DEPARTMENT OF THE AIR FORCE

AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

AFIT/GEE/ENV/95D-09

EFFECTS OF AMMONIUM PERCHLORATE ON THE THYROID HORMONE LEVELS OF THE SPRAGUE-DAWLEY RAT

THESIS

James H. King, Jr., Captain, USAF

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James H. King, Jr.

Presented to the Faculty of the School of Engineering

of the Air Force Institute of Technology

In Partial Fulfillment of the

Requirements for the Degree of

Masters of Science in Engineering and Environmental Management

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Preface

The purpose of this study was to determine the estimated threshold dose of ammonium perchlorate (AP) in the Sprague Dawley rat. This threshold dose could then aid the EPA, Department of Defense, NASA, and their contractors in determining a human threshold dose and subsequently, clean-up levels of AP released into the environment. The threshold could not be determined because the data did not allow for an adequate fit to the sigmoidal function. However, a NOAEL for AP's affect on TSH levels was determined to be .44 mg/kg/day in male rats and .124 mg/kg/day for female rats. The NOAELs were determined by exposing rats to incremental doses of AP in their drinking water for two weeks. At the completion of the exposure period, the rats were sacrificed, blood drawn and their thyroid hormone levels measured using radioimmunoassay. Univariate, multivariate analysis, and maximum likelihood estimation were then implemented in an attempt to determine the threshold dose for the exposed rats.

This research effort would not have been possible without the contribution and support of many individuals. I would like to express special thanks to my faculty advisor, Dr. Daniel J. Caldwell whose knowledge and experience made this and outstanding learning experience. I would also like to thank Ed Kinkead and Robin Wolfe for their instruction in the "Pathtox" software, Brenda Schimmel for her instruction in animal handling, Peggy Parrish and Jerry Nichols for their expertise and instruction in necropsy, Latha Narayanan for her expertise with the radioimmunoassays, Lt Col Michael Shelley for his instruction in risk analysis, and Dan Reynolds and Carlyle Flemming for their instruction in the statistical realm. I would also like to give special thanks to my wife, Cheryl, and daughters, Jennifer, Kathleen and Rachel, for their love and support throughout the thesis effort and master degree program.

James H. King, Jr.

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Abstract

The purpose of this research was to determine the threshold dose for ammonium perchlorate (AP) in the Sprague-Dawley rat. No dose response data exist for AP and the EPA has studied literature on the subject of perchlorates to determine a provisional reference dose. The Perchlorate Group, a consortium of DoD and industry representatives, believes this provisional reference dose is too conservative. This experiment was executed to provide dose response data on which to base a more accurate reference dose. The study consisted of eight groups of 12 Sprague-Dawley rats, six male and six female, which were exposed to incremental doses of AP in their drinking water.

The results indicated that Triiodothyronine (T3) levels in male and female rats fell, Thyroxine (T4) levels in male rats remained relatively unchanged. Reverse Triiodothyronine (rT3), Thyrotropin (TSH), and Thyroglobulin (Tg) all increased in both males and females. These results imply that the AP anion blocked the uptake of iodine in the thyroid. Although an estimated threshold could not be determined, the NOAEL for AP on the TSH levels was .44 mg/kg/day for the male rats and .124 mg/kg/day for the female rats. These NOAELs are consistent with the assumptions made by the EPA, which estimated a NOAEL of .14 mg/kg/day for perchlorates.

EFFECTS OF AMMONIUM PERCHLORATE ON THE THYROID HORMONE LEVELS OF THE SPRAGUE-DAWLEY RAT

I. Introduction

Objective

The primary objective of this research is to determine toxicity information to establish permissible exposure levels of ammonium perchlorate.

Background

Ammonium perchlorate (AP), [NH4+] [ClO4-], is a white, crystalline solid anion which is used as an oxidant in solid propellants for rockets and missiles. It is also used in explosive mixtures, mines, shells, timing devices, other pyrotechnics, and as a chemical raw material (CPIA, 1989). In addition to being listed by the Environmental Protection Agency (EPA) as a class B2 carcinogen (Table 1-1), when AP is used as an oxidizer in solid propellants, 20% of the exhaust produced is HCl by weight. Therefore, clean propellants are being sought to replace AP because of the impact of large quantities

Group		Criteria for Classification
A	-Human carcinogen	-Sufficient evidence from epidemiologic studies
B	-Probable human carcinogen (two subgroups)	-Limited evidence from epidemiologic studies and sufficient evidence from animal studies (B1); or inadequate evidence from epidemiologic studies (or no data) and sufficient evidence from animal studies (B2)
C	-Possible human carcinogen	-Limited evidence from animal studies and no human data
D	-Not classifiable as to human carcinogenicity	-Inadequate human and animal data or no data
E	-Evidence of noncarcinogenicity in humans	-No evidence of carcinogenicity from adequate human and animal studies

Table 1-1. Categorization of Evidence of Carcinogenicity
SOURCE: Science and Judgment in Risk Assessment (1994) adapted from EPA, 1987a

of HCl on the launch locale and long-range acid rain implications (Nieder et al., 1990). However, even if AP is replaced, it will remain necessary to clean-up contaminated areas to acceptable levels.

Currently, the EPA has established a provisional reference dose (RfD) for AP of 1.4×10^{-4} mg/kg/day (Dollarhide, 1992). Establishing a RfD is just one step in a four step process that is generally used in assessing human health risks associated with exposure to toxic substances. The first step is identifying the contaminant and the specific forms of toxicity. The next step is to evaluate the conditions under which the toxic properties of a chemical might be manifested in exposed people, with particular emphasis on the quantitative relation between the dose and the toxic response. Step three involves specifying the population that might be exposed to the agent of concern, identifying the routes through which exposure can occur, and estimating the magnitude, duration and timing of the doses that people might receive as a result of their exposure. The last step involves integration of information from the first three steps to develop a qualitative or quantitative estimate of the likelihood that any of the hazards associated with the agent of concern will be realized in exposed people (S&J, 1994).

After a quantitative relation between the dose and the toxic response is established, the lowest observable adverse effect level (LOAEL) and the no observed adverse effect level (NOAEL) are determined. The LOAEL is the lowest dose which triggers an adverse toxic response, while the NOAEL is the dose which does not trigger a toxic response. Once the NOAEL is determined, it is divided by one or more uncertainty factors to arrive at a "safe" exposure or RfD (S&J, 1994).

Traditionally, a RfD is associated with non-carcinogens. With the exception of carcinogens, it is generally assumed that every organism has the capacity to adapt to or otherwise tolerate some exposure to any substance until a threshold amount is reached. With regard to carcinogens, modern dose-response models are based on the premise that exposure to even one molecule of a carcinogen poses a small but non-zero increased risk of tumor formation (S&J, 1994). What sets AP (and other agents which inhibit iodide uptake by the thyroid gland) apart from other B2 carcinogens is the mechanism of action which leads to the toxic response of an excessive development of the thyroid (hypertrophy), an abnormal increase in the number of tissue cells (hyperplasia), followed by the formation of tumors (neoplasia). It is not exposure to the chemical itself which leads to hyperplasia, but the body's physiological response to the chemical. AP blocks iodide uptake, which leads to a decrease in blood hormone levels of Thyroxine (T4) and Triiodothyronine (T3). Subsequently, the pituitary gland increases production of thyroid stimulating hormone (TSH). This chain of events has been shown to elicit the toxic response of hypertrophy, followed by hyperplasia, which is then followed by neoplasia (Hill et al., 1989; Paynter et al., 1988). Therefore, if a threshold can be found for which there is no decrease in T3 or T4 levels followed by an increase in TSH levels, carcinogenesis should not occur.

Dollarhide (1992) based her provisional RfD on a study conducted in 1952 using patients suffering from Graves' disease, a disease characterized by the enlargement of the thyroid gland and its increased metabolic rate (Stanbury and Wyngaarden). These patients were administered potassium perchlorate (KP) under various conditions. The

study revealed that 100 mg of KP (1.4 mg/kg, using the standard EPA body weight of 70 kg) was the LOAEL and 10mg of KP (0.14 mg/kg) was the NOAEL. Dollarhide then divided the NOAEL by three separate uncertainty factors to arrive at the provisional RfD.

Goal

The Perchlorate Study Group (PSG), composed of DoD and contractor members, was set up to evaluate the impending RfD established by the EPA and the problems associated with it. They believe that this RfD is too conservative because it was based on a study that did not accurately reflect the dose response relationship of AP (ERM, 1995). In addition, at the time the provisional RfD was established, the available subchronic and chronic animal studies with perchlorate salts did not adequately describe thresholds for adverse effects on the production of thyroid hormones (Dollarhide, 1992).

The mechanism by which AP exerts its effect is well known and current literature suggests that the RfD for perchlorate should be higher than the one established by the EPA (Hill et al, 1989; Capen, 1992).

Once the provisional RfD is in effect, the U.S. Air Force and its contractors will become liable for the clean up of contaminated sites. Current methods for the disposal of AP include open burning and open detonation. However, these methods cause environmental pollution. An inexpensive method using a biodegradation process is being developed by Armstrong Laboratory's Environics Directorate at Tyndall AFB, FL, which will not only clean up contaminated sites, but will recover and reuse the AP (Gooden, 1994). An accurate estimate for the cost of this method will not be realized until the summer of 1996. Clean-up of contaminated sites is a very expensive process. At one site

alone, the U.S. Air Force spent $80 million using a pump-and-treat method to return TCE to acceptable levels (Hurley, 1995).

In order for the EPA to establish a more accurate RfD for AP, dose-response data is needed (Dollarhide, 1992). Since there is minimal dose response data for AP in the literature, this requirement provides the basis for this research.

This thesis will analyze data from a 14-day acute study using Sprague-Dawley rats to acquire dose-response data on AP. This data will be analyzed using univariate and multivariate analysis and maximum likelihood estimation techniques to estimate the threshold dose for AP in the rat.

Possible Benefits

The dose-response data derived from this research could be used by the EPA to more accurately characterize a RfD dose for AP. Since it is now known that the rat thyroid is more sensitive than the human (Capen, 1992), this threshold would not need to be adjusted with the same conservatism as the provisional RfD (i.e. there would be no need to divide the threshold by 1000 to derive the RfD). If AP concentrations in groundwater or contaminated soil did not have to be reduced to such low levels, which are overly conservative because of insufficient information, the DoD, NASA and their contractors could save a significant amount of resources which could be utilized elsewhere.

Overview

This thesis consists of four more chapters. The following chapter is a review of the literature concerning AP and the mechanism by which it exerts its effect. Chapter

three will describe the methodology used to obtain the dose-response data for this research effort, and the results will be presented and discussed in chapter four. Finally, chapter five will draw conclusions, discuss uncertainties, and provide recommendations for refinement and further study.

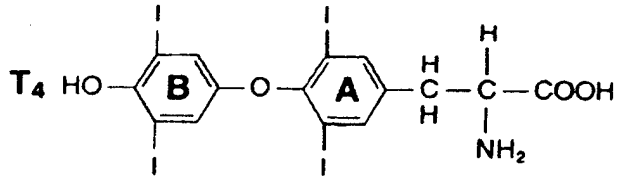
II. Literature Review

The purpose of the literature review is to emphasize the need for this research by describing the lack of dose response data for AP. Furthermore, it is intended to familiarize the reader with the mechanism by which thyroid hormone levels are inhibited. Hormones are products of living cells that circulate in body fluids and produce a specific effect on the activity of cells remote from their point of origin. This inability of the thyroid gland to produce thyroid hormones, if not corrected, leads to excessive development of thyroid tissue cells (hypertrophy), an unusual increase in the number of tissue cells (hyperplasia) and the formation of abnormal masses of tissue (tumors) that possess no physiologic function (neoplasia) in experimental rodents (Capen, 1992; Hill et al., 1989; Paynter et al., 1988).

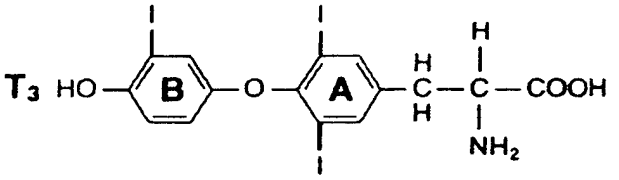
The Thyroid Gland

This section explains the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated.

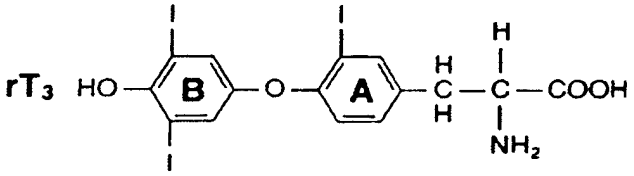
Thyroxine (T4) and triiodothyronine (T3) are classically regarded as the two hormones produced by the thyroid gland. They contain 4 and 3 atoms of iodine, respectively, and are abbreviated as T4 and T3 because of their iodine content (Fig. 2-1). The thyroid hormones are synthesized in the thyroid gland by iodinating thyroglobulin (Tg), an iodine containing protein stored in the thyroid (Goodman and van Middlesworth, 1980). The first stage in the synthesis of thyroid hormones is the uptake of iodide from the blood by the thyroid gland (Fig. 2-2).



Thyroxine (3,5,3′ 5′-tetraiodo-L-thyronine)



Triiodothyronine (3,5,3′-triiodo-L-thyronine)



Triiodothyronine (3′,5′,3 -triiodo-L-thyronine)

Figure 2-1. Structural formulas of thyroxine, triiodothyronine, and "reverse" triiodothyronine. Source: Goodman and van Middlesworth (1980).

This process can be divided into four steps. First the iodide is trapped, then it is believed to be combined with oxygen (oxidized). Once it is oxidized, iodine is added to Tg molecules (iodinated). This iodination produces monoiodotyrosine (MIT) and diiodotyrosine (DIT). Finally, either two DIT molecules or one DIT and one MIT molecule are coupled to form T4 and T3 respectively (Fig. 2-2).

Under normal conditions the thyroid may concentrate iodide up to 25 times higher than the concentration in blood, and this ratio may be considerably higher when the

thyroid is active (250:1). Iodide uptake may be blocked by several anions, one of which is perchlorate (Goodman and van Middlesworth, 1980).

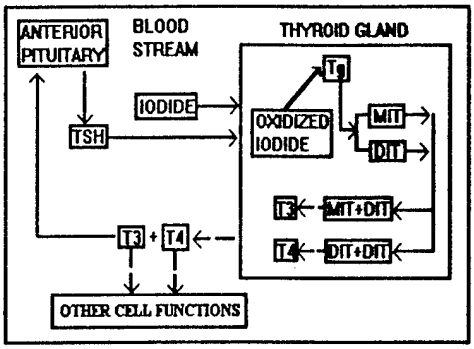


Figure 2-2. Schematic representation of thyroid hormone biosynthesis and secretion. Source: Adapted from Stevens (1985).

T4 is the major hormone secreted from the thyroid and is converted to more active T3 in a variety of peripheral tissues, including the pituitary gland. T4 is also metabolized to rT3 (Fig. 2-1) which is hormonally inactive and has no know function, except perhaps as an inhibitor of the conversion of T4 to T3 (Hill et al., 1989; Stevens, 1985, Goodman and van Middlesworth, 1980).

Homeostatic control of thyroid hormone synthesis and secretion in the thyroid gland is effected by a sensitive feedback mechanism that responds to changes in circulating levels of the thyroid hormones T4 and T3. The mechanism involves the anterior pituitary of the brain (Fig. 2-3) (Hill et al., 1989; Paynter et al., 1988; Houk, 1980). Thyroid-stimulating hormone (TSH, thyrotropin), which is secreted by the anterior pituitary gland and causes the thyroid to create new thyroid hormones, is very important in the feedback mechanism. It independently promotes iodine trapping and iodination of Tg. The rate of release of TSH from the pituitary is controlled by the circulating levels of T4 and T3.

If for any reason there is a decrease in circulating levels of thyroid hormones, TSH is secreted and thyroid function is increased. If exogenous thyroid hormone is administered, eventually the thyroid gland becomes inactive and regresses. The blood concentrations of both T4 and T3 are important factors in the release of TSH (Capen, 1992; Hill et al., 1989; Paynter et al., 1988; Goodman and van Middlesworth, 1980).

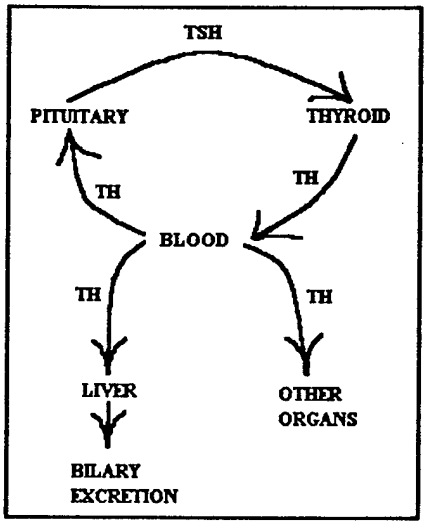


Figure 2-3. Hypothalmic-pituitary-thyroid-peripheral organ relationships. TSH, thyroid-stimulating hormone; TH, thyroid hormones.
Source: Adapted from Hill et al. (1989).

According to Goodman and van Middlesworth (1980), an exact description of the role of the thyroid hormones is not yet possible. However, they discuss several studies which indicate that if the hormones are not present in the early stages of life, mental maturation, bone development, and the central nervous system are negatively affected. In some instances, the lack of bone development can be corrected by administering T4. However, administration of even tremendous amount of T4 does nothing to correct

mental retardation, suggesting that the hormones must be present during critical periods in order for normal development to occur.

Thyroid Gland Neoplasia

Hill et al. explains that thyroid neoplasia may be induced by exposure of experimental animals to a variety of treatment regiments, chemicals produced outside the body (exogenous), or physical agents. "It has been recognized for some time that neoplasms induced in experimental animals by a number of these treatments result from thyroid gland dysfunction, in particular, [enlargement of the thyroid gland and increased metabolic rate] hypothyroidism." Factors inducing hypothyroidism include iodine deficiency, surgically removing part of the thyroid gland, and the transplantation of TSH-secreting pituitary tumors. "The one factor common to each of these conditions is that they all lead to increased production of TSH and prolonged stimulation of the thyroid gland by "excess" TSH." Whatever the cause (i.e. low iodine diet, blocked iodide uptake by an anion), prolonged stimulation of the thyroid-pituitary feedback mechanism that results in the release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia. However, thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone or by surgically removing the pituitary gland (hypophysectomy) (Hill et al., 1989).

A recent review of chemical injury of the thyroid (Capen, 1992) showed that rodents treated with agents that directly interfere with thyroid hormone production in the thyroid gland depress T3 and T4 levels resulting in a compensatory increase of TSH.

This TSH stimulation of the thyroid gland leads to hypertrophy, hyperplasia, and neoplasia in rodents. Capen states that this chronic hypersecretion of TSH places the rodent thyroid gland at greater risk to develop tumors through a secondary mechanism. "In the secondary mechanism of thyroid oncogenesis [the formation of tumors] in rodents the specific xenobiotic [compound foreign to the body] or physiologic perturbation evokes another stimulus (e.g. chronic hypersecretion of TSH) that promotes the development of nodular lesions (initially hypertrophy, followed by hyperplasia, subsequently adenomas [benign tumors], infrequently carcinomas [malignant tumors]) derived from [thyroid] cells." In addition, this excessive secretion of TSH alone, without any chemical exposure, produces a high incidence of thyroid tumors in rodents. Capen concluded that thresholds for agents which inhibit iodine uptake by the thyroid can be established by determining the dose that fails to elicit an elevation in the circulating level of TSH. Hence, the threshold concentration of perchlorate, i.e., the perchlorate concentration below which there is no depression of T3 and T4 accompanied by TSH elevation, is completely protective against carcinogenesis.

Ammonium Perchlorate Related Research

The perchlorate study group (PSG) cites five major issues concerning the provisional RfD established by the EPA for AP:

1) Sparse animal data exist for potassium perchlorate and there are minimal data on ammonium perchlorate in the literature (Dollarhide, 1992).
2) The provisional RfD is based on a human clinical study in which patients with hyperthyroidism were treated with potassium perchlorate (Stanbury & Wyngaarden; 1952). Ammonium perchlorate is known to affect thyroid function.
3) Existing animal data on AP have been derived from studies using single, high

level doses (Dollarhide, 1992). No dose-response data exist.

4) The rat is now considered to be more sensitive to thyroid hormone fluctuations than is man (Capen, 1992, Hill et al., 1989). The provisional RfD assumed the opposite.
5) The application of standard default uncertainty factors (i.e., division of the apparent safe level by 1000) by the EPA appears unjustified (Dollarhide, 1992). The biochemical mechanism by which perchlorate exerts its effect in humans is well understood, alleviating the need for application of safety factors normally associated with unknown xenobiotics. (Caldwell, 1995)

Human Data. Brabant et al. conducted a study in which 5 healthy males were exposed to an oral treatment of 300 mg of perchlorate 3 times daily over a 4-week period. Mean serum TSH levels actually decreased slightly and the thyroid volumes were unaltered. The body weights of the volunteers were not provided. However, using the standard 70 kg default body weight for risk assessment leads to a dose of 12.86 mg/kg/day. This would suggest that the threshold in healthy humans is higher than 12.86 mg/kg/day.

Burgi et al. (1974) administered 200 mg of perchlorate 3 times daily to three healthy females and two healthy males for 8 days. The average dose for the females was 11.04 mg/kg/day and the average for the males was 8.22 mg/kg/day. These doses were sufficient to completely block iodide uptake by the thyroid as measured in the urine. However, thyroid hormone levels were not measured in order to determine if this dose produced a decrease in T3 and T4 or and increase in TSH levels.

These two studies contradict the mechanistic conceptual model. Brabant et al. found that a dose of 12.86 mg/kg/day did not increase TSH levels while Burgi et al. found that a lower dose of 8.22 mg/kg/day was sufficient to completely block iodide uptake by the thyroid.

As described in the first chapter, a study was found that used potassium perchlorate (KP) to displace iodide from the thyroid gland (Stanbury and Wyngaarden, 1952). This study was used as the basis for deriving the EPA's provisional RfD. Standbury and Wyngaarden found that .14 mg/kg/day (assuming a body weight of 70 kg) was not sufficient to completely block iodide uptake. However, 1.4 mg/kg/day was sufficient to block 90% of the measured iodide. The study did not evaluate the effects of KP on the thyroid hormone levels and there were only three doses given. The previous three studies are summarized in Table 2-1.

Study	Exposure Conditions	Conclusions
Brabant et al.	-12.86 mg/kg/day for four weeks	-TSH levels decreased slightly
Burgi et al.	-11.04 mg/kg/day for four weeks (males) -8.22 mg/kg/day for four weeks (females)	-Sufficient to completely block iodide uptake by the thyroid as measured in urine
Stanbury and Wyngaarden	-0.14 mg/kg (once) -1.4 mg/kg (once)	-55% of initially accumulated radioactive iodide was present in the neck -15% of initially accumulated radioactive iodide was present in the neck

Table 2-1. Summary of human studies using perchlorates.

Animal Data. With regard to animal data, Shigan conducted a study in which 'white rats' were given AP under various conditions (1963, translated from Russian, 1994). The rats were treated with doses ranging from 2500 to 6500 mg/kg and observed for 15 days. Even though most of the animals died during the first 3 days, Shigan was able to calculate the dose of AP which killed fifty percent of the total experimental population (LD_{50}) (Table 2-2). 'White rats' were also exposed to AP under two other conditions described in Table 2-2.

Exposure Conditions	Conclusions
4200 mg/kg (once)	-LD_{50}
650 mg/kg/day for one month	-No noticeable cumulative properties
190 mg/kg/day for three months	-Affects the regulation of the involuntary nervous system -Causes a statistically reliable change in the protein fractions of the blood serum -Disrupts the liver's ability to produce glycogen for carbohydrate storage

Table 2-2. Results of Experiments on 'white rats'
(Adapted from Shigan, 1963)

These results do not provide any insight in determining a threshold because there were no doses given at low concentrations.

Mannisto et al. (1979) studied the effects of Potassium Perchlorate (KP) on the thyroid of the Sprague-Dawley rat. He found that doses of KP from 7.6 to 15.3 mg/kg/day administered over a 4 day period reduced serum triiodothyronine (T3) and thyroxin (T4) levels and increased thyroid stimulating hormone (TSH) levels.

Conclusion

These experiments do not provide enough information on which to base an accurate RfD. Therefore, in an effort to provide more toxicological evidence, Caldwell (1995) has designed a study in which thyroid hormone levels and thyroglobulin (Tg) levels in the Sprague-Dawley rat were determined. This study provided specific dose-response data, from which a threshold level for thyroid hormone effects of AP can be determined. Since the EPA based their provisional perchlorate RfD on the Stanbury and Wyngaarden study and predicted that chronic administration of perchlorate at this dose would likely have resulted in lowering of the patients T3 and T4 levels, with subsequent

increases in the levels of TSH, "this hypothesis will be tested with this protocol" (Caldwell, 1995).

Since increased levels of TSH are a sign that the thyroid has been disturbed, if a dose of perchlorate can be found from which there is no observed statistically significant increase in the amount of TSH in the blood, this dose can be considered at or below the threshold dose (Capen, 1992). For thyroid hyperplasia it is inferred that this is a precursor to cancer. Subsequently, this dose can be used in deriving a RfD.

III. Methodology

Introduction

This section describes the process by which this research was conducted. The design will be explained, followed by a description of the execution and the analysis techniques. It concludes with a summary of the chapter.

Design

The 14-day pilot study included 96 rats, 48 male and 48 female. They were divided into eight dose groups, including a control (Table 3-1). The male rats were estimated to consume water at the rate of 45 ml/day/rat and have an estimated body weight of 450 grams. The female rats were estimated to consume water at the rate of 27 ml/day/rat and have an estimated body weight of 270 grams. The target doses are specified in Table 2. These target doses were designed around an estimated threshold dose of 10 mg/kg/day based on the available literature (Caldwell, 95).

Group	No. of Animals		AP Conc. (mg/L)	AP Target Dose (mg/kg/day)
	Males	Females		
Control	6	6	0.00	0.0
Very Low	6	6	1.25	0.125
Low	6	6	5.00	0.5
Med. Low	6	6	12.50	1.25
Medium	6	6	25.00	2.5
Med. High	6	6	50.00	5.0
High	6	6	125.00	12.5
Very High	6	6	250.00	25.0

Table 3-1. Pilot Study Dose Groups, Concentrations and Target Doses
Source: Caldwell (1995).

Execution

The study began with 100 rats, 50 male and 50 female. These rats were single housed throughout the study. The quarantine period lasted for 14 days. During the last 7 days of quarantine, the water consumption rates and body weights were measured as baseline data. On the last day of the quarantine the rats were randomly assigned, using the PATH/TOX (XMSC, 1993) randomization algorithm, to 8 groups, each containing 6 males and 6 females.

The dosing solutions were prepared in eight 20 liter containers on day 14 of the quarantine and samples were taken to verify accurate concentrations and stability. The dosing period began the day following the quarantine period. The animals were dosed for 14 days according to table 3-1. The animals were weighed on days 8 and 14 of the quarantine and days 7 and 15 or 16 of the study. Water consumption was measured on day 12 of the quarantine and days 1, 4, 7, 10 and 14 of the study and averaged over the respective periods. This data was used to determine if the dosing solutions affected water consumption or body weight, and calculate the actual dose consumed by the animals.

Following the dosing period, the rats were euthanised via CO_2 inhalation on day 15 or 16. Blood was immediately drawn from the vena cava at necropsy and centrifuged. The serum was stored at -20° C until analyzed for the hormone levels listed above using commercially available radioimmunoassay kits.

Statistical Analysis

The results were analyzed using two-factor Analysis of Variance (ANOVA) (Statistix 4.1), Multivariate Analysis of Variance (MANOVA) (SAS), Tukey's method for multiple comparisons (Statistix 4.1), and Maximum Likelihood Estimation (MLE) (SAS). Two-factor ANOVA was used to determine if there were statistically significant differences between dose groups and the male and female rats. Two-factor MANOVA was used to determine if there were statistically significant differences between dose groups and the male and female rats. If the males and females responded similarly to the dosing, Tukey's method for multiple comparisons was used to determine which dose groups differed.

Capen (1992) concluded that if a dose can be found for which there is no decrease in T3 or T4 accompanied by an increase in TSH, that dose can be considered the threshold. Therefore, the sigmoid function was used to fit the data points for T3 and TSH. The sigmoid function was not used to fit the T4 data because there was no dose response relationship. Maximum Likelihood Estimation (MLE) was then used to determine the parameter point estimates for the sigmoid function. Once the parameter estimates were determined, the function was evaluated using the F-test for lack of fit (LOF). If the functions passed the LOF test, this would mean that they accurately characterized the relationship between dose and hormone levels. These functions, which serve as conservative estimations of the dose-response relationship, could subsequently be used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

Two-Factor ANOVA (Devore, 1991):

The two-factor ANOVA was used to determine whether there were statistically significant differences between dose/sex group means. This is done by first determining the grand mean (the mean of all dose/sex groups) and then comparing each data value to the grand mean. The differences between each data value and the grand mean are then squared and summed to determine the sum of squares for the total samples (SST). Next, the mean for each dose group is determined and compared against the grand mean. The differences are squared and summed to determine the sums of squares for dose group (factor A, SSA). SSB (factor B), or the sums of squares for the sex factor, is determined by holding the dose factor constant and determining the mean across sex for each sex and comparing them to the grand mean. SSAB (interaction sum of squares) is determined by computing the mean for each dose/sex group and subtracting the means of the dose groups and sexes and adding the grand mean. SSE (error sum of squares) is determined by each data value to the dose/sex mean. Each sums of squares is then divided by its degrees of freedom to obtain the mean square for each factor (e.g. MSA, MSB, MSAB). These mean square values are divided by the mean square error (MSE) to determine the f-ratio (f has a certain distribution when the null hypothesis is true). If the computed f-value is greater than the value chosen for alpha (the level at which any percentage above is grounds to reject the null hypothesis and subject to type-I error or the error of rejecting the null when it is true), then the data cannot be considered to be from the distribution and the null hypothesis is rejected. The statistical analysis package Statistix 4.1 was used to perform the two-factor ANOVA. The research problems were as follows:

Problem 1 (Average Water Consumption)

(1) Does the dose of AP affect average water consumption?

(2) Does the sex of the rat affect average water consumption?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Problem 2 (Body Weight Gain)

(1) Does the dose of AP affect average body weight gain?

(2) Does the sex of the rat affect average body weight gain?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Problem 3 (Thyroid/Body Weight Ratio)

(1) Does the dose of AP affect thyroid/body weight ratio?

(2) Does the sex of the rat affect thyroid/body weight ratio?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Research Null Hypotheses:

Hypothesis 1 (Average Water Consumption)

(1) The dose of AP does not affect average water consumption.

(2) The sex of the rat does not affect average water consumption.

(3) There is no interaction between the dose of AP and the sex of the rat.

Hypothesis 2 (Body Weight Gain)

(1) The dose of AP does not affect body weight gain.

(2) The sex of the rat does not affect body weight gain.

(3) There is no interaction between the dose of AP and the sex of the rat.

Hypothesis 3 (Thyroid/Body Weight Ratio)

(1) The dose of AP does not affect thyroid/body weight ratio.

(2) The sex of the rat does not affect thyroid/body weight ratio.

(3) There is no interaction between the dose of AP and the sex of the rat.

An answer which contradicted any one of the previous hypotheses resulted in rejecting the respective null hypothesis in favor of the alternate hypothesis which states that there is an affect.

Two-Factor MANOVA (Barcikowski, 1983; Hair et al., 1979):

Two-factor MANOVA is similar to ANOVA, except there are several dependent variables (e.g. T3, T4, TSH, rT3 and Tg) instead of one dependent variable (e.g. average water consumption or body weight gain). While ANOVA and MANOVA both test for differences among groups using sums-of-squares, MANOVA finds them using the covariance structure of the dependent variables. The F-ratio tests for equality among dose groups based on their vector means. The statistical analysis package SAS was used to perform the MANOVA. The research problem was as follows:

Problem

(1) Does the dose of AP affect Tg, T3, rT3, T4, and TSH thyroid hormone levels?

(2) Does the sex of the rat affect Tg, T3, rT3, T4, and TSH thyroid hormone levels?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Research Null Hypotheses

(1) The dose of AP has no affect on Tg, T3, rT3, T4, and TSH thyroid hormone levels.

(2) The sex of the rat has no affect Tg, T3, rT3, T4, and TSH thyroid hormone levels.

(3) There is no interaction between the dose of AP and the sex of the rat.

An answer which contradicted any of the three previous null hypotheses resulted in rejecting the respective null hypothesis.

Maximum Likelihood Estimation

Maximum likelihood estimation (MLE) was used to obtain point estimates of the parameters of the sigmoid function. The sigmoid function was used because it approaches a threshold affect at low doses (Fig. 3-1). The sigmoid function is as follows:

$$\text{HormLevel}(\text{Dose}, B0, B1) := \frac{B0 \cdot \text{Dose}^{B1}}{\left[\text{Dose}^{B1} + \left(\frac{B0}{2}\right)^{B1}\right]}$$

Where B0 is the highest observed response value (highest observed hormone level) and B1 is an exponent parameter which controls the function shape.

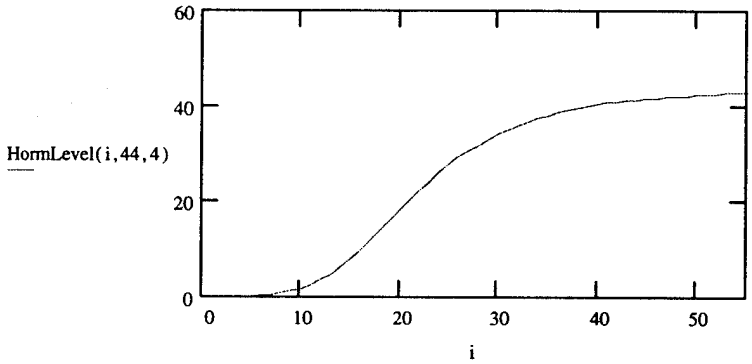


Figure 3-1. The Sigmoid function.

The MLE method assumes that all the hormone levels are normally distributed, have equal variances and are independent. The likelihood function:

$$L(\mu_i,\sigma) = \prod_{i=1}^{n} \frac{1}{(2\cdot\pi\cdot\sigma^2)^{\frac{1}{2}}} \cdot e^{\left[-\frac{1}{2\cdot\sigma^2}\cdot(Y_i-\mu_i)^2\right]}$$

where the expected value of the sigmoid function, $E(f(x_i,B0,B1)) = \mu_i$ (the population mean) and the expected value of the difference between each observed value of hormone level (y_i) and the μ_i squared, $E(y_i - \mu_i)^2 = \sigma^2$. The expected value of the sigmoid function is substituted in the likelihood function for μ_i and $E(y_i - \mu_i)^2$ for σ^2. Subsequently, the values of B0 and B1 that maximize the likelihood function are the maximum likelihood estimators.

Once the maximum likelihood estimators are determined, the sigmoid function can be tested to see how well it characterizes the data with an F-test for lack of fit (LOF). Once the function passes the LOF test, it can be used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

F Test for Lack of Fit

The F test for lack of fit assumes that the observations Y (e.g. hormone levels) for given X (e.g. dose) are independent and normally distributed, and the distributions of Y have the same variance. The test is accomplished by first determining the pure error sum of squares (SSPE) and the residual sum of squares (SSRes) which are computed in the ANOVA table. The difference between these two error sums of squares is called the lack of fit sum of squares (SSLF). After dividing the SSPE and SSLF by their appropriate degrees of freedom, the F statistic can then be expressed as follows:

$$F = \frac{MSLF}{MSPE}$$

The research problem for each data set was as follows:

Problem

Does the function accurately characterize the data?

Research Null Hypothesis

The function accurately characterizes the data.

If the value for the computed F is less than or equal to the critical value for the level of significance (α=.05), then the null hypothesis holds.

Tukey's Method for Multiple Comparisons

When the no-interaction hypothesis was not rejected and at least one of the two main effect null hypotheses was rejected, Tukey's method was used to identify significant differences between dose groups. For identifying differences among the means when the null hypothesis was rejected,

1. Obtain the value of the upper-tail α from the studentized t-distribution, above which the null is rejected (Q).

2. Compute $w = Q * (MSE/(JK))^{1/2}$, where MSE is the mean squared error obtained from the ANOVA table and JK is the number of observations averaged to obtain each of the sample means compared in step 3.

3. Order the sample means from smallest to largest and underscore all pairs that differ by less than w. Pairs not underscored correspond to significantly different levels for the factor under consideration.

Summary

To determine whether there was a dose-response relationship for body weight gain, average water consumption and thyroid/body weight ratio, two-factor ANOVA was used. If no interaction was observed, Tukey's method for multiple comparisons was used to determine which groups differed by dose. Two-factor MANOVA was used to determine statistically significant differences between dose groups with five dependent variables. MLE was then used to find the point estimates for the parameters of the sigmoid curve which was used to fit the data. Once the parameters which maximized the likelihood function were determined, the F-test for lack of fit was used to determine if the functions accurately characterized the data sets. The results are presented in Chapter 4.

IV. Data Description and Analysis

Introduction

This chapter presents and analyzes the raw data obtained from this study. Two-factor ANOVA was used to determine statistically significant differences between dose groups based on weight gain, water consumption, thyroid/body weight ratio. Two-factor MANOVA was used to determine significant differences between dose groups and sex based on thyroid hormone levels. When a null hypothesis was not rejected the analysis was terminated. However, when the no-interaction hypothesis was not rejected and at least one of the two main effect null hypotheses was rejected, Tukey's method was used to identify which levels differed from the control. Maximum Likelihood Estimation (MLE) was then used to determine the point estimates for the parameters of the sigmoid function used to fit the data. The F-test for lack-of-fit was then used to determine if the function accurately characterized the data.

The data was analyzed in this order. First, the water consumption data was analyzed to determine whether there was any statistically significant difference between dose groups and what the actual doses were. Next, body weight gain and thyroid/body weight ratio was analyzed to determine whether there was a difference between dose groups. The effect of dose, if any, on thyroid hormone levels was then evaluated. If an affect was noted, maximum likelihood estimation was used to maximize the parameters for the sigmoid function. The function was then used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

Water Consumption and Dose

The average water consumed by dose group is shown in figures 4-1 and 4-2. Two-factor ANOVA revealed that there were no statistically significant differences

ANALYSIS OF VARIANCE TABLE FOR AVCONSMP

SOURCE	DF	SS	MS	F	P
GROUP (A)	7	280.870	40.1243	1.07	0.3900
SEX (B)	1	4423.51	4423.51	118.07	0.0000
A*B	7	195.063	27.8662	0.74	0.6372
RESIDUAL	80	2997.26	37.4658		
TOTAL	95	7896.71			

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF AVCONSMP BY GROUP

DOSE GROUP	MEAN	HOMOGENEOUS GROUPS
5	37.612	I
4	33.929	I
8	33.883	I
2	33.622	I
7	33.522	I
6	33.337	I
3	32.667	I
1	31.097	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL Q VALUE	4.401	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	7.7762		
STANDARD ERROR FOR COMPARISON	2.4989		
ERROR TERM USED: RESIDUAL, 80 DF			

Table 4-1. Results of Two-factor ANOVA for average water consumption.

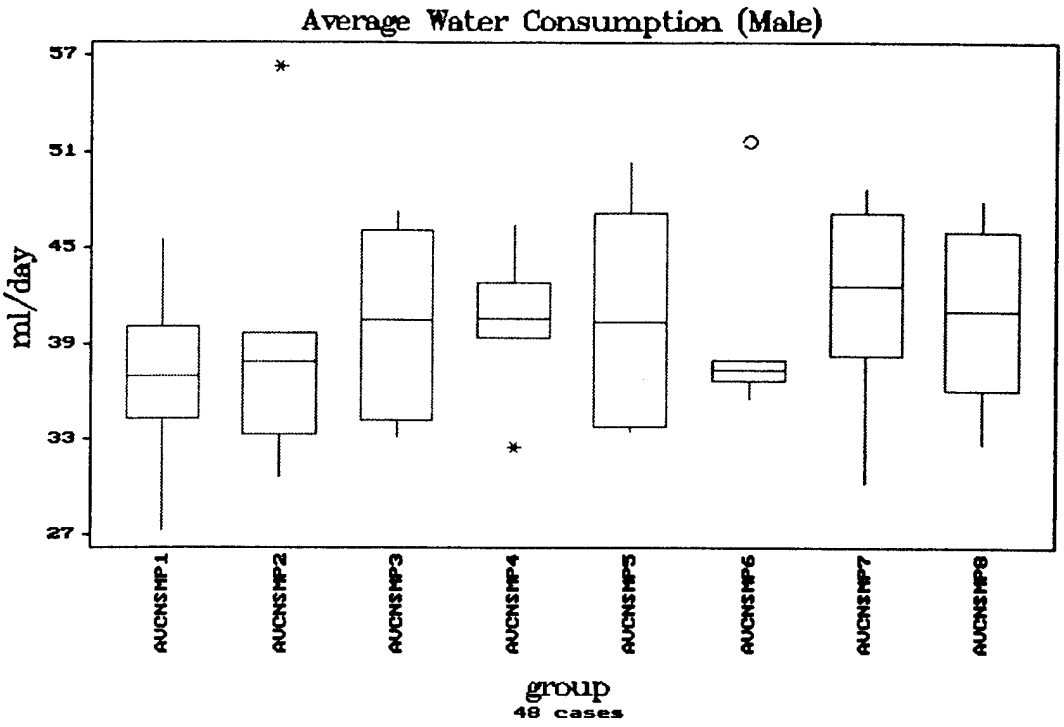


Figure 4-1. Boxplots of male rat average water consumption by dose group.

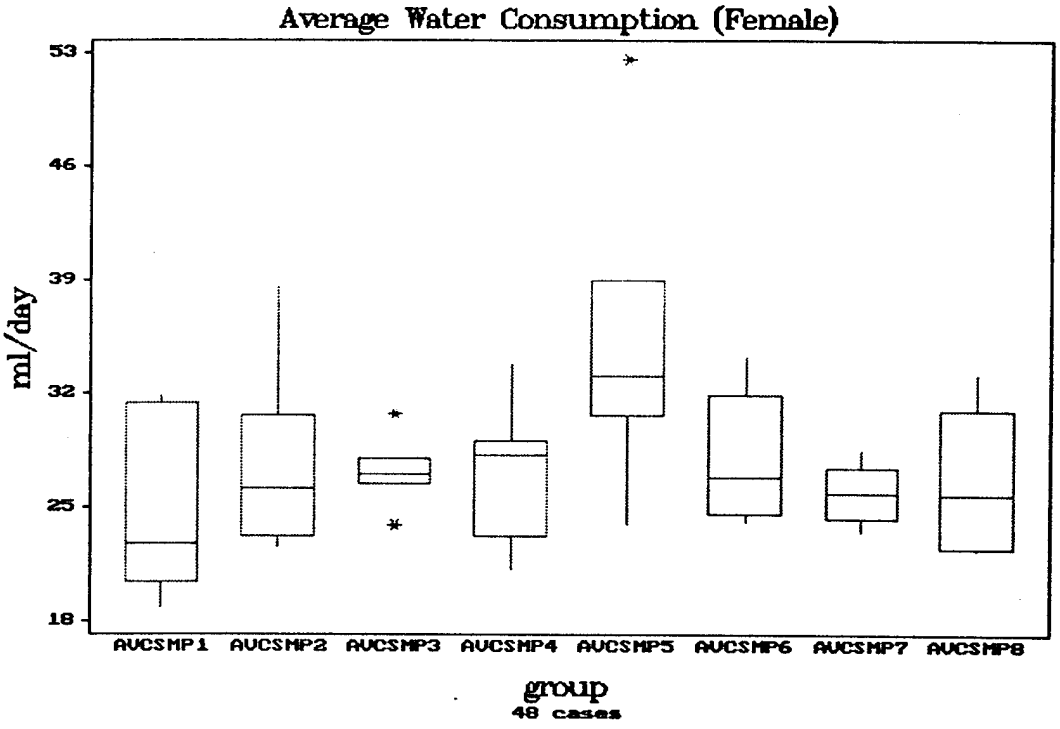


Figure 4-2. Boxplots of female rat average water consumption by dose group.

between the groups with regard to dose, indicating that the concentration of AP in the water was not sufficient to decrease the consumption rate. However there were differences with regard to sex (Table 4-1). The P value for sex was much less than .05 indicating that the male consumption rate is different from that of the female. The average water consumption was used to determine the actual dose administered (Table 4-2).

Group	No. of Animals Males	Females	AP Conc. (mg/L)	AP Target Dose (mg/kg/day)	AP Dose (mg/kg/day) Males	Females
Control	6	6	0.00	0.0	0.0	0.0
Very Low	6	6	1.25	0.125	0.110	0.124
Low	6	6	5.00	0.5	0.443	0.466
Med. Low	6	6	12.50	1.25	1.112	1.232
Medium	6	6	25.00	2.5	2.263	3.063
Med. High	6	6	50.00	5.0	4.321	4.912
High	6	6	125.00	12.5	11.443	11.469
Very High	6	6	250.00	25.0	22.157	24.863

TABLE 4-2. Ammonium perchlorate dose by group.

Body Weight Gain

The body weight gained per dose group is shown in figures 4-3 and 4-4. The two-factor ANOVA indicated that there were no statistically significant differences between dose groups, indicating that the concentrations of AP did not affect appetite. However there were differences with regard to sex. The P value of .0001 for sex was much less than α=.05. The male rats gained more weight than the female rats. No interaction between dose and sex was observed indicating that both sexes responded in the same way to the dosing.

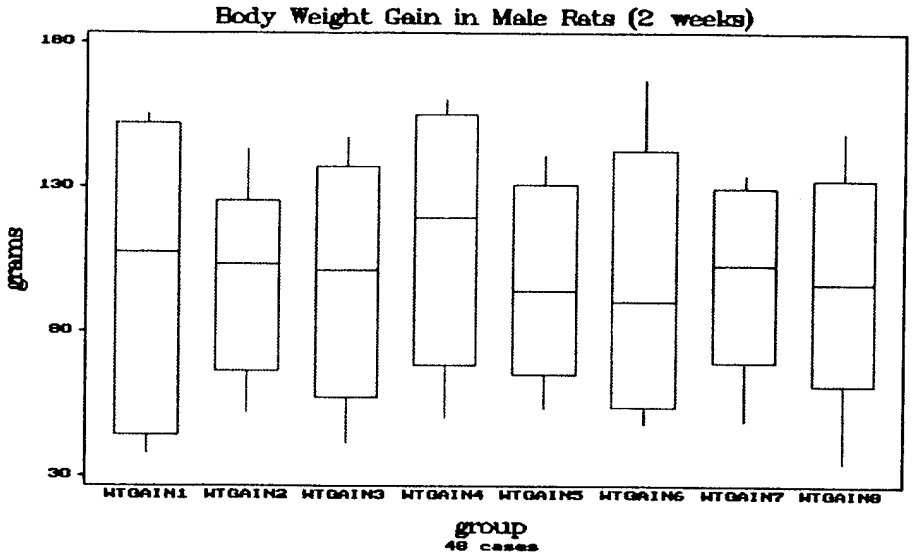


Figure 4-3. Boxplots of male rat body weight gained by dose group.

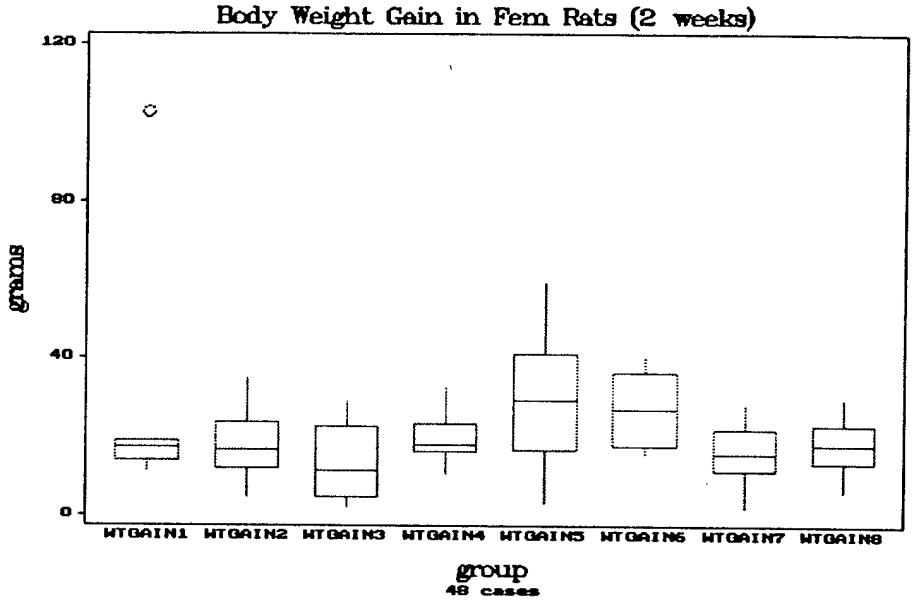


Figure 4-4. Boxplots of female rat body weight gained by dose group.

ANALYSIS OF VARIANCE TABLE FOR WTGAIN

SOURCE	DF	SS	MS	F	P
SEX (A)	1	1.463E+05	1.463E+05	129.86	0.0000
GROUP (B)	7	1515.72	216.532	0.19	0.9851
A*B	7	1476.96	210.995	0.19	0.9861
RESIDUAL	80	90147.9	1126.85		
TOTAL	95	2.395E+05			

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF WTGAIN BY GROUP

DOSE GROUP	MEAN	HOMOGENEOUS GROUPS
4	66.033	I
1	65.608	I
5	63.192	I
6	63.000	I
2	58.575	I
7	56.883	I
8	56.575	I
3	55.583	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL Q VALUE	4.401	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	42.646		
STANDARD ERROR FOR COMPARISON	13.704		

ERROR TERM USED: RESIDUAL, 80 DF

Table 4-3. Results of Two-factor ANOVA for body weight gain.

Thyroid/Body Weight Ratio

Thyroid/body weight ratio data are shown in figures 4-5 and 4-6. Two-factor ANOVA indicated that there were differences among the means with respect to dose and sex. No interaction was observed indicating that males and females reacted similarly to the dosing (Table 4-4). Tukey's test for multiple comparisons revealed that the ratio for dose groups 7 and 8 increased and were statistically significantly different from the control group (Table 4-4).

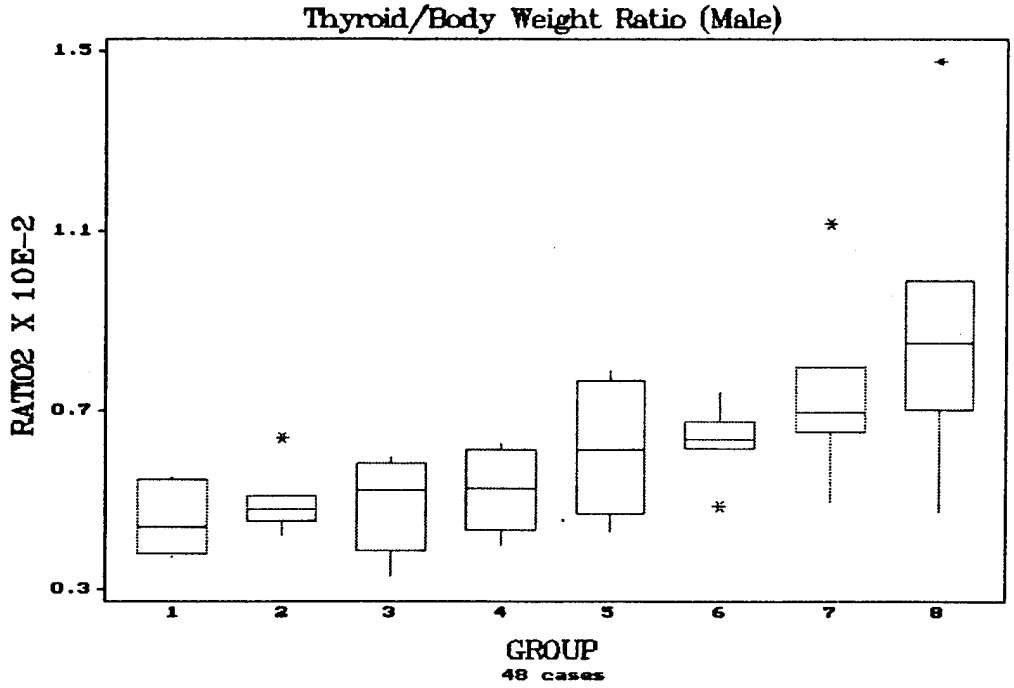


Figure 4-5. Boxplots of male thyroid/body weight ratios by dose group.

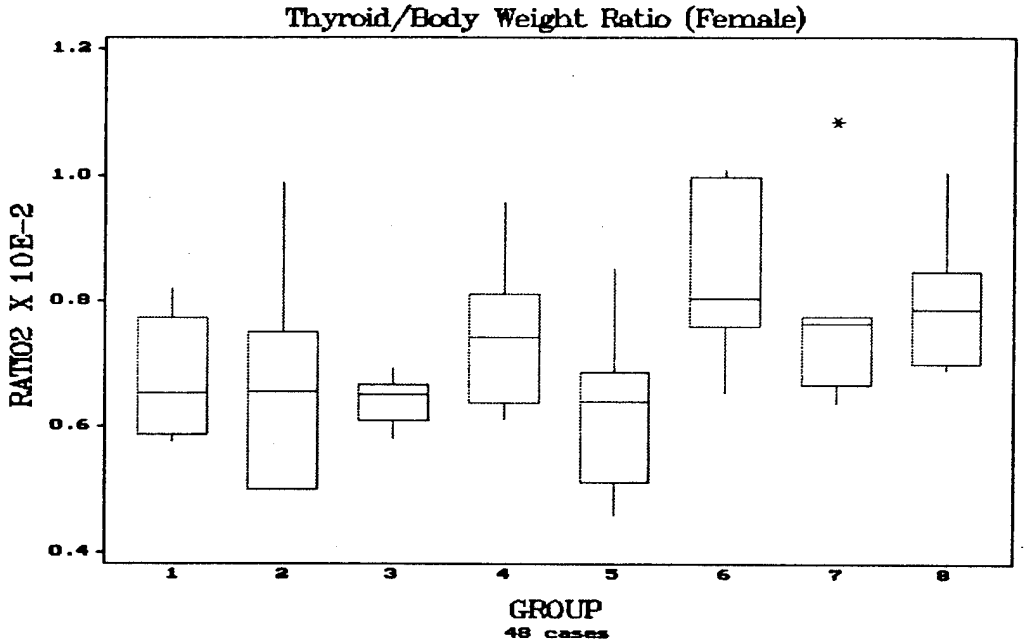


Figure 4-6. Boxplots of female thyroid/body weight ratios by dose group.

ANALYSIS OF VARIANCE TABLE FOR RATIO

SOURCE	DF	SS	MS	F	P
GROUP (A)	7	9.128E-07	1.304E-07	5.76	0.0000
SEX (B)	1	3.425E-07	3.425E-07	15.14	0.0002
A*B	7	2.841E-07	4.059E-08	1.79	0.0992
RESIDUAL	80	1.810E-06	2.262E-08		
TOTAL	95	3.349E-06			

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF RATIO BY GROUP

DOSE GROUP	MEAN	HOMOGENEOUS GROUPS
8	8.47E-04	I
7	7.64E-04	I I
6	7.36E-04	I I I
4	6.36E-04	.. I I
5	6.24E-04	.. I I
2	5.87E-04	.. I I
3	5.67E-04	 I
1	5.66E-04	 I

THERE ARE 3 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.401	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	1.911E-04		
STANDARD ERROR FOR COMPARISON	6.140E-05		

ERROR TERM USED: RESIDUAL, 80 DF

Table 4-4. Results of two-factor ANOVA for thyroid/body weight ratio.

Thyroid Hormone Levels

Two-factor MANOVA was used to determine relationships between thyroid hormone levels. The results of the MANOVA are condensed in table 4-5.

Thyroid Hormone	P-Value (Dose)	P-Value (Sex)	P-Value (Dose*Sex)
Tg	.0001	.2321	.0001
rT3	.0001	.6923	.4104
T3	.0001	.0001	.0001
TSH	.0001	.0001	.0001
T4	.0006	.0001	.2909

Table 4-5. Results of two-factor MANOVA.

The results show that the null hypothesis for dose is rejected for every hormone indicating that dose does have a statistically significant impact on their levels. The sexes within dose groups were statistically significantly different in T3, TSH, and T4. In addition, the null hypotheses for interaction for Tg, T3 and TSH were rejected, indicating that the sexes were not similarly affected by the dosing. Therefore, MANOVA was used to evaluate the sexes separately.

Males

The correlation matrix for males was as follows (Fig. 4-7) (SAS output):

Correlation Analysis/Pearson Correlation Coefficients

	HTG	RT3	T3	TSH	T4
HTG	1.00000				
	0.0				
RT3	0.82190	1.00000			
	0.0001	0.0			
T3	-0.77049	-0.76134	1.00000		
	0.0001	0.0001	0.0		
TSH	0.83526	0.80132	-0.88490	1.00000	
	0.0001	0.0001	0.0001	0.0	
T4	-0.45956	-0.35538	0.21192	-0.34479	1.00000
	0.0010	0.0132	0.1482	0.0164	0.0

Figure 4-7. Correlation matrix for male rats.

The correlation matrix showed a strong negative relationship between T3 and TSH. This supports the literature findings.

Females

The correlation matrix for females was as follows (SAS output):

Correlation Analysis/Pearson Correlation Coefficients

	HTG	RT3	T3	TSH	T4
HTG	1.00000				
	0.0				
RT3	0.66152	1.00000			
	0.0001	0.0			
T3	-0.74760	-0.62200	1.00000		
	0.0001	0.0001	0.0		
TSH	0.95962	0.65659	-0.67418	1.00000	
	0.0001	0.0001	0.0001	0.0	
T4	-0.00165	0.14767	-0.20017	-0.02562	1.00000
	0.9911	0.3165	0.1725	0.8627	0.0

Figure 4-8. Correlation matrix for female rats.

The correlation matrix for females showed a strong negative relationship between T3 and TSH. This also supports the literature findings.

The MANOVA revealed that there was a statistically significant relationship between dose and hormone levels and that the sexes were affected differently. Based on this information sigmoid functions were used to fit the data as closely as possible. These models were then tested for lack of fit.

All the thyroid hormone levels were affected by dose (Table 4-5). Capen (1992) stated that the lowest dose which lowers T3 and/or T4 and simultaneously increases TSH could be considered the threshold dose. Therefore, although all the hormone levels are discussed in the next few sections, only those functions used to fit the data for T3 and TSH will be discussed. T4 showed a statistically significant affect from dose, but there was no dose-response relationship.

Thyroglobulin (Tg)

Tg increased consistently with dose (Figs. 4-9 and 4-10). Since iodized Tg is needed to make MIT and DIT, which combine to form T3 and T4, the negative feedback mechanism could have triggered a response to produce more Tg based on declining T3 levels.

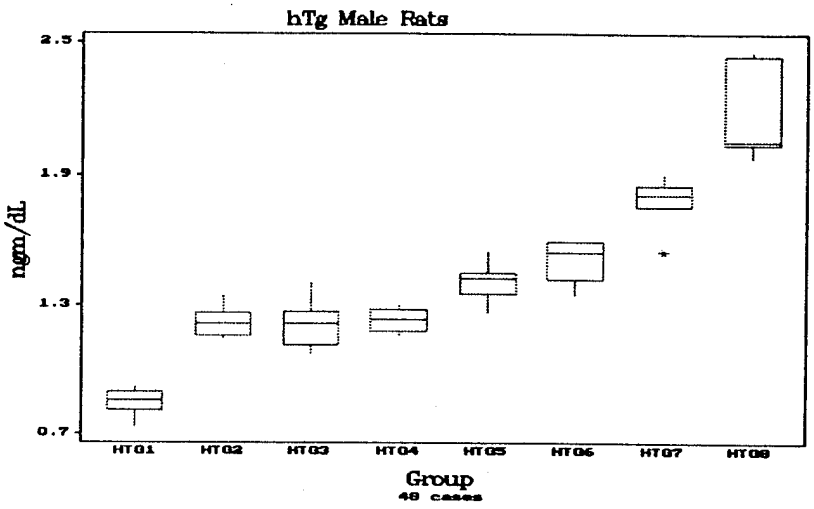


Figure 4-9. Boxplots of male Tg levels by dose group.

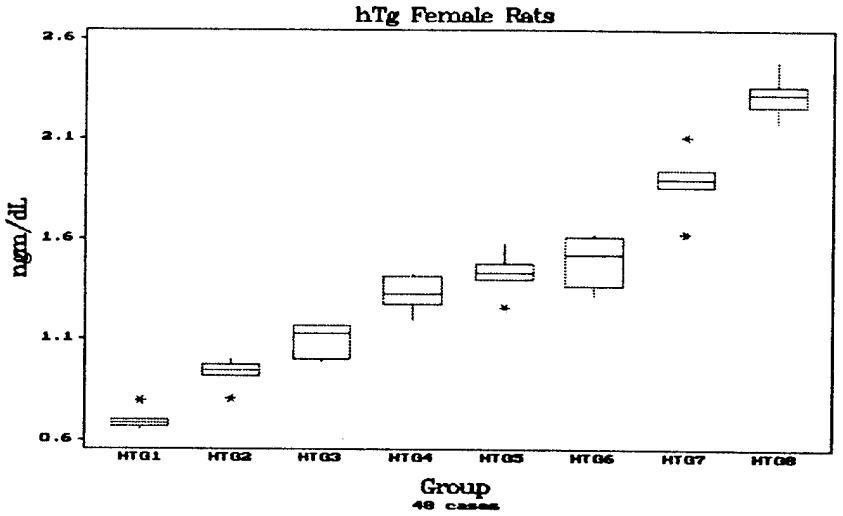


Figure 4-10. Boxplots of female Tg levels by dose group.

Reverse Triiodothyronine (rT3)

rT3 also increased consistently with dose (Figs. 4-11 and 4-12). Since rT3 is formed from T4 in the peripheral tissues and has no known function, except to prevent the formation of T3. An increase in rT3 could have contributed to the depletion of T3.

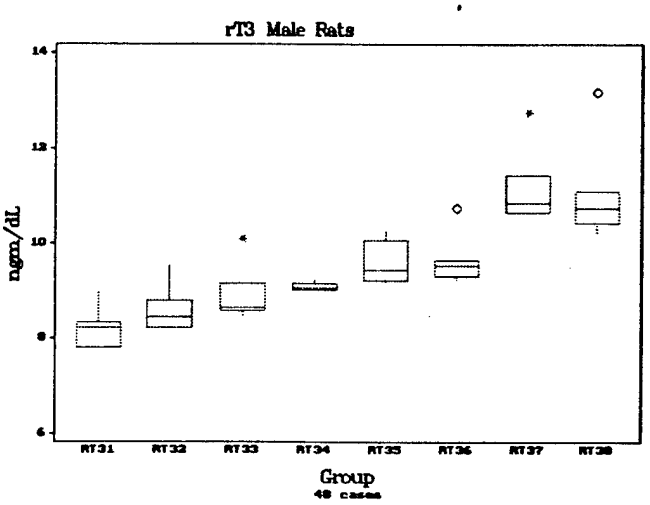


Figure 4-11. Boxplots of male rT3 levels by dose group.

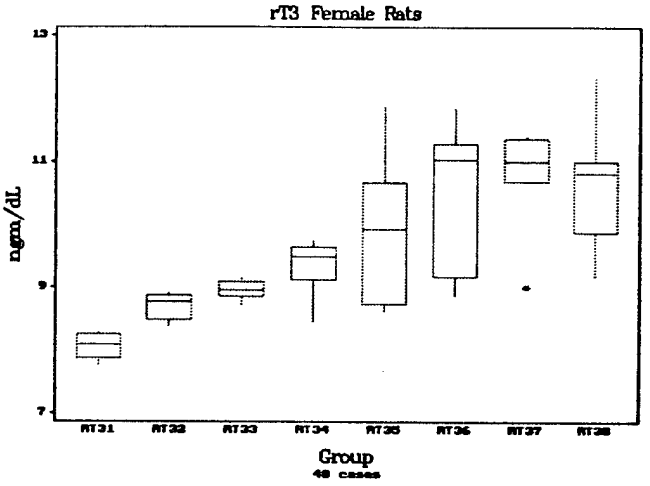


Figure 4-12. Boxplots of female rT3 levels by dose group.

Thyroxine (T4)

Although T4 showed a statistically significant effect, there was no dose-response relationship (Figs. 4-13 and 4-14).

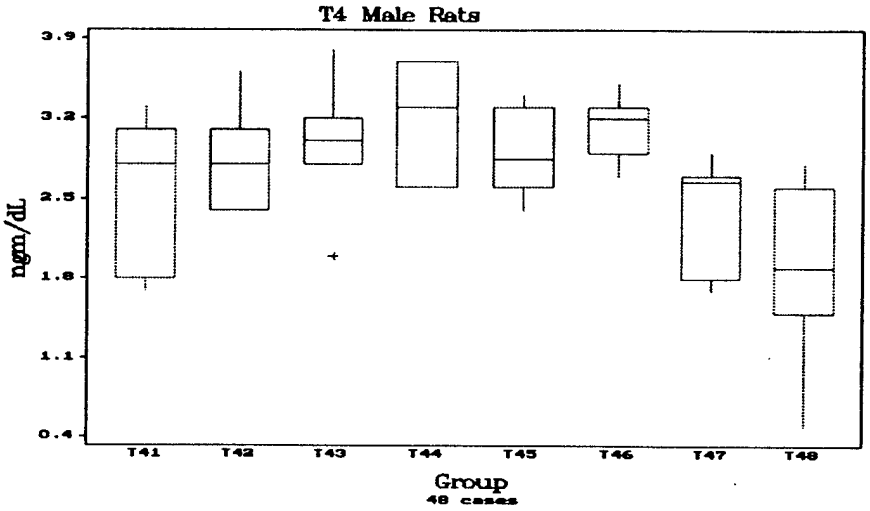


Figure 4-13. Boxplots of male T4 levels by dose group.

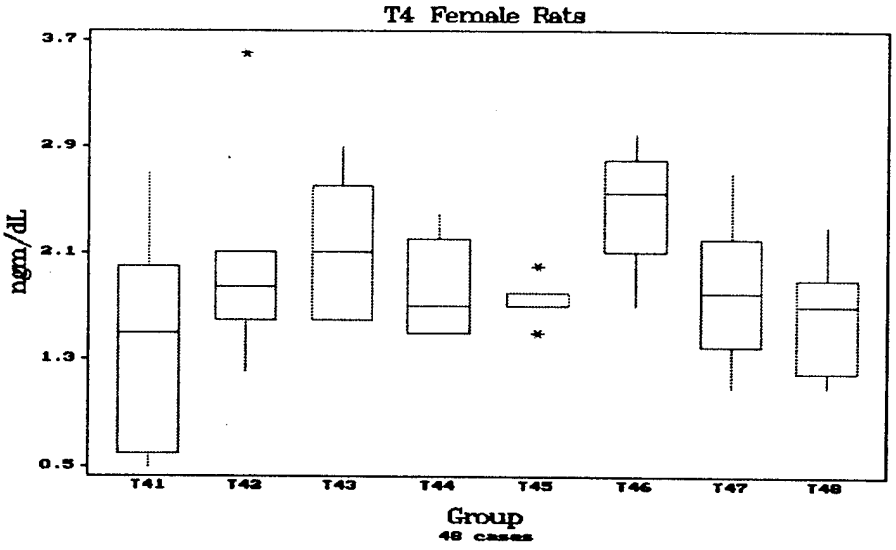


Figure 4-14. Boxplots of female T4 levels by dose group.

Triiodothyronine (T3)

T3 decreased with dose (Figs. 4-15 and 4-16). The mean value for T3 in the control group for females was 128.51 ngm/ml with a standard deviation of 8.99. The mean value for T3 in the control group for males was 132.87 ngm/ml for females with a standard deviation of 11.71.

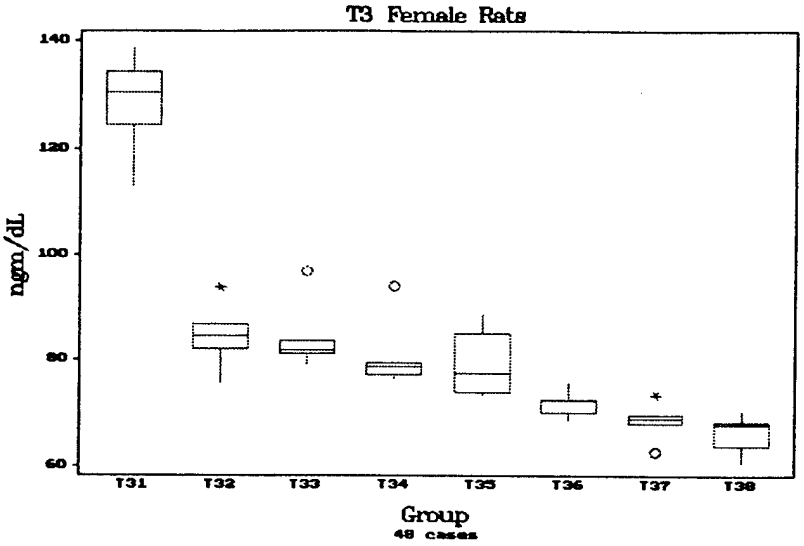


Figure 4-15. Boxplots of female T3 levels by dose group.

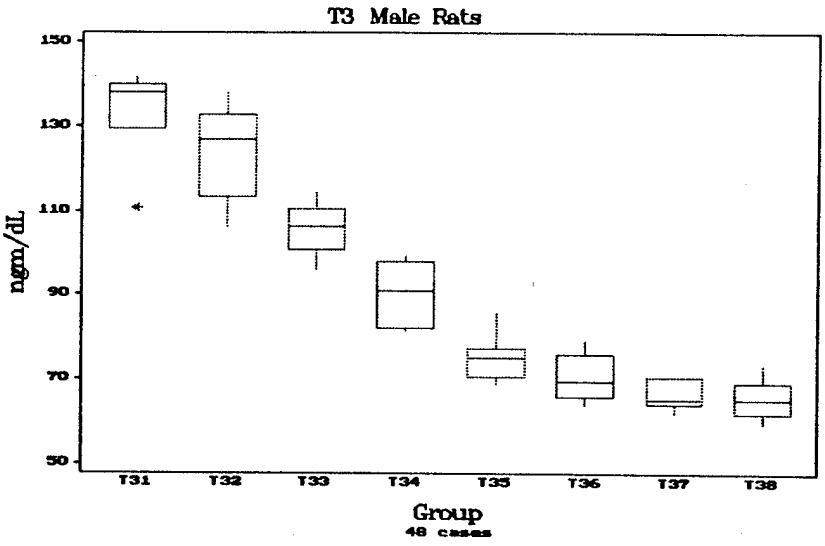


Figure 4-16. Boxplots of male T3 levels by dose group.

Females

The MLE sigmoid function for female T3 levels was as follows:

$$T3Female(Dose) = 138.364 - \frac{132.05\,Dose^{.119}}{\left[Dose^{.119} + \left(\frac{132.05}{2}\right)^{.119}\right]}$$

The relationship of the function to the data is displayed in figure 4-17.

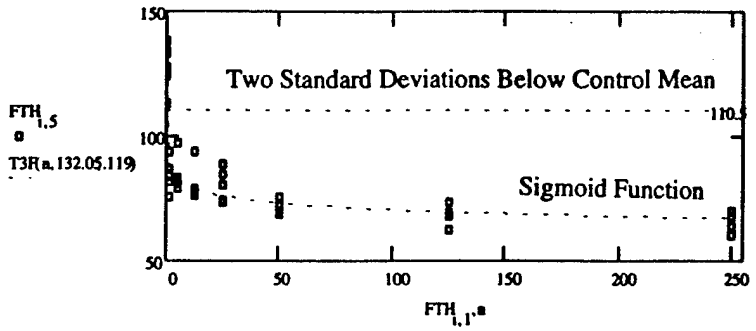


Figure 4-17. T3 dose-response data for females with fitted sigmoid function.

The lack of fit test for the female T3 sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha, v1, v2}$
SS Pure Error	40	1369.845284	34.2461321	3.709	2.34
SS Lack of Fit	6	762.156736	127.026		
SS Error	46	2132.00202			

Table 4-6. Table for T3 sigmoid function lack of fit test (females)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 3.709 > 2.34, the null hypothesis was rejected in favor of the alternate. Therefore, the function did not accurately characterize the data and could not be used to obtain a 95% confidence interval.

Males

The MLE sigmoid function for male T3 levels was as follows:

$$T3Male(Dose) = 141.536 - \frac{136.68\,Dose^{.359}}{\left[Dose^{.359} + \left(\frac{136.68}{2}\right)^{.359}\right]}$$

The relationship of the function to the data is displayed in figures 4-18.

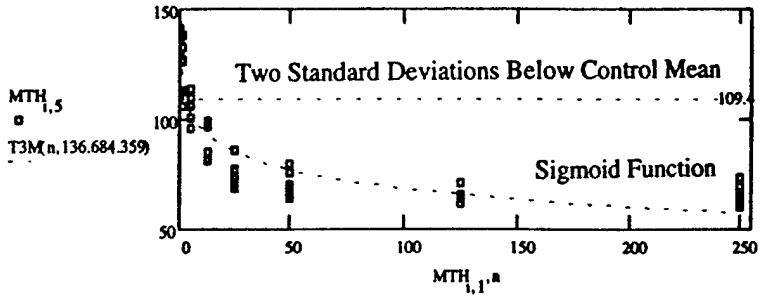


Figure 4-18. T3 dose-response data for males with fitted sigmoid function.

The lack of fit test for the male T3 sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	2529.145352	63.2286	5.96	2.34
SS Lack of Fit	6	2262.14176	377.0236		
SS Error	46	4791.28711			

Table 4-7. Table for T3 sigmoid function lack of fit test (males)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 5.96 > 2.34, the null hypothesis was rejected in favor of the alternate. As with the females, the function did not accurately characterize the data and could not be used to calculate an upper 95% confidence interval.

Thyroid Stimulating Hormone (TSH)

A very clear relationship between dose and TSH levels was observed (Figs. 4-19 and 4-20). The mean value for TSH in the control group for females was 11.251 ngm/ml with a standard deviation of .4780. The mean value for TSH in the control group for males was 14.472 ngm/ml with a standard deviation of 1.1547.

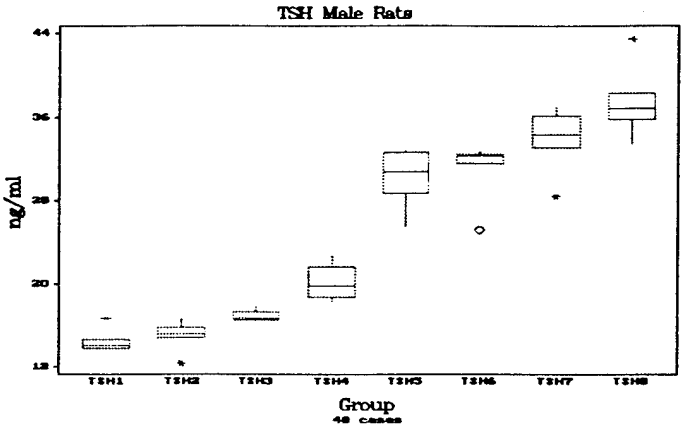


Figure 4-19. Boxplots of male TSH levels by dose group.

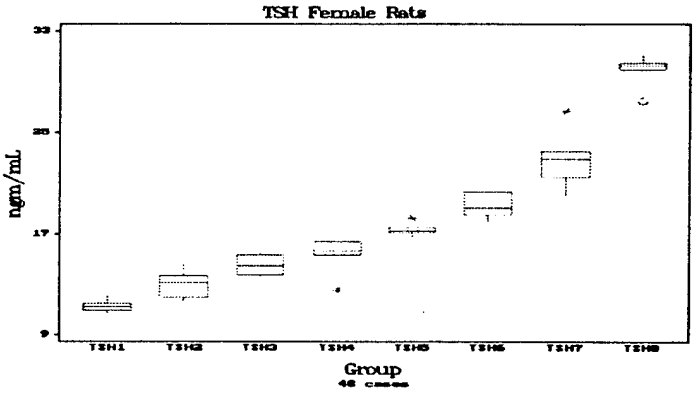


Figure 4-20. Boxplots of female TSH levels by dose group.

Females

The MLE sigmoidal function for female TSH is as follows:

$$TSHFemale(Dose) := 11.248 + \frac{12.885\,Dose^{.7025}}{\left[Dose^{.7025} + \left(\frac{12.885}{2}\right)^{.7025}\right]}$$

The relationship of the function to the data is displayed in figure 4-21.

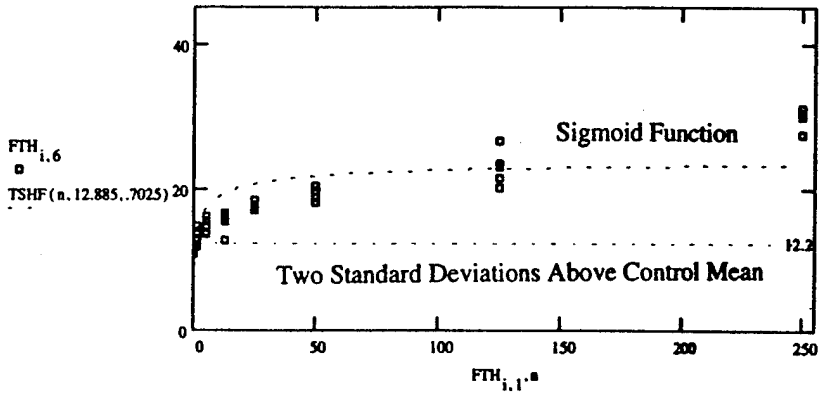


Figure 4-21. TSH dose-response data for females with fitted sigmoid function.

The lack of fit test for the female TSH sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	60.9892939	1.5247	54.72	2.34
SS Lack of Fit	6	500.556979	83.4261632		
SS Error	46	561.5462732			

Table 4-8. Table for TSH sigmoid function lack of fit test (females)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 54.72 > 2.34, the null hypothesis was rejected in favor of the alternate. Once again, the function did not accurately characterize the data. This time it was soundly rejected.

Males

The sigmoidal function for male TSH is as follows:

$$TSHMale(Dose) := 14.473 + \frac{20.257 \cdot Dose^{1.417}}{\left[Dose^{1.417} + \left(\frac{20.257}{2}\right)^{1.417}\right]}$$

The relationship of the function to the data is displayed in figure 4-22.

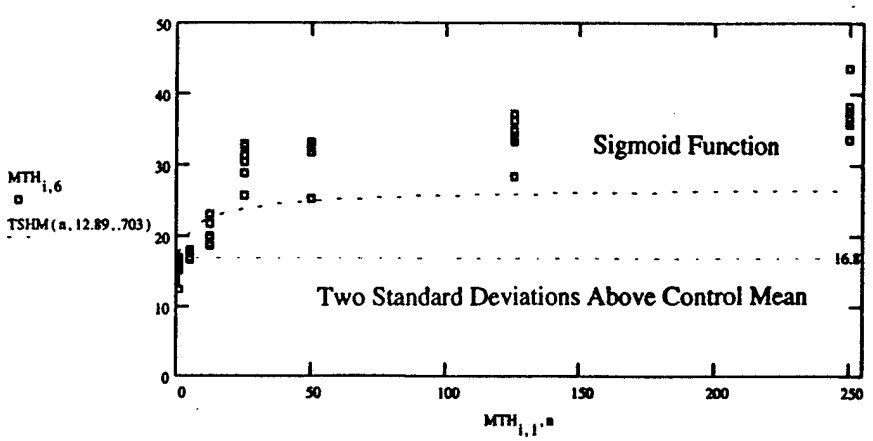


Figure 4-22. TSH dose-response data for males with fitted sigmoid function.

The lack of fit test for the male TSH sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	218.8194058	11.911	10.0262	2.34
SS Lack of Fit	6	329.090418	54.848403		
SS Error	46	547.9098236			

Table 4-9. Table for TSH sigmoid function lack of fit test (males)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 10.02 > 2.34, the null hypothesis was rejected in favor of the alternate. Therefore, the function did not accurately characterize the data.

An upper 95% confidence interval for the sigmoid function could not be determined because the functions were not able to accurately characterize the data.

Therefore, a threshold value could not be determined with the data from this study. However, Tukey's method for multiple comparisons was performed in order to determine the NOAEL values for T3 and TSH. The results are as follows:

T3

Tukey's test for multiple comparisons for female T3 hormone levels revealed that group one was statistically significantly different from the other dose groups (Table 4-10).

STATISTIX 4.1 HORMFEM

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF T3 BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
0	128.51	I
1	84.600	.. I
5	84.074	.. I
12	80.676	.. II
25	79.300	.. III
50	71.860	 III
125	68.548	 II
250	66.401	 I

THERE ARE 5 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	10.799		
STANDARD ERROR FOR COMPARISON	3.3787		

Table 4-10. Tukey's test for multiple comparisons for female T3 levels.

Therefore, the NOAEL in this experiment for T3 in female rats is the control.

Tukey's test for multiple comparisons for male T3 levels revealed that groups one and two were statistically the same (Table 4-11).

STATISTIX 4.1 HORMMALE

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF T3 BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
0	132.87	I
1	124.02	I
5	105.67	.. I
12	90.459	 I
25	75.417	 I
50	70.690	 I
125	66.465	 I
250	65.936	 I

THERE ARE 4 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	14.674		
STANDARD ERROR FOR COMPARISON	4.5909		

Table 4-11. Tukey's test for multiple comparisons for male T3 levels.

The NOAEL in this experiment for T3 in male rats was .11 mg/kg/day.

TSH

Tukey's test for multiple comparisons for female rats revealed that groups one and two were statistically the same (Table 4-12).

STATISTIX 4.1 HORMFEM

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF TSH BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
250	29.926	I
125	22.905	.. I
50	19.254	 I
25	17.385	 II
12	15.358	 II
5	14.584	 II
1	13.051	 II
0	11.251	 I

THERE ARE 7 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	2.2787		
STANDARD ERROR FOR COMPARISON	0.7129		

Table 4-12. Tukey's test for multiple comparisons for female TSH levels

Therefore, the NOAEL in this experiment for TSH in female rats is .124 mg/kg/day.

Tukey's test for multiple comparisons for male rats revealed that dose groups one, two and three were statistically the same (Table 4-13).

STATISTIX 4.1 HORMMALE

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF TSH BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
250	37.444	I
125	33.960	II
50	31.147	.. I
25	30.236	.. I
12	20.250	 I
5	16.919	 II
1	15.022	 I
0	14.472	 I

THERE ARE 4 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	4.3162		
STANDARD ERROR FOR COMPARISON	1.3504		

Table 4-13. Tukey's test for multiple comparisons for male TSH levels.

Therefore, the NOAEL in this experiment for TSH in male rats is .44 mg/kg/day. A summary of the NOAELs is presented in table 4-14.

Dose Groups Statistically Significantly Equal to the Control		
	MALE	FEMALE
T3	.11 mg/kg/day	None
TSH	.44 mg/kg/day	.124 mg/kg/day

Table 4-14. Summary of NOAELs for T3 and TSH.

A summary of the results, conclusions and recommendations are presented in chapter 5.

V. Conclusions and Recommendations

Overview

The primary objective of this research was to determine toxicity information to establish permissible exposure levels of ammonium perchlorate (AP). Sprague Dawley rats were exposed to varying concentrations of AP in their drinking water for a two-week period. The rats were then sacrificed and their hormone levels were measured via radioimmunoassay. The goal of the statistical analysis was to determine a threshold dose based on the hormone level data. The sigmoidal function did not accurately characterize the data. However, the NOAEL for AP on TSH levels was 0.443 mg/kg/day for the male rats and 0.124 mg/kg/day for the female rats.

Summary of Findings

Average water consumption

AP did not have a statistically significant affect on the average water consumption of either sex at the concentrations administered.

Body weight gain

AP did not have a statistically significant affect on the body weight gain of either sex. Both sexes gained weight in the same manner over the two-week period.

Thyroid/Body weight ratio

AP had a statistically significant affect on the thyroid/body weight ratios of the rats exposed 11.4 mg/kg/day and higher. The thyroid/body weights in these dose groups experienced an increase in thyroid body weight ratios as compared with the control group.

Thyroid hormone levels

AP had a statistically significant affect on the thyroid hormone levels in both sexes and the sexes were not affected in the same way. Triiodothyronine (T3) levels in male and female rats fell, Thyroxine (T4) levels remained relatively unchanged. Reverse Triiodothyronine (rT3), Thyrotropin (TSH), and Thyroglobulin (Tg) all increased in both males and females.

Data

The data derived from the two-week study could not be used to establish a dose-response function in order to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group. It appeared that the dose range was not optimum and that lower doses were needed. However, a Tukey comparison of means revealed a NOAEL of .44 mg/kg/day for the male rats and .124 mg/kg/day for the female rats. These results are consistent with the assumption made by Dollarhide (1992) that .14 mg/kg/day was the NOAEL for perchlorate. Based on these NOAELs a RfD of 4.133×10^{-4} is recommended. This reference dose proposes an uncertainty factor of 300. Ten for the use of less than a chronic study, ten for the protection of sensitive individuals and three for the application of animal data to humans.

Since Capen (1992) has shown that the rat thyroid is more sensitive than the human thyroid, the later uncertainty factor was halved on a log scale to 3.

Future Research

Although there was enough data to establish a NOAEL for AP, more research is needed in order to determine the estimated threshold dose. Future studies should establish dose ranges around an estimated threshold between .124 mg/kg/day and .46 mg/kg/day. In addition, the NOAEL should not be divided by overly conservative safety factors in light of this study's NOAEL which confirms the range assumed by the EPA and because it is now known that the rat thyroid is more sensitive than the human (Capen, 1992).

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Vita

Captain James H. King, Jr. was born August 20, 1966, in Port O'Spain, Trinidad. He graduated from Miami Southridge High School in Miami, Florida in 1984. Enlisting in the United States Army upon graduation, his first permanent duty assignment was as a Power Generation Repair Specialist with the 820th Ordnance Company at Bitburg AB, West Germany from January 1985 to July 1986. Returning from overseas, he enlisted in the United States Air Force Reserves and was assigned to the 482 Consolidated Aircraft Maintenance Squadron, Homestead AFB, Florida, and served as an Aircraft Fuel Systems Mechanic repairing and maintaining F/A-4D Phantom II aircraft. While serving in the reserves, he attended college full-time. He attended Florida International University in Miami, Florida, graduating with a Bachelor of Science Degree in Industrial and Systems Engineering. Following graduation, he earned a commission in the United States Air Force and was assigned to the 56th Civil Engineering Squadron at MacDill AFB, Florida. During his tour at MacDill, he served as Chief of Plans and Programs and Chief of Air Base Operability. He was further assigned as Assistant Chief of Simplified Acquisition of Base Engineering Requirements (SABER) until entering the School of Engineering, Air Force Institute of Technology, in May of 1994.

REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 1995	Master's Thesis

4. TITLE AND SUBTITLE

EFFECTS OF AMMONIUM PERCHLORATE ON THE THYROID HORMONE LEVELS OF THE SPRAGUE-DAWLEY RAT

5. FUNDING NUMBERS

PE 62202F
WU 6302A010

6. AUTHOR(S)

James H. King, Jr., Capt, USAF

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Air Force Institute of Technology,
WPAFB OH 45433-6583

8. PERFORMING ORGANIZATION REPORT NUMBER

AFIT/ENV/GEE/95D-09

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Armstrong Laboratory Toxicology Division AL/OET
2856 G St Bldg 79
WPAFB OH 45433-7400

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The purpose of this research was to determine the threshold dose for ammonium perchlorate (AP) in the Sprague-Dawley rat. No dose response data exist for AP and the EPA has studied literature on the subject of perchlorates to determine a provisional reference dose. The Perchlorate Group, a consortium of DoD and industry representatives, believes this provisional reference dose is too conservative. This experiment was executed to provide dose response data on which to base a more accurate reference dose. The study consisted of eight groups of 12 Sprague-Dawley rats, six male and six female, which were exposed to incremental doses of AP in their drinking water.
The results indicated that Triiodothyronine (T3) levels in male and female rats fell, Thyroxine (T4) levels in male rats remained relatively unchanged. Reverse Triiodothyronine (rT3), Thyrotropin (TSH), and Thyroglobulin (Tg) all increased in both males and females. These results imply that the AP anion blocked the uptake of iodine in the thyroid. Although an estimated threshold could not be determined, the NOAEL for AP on the TSH levels was .44 mg/kg/day for the male rats and .124 mg/kg/day for the female rats. These NOAELs are consistent with the assumptions made by the EPA, which estimated a NOAEL of .14 mg/kg/day for perchlorates.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Ammonium Perchlorate, Ammonium Compounds, Perchlorates, Thyroid, Thyroid Gland, Thyroid Hormones, Thyroglobulin, Thyroxine, Thyrotropin	65
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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