463	0.157	4.3320	4.9606	-0.5963

---------- TMPT=15-18 ----------

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

39	113.0	153.2	4.412	144.3	162.0	-40.1662
40	147.2	153.2	4.412	144.3	162.0	-5.9262
41	128.8	153.2	4.412	144.3	162.0	-24.3262
42	145.9	153.2	4.412	144.3	162.0	-7.2562

---------- 2 ---- TMPT=15-18 ----------

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
43	115.3	153.2	4.412	144.3	162.0	-37.8762
44	140.0	124.4	5.698	112.9	135.8	15.5825
45	122.8	124.4	5.698	112.9	135.8	-1.5875
46	109.9	124.4	5.698	112.9	135.8	-14.4375
47	121.8	124.4	5.698	112.9	135.8	-2.5675
48	117.3	124.4	5.698	112.9	135.8	-7.0775
49	127.9	124.4	5.698	112.9	135.8	3.5125
50	138.2	124.4	5.698	112.9	135.8	13.8525
51	108.5	124.4	5.698	112.9	135.8	-15.8675
52	135.8	124.4	5.698	112.9	135.8	11.3925
53	129.1	124.4	5.698	112.9	135.8	4.6925

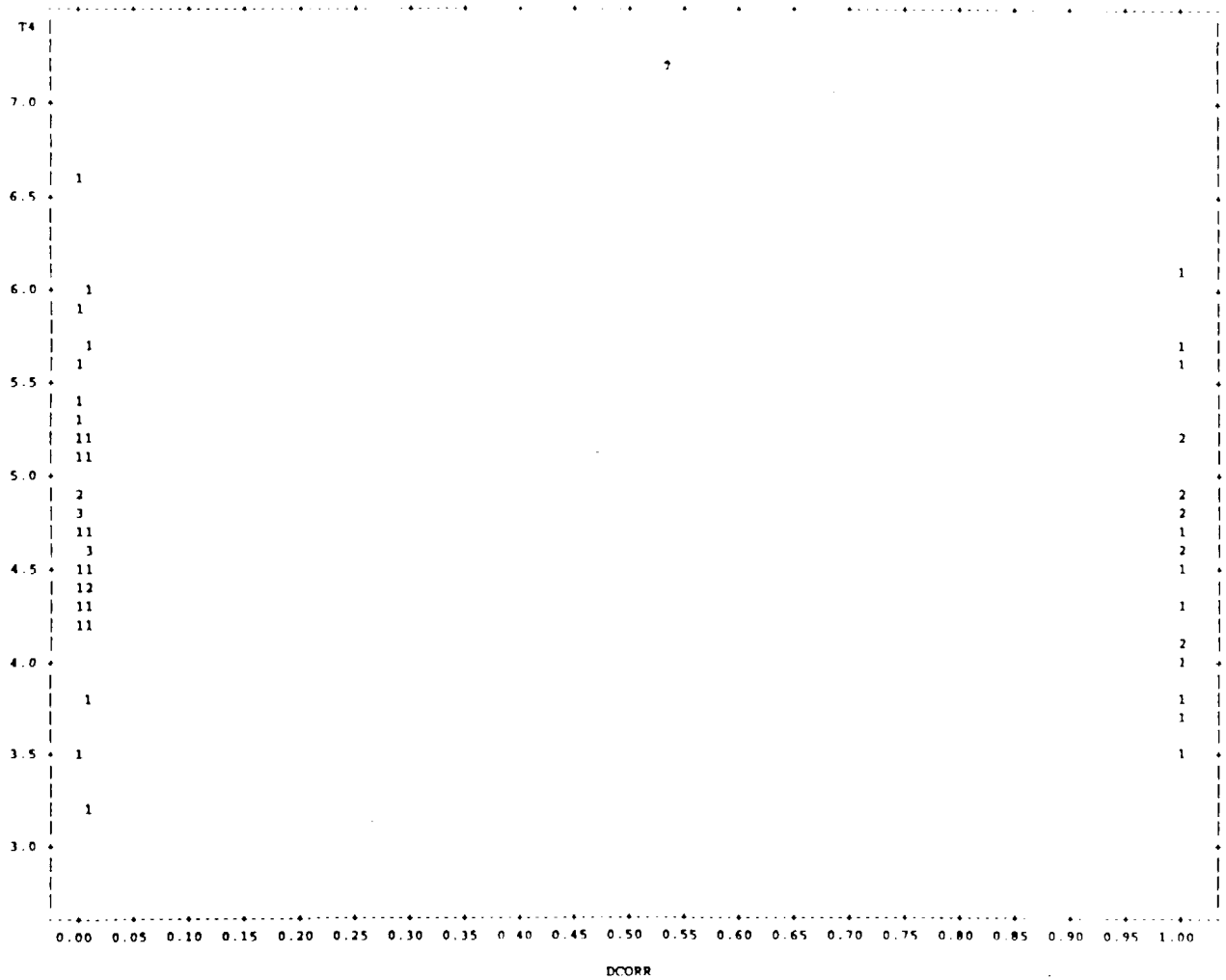
Sum of Residuals	0
Sum of Squared Residuals	33114.8027
Predicted Resid SS (Press)	35338.4234

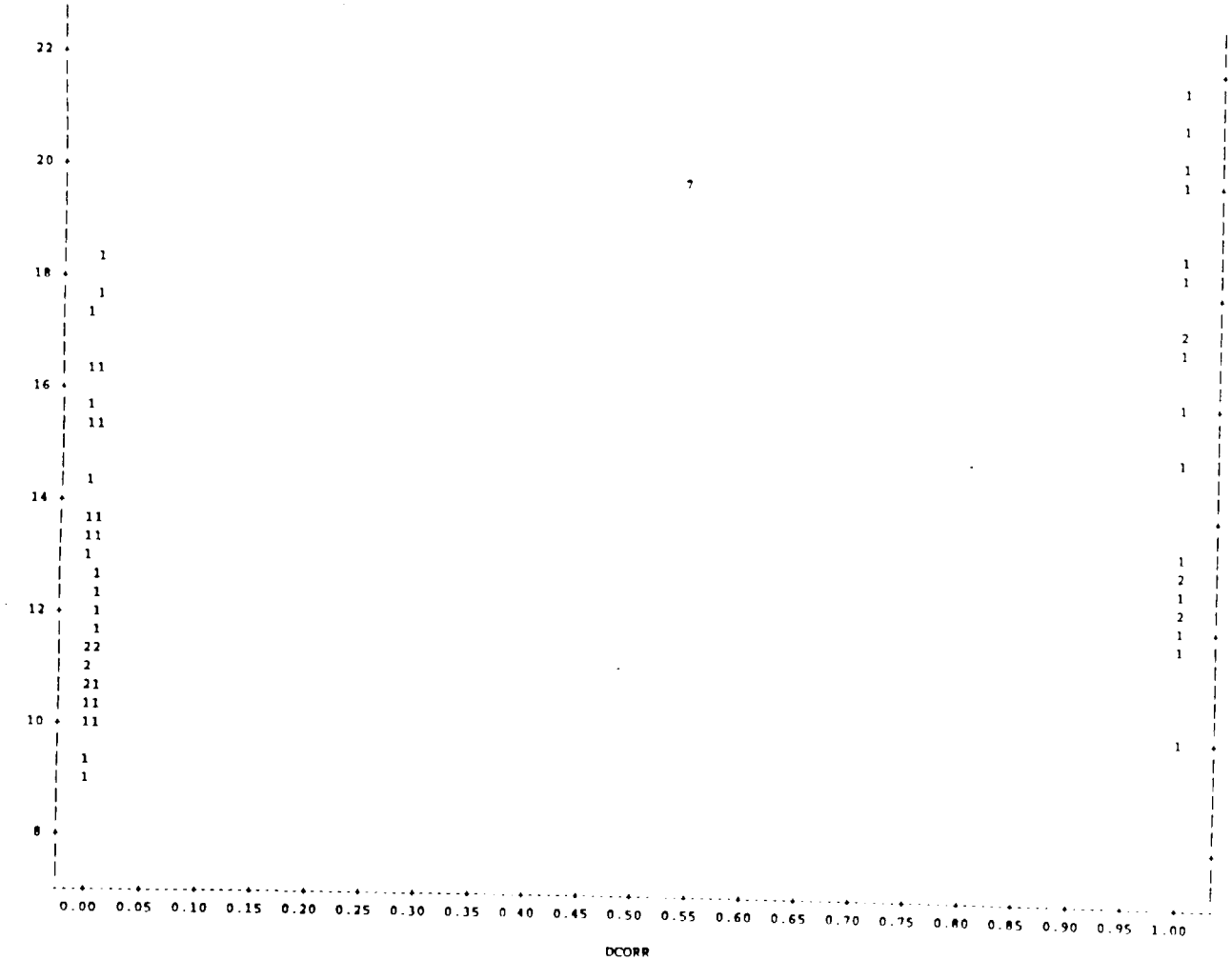
Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	13.0800	12.7408	0.519	11.6993	13.7822	0.3392
2	13.7200	12.7408	0.519	11.6993	13.7822	0.9792
3	14.2000	12.7408	0.519	11.6993	13.7822	1.4592
4	16.3800	12.7408	0.519	11.6993	13.7822	3.6392
5	13.2100	12.7408	0.519	11.6993	13.7822	0.4692
6	15.5200	12.7408	0.519	11.6993	13.7822	2.7792
7	17.4500	12.7408	0.519	11.6993	13.7822	4.7092
8	15.3000	12.7408	0.519	11.6993	13.7822	2.5592
9	15.2200	12.7703	0.514	11.7393	13.8014	2.4497
10	12.7600	12.7703	0.514	11.7393	13.8014	-0.0103
11	17.6300	12.7703	0.514	11.7393	13.8014	4.8597
12	18.4100	12.7703	0.514	11.7393	13.8014	5.6397
13	16.4900	12.7703	0.514	11.7393	13.8014	3.7197
14	19.9300	15.6976	0.663	14.3659	17.0292	4.2324
15	20.2700	15.6976	0.663	14.3659	17.0292	4.5724
16	17.3900	15.6976	0.663	14.3659	17.0292	1.6924
17	17.0300	15.6976	0.663	14.3659	17.0292	1.3324

1 Subchronic Rat Perchlorate Study, Correlations with external dose 52

15:56 Thursday, February 4, 1999

TMPT=15-18




22
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DCORR

INTERCEP	1	162.893104	2.98723098	54.530	0.0001
DCORR	1	-40.884017	5.26374877	-7.767	0.0001

1 Subchronic Rat Perchlorate Study, Correlations with external dose 56
15:56 Thursday, February 4, 1999

TMPT=92-95

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	41.81414	41.81414	16.250	0.0002
Error	57	146.66803	2.57312		
C Total	58	188.48216			

Root MSE	1.60410	R-square	0.2218
Dep Mean	17.15153	Adj R-sq	0.2082
C.V.	9.35250		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	16.562289	0.25490776	64.974	0.0001
DCORR	1	1.810676	0.44916861	4.031	0.0002

1 Subchronic Rat Perchlorate Study, Correlations with external dose 57
15:56 Thursday, February 4, 1999

TMPT=92-95

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.6100	4.3516	0.098	4.1564	4.5469	0.2584
2	5.0200	4.3516	0.098	4.1564	4.5469	0.6684
3	4.9600	4.3516	0.098	4.1564	4.5469	0.6084
4	4.6300	4.3516	0.098	4.1564	4.5469	0.2784
5	6.3500	4.3516	0.098	4.1564	4.5469	1.9984
6	5.1900	4.3516	0.098	4.1564	4.5469	0.8384
7	5.1200	4.3516	0.098	4.1564	4.5469	0.7684
8	4.3700	4.3516	0.098	4.1564	4.5469	0.0184
9	4.9700	4.3516	0.098	4.1564	4.5469	0.6184
10	5.3800	4.3516	0.098	4.1564	4.5469	1.0284

53	3.4100	3.3284	0.141	3.0465	3.6103	0.0816
54	2.9000	3.3284	0.141	3.0465	3.6103	-0.4284
55	3.7800	3.3284	0.141	3.0465	3.6103	0.4516
56	3.4300	3.3284	0.141	3.0465	3.6103	0.1016
57	3.1800	3.3284	0.141	3.0465	3.6103	-0.1484
58	2.7900	3.3284	0.141	3.0465	3.6103	-0.5384
59	3.0400	3.3284	0.141	3.0465	3.6103	-0.2884

Sum of Residuals	0
Sum of Squared Residuals	21.4585
Predicted Resid SS (Press)	22.7137

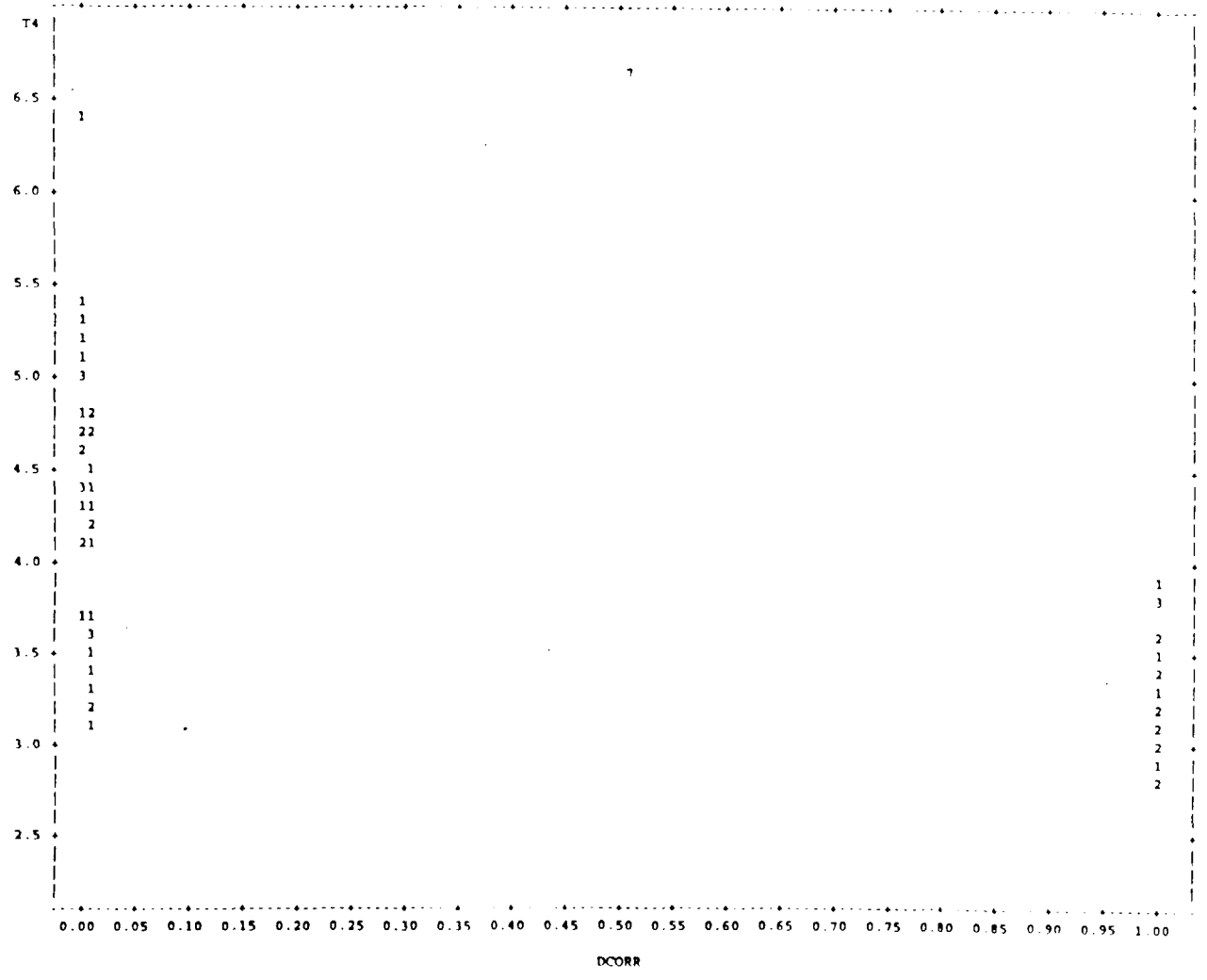
Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	204.7	162.9	2.987	156.9	168.9	41.7969
2	182.7	162.9	2.987	156.9	168.9	19.7569
3	158.2	162.9	2.987	156.9	168.9	-4.6631
4	156.9	162.9	2.987	156.9	168.9	-6.0031
5	189.8	162.9	2.987	156.9	168.9	26.9469
6	179.3	162.9	2.987	156.9	168.9	16.3869
7	215.3	162.9	2.987	156.9	168.9	52.4369
8	160.9	162.9	2.987	156.9	168.9	-2.0331
9	171.1	162.9	2.987	156.9	168.9	8.1969
10	179.5	162.9	2.987	156.9	168.9	16.6169
11	158.2	162.5	2.957	156.6	168.4	-4.3343
12	155.4	162.5	2.957	156.6	168.4	-7.0843
13	136.2	162.5	2.957	156.6	168.4	-26.2943
14	152.6	162.5	2.957	156.6	168.4	-9.8743
15	154.1	162.5	2.957	156.6	168.4	-8.4243
16	144.1	162.5	2.957	156.6	168.4	-18.4243
17	186.9	162.5	2.957	156.6	168.4	24.3757
18	149.5	162.5	2.957	156.6	168.4	-12.9743
19	151.6	162.5	2.957	156.6	168.4	-10.8743
20	186.2	162.5	2.957	156.6	168.4	23.7057
21	134.5	122.0	4.312	113.4	130.6	12.5209
22	133.3	122.0	4.312	113.4	130.6	11.2709
23	116.0	122.0	4.312	113.4	130.6	-6.0291
24	112.2	122.0	4.312	113.4	130.6	-9.8491
25	103.3	122.0	4.312	113.4	130.6	-18.6791
26	112.8	122.0	4.312	113.4	130.6	-9.2291
27	138.7	122.0	4.312	113.4	130.6	16.6809
28	129.2	122.0	4.312	113.4	130.6	7.1809
29	114.3	122.0	4.312	113.4	130.6	-7.7391
30	123.7	122.0	4.312	113.4	130.6	1.7009
31	208.4	162.9	2.987	156.9	168.9	45.5169
32	176.3	162.9	2.987	156.9	168.9	13.4069

6	15.3900	16.5623	0.255	16.0518	17.0727	-1.1723
7	17.6100	16.5623	0.255	16.0518	17.0727	1.0477
8	14.9800	16.5623	0.255	16.0518	17.0727	-1.5823
9	14.7900	16.5623	0.255	16.0518	17.0727	-1.7723
10	18.6100	16.5623	0.255	16.0518	17.0727	2.0477
11	16.2700	16.5804	0.252	16.0751	17.0857	0.3104
12	16.8700	16.5804	0.252	16.0751	17.0857	0.2896
13	15.9500	16.5804	0.252	16.0751	17.0857	-0.6304
14	17.5400	16.5804	0.252	16.0751	17.0857	0.9596
15	15.4000	16.5804	0.252	16.0751	17.0857	-1.1804
16	15.4600	16.5804	0.252	16.0751	17.0857	-1.1204
17	14.9600	16.5804	0.252	16.0751	17.0857	-1.6204
18	20.1400	16.5804	0.252	16.0751	17.0857	3.5596
19	19.5100	16.5804	0.252	16.0751	17.0857	2.9296
20	16.3100	16.5804	0.252	16.0751	17.0857	-0.2704

---------- TMPT=92-95 ----------

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
21	17.3100	18.3730	0.368	17.6361	19.1099	-1.0630
22	17.5000	18.3730	0.368	17.6361	19.1099	-0.8730
23	20.2700	18.3730	0.368	17.6361	19.1099	1.8970
24	18.9800	18.3730	0.368	17.6361	19.1099	0.6070
25	21.5200	18.3730	0.368	17.6361	19.1099	3.1470
26	20.0600	18.3730	0.368	17.6361	19.1099	1.6870
27	17.4200	18.3730	0.368	17.6361	19.1099	-0.9530
28	18.9600	18.3730	0.368	17.6361	19.1099	0.5870
29	18.9700	18.3730	0.368	17.6361	19.1099	0.5970
30	19.4100	18.3730	0.368	17.6361	19.1099	1.0370
31	17.3200	16.5623	0.255	16.0518	17.0727	0.7577
32	17.2700	16.5623	0.255	16.0518	17.0727	0.7077
33	16.9700	16.5623	0.255	16.0518	17.0727	0.4077
34	13.6500	16.5623	0.255	16.0518	17.0727	-2.9123
35	16.0600	16.5623	0.255	16.0518	17.0727	-0.5023
36	16.5100	16.5623	0.255	16.0518	17.0727	-0.0523
37	17.1500	16.5623	0.255	16.0518	17.0727	0.5877
38	15.3100	16.5623	0.255	16.0518	17.0727	-1.2523
39	19.1400	16.5623	0.255	16.0518	17.0727	2.5777
40	15.4100	16.5623	0.255	16.0518	17.0727	-1.1523
41	17.8500	16.5804	0.252	16.0751	17.0857	1.2696
42	15.7600	16.5804	0.252	16.0751	17.0857	-0.8204
43	17.4700	16.5804	0.252	16.0751	17.0857	0.8896
44	14.8000	16.5804	0.252	16.0751	17.0857	-1.7804
45	19.7600	16.5804	0.252	16.0751	17.0857	3.1796
46	19.4800	16.5804	0.252	16.0751	17.0857	2.8996

TMPT=92 95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4.5
4.0
3.5
3.0
2.5
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DCORR

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TMPT=92 95

45	F	15-18	5	18	3.45	122.78	12.33	n	0	-9.0	1.00
46	F	15-18	5	17	4.30	109.93	10.13	n	0	-9.0	1.00
47	F	15-18	5	16	4.46	121.80	12.02	n	0	-9.0	1.00
48	F	15-18	5	18	3.67	117.29	13.48	n	0	-9.0	1.00
49	F	15-18	5	18	3.81	127.88	11.70	n	0	-9.0	1.00
50	F	15-18	5	16	4.60	138.22	12.48	n	0	-9.0	1.00
51	F	15-18	5	15	4.61	108.50	13.14	n	0	-9.0	1.00
52	F	15-18	5	17	4.05	135.76	14.91	n	0	-9.0	1.00
53	F	15-18	5	15	4.07	129.06	12.63	n	0	-9.0	1.00
54	M	92-95	1	92	4.61	204.69	15.71	n	0	0.0	0.00
55	M	92-95	1	92	5.02	182.65	14.38	n	0	0.0	0.00
56	M	92-95	1	92	4.96	158.23	15.18	n	0	0.0	0.00

1 T4, T3, TSH data from Subchronic Perchlorate study 10:02 Monday, February 8, 1999 2

OBS	SEX	TMPT	DOSE	AGE	T4	T3	TSH	CODE	FOLL	INTDOSE	DCORR
57	M	92-95	1	93	4.63	156.89	17.72	n	0	0.0	0.00
58	M	92-95	1	93	6.35	189.84	17.50	n	0	0.0	0.00
59	M	92-95	1	94	5.19	179.28	15.39	n	0	0.0	0.00
60	M	92-95	1	94	5.12	215.33	17.61	ab	1	0.0	0.00
61	M	92-95	1	94	4.37	160.86	14.98	ab	1	0.0	0.00
62	M	92-95	1	95	4.97	171.09	14.79	n	0	0.0	0.00
63	M	92-95	1	95	5.38	179.51	18.61	n	0	0.0	0.00
64	M	92-95	2	92	3.22	158.15	16.27	n	0	5.1	0.01
65	M	92-95	2	92	4.22	155.40	16.87	n	0	5.1	0.01
66	M	92-95	2	92	4.70	136.19	15.95	n	0	5.1	0.01
67	M	92-95	2	93	4.23	152.61	17.54	n	0	5.1	0.01
68	M	92-95	2	93	4.12	154.06	15.40	n	0	5.1	0.01
69	M	92-95	2	94	4.76	144.06	15.46	n	0	5.1	0.01
70	M	92-95	2	94	4.38	186.86	14.96	n	0	5.1	0.01
71	M	92-95	2	94	4.49	149.51	20.14	n	0	5.1	0.01
72	M	92-95	2	95	4.65	151.61	19.51	n	0	5.1	0.01
73	M	92-95	2	95	4.79	186.19	16.31	n	0	5.1	0.01
74	M	92-95	5	92	3.87	134.53	17.31	n	0	-9.0	1.00
75	M	92-95	5	92	3.14	133.28	17.50	n	0	-9.0	1.00
76	M	92-95	5	92	3.84	115.98	20.27	n	0	-9.0	1.00
77	M	92-95	5	93	3.32	112.16	18.98	n	0	-9.0	1.00
78	M	92-95	5	93	3.59	103.33	21.52	n	0	-9.0	1.00
79	M	92-95	5	94	2.79	112.78	20.06	ab	1	-9.0	1.00
80	M	92-95	5	94	3.20	138.69	17.42	n	0	-9.0	1.00
81	M	92-95	5	94	3.61	129.19	18.96	n	0	-9.0	1.00
82	M	92-95	5	95	3.82	114.27	18.97	n	0	-9.0	1.00
83	M	92-95	5	95	3.49	123.71	19.41	n	0	-9.0	1.00
84	F	92-95	1	92	3.73	208.41	17.32	n	0	0.0	0.00
85	F	92-95	1	92	4.39	176.30	17.27	n	0	0.0	0.00
86	F	92-95	1	93	4.11	156.30	16.97	n	0	0.0	0.00
87	F	92-95	1	93	4.38	184.05	13.65	n	0	0.0	0.00
88	F	92-95	1	93	4.70	162.70	16.06	n	0	0.0	0.00
89	F	92-95	1	94	4.10	174.56	16.51	n	0	0.0	0.00

22	2	19534	4.19	3.43	92.3	-17.5	0.1
23	2	19536	4.62	.	.	-17.5	0.1
24	2	19542	4.94	3.30	77.9	-17.5	0.1
25	2	19562	4.71	.	.	-17.5	0.1
26	2	19575	4.20	3.23	90.9	-17.5	0.1
27	2	19576	4.09	3.05	83.3	-17.5	0.1
28	2	19590	4.30	3.84	74.5	-17.5	0.1
29	2	19593	4.76	3.03	94.9	-17.5	0.1
30	2	19594	4.89	.	.	-17.5	0.1
31	2	19605	5.25	3.67	86.1	-17.5	0.1
32	3	19504	4.56	3.69	74.3	-9.0	1.0
33	3	19521	4.74	3.50	89.5	-9.0	1.0
34	3	19535	4.04	2.92	91.3	-9.0	1.0
35	3	19538	5.13	2.91	75.5	-9.0	1.0
36	3	19539	4.40	3.15	80.9	-9.0	1.0
37	3	19547	4.51	3.11	78.1	-9.0	1.0
38	3	19568	4.39	3.31	.	-9.0	1.0
39	3	19579	4.38	.	.	-9.0	1.0
40	3	19584	4.89	2.71	70.8	-9.0	1.0
41	3	19586	4.22	.	.	-9.0	1.0
42	3	19587	5.61	2.98	78.9	-9.0	1.0
43	3	19588	4.98	.	.	-9.0	1.0
44	3	19589	5.63	.	.	-9.0	1.0
45	3	19592	4.78	3.18	71.0	-9.0	1.0
46	3	19596	5.28	2.84	83.5	-9.0	1.0
47	3	19597	4.03	.	.	-9.0	1.0
48	3	19603	4.88	3.33	.	-9.0	1.0
49	3	19616	5.11	3.15	.	-9.0	1.0
50	4	19522	4.34	.	.	3.0	3.0
51	4	19522	4.73	2.46	40.4	3.0	3.0
52	4	19525	5.01	2.28	38.9	3.0	3.0
53	4	19537	4.13	2.92	38.0	3.0	3.0
54	4	19544	5.58	2.73	46.1	3.0	3.0
55	4	19554	3.76	2.37	37.2	3.0	3.0

1 T4, T3, TSH data from Dev NT, PND 5Perchlorate study including approx of internal dose 4

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OBS	DOSE	LITTER	TSH	T4	T3	INTDOSE	DCORR
56	4	19557	5.03	2.52	42.4	3	3
57	4	19569	4.99	3.12	40.0	3	3
58	4	19573	3.97	.	.	3	3
59	4	19577	4.92	2.78	37.3	3	3
60	4	19598	4.60	2.76	34.5	3	3
61	4	19600	5.29	2.90	35.1	3	3
62	4	19604	4.95	.	43.4	3	3
63	4	19607	4.92	.	.	3	3
64	4	19612	4.95	.	.	3	3
65	4	19617	5.10	2.64	36.7	3	3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	1.16630	1.16630	7.700	0.0086
Error	37	5.60433	0.15147		
C Total	38	6.77064			

Root MSE	0.38919	R-square	0.1723
Dep Mean	3.10795	Adj R-sq	0.1499
C.V.	12.52239		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	2.981748	0.07715057	38.648	0.0001
INTDOSE	1	-0.021215	0.00764529	-2.775	0.0086

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	7141.94275	7141.94275	25.691	0.0001
Error	37	10285.95468	277.99878		
C Total	38	17427.89744			

Root MSE	16.67330	R-square	0.4098
Dep Mean	70.94872	Adj R-sq	0.3938
C.V.	23.50049		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	61.073094	3.30521267	18.478	0.0001
INTDOSE	1	-1.660126	0.32753252	-5.069	0.0001

Dependent Variable: TSH

23	.	3.3530	0.108	3.1340	3.5720	.
24	3.3000	3.3530	0.108	3.1340	3.5720	-0.0530
25	.	3.3530	0.108	3.1340	3.5720	.
26	3.2300	3.3530	0.108	3.1340	3.5720	-0.1230
27	3.0500	3.3530	0.108	3.1340	3.5720	-0.3030
28	3.8400	3.3530	0.108	3.1340	3.5720	0.4870
29	3.0300	3.3530	0.108	3.1340	3.5720	-0.3230
30	.	3.3530	0.108	3.1340	3.5720	.
31	3.6700	3.3530	0.108	3.1340	3.5720	0.3170
32	3.6900	3.1727	0.067	3.0379	3.3075	0.5173
33	3.5000	3.1727	0.067	3.0379	3.3075	0.3273
34	2.9200	3.1727	0.067	3.0379	3.3075	-0.2527
35	2.9100	3.1727	0.067	3.0379	3.3075	-0.2627
36	3.1500	3.1727	0.067	3.0379	3.3075	-0.0227
37	3.1100	3.1727	0.067	3.0379	3.3075	-0.0627
38	3.3100	3.1727	0.067	3.0379	3.3075	0.1373
39	.	3.1727	0.067	3.0379	3.3075	.
40	2.7100	3.1727	0.067	3.0379	3.3075	-0.4627
41	.	3.1727	0.067	3.0379	3.3075	.
42	2.9800	3.1727	0.067	3.0379	3.3075	-0.1927
43	.	3.1727	0.067	3.0379	3.3075	.
44	.	3.1727	0.067	3.0379	3.3075	.
45	3.1800	3.1727	0.067	3.0379	3.3075	0.00732
46	2.8400	3.1727	0.067	3.0379	3.3075	-0.3327
47	.	3.1727	0.067	3.0379	3.3075	.
48	3.3300	3.1727	0.067	3.0379	3.3075	0.1573
49	3.1500	3.1727	0.067	3.0379	3.3075	-0.0227
50	.	2.9181	0.093	2.7306	3.1056	.
51	2.4600	2.9181	0.093	2.7306	3.1056	-0.4581
52	2.2800	2.9181	0.093	2.7306	3.1056	-0.6381
53	2.9200	2.9181	0.093	2.7306	3.1056	0.00190
54	2.7300	2.9181	0.093	2.7306	3.1056	-0.1881

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
55	2.3700	2.9181	0.093	2.7306	3.1056	-0.5481
56	2.5200	2.9181	0.093	2.7306	3.1056	-0.3981
57	3.1200	2.9181	0.093	2.7306	3.1056	0.2019
58	.	2.9181	0.093	2.7306	3.1056	.
59	2.7800	2.9181	0.093	2.7306	3.1056	-0.1381
60	2.7600	2.9181	0.093	2.7306	3.1056	-0.1581
61	2.9000	2.9181	0.093	2.7306	3.1056	-0.0181
62	.	2.9181	0.093	2.7306	3.1056	.
63	.	2.9181	0.093	2.7306	3.1056	.
64	.	2.9181	0.093	2.7306	3.1056	.
65	2.6400	2.9181	0.093	2.7306	3.1056	-0.2781

34	91.3000	76.0142	2.851	70.2380	81.7905	15.2858
35	75.5000	76.0142	2.851	70.2380	81.7905	-0.5142
36	80.9000	76.0142	2.851	70.2380	81.7905	4.8858
37	78.1000	76.0142	2.851	70.2380	81.7905	2.0858
38	.	76.0142	2.851	70.2380	81.7905	.
39	.	76.0142	2.851	70.2380	81.7905	.
40	70.8000	76.0142	2.851	70.2380	81.7905	-5.2142
41	.	76.0142	2.851	70.2380	81.7905	.
42	78.9000	76.0142	2.851	70.2380	81.7905	2.8858
43	.	76.0142	2.851	70.2380	81.7905	.
44	.	76.0142	2.851	70.2380	81.7905	.
45	71.0000	76.0142	2.851	70.2380	81.7905	-5.0142
46	83.5000	76.0142	2.851	70.2380	81.7905	7.4858
47	.	76.0142	2.851	70.2380	81.7905	.
48	.	76.0142	2.851	70.2380	81.7905	.
49	.	76.0142	2.851	70.2380	81.7905	.
50	.	56.0927	3.965	48.0595	64.1260	.
51	40.4000	56.0927	3.965	48.0595	64.1260	-15.6927
52	38.9000	56.0927	3.965	48.0595	64.1260	-17.1927
53	38.0000	56.0927	3.965	48.0595	64.1260	-18.0927
54	46.1000	56.0927	3.965	48.0595	64.1260	-9.9927
55	37.2000	56.0927	3.965	48.0595	64.1260	-18.8927
56	42.4000	56.0927	3.965	48.0595	64.1260	-13.6927
57	40.0000	56.0927	3.965	48.0595	64.1260	-16.0927
58	.	56.0927	3.965	48.0595	64.1260	.
59	37.3000	56.0927	3.965	48.0595	64.1260	-18.7927
60	34.5000	56.0927	3.965	48.0595	64.1260	-21.5927
61	35.1000	56.0927	3.965	48.0595	64.1260	-20.9927
62	43.4000	56.0927	3.965	48.0595	64.1260	12.6927
63	.	56.0927	3.965	48.0595	64.1260	.
64	.	56.0927	3.965	48.0595	64.1260	.
65	36.7000	56.0927	3.965	48.0595	64.1260	19.3927
66	.	56.0927	3.965	48.0595	64.1260	.

Sum of Residuals	0
Sum of Squared Residuals	10285.9547
Predicted Resid SS (Press)	11337.6685

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.1500	4.7424	0.095	4.5497	4.9350	-0.5924
2	4.2300	4.7424	0.095	4.5497	4.9350	-0.5124
3	4.3600	4.7424	0.095	4.5497	4.9350	-0.3824
4	4.0600	4.7424	0.095	4.5497	4.9350	-0.6824
5	4.1200	4.7424	0.095	4.5497	4.9350	-0.6224
6	4.6800	4.7424	0.095	4.5497	4.9350	-0.0624
7	4.1900	4.7424	0.095	4.5497	4.9350	-0.5524

51	4.7300	4.7702	0.114	4.5391	5.0013	-0.0402
52	5.0100	4.7702	0.114	4.5391	5.0013	0.2398
53	4.1300	4.7702	0.114	4.5391	5.0013	-0.6402
54	5.5800	4.7702	0.114	4.5391	5.0013	0.8098
55	3.7600	4.7702	0.114	4.5391	5.0013	1.0102
56	5.0300	4.7702	0.114	4.5391	5.0013	0.2598
57	4.9900	4.7702	0.114	4.5391	5.0013	0.2198
58	3.9700	4.7702	0.114	4.5391	5.0013	-0.8002
59	4.9200	4.7702	0.114	4.5391	5.0013	0.1498
60	4.6000	4.7702	0.114	4.5391	5.0013	-0.1702
61	5.2900	4.7702	0.114	4.5391	5.0013	0.5198
62	4.9500	4.7702	0.114	4.5391	5.0013	0.1798
63	4.9200	4.7702	0.114	4.5391	5.0013	0.1498
64	4.9500	4.7702	0.114	4.5391	5.0013	0.1798
65	5.1000	4.7702	0.114	4.5391	5.0013	0.3298
66	4.8900	4.7702	0.114	4.5391	5.0013	0.1198

Sum of Residuals	0
Sum of Squared Residuals	8.5117
Predicted Resid SS (Press)	9.4559

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 14

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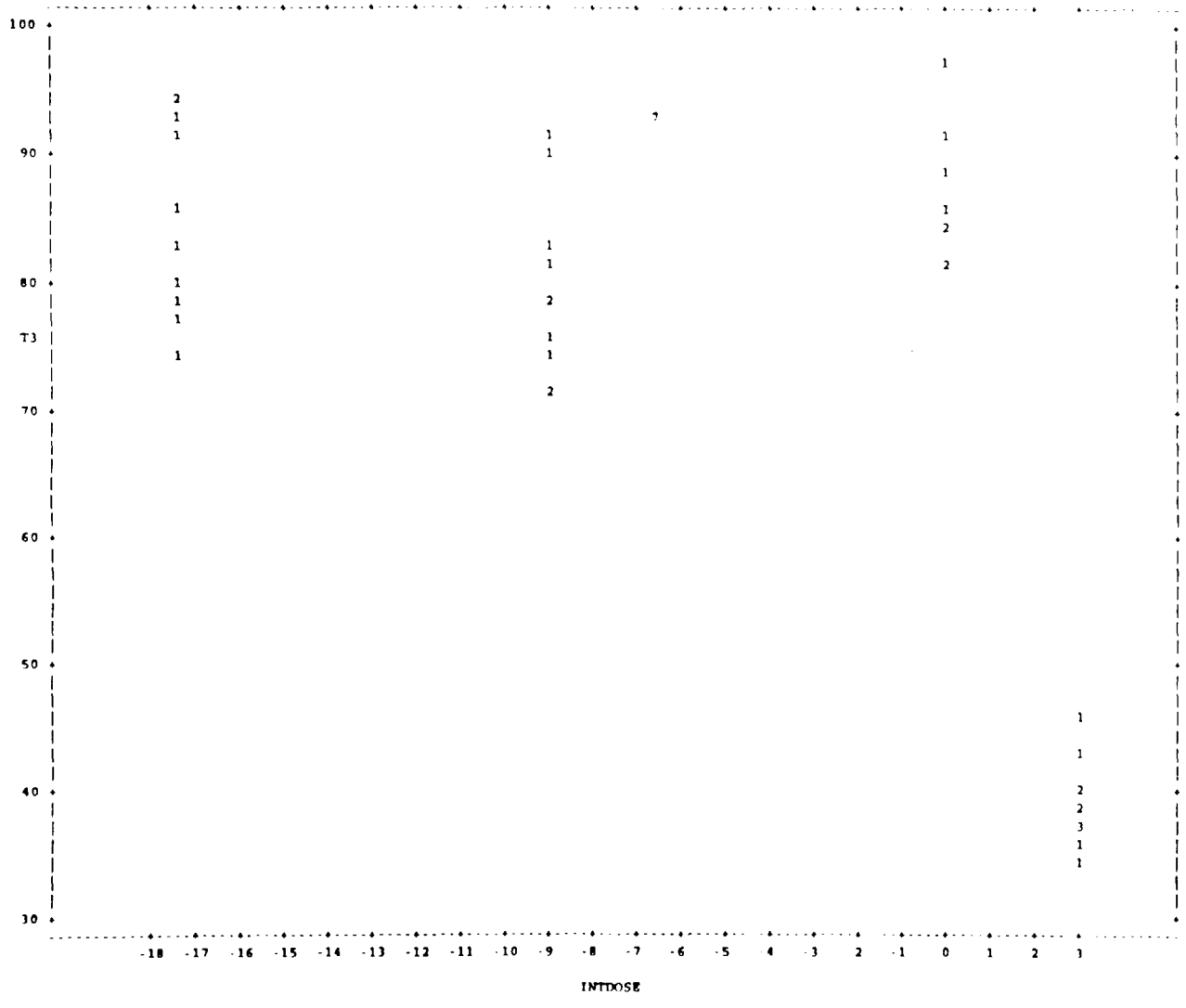


Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	3.28404	3.28404	34.850	0.0001
Error	37	3.48660	0.09423		
C Total	38	6.77064			

Root MSE	0.30697	R-square	0.4850
Dep Mean	3.10795	Adj R-sq	0.4711
C.V.	9.87703		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	3.373094	0.06658424	50.659	0.0001
DCORR	1	-0.235015	0.03980993	-5.903	0.0001

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	15597.22790	15597.22790	315.238	0.0001
Error	37	1830.66953	49.47755		
C Total	38	17427.89744			

Root MSE	7.03403	R-square	0.8950
Dep Mean	70.94872	Adj R-sq	0.8921
C.V.	9.91424		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	89.221433	1.52572225	58.478	0.0001
DCORR	1	-16.196270	0.91221122	-17.755	0.0001

20	3.6700	3.3496	0.064	3.2200	3.4792	0.3204
21	2.8700	3.3496	0.064	3.2200	3.4792	-0.4796
22	3.4300	3.3496	0.064	3.2200	3.4792	0.0804
23	.	3.3496	0.064	3.2200	3.4792	.
24	3.3000	3.3496	0.064	3.2200	3.4792	-0.0496
25	.	3.3496	0.064	3.2200	3.4792	.
26	3.2300	3.3496	0.064	3.2200	3.4792	-0.1196
27	3.0500	3.3496	0.064	3.2200	3.4792	-0.2996
28	3.8400	3.3496	0.064	3.2200	3.4792	0.4904
29	3.0300	3.3496	0.064	3.2200	3.4792	-0.3196
30	.	3.3496	0.064	3.2200	3.4792	.
31	3.6700	3.3496	0.064	3.2200	3.4792	0.3204
32	3.6900	3.1381	0.049	3.0379	3.2382	0.5519
33	3.5000	3.1381	0.049	3.0379	3.2382	0.3619
34	2.9200	3.1381	0.049	3.0379	3.2382	-0.2181
35	2.9100	3.1381	0.049	3.0379	3.2382	-0.2281
36	3.1500	3.1381	0.049	3.0379	3.2382	0.0119
37	3.1100	3.1381	0.049	3.0379	3.2382	-0.0281
38	3.3100	3.1381	0.049	3.0379	3.2382	0.1719
39	.	3.1381	0.049	3.0379	3.2382	.
40	2.7100	3.1381	0.049	3.0379	3.2382	-0.4281
41	.	3.1381	0.049	3.0379	3.2382	.
42	2.9800	3.1381	0.049	3.0379	3.2382	-0.1581
43	.	3.1381	0.049	3.0379	3.2382	.
44	.	3.1381	0.049	3.0379	3.2382	.
45	3.1800	3.1381	0.049	3.0379	3.2382	0.0419
46	2.8400	3.1381	0.049	3.0379	3.2382	-0.2981
47	.	3.1381	0.049	3.0379	3.2382	.
48	3.3300	3.1381	0.049	3.0379	3.2382	0.1919
49	3.1500	3.1381	0.049	3.0379	3.2382	0.0119
50	.	2.6680	0.089	2.4872	2.8489	.
51	2.4600	2.6680	0.089	2.4872	2.8489	-0.2080
52	2.2800	2.6680	0.089	2.4872	2.8489	-0.3880
53	2.9200	2.6680	0.089	2.4872	2.8489	0.2520
54	2.7300	2.6680	0.089	2.4872	2.8489	0.0620
55	2.3700	2.6680	0.089	2.4872	2.8489	-0.2980

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
56	2.5200	2.6680	0.089	2.4872	2.8489	-0.1480
57	3.1200	2.6680	0.089	2.4872	2.8489	0.4520
58	.	2.6680	0.089	2.4872	2.8489	.
59	2.7800	2.6680	0.089	2.4872	2.8489	0.1120
60	2.7600	2.6680	0.089	2.4872	2.8489	0.0920
61	2.9000	2.6680	0.089	2.4872	2.8489	0.2320
62	.	2.6680	0.089	2.4872	2.8489	.
63	.	2.6680	0.089	2.4872	2.8489	.

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
36	80.9000	73.0252	1.132	70.7307	75.3196	7.8748
37	78.1000	73.0252	1.132	70.7307	75.3196	5.0748
38	.	73.0252	1.132	70.7307	75.3196	.
39	.	73.0252	1.132	70.7307	75.3196	.
40	70.8000	73.0252	1.132	70.7307	75.3196	-2.2252
41	.	73.0252	1.132	70.7307	75.3196	.
42	78.9000	73.0252	1.132	70.7307	75.3196	5.8748
43	.	73.0252	1.132	70.7307	75.3196	.
44	.	73.0252	1.132	70.7307	75.3196	.
45	71.0000	73.0252	1.132	70.7307	75.3196	-2.0252
46	83.5000	73.0252	1.132	70.7307	75.3196	10.4748
47	.	73.0252	1.132	70.7307	75.3196	.
48	.	73.0252	1.132	70.7307	75.3196	.
49	.	73.0252	1.132	70.7307	75.3196	.
50	.	40.6326	2.046	36.4880	44.7772	.
51	40.4000	40.6326	2.046	36.4880	44.7772	-0.2326
52	38.9000	40.6326	2.046	36.4880	44.7772	-1.7326
53	38.0000	40.6326	2.046	36.4880	44.7772	-2.6326
54	46.1000	40.6326	2.046	36.4880	44.7772	5.4674
55	37.2000	40.6326	2.046	36.4880	44.7772	-3.4326
56	42.4000	40.6326	2.046	36.4880	44.7772	1.7674
57	40.0000	40.6326	2.046	36.4880	44.7772	-0.6326
58	.	40.6326	2.046	36.4880	44.7772	.
59	37.3000	40.6326	2.046	36.4880	44.7772	-3.3326
60	34.5000	40.6326	2.046	36.4880	44.7772	-6.1326
61	35.1000	40.6326	2.046	36.4880	44.7772	-5.5326
62	43.4000	40.6326	2.046	36.4880	44.7772	2.7674
63	.	40.6326	2.046	36.4880	44.7772	.
64	.	40.6326	2.046	36.4880	44.7772	.
65	36.7000	40.6326	2.046	36.4880	44.7772	-3.9326
66	.	40.6326	2.046	36.4880	44.7772	.

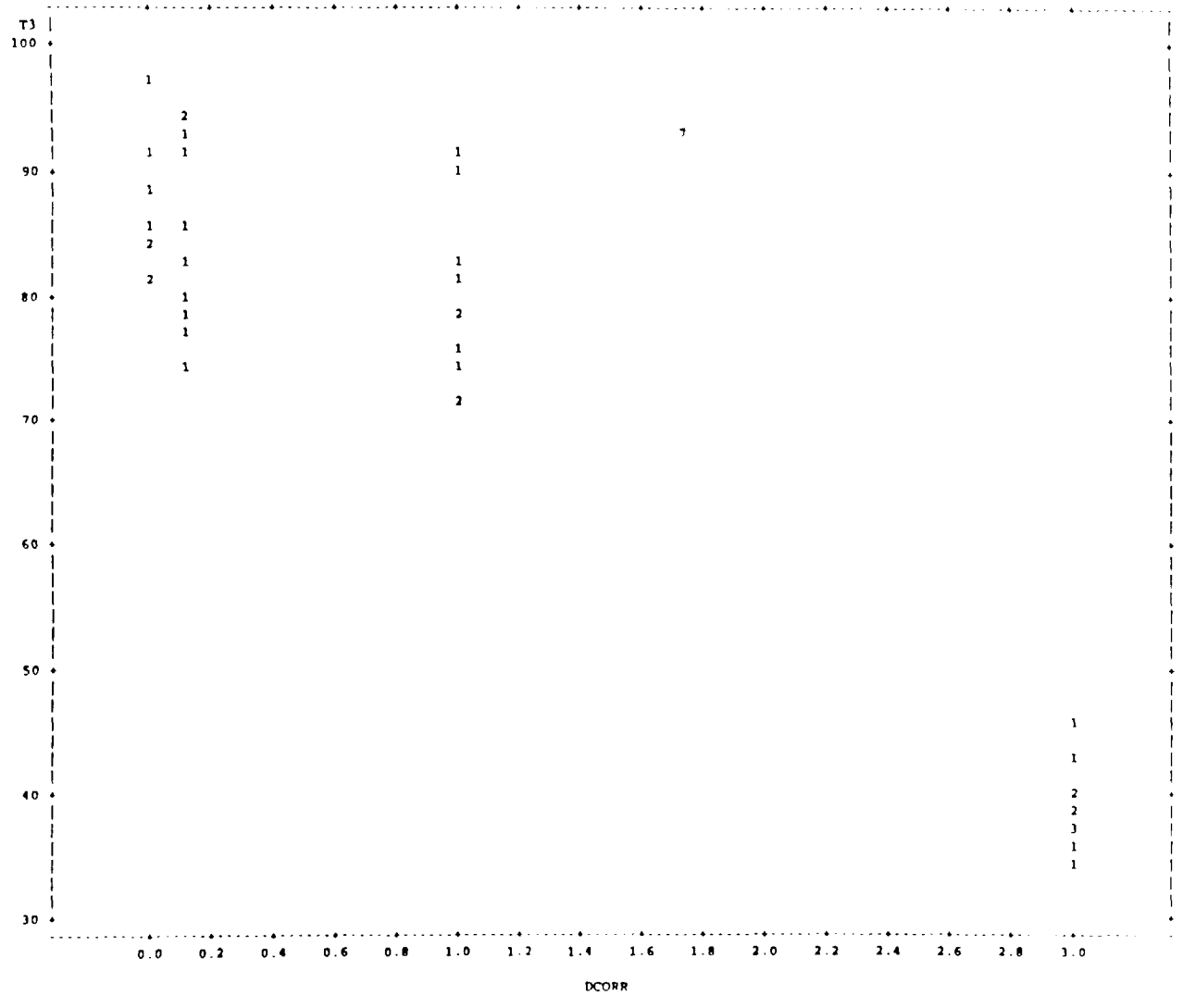
Sum of Residuals	0
Sum of Squared Residuals	1830.6695
Predicted Resid SS (Press)	1984.5367

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.1500	4.5800	0.102	4.3732	4.7869	-0.4300
2	4.2300	4.5800	0.102	4.3732	4.7869	-0.3500
3	4.3600	4.5800	0.102	4.3732	4.7869	-0.2200
4	4.0600	4.5800	0.102	4.3732	4.7869	-0.5200
5	4.1200	4.5800	0.102	4.3732	4.7869	-0.4600
6	4.6800	4.5800	0.102	4.3732	4.7869	0.1000

51	4.7300	4.8649	0.137	4.5876	5.1423	-0.1349
52	5.0100	4.8649	0.137	4.5876	5.1423	0.1451
53	4.1300	4.8649	0.137	4.5876	5.1423	-0.7349
54	5.5800	4.8649	0.137	4.5876	5.1423	0.7151
55	3.7600	4.8649	0.137	4.5876	5.1423	-1.1049
56	5.0300	4.8649	0.137	4.5876	5.1423	0.1651
57	4.9900	4.8649	0.137	4.5876	5.1423	0.1251
58	3.9700	4.8649	0.137	4.5876	5.1423	0.8949
59	4.9200	4.8649	0.137	4.5876	5.1423	0.0551
60	4.6000	4.8649	0.137	4.5876	5.1423	-0.2649
61	5.2900	4.8649	0.137	4.5876	5.1423	0.4251
62	4.9500	4.8649	0.137	4.5876	5.1423	0.0851
63	4.9200	4.8649	0.137	4.5876	5.1423	0.0551
64	4.9500	4.8649	0.137	4.5876	5.1423	0.0851
65	5.1000	4.8649	0.137	4.5876	5.1423	0.2351
66	4.8900	4.8649	0.137	4.5876	5.1423	0.0251

Sum of Residuals	0
Sum of Squared Residuals	8.1984
Predicted Resid SS (Press)	9.1615

1 DEV NT STUDY, PNDS PUPS, CORRELATIONS WITH EXTERNAL DOSE 10:02 Monday, February 8, 1999 23



---------- TMPT=15-18 ----------

Correlation Analysis

5 'VAR' Variables: DCORR INTDOSE T4 T3 TSH

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DCORR	53	0.380189	0.487156	20.150000	0	1.000000
INTDOSE	53	-1.952830	5.896596	-103.500000	-9.000000	5.100000
T4	53	4.742075	0.697466	251.330000	3.240000	6.640000
T3	53	142.397547	28.942102	7547.070000	101.610000	234.210000
TSH	53	13.864906	3.271930	734.840000	9.000000	21.750000

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 53

	DCORR	INTDOSE	T4	T3	TSH
DCORR	1.00000 0.0	-0.93648 0.0001	-0.10798 0.4415	-0.48964 0.0002	0.44024 0.0010
INTDOSE	-0.93648 0.0001	1.00000 0.0	0.04070 0.7723	0.37723 0.0054	-0.38054 0.0049
T4	-0.10798 0.4415	0.04070 0.7723	1.00000 0.0	0.38512 0.0044	0.52428 0.0001
T3	-0.48964 0.0002	0.37723 0.0054	0.38512 0.0044	1.00000 0.0	0.08258 0.5566
TSH	0.44024 0.0010	-0.38054 0.0049	0.52428 0.0001	0.08258 0.5566	1.00000 0.0

---------- TMPT=92-95 ----------

Correlation Analysis

5 'VAR' Variables: DCORR INTDOSE T4 T3 TSH

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.751477	0.10191847	46.620	0.0001
INTDOSE	1	0.004815	0.01654918	0.291	0.7723

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6198.44222	6198.44222	8.462	0.0054
Error	51	37359.11316	732.53163		
C Total	52	43557.55538			

Root MSE	27.06532	R-square	0.1423
Dep Mean	142.39755	Adj R-sq	0.1255
C.V.	19.00687		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	146.013333	3.92000322	37.248	0.0001
INTDOSE	1	1.851562	0.63651717	2.909	0.0054

---------- TMPT=15-18 ----------

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	80.61296	80.61296	8.636	0.0049
Error	51	476.07436	9.33479		
C Total	52	556.68732			

Root MSE	3.05529	R-square	0.1448

31	4.3600	4.7515	0.102	4.5469	4.9561	-0.3915
32	4.7500	4.7515	0.102	4.5469	4.9561	-0.00148
33	4.7200	4.7515	0.102	4.5469	4.9561	-0.0315
34	4.4800	4.7760	0.152	4.4718	5.0803	0.2960
35	4.3900	4.7760	0.152	4.4718	5.0803	-0.3860
36	4.5900	4.7760	0.152	4.4718	5.0803	-0.1860
37	4.5800	4.7760	0.152	4.4718	5.0803	0.1960
38	4.7400	4.7760	0.152	4.4718	5.0803	-0.0360
39	4.6000	4.7760	0.152	4.4718	5.0803	-0.1760
40	3.2400	4.7760	0.152	4.4718	5.0803	-1.5360
41	4.4400	4.7760	0.152	4.4718	5.0803	-0.3360
42	3.8300	4.7760	0.152	4.4718	5.0803	-0.9460
43	4.3400	4.7760	0.152	4.4718	5.0803	-0.4360
44	4.0200	4.7081	0.151	4.4041	5.0122	-0.6881
45	3.4500	4.7081	0.151	4.4041	5.0122	1.2581
46	4.3000	4.7081	0.151	4.4041	5.0122	-0.4081
47	4.4600	4.7081	0.151	4.4041	5.0122	-0.2481
48	3.6700	4.7081	0.151	4.4041	5.0122	-1.0381
49	3.8100	4.7081	0.151	4.4041	5.0122	-0.8981
50	4.6000	4.7081	0.151	4.4041	5.0122	-0.1081
51	4.6100	4.7081	0.151	4.4041	5.0122	-0.0981
52	4.0500	4.7081	0.151	4.4041	5.0122	-0.6581

TMPT=15-18

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
53	4.0700	4.7081	0.151	4.4041	5.0122	-0.6381

Sum of Residuals	0
Sum of Squared Residuals	25.2540
Predicted Resid SS (Press)	27.2588

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	200.1	146.0	3.920	138.1	153.9	54.1267
2	208.5	146.0	3.920	138.1	153.9	62.4367
3	186.2	146.0	3.920	138.1	153.9	40.2167
4	234.2	146.0	3.920	138.1	153.9	88.1967
5	205.1	146.0	3.920	138.1	153.9	59.0667
6	178.5	146.0	3.920	138.1	153.9	32.5167
7	170.2	146.0	3.920	138.1	153.9	24.1467
8	187.1	146.0	3.920	138.1	153.9	41.0967
9	173.2	155.5	5.829	143.8	167.2	17.7737

51	108.5	129.3	5.826	117.7	141.0	-20.8493
52	135.8	129.3	5.826	117.7	141.0	6.4107
53	129.1	129.3	5.826	117.7	141.0	-0.2893

Sum of Residuals	0
Sum of Squared Residuals	37359.1132
Predicted Resid SS (Press)	39573.3004

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	13.0800	13.4526	0.443	12.5642	14.3409	-0.3726
2	13.7200	13.4526	0.443	12.5642	14.3409	0.2674
3	14.2000	13.4526	0.443	12.5642	14.3409	0.7474
4	16.3800	13.4526	0.443	12.5642	14.3409	2.9274
5	13.2100	13.4526	0.443	12.5642	14.3409	-0.2426
6	15.5200	13.4526	0.443	12.5642	14.3409	2.0674
7	17.4500	13.4526	0.443	12.5642	14.3409	3.9974
8	15.3000	13.4526	0.443	12.5642	14.3409	1.8474
9	15.2200	12.3757	0.658	11.0547	13.6966	2.8443
10	12.7600	12.3757	0.658	11.0547	13.6966	0.3843
11	17.6300	12.3757	0.658	11.0547	13.6966	5.2543
12	18.4100	12.3757	0.658	11.0547	13.6966	6.0343
13	16.4900	12.3757	0.658	11.0547	13.6966	4.1143
14	19.9300	15.3529	0.658	14.0326	16.6733	4.5771
15	20.2700	15.3529	0.658	14.0326	16.6733	4.9171
16	17.3900	15.3529	0.658	14.0326	16.6733	2.0371
17	17.0300	15.3529	0.658	14.0326	16.6733	1.6771
18	20.8700	15.3529	0.658	14.0326	16.6733	5.5171
19	18.7200	15.3529	0.658	14.0326	16.6733	3.3671
20	21.7500	15.3529	0.658	14.0326	16.6733	6.3971
21	16.1100	15.3529	0.658	14.0326	16.6733	0.7571
22	17.4800	15.3529	0.658	14.0326	16.6733	2.1271
23	18.4500	15.3529	0.658	14.0326	16.6733	3.0971
24	9.8900	13.4526	0.443	12.5642	14.3409	-3.5626
25	9.4900	13.4526	0.443	12.5642	14.3409	-3.9626
26	9.0000	13.4526	0.443	12.5642	14.3409	-4.4526
27	10.5100	13.4526	0.443	12.5642	14.3409	-2.9426
28	10.8600	13.4526	0.443	12.5642	14.3409	-2.5926
29	11.3000	13.4526	0.443	12.5642	14.3409	-2.1526
30	10.2700	13.4526	0.443	12.5642	14.3409	-3.1826
31	11.1500	13.4526	0.443	12.5642	14.3409	-2.3026
32	10.5700	13.4526	0.443	12.5642	14.3409	-2.8826

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 31

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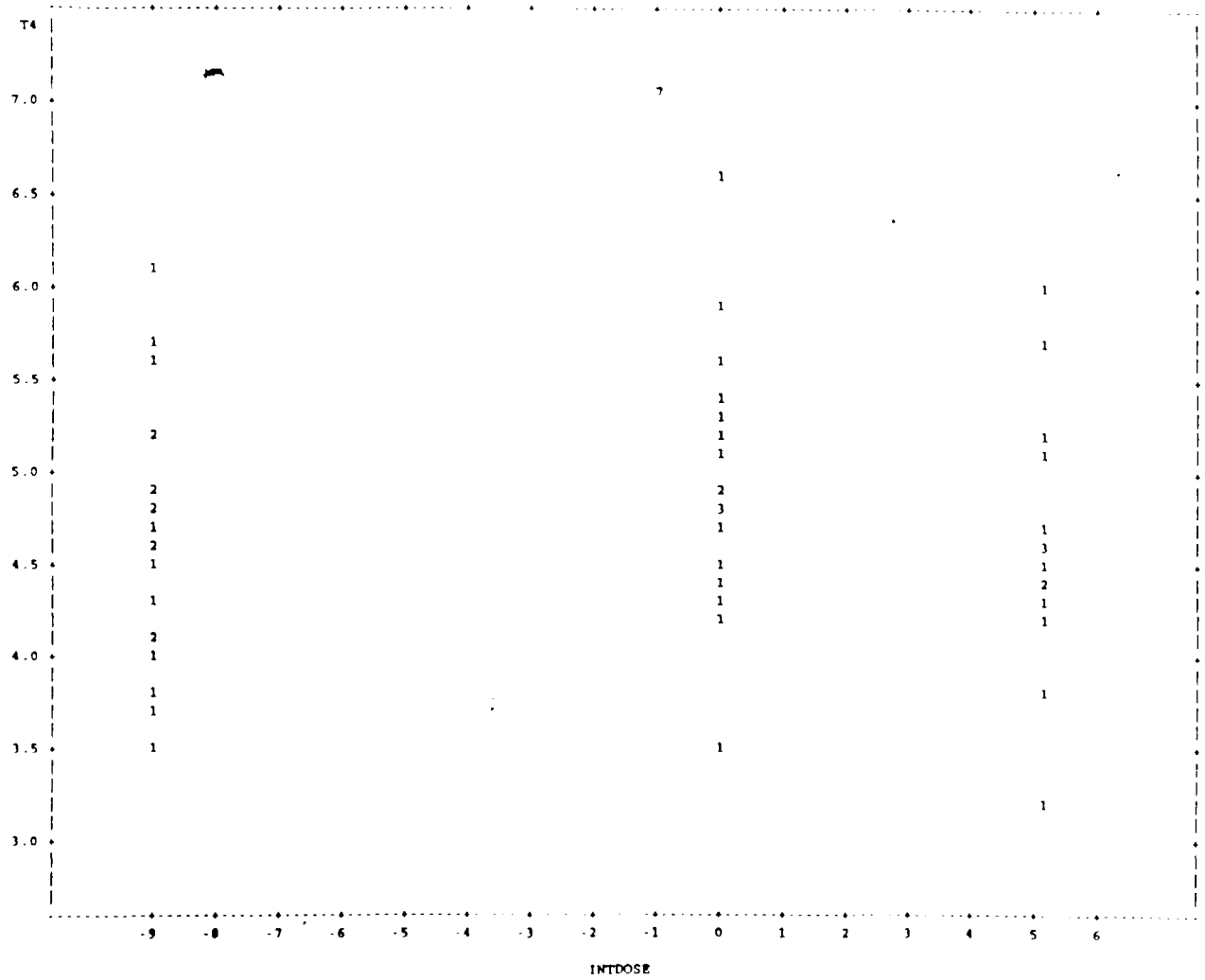
TMPT=15-18



1 Subchronic Rat Perchlorate Study, Correlations with int. dose 35
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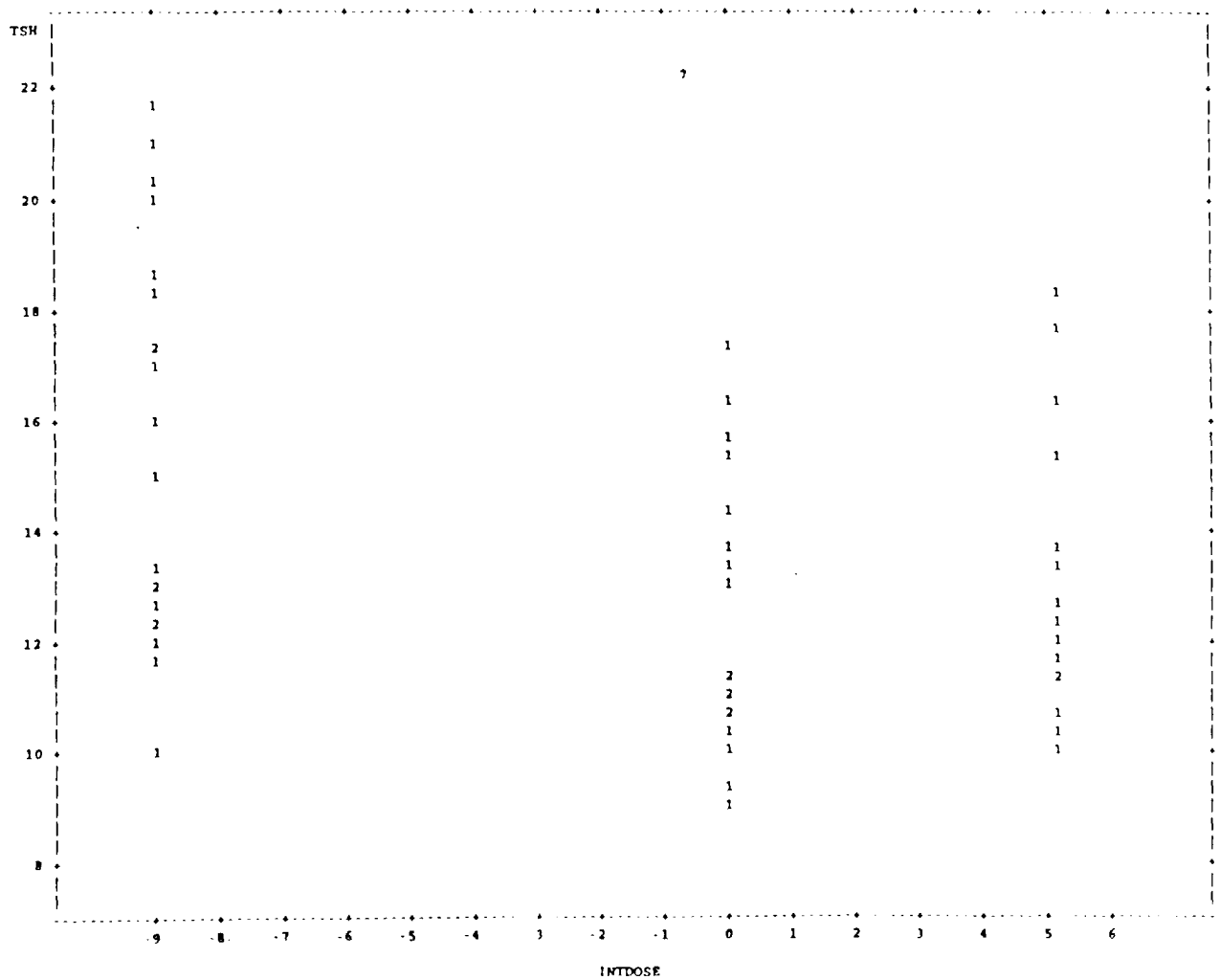
TMPT=15 18



INTERCEP	1	152.405922	3.04588710	50.037	0.0001
INTDOSE	1	2.409122	0.51557345	4.673	0.0001

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 37
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TMPT=92-95

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	29.86510	29.86510	10.732	0.0018
Error	57	158.61706	2.78276		
C Total	58	188.48216			

Root MSE	1.66816	R-square	0.1585
Dep Mean	17.15153	Adj R-sq	0.1437
C.V.	9.72601		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	17.007841	0.22156043	76.764	0.0001
INTDOSE	1	-0.122861	0.03750325	-3.276	0.0018

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 38
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TMPT=92-95

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.6100	4.0832	0.094	3.8941	4.2722	0.5268
2	5.0200	4.0832	0.094	3.8941	4.2722	0.9368
3	4.9600	4.0832	0.094	3.8941	4.2722	0.8768
4	4.6300	4.0832	0.094	3.8941	4.2722	0.5468
5	6.3500	4.0832	0.094	3.8941	4.2722	2.2668
6	5.1900	4.0832	0.094	3.8941	4.2722	1.1068
7	5.1200	4.0832	0.094	3.8941	4.2722	1.0368
8	4.3700	4.0832	0.094	3.8941	4.2722	0.2868
9	4.9700	4.0832	0.094	3.8941	4.2722	0.8868
10	5.3800	4.0832	0.094	3.8941	4.2722	1.2968

53	3.4100	3.5867	0.156	3.2751	3.8983	-0.1767
54	2.9000	3.5867	0.156	3.2751	3.8983	-0.6867
55	3.7800	3.5867	0.156	3.2751	3.8983	0.1933
56	3.4300	3.5867	0.156	3.2751	3.8983	-0.1567
57	3.1800	3.5867	0.156	3.2751	3.8983	-0.4067
58	2.7900	3.5867	0.156	3.2751	3.8983	-0.7967
59	3.0400	3.5867	0.156	3.2751	3.8983	-0.5467

Sum of Residuals	0
Sum of Squared Residuals	28.7923
Predicted Resid SS (Press)	30.4899

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	204.7	152.4	3.046	146.3	158.5	52.2841
2	182.7	152.4	3.046	146.3	158.5	30.2441
3	158.2	152.4	3.046	146.3	158.5	5.8241
4	156.9	152.4	3.046	146.3	158.5	4.4841
5	189.8	152.4	3.046	146.3	158.5	37.4341
6	179.3	152.4	3.046	146.3	158.5	26.8741
7	215.3	152.4	3.046	146.3	158.5	62.9241
8	160.9	152.4	3.046	146.3	158.5	8.4541
9	171.1	152.4	3.046	146.3	158.5	18.6841
10	179.5	152.4	3.046	146.3	158.5	27.1041
11	158.2	164.7	4.400	155.9	173.5	-6.5424
12	155.4	164.7	4.400	155.9	173.5	-9.2924
13	136.2	164.7	4.400	155.9	173.5	-28.5024
14	152.6	164.7	4.400	155.9	173.5	-12.0824
15	154.1	164.7	4.400	155.9	173.5	-10.6324
16	144.1	164.7	4.400	155.9	173.5	-20.6324
17	186.9	164.7	4.400	155.9	173.5	22.1676
18	149.5	164.7	4.400	155.9	173.5	-15.1824
19	151.6	164.7	4.400	155.9	173.5	-13.0824
20	186.2	164.7	4.400	155.9	173.5	21.4976
21	134.5	130.7	5.021	120.7	140.8	3.8062
22	133.3	130.7	5.021	120.7	140.8	2.5562
23	116.0	130.7	5.021	120.7	140.8	-14.7438
24	112.2	130.7	5.021	120.7	140.8	-18.5638
25	103.3	130.7	5.021	120.7	140.8	-27.3938
26	112.8	130.7	5.021	120.7	140.8	-17.9438
27	138.7	130.7	5.021	120.7	140.8	7.9662
28	129.2	130.7	5.021	120.7	140.8	-1.5338
29	114.3	130.7	5.021	120.7	140.8	-16.4538
30	123.7	130.7	5.021	120.7	140.8	-7.0138
31	208.4	152.4	3.046	146.3	158.5	56.0041
32	176.3	152.4	3.046	146.3	158.5	23.8941

6	15.3900	17.0078	0.222	16.5642	17.4515	-1.6178
7	17.6100	17.0078	0.222	16.5642	17.4515	0.6022
8	14.9800	17.0078	0.222	16.5642	17.4515	-2.0278
9	14.7900	17.0078	0.222	16.5642	17.4515	-2.2178
10	18.6100	17.0078	0.222	16.5642	17.4515	1.6022
11	16.2700	16.3813	0.320	15.7403	17.0222	-0.1113
12	16.8700	16.3813	0.320	15.7403	17.0222	0.4887
13	15.9500	16.3813	0.320	15.7403	17.0222	0.4313
14	17.5400	16.3813	0.320	15.7403	17.0222	1.1587
15	15.4000	16.3813	0.320	15.7403	17.0222	-0.9813
16	15.4600	16.3813	0.320	15.7403	17.0222	-0.9213
17	14.9600	16.3813	0.320	15.7403	17.0222	-1.4213
18	20.1400	16.3813	0.320	15.7403	17.0222	3.7587
19	19.5100	16.3813	0.320	15.7403	17.0222	3.1287
20	16.3100	16.3813	0.320	15.7403	17.0222	-0.0713

---------- TMPT=92-95 ----------

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
21	17.3100	18.1136	0.365	17.3822	18.8450	-0.8036
22	17.5000	18.1136	0.365	17.3822	18.8450	-0.6136
23	20.2700	18.1136	0.365	17.3822	18.8450	2.1564
24	18.9800	18.1136	0.365	17.3822	18.8450	0.8664
25	21.5200	18.1136	0.365	17.3822	18.8450	3.4064
26	20.0600	18.1136	0.365	17.3822	18.8450	1.9464
27	17.4200	18.1136	0.365	17.3822	18.8450	-0.6936
28	18.9600	18.1136	0.365	17.3822	18.8450	0.8464
29	18.9700	18.1136	0.365	17.3822	18.8450	0.8564
30	19.4100	18.1136	0.365	17.3822	18.8450	1.2964
31	17.3200	17.0078	0.222	16.5642	17.4515	0.3122
32	17.2700	17.0078	0.222	16.5642	17.4515	0.2622
33	16.9700	17.0078	0.222	16.5642	17.4515	-0.0378
34	13.6500	17.0078	0.222	16.5642	17.4515	-3.3578
35	16.0600	17.0078	0.222	16.5642	17.4515	-0.9478
36	16.5100	17.0078	0.222	16.5642	17.4515	-0.4978
37	17.1500	17.0078	0.222	16.5642	17.4515	0.1422
38	15.3100	17.0078	0.222	16.5642	17.4515	-1.6978
39	19.1400	17.0078	0.222	16.5642	17.4515	2.1322
40	15.4100	17.0078	0.222	16.5642	17.4515	-1.5978
41	17.8500	16.3813	0.320	15.7403	17.0222	1.4687
42	15.7600	16.3813	0.320	15.7403	17.0222	-0.6213
43	17.4700	16.3813	0.320	15.7403	17.0222	1.0887
44	14.8000	16.3813	0.320	15.7403	17.0222	-1.5813
45	19.7600	16.3813	0.320	15.7403	17.0222	3.3787
46	19.4800	16.3813	0.320	15.7403	17.0222	3.0987

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 42

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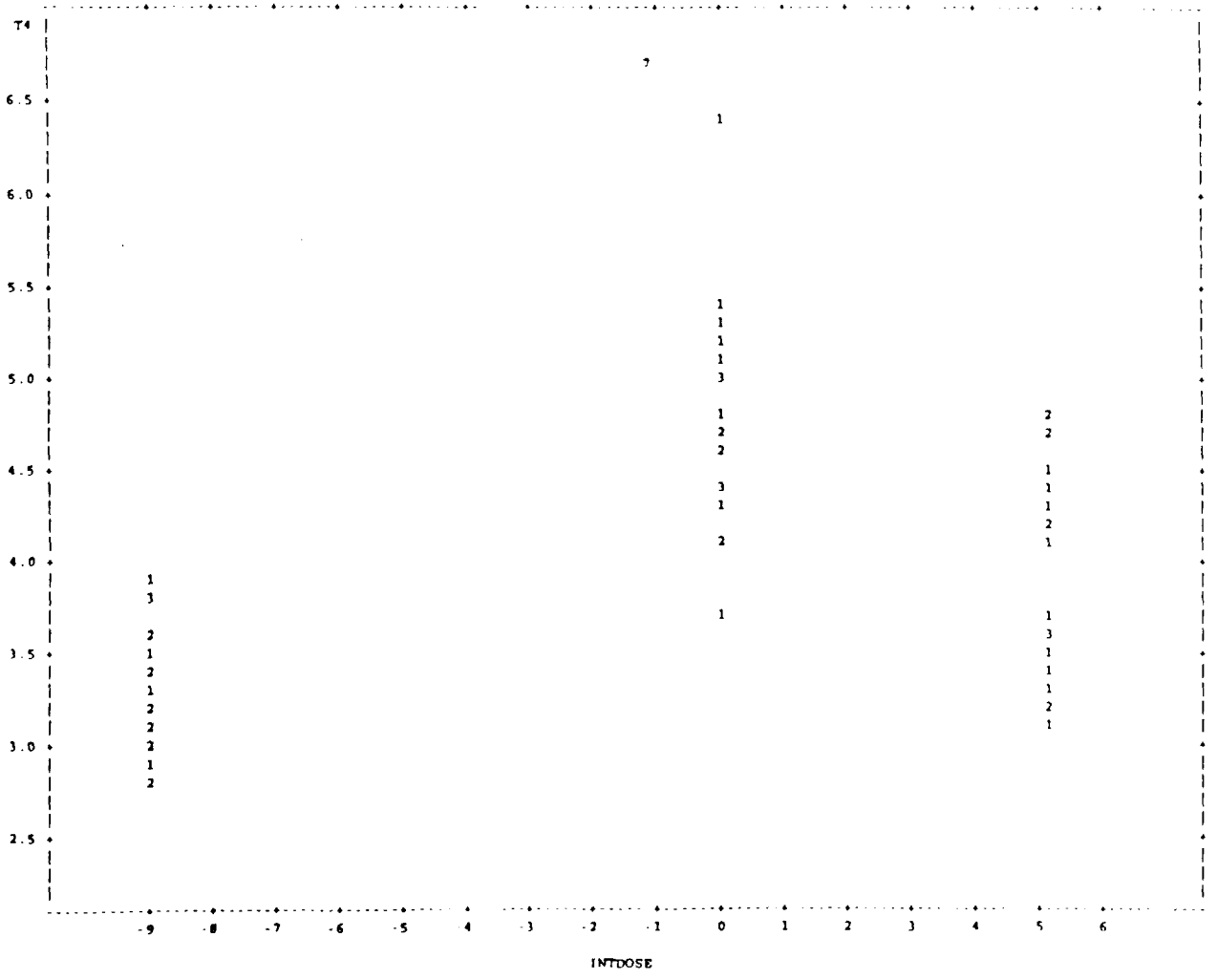
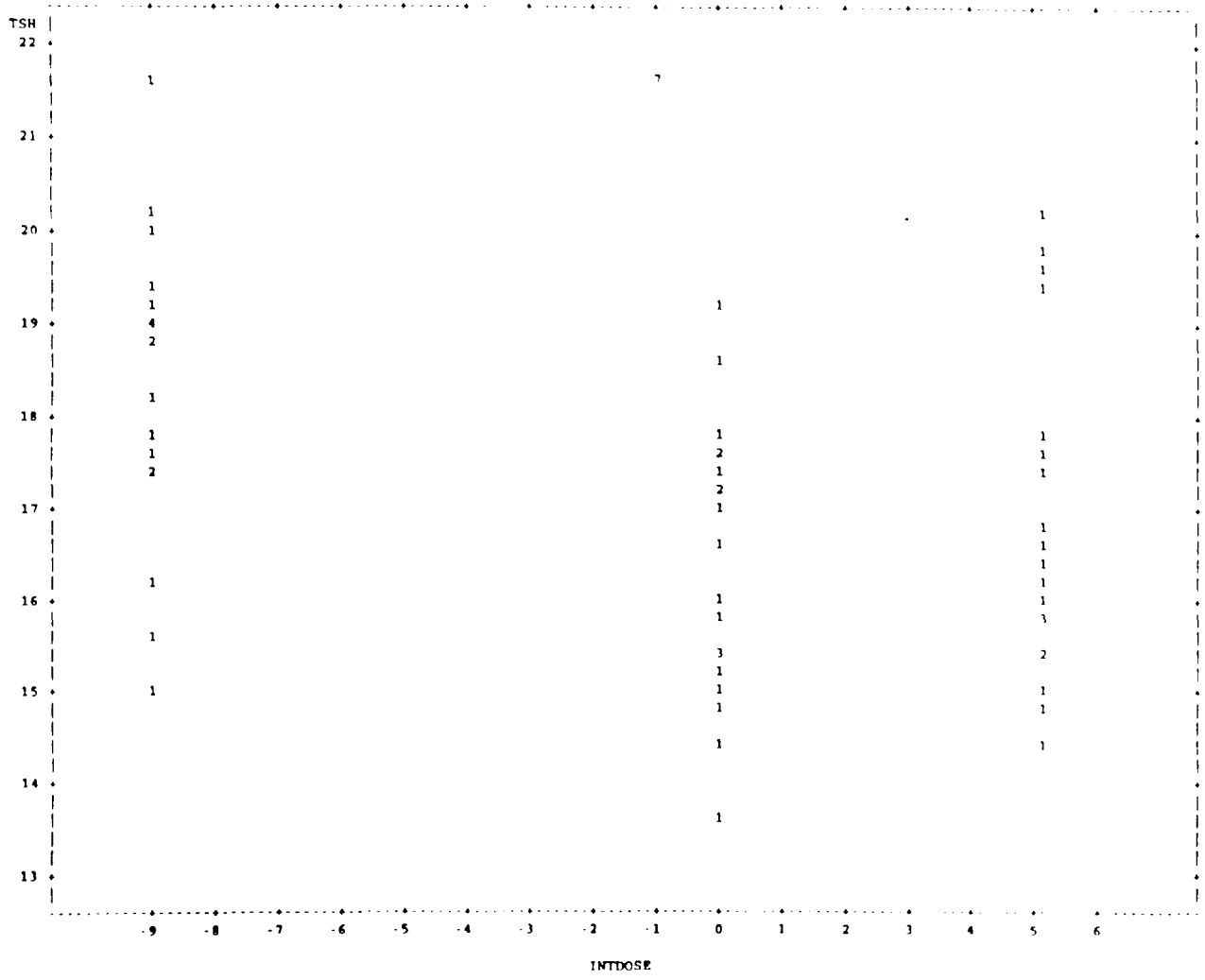
TMPT=92-95



1 Subchronic Rat Perchlorate Study, Correlations with int. dose 44

10:02 Monday, February 8, 1999

TMPT=92-95



INTERCEP	1	153.457081	4.45604412	34.438	0.0001
DCORR	1	-29.089592	7.25364227	-4.010	0.0002

1 Subchronic Rat Perchlorate Study, Correlations with external dose 46
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---------- TMPT=15-18 ----------

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	107.89105	107.89105	12.260	0.0010
Error	51	448.79628	8.79993		
C Total	52	556.68732			

Root MSE	2.96647	R-square	0.1938
Dep Mean	13.86491	Adj R-sq	0.1780
C.V.	21.39551		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	12.740761	0.51875586	24.560	0.0001
DCORR	1	2.956806	0.84444170	3.501	0.0010

1 Subchronic Rat Perchlorate Study, Correlations with external dose 47
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---------- TMPT=15-18 ----------

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	5.1000	4.8009	0.122	4.5550	5.0467	0.2991
2	5.6400	4.8009	0.122	4.5550	5.0467	0.8391
3	6.6400	4.8009	0.122	4.5550	5.0467	1.8391
4	5.8800	4.8009	0.122	4.5550	5.0467	1.0791
5	4.8400	4.8009	0.122	4.5550	5.0467	0.0391
6	5.4200	4.8009	0.122	4.5550	5.0467	0.6191
7	5.2900	4.8009	0.122	4.5550	5.0467	0.4891
8	5.1900	4.8009	0.122	4.5550	5.0467	0.3891
9	5.1000	4.7993	0.121	4.5560	5.0427	0.3007
10	4.2300	4.7993	0.121	4.5560	5.0427	-0.5693

53	4.0700	4.6463	0.157	4.3320	4.9606	0.5763

Sum of Residuals	0
Sum of Squared Residuals	25.0009
Predicted Resid SS (Press)	26.9981

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	200.1	153.5	4.456	144.5	162.4	46.6829
2	208.5	153.5	4.456	144.5	162.4	54.9929
3	186.2	153.5	4.456	144.5	162.4	32.7729
4	234.2	153.5	4.456	144.5	162.4	80.7529
5	205.1	153.5	4.456	144.5	162.4	51.6229
6	178.5	153.5	4.456	144.5	162.4	25.0729
7	170.2	153.5	4.456	144.5	162.4	16.7029
8	187.1	153.5	4.456	144.5	162.4	33.6529
9	173.2	153.2	4.412	144.3	162.0	20.0638
10	170.5	153.2	4.412	144.3	162.0	17.3238
11	154.8	153.2	4.412	144.3	162.0	1.6438
12	136.3	153.2	4.412	144.3	162.0	-16.8662
13	183.3	153.2	4.412	144.3	162.0	30.1338
14	122.4	124.4	5.698	112.9	135.8	-1.9775
15	130.7	124.4	5.698	112.9	135.8	6.3425
16	139.6	124.4	5.698	112.9	135.8	15.1925
17	137.4	124.4	5.698	112.9	135.8	13.0725
18	133.2	124.4	5.698	112.9	135.8	8.8125
19	101.6	124.4	5.698	112.9	135.8	-22.7575
20	125.9	124.4	5.698	112.9	135.8	1.5425
21	109.9	124.4	5.698	112.9	135.8	-14.4375
22	124.6	124.4	5.698	112.9	135.8	0.2025
23	112.3	124.4	5.698	112.9	135.8	-12.0975
24	133.3	153.5	4.456	144.5	162.4	-20.1771
25	147.0	153.5	4.456	144.5	162.4	-6.4771
26	148.5	153.5	4.456	144.5	162.4	-4.9371
27	113.0	153.5	4.456	144.5	162.4	-40.4471
28	141.8	153.5	4.456	144.5	162.4	-11.6671
29	115.1	153.5	4.456	144.5	162.4	-38.3371
30	125.5	153.5	4.456	144.5	162.4	-27.9971
31	155.3	153.5	4.456	144.5	162.4	1.8229
32	117.3	153.5	4.456	144.5	162.4	-36.1871
33	133.2	153.5	4.456	144.5	162.4	-20.2171
34	144.3	153.2	4.412	144.3	162.0	-8.8462
35	134.1	153.2	4.412	144.3	162.0	-19.0262
36	134.2	153.2	4.412	144.3	162.0	-19.0062
37	134.9	153.2	4.412	144.3	162.0	-18.2762
38	142.6	153.2	4.412	144.3	162.0	-10.6162

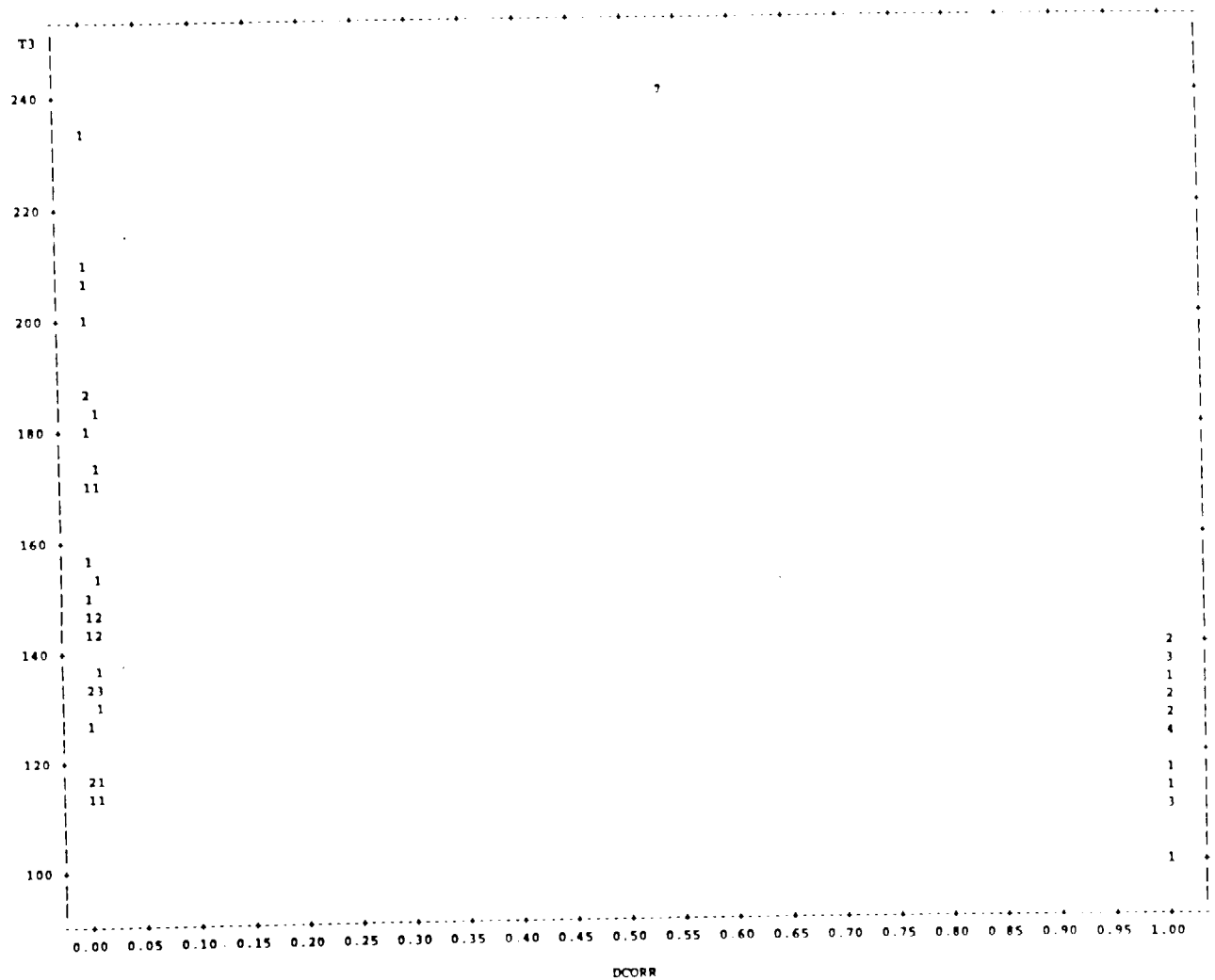
18	20.8700	15.6976	0.663	14.3659	17.0292	5.1724
19	18.7200	15.6976	0.663	14.3659	17.0292	3.0224
20	21.7500	15.6976	0.663	14.3659	17.0292	6.0524
21	16.1100	15.6976	0.663	14.3659	17.0292	0.4124
22	17.4800	15.6976	0.663	14.3659	17.0292	1.7824
23	18.4500	15.6976	0.663	14.3659	17.0292	2.7524
24	9.8900	12.7408	0.519	11.6993	13.7822	-2.8508
25	9.4900	12.7408	0.519	11.6993	13.7822	-3.2508
26	9.0000	12.7408	0.519	11.6993	13.7822	-3.7408
27	10.5100	12.7408	0.519	11.6993	13.7822	-2.2308
28	10.8600	12.7408	0.519	11.6993	13.7822	-1.8808
29	11.3000	12.7408	0.519	11.6993	13.7822	-1.4408
30	10.2700	12.7408	0.519	11.6993	13.7822	-2.4708
31	11.1500	12.7408	0.519	11.6993	13.7822	-1.5908
32	10.5700	12.7408	0.519	11.6993	13.7822	-2.1708

---------- TMPT=15-18 ----------

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
33	11.3600	12.7408	0.519	11.6993	13.7822	-1.3808
34	11.8600	12.7703	0.514	11.7393	13.8014	-0.9103
35	11.8300	12.7703	0.514	11.7393	13.8014	-0.9403
36	10.3300	12.7703	0.514	11.7393	13.8014	-2.4403
37	10.0300	12.7703	0.514	11.7393	13.8014	-2.7403
38	13.4700	12.7703	0.514	11.7393	13.8014	0.6997
39	10.7800	12.7703	0.514	11.7393	13.8014	-1.9903
40	13.7100	12.7703	0.514	11.7393	13.8014	0.9397
41	11.4600	12.7703	0.514	11.7393	13.8014	-1.3103
42	11.3600	12.7703	0.514	11.7393	13.8014	-1.4103
43	12.3500	12.7703	0.514	11.7393	13.8014	-0.4203
44	13.0700	15.6976	0.663	14.3659	17.0292	-2.6276
45	12.3300	15.6976	0.663	14.3659	17.0292	-3.3676
46	10.1300	15.6976	0.663	14.3659	17.0292	-5.5676
47	12.0200	15.6976	0.663	14.3659	17.0292	-3.6776
48	13.4800	15.6976	0.663	14.3659	17.0292	-2.2176
49	11.7000	15.6976	0.663	14.3659	17.0292	-3.9976
50	12.4800	15.6976	0.663	14.3659	17.0292	-3.2176
51	13.1400	15.6976	0.663	14.3659	17.0292	-2.5576
52	14.9100	15.6976	0.663	14.3659	17.0292	-0.7876
53	12.6300	15.6976	0.663	14.3659	17.0292	-3.0676

Sum of Residuals	0
Sum of Squared Residuals	448.7963
Predicted Resid SS (Press)	487.8609

TMPT=15-18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180
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DCORR

TMPT=92-95

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	13.35356	13.35356	35.471	0.0001
Error	57	21.45854	0.37647		
C Total	58	34.81209			

Root MSE	0.61357	R-square	0.3836
Dep Mean	4.01864	Adj R-sq	0.3728
C.V.	15.26803		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.351631	0.09750241	44.631	0.0001
DCORR	1	-1.023240	0.17180734	-5.956	0.0001

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	21318.12087	21318.12087	60.328	0.0001
Error	57	20142.20249	353.37197		
C Total	58	41460.32336			

Root MSE	18.79819	R-square	0.5142
Dep Mean	149.58847	Adj R-sq	0.5057
C.V.	12.56660		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|

11	3.2200	4.3414	0.097	4.1481	4.5347	-1.1214
12	4.2200	4.3414	0.097	4.1481	4.5347	-0.1214
13	4.7000	4.3414	0.097	4.1481	4.5347	0.3586
14	4.2300	4.3414	0.097	4.1481	4.5347	-0.1114
15	4.1200	4.3414	0.097	4.1481	4.5347	-0.2214
16	4.7600	4.3414	0.097	4.1481	4.5347	0.4186
17	4.3800	4.3414	0.097	4.1481	4.5347	0.0386
18	4.4900	4.3414	0.097	4.1481	4.5347	0.1486
19	4.6500	4.3414	0.097	4.1481	4.5347	0.3086
20	4.7900	4.3414	0.097	4.1481	4.5347	0.4486
21	3.8700	3.3284	0.141	3.0465	3.6103	0.5416
22	3.1400	3.3284	0.141	3.0465	3.6103	-0.1884
23	3.8400	3.3284	0.141	3.0465	3.6103	0.5116
24	3.3200	3.3284	0.141	3.0465	3.6103	-0.00839
25	3.5900	3.3284	0.141	3.0465	3.6103	0.2616
26	2.7900	3.3284	0.141	3.0465	3.6103	-0.5384
27	3.2000	3.3284	0.141	3.0465	3.6103	-0.1284
28	3.6100	3.3284	0.141	3.0465	3.6103	0.2816
29	3.8200	3.3284	0.141	3.0465	3.6103	0.4916
30	3.4900	3.3284	0.141	3.0465	3.6103	0.1616
31	3.7300	4.3516	0.098	4.1564	4.5469	-0.6216
32	4.3900	4.3516	0.098	4.1564	4.5469	0.0384
33	4.1100	4.3516	0.098	4.1564	4.5469	-0.2416
34	4.3800	4.3516	0.098	4.1564	4.5469	0.0284
35	4.7000	4.3516	0.098	4.1564	4.5469	0.3484
36	4.1000	4.3516	0.098	4.1564	4.5469	0.2516
37	5.2800	4.3516	0.098	4.1564	4.5469	0.9284
38	4.7500	4.3516	0.098	4.1564	4.5469	0.3984
39	4.2500	4.3516	0.098	4.1564	4.5469	-0.1016
40	4.7200	4.3516	0.098	4.1564	4.5469	0.3684
41	3.6100	4.3414	0.097	4.1481	4.5347	-0.7314
42	3.7300	4.3414	0.097	4.1481	4.5347	-0.6114
43	3.6000	4.3414	0.097	4.1481	4.5347	0.7414
44	3.3600	4.3414	0.097	4.1481	4.5347	-0.9814
45	3.1800	4.3414	0.097	4.1481	4.5347	-1.1614
46	3.3000	4.3414	0.097	4.1481	4.5347	1.0414
47	3.1000	4.3414	0.097	4.1481	4.5347	-1.2414
48	3.4600	4.3414	0.097	4.1481	4.5347	-0.8814
49	4.2900	4.3414	0.097	4.1481	4.5347	-0.0514
50	3.5800	4.3414	0.097	4.1481	4.5347	-0.7614
51	3.0200	3.3284	0.141	3.0465	3.6103	-0.3084
52	3.1000	3.3284	0.141	3.0465	3.6103	-0.2284

TMPT=92-95

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

33	156.3	162.9	2.987	156.9	168.9	-6.5931
34	184.1	162.9	2.987	156.9	168.9	21.1569
35	162.7	162.9	2.987	156.9	168.9	-0.1931
36	174.6	162.9	2.987	156.9	168.9	11.6669

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TMPT=92-95

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
37	178.4	162.9	2.987	156.9	168.9	15.4869
38	157.3	162.9	2.987	156.9	168.9	-5.5531
39	154.0	162.9	2.987	156.9	168.9	-8.8631
40	148.7	162.9	2.987	156.9	168.9	-14.1731
41	161.1	162.5	2.957	156.6	168.4	-1.3843
42	133.7	162.5	2.957	156.6	168.4	-28.7843
43	153.4	162.5	2.957	156.6	168.4	-9.0643
44	140.3	162.5	2.957	156.6	168.4	-22.1943
45	122.9	162.5	2.957	156.6	168.4	-39.5843
46	126.2	162.5	2.957	156.6	168.4	-36.2543
47	136.1	162.5	2.957	156.6	168.4	-26.3843
48	158.9	162.5	2.957	156.6	168.4	-3.6343
49	134.9	162.5	2.957	156.6	168.4	-27.5443
50	163.8	162.5	2.957	156.6	168.4	1.2957
51	122.9	122.0	4.312	113.4	130.6	0.8509
52	116.1	122.0	4.312	113.4	130.6	-5.9191
53	116.6	122.0	4.312	113.4	130.6	-5.4191
54	107.7	122.0	4.312	113.4	130.6	-14.2791
55	135.3	122.0	4.312	113.4	130.6	13.2909
56	117.3	122.0	4.312	113.4	130.6	-4.7191
57	137.2	122.0	4.312	113.4	130.6	15.1809
58	124.0	122.0	4.312	113.4	130.6	2.0209
59	125.6	122.0	4.312	113.4	130.6	3.6009

Sum of Residuals	0
Sum of Squared Residuals	20142.2025
Predicted Resid SS (Press)	21311.0806

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	15.7100	16.5623	0.255	16.0518	17.0727	-0.8523
2	14.3800	16.5623	0.255	16.0518	17.0727	-2.1823
3	15.1800	16.5623	0.255	16.0518	17.0727	-1.3823
4	17.7200	16.5623	0.255	16.0518	17.0727	1.1577
5	17.5000	16.5623	0.255	16.0518	17.0727	0.9377

47	15.8900	16.5804	0.252	16.0751	17.0857	-0.6904
48	16.6400	16.5804	0.252	16.0751	17.0857	0.0596
49	14.4500	16.5804	0.252	16.0751	17.0857	-2.1304
50	15.7300	16.5804	0.252	16.0751	17.0857	-0.8504
51	17.7500	18.3730	0.368	17.6361	19.1099	-0.6230
52	18.8100	18.3730	0.368	17.6361	19.1099	0.4370
53	16.2600	18.3730	0.368	17.6361	19.1099	-2.1130
54	19.2000	18.3730	0.368	17.6361	19.1099	0.8270
55	18.7600	18.3730	0.368	17.6361	19.1099	0.3870
56	15.6900	18.3730	0.368	17.6361	19.1099	-2.6830
57	18.2700	18.3730	0.368	17.6361	19.1099	-0.1030
58	14.9700	18.3730	0.368	17.6361	19.1099	-3.4030
59	18.9300	18.3730	0.368	17.6361	19.1099	0.5570

Sum of Residuals	0
Sum of Squared Residuals	146.6680
Predicted Resid SS (Press)	157.1575

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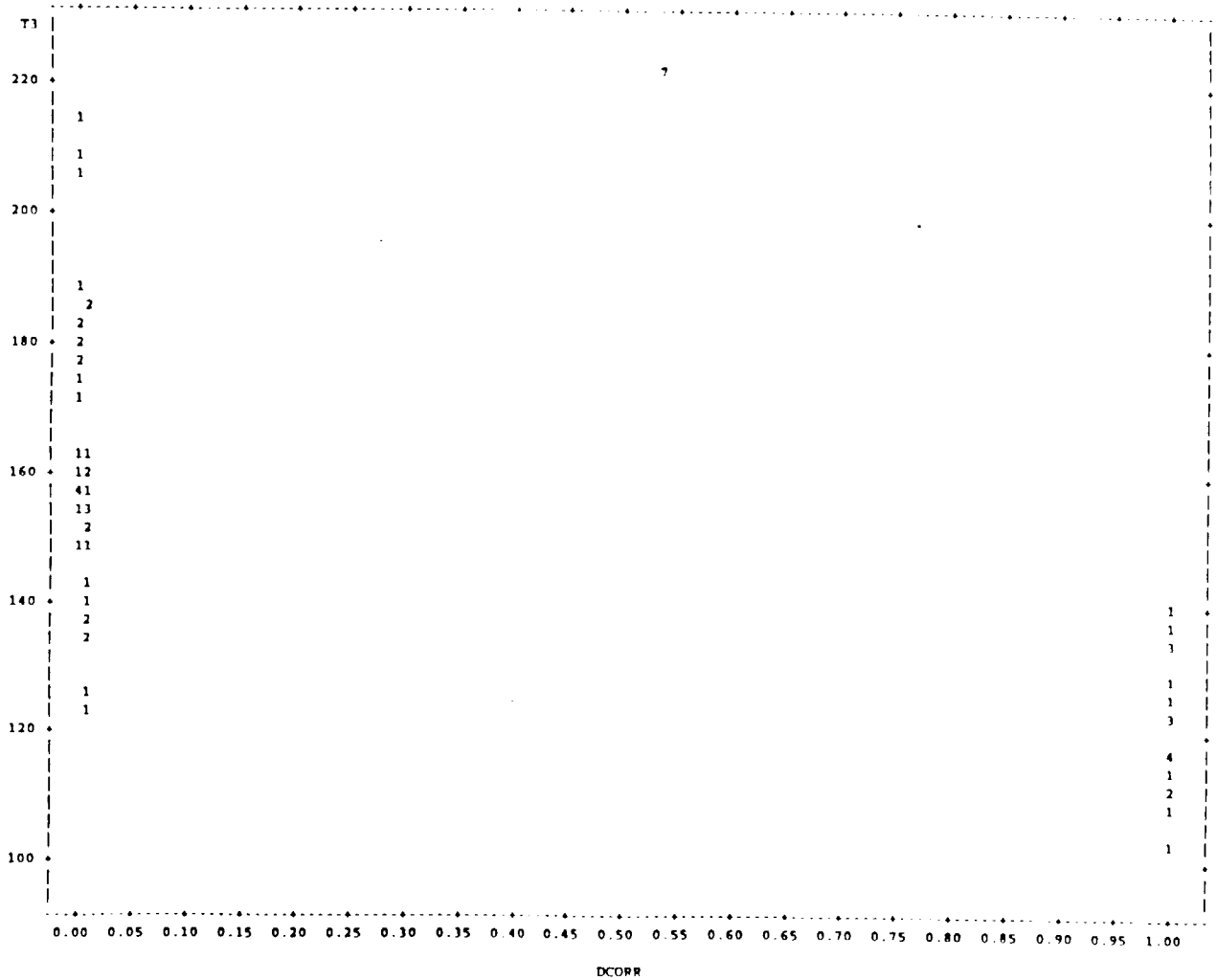
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