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# EFFECT OF SODIUM FLUORIDE AND AMOX ($NF_3O$) ON GROWTH AND THYROID FUNCTION IN THE RAT

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# Foreword

This report was prepared under Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology-Biochemistry," in the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory of the Aerospace Medical Research Laboratories. The research was begun in March 1967 and was completed in May 1967.

Acknowledgment is made of the assistance of A2C Doyle Manion, TSgt Edgar Hagen, Miss Marilyn George, and Mr. Mark Haller.

This technical report has been reviewed and is approved.

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# Abstract

Intact and thyroidectomized growing rats were treated daily for 60 days with thyroxine, potassium iodide, sodium fluoride, or $NF_3O$, an oxidizing substance. Results indicated that treatment with NaF or $NF_3O$ at doses which were molar equivalents with respect to fluoride to a thyrotoxic dose of iodide had no effect on thyroid function measured by growth rate, $^{131}I$-uptake, and adrenal weight. A sparing effect of thyroid on $NF_3O$ toxicity was suggested.

## SECTION I.

# Introduction

Lee (1966) has investigated the toxicity of $NF_3O$ (AMOX) in rats and mice. The compound, a gas at standard temperature and pressure, is a strong oxidizing agent. The median lethal dose ($LD_{50}$) of AMOX for rats 7 days following a single intraperitoneal injection was 38.4 mg (10.8 ml) per kg of body weight for females and 51.6 mg (14.5 ml) per kg of body weight for males. Tissue analyses for fluorine (reported as fluoride) were performed by a method which destroyed the chemical identity of the fluorine compounds present. The thyroid was found to concentrate and retain fluoride to a greater extent than the blood. Significant thyroid concentrations of fluoride were observed by Lee (1966) to persist for 4-6 weeks following exposure.

The present study was designed to investigate the possibility that increased concentrations of fluorides or AMOX might result in functional changes in the thyroid.

## SECTION II.
# Methods

One hundred twenty-eight albino rats of a derivative of the Sprague-Dawley strain[1] were used. Thirty-two each of males and females were subjected to surgical thyroidectomy at 28 days of age. A similar group was left intact. The rats were divided into 16 treatment groups containing 8 rats each. The rats were separated according to sex and treatment group and housed in groups of 4 rats per cage.

Each rat was weighed and treated individually daily for 60 days. Day 1 of the experiment was the second day following surgery, when the rats were 30 days old. The treatment groups and dosages are identified in Table I. Odd-numbered groups contained only intact rats and even-numbered groups contained only thyroidectomized rats.

All treatments were given as intraperitoneal injections. The potassium iodide and sodium fluoride were dissolved in 5% dextrose in water to concentrations of 6.54 g/l and 1.7 g/l, respectively, which provided the proper dose when injected at a rate of 10.0 ml/kg of body weight. The thyroxine[2] was dissolved in 5% dextrose in water to a concentration of 2.5 mg/l which provided the proper dose when injected at a rate of 0.2 ml/rat. Control animals were injected with a 5% dextrose solution. The AMOX was injected as the dry gas. It was transferred from a stainless steel cylinder to a 50-ml, inert plastic, disposable syringe. The gas for immediate administration was transferred from the large syringe to a similar syringe of 1.00-ml capacity. All handling of the gas outside the steel cylinder was at 1 atmosphere. The conversion of the molar dosage of AMOX to the volume dosage was by means of the perfect gas law ($PV = nRT$) assuming 1 atmosphere of pressure. Thus, one millimole (mmole) of AMOX would occupy 22.4 ml. The volume dose rate was 3.33 ml/kg body weight/day.

TABLE I

TREATMENT GROUPS

| *Group Number* | *Treatment* | *Dosage* |
|---|---|---|
| 1, 2 | 5% dextrose injection | 10.0 ml/kg |
| 3, 4 | 5% dextrose injection | 10.0 ml/kg |
| 5, 6 | Thyroxine, Na salt ($T_4$) | 0.0005 mg/rat/day |
| 7, 8 | Potassium iodide | 65 mg/kg (0.4 mM/kg) |
| 9, 10 | Sodium fluoride | 17 mg/kg (0.4 mM/kg) |
| 11, 12 | AMOX ($NF_3O$) | 12 mg/kg (0.013 mM/kg) |
| 13, 14 | Thyroxine, Na salt | 0.0005 mg/rat/day |
| | Sodium fluoride | 17 mg/kg (0.4 mM/kg) |
| 15, 16 | Thyroxine, Na salt | 0.0005 mg/rat/day |
| | AMOX | 12 mg/kg (0.013 mM/kg) |

[1] Charles River Breeding Laboratories, Wilmington, Mass. 01887
[2] L-thyroxine, Na salt • $5H_2O$, grade II (pfs), Sigma Chemical Co., St. Louis, Mo. 63118

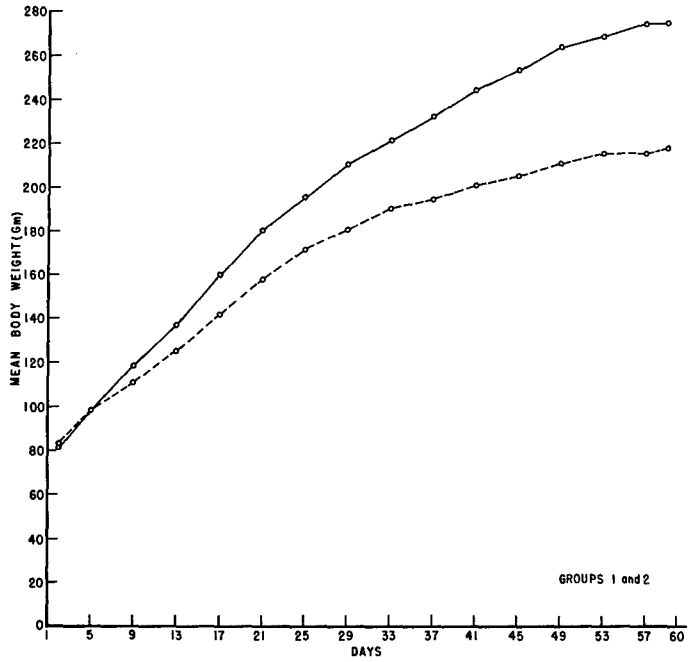


**Figure 1. Growth curves of intact (unbroken line) and thyroidectomized (broken line) rats which were fed a complete commercial rat diet[3] and given daily 5% dextrose injections.**

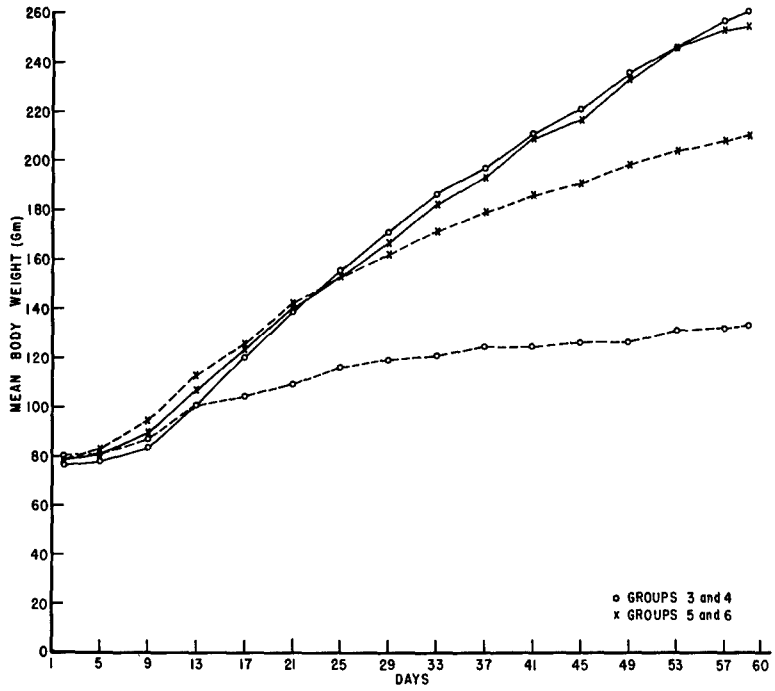


**Figure 2. Growth curves of intact (unbroken line) and thyroidectomized (broken line) rats which were fed a low-iodine diet. Groups 3 and 4 were given daily 5% dextrose injections and groups 5 and 6 were given daily thyroxine injections.**

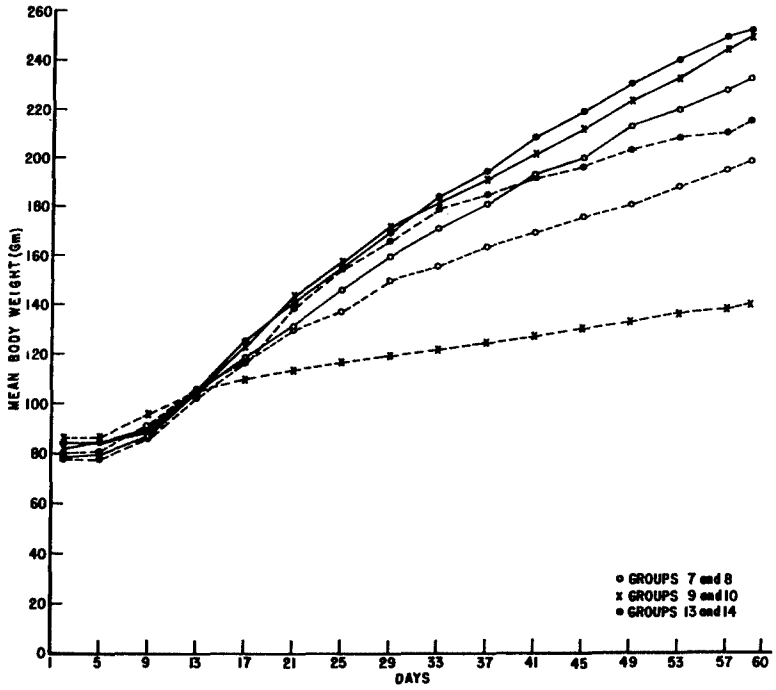


**Figure 3. Growth curves of intact (unbroken lines) and thyroidectomized (broken lines) rats which were fed a low-iodine diet. Groups 7 and 8 were given daily injections of KI, groups 9 and 10 were given daily injections of NaF, and groups 13 and 14 were given daily injections of both NaF and thyroxine.**

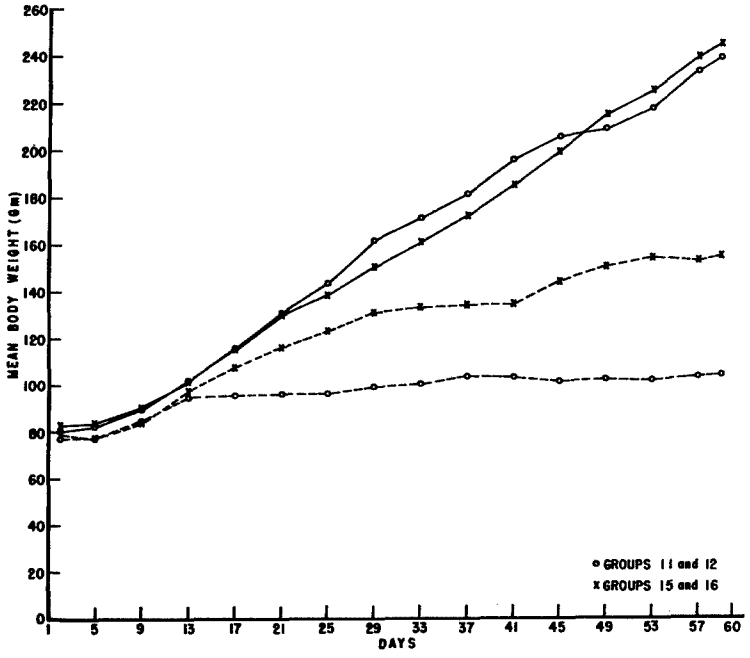


**Figure 4. Growth curves of intact (unbroken lines) and thyroidectomized (broken lines) rats which were fed a low-iodine diet. Groups 11 and 12 were given daily injections of AMOX ($NF_3O$) and groups 15 and 16 were given daily injections of both AMOX and thyroxine.**

Groups 1 and 2 were fed a commercial laboratory ration[3] and the remainder were fed the low-iodine Remington (1937) diet[4]. The rations of all of the rats on the Remington diet were supplemented with approximately 175 mg/rat/day of a vitamin-mineral mixture[5] containing no added iodine. All rats received raw carrots weekly. All rats received 1% calcium acetate in distilled water as the sole source of drinking water for the first week. Distilled water only was used for the remainder of the experiment.

On the 61st day of the experiment each rat was given an intraperitoneal injection of a tracer dose (0.6 $\mu$c) of $^{131}$I as sodium iodide[6] in 5% dextrose in water. Six hours later each was rapidly killed by the intraperitoneal injection of 1 ml of a saturated solution of magnesium acetate. Laryngeal and perilaryngeal tissue was removed, weighed, and the radioactivity measured in a well scintillation detector. An equal mass of tissue was removed from the femoral muscle mass. The difference was recorded as the thyroid uptake of radioiodide in count/min.

The equality of the mean weights of all combinations of two treatment groups (120 combinations), assuming independent samples and equal variances, was tested for each day by means of the 2-tailed Student's t-test (Freund, 1960). The equality of the mean $^{131}$I-uptakes and equality of the mean combined adrenal weights were also tested by means of the t-test.

The growth curves of the rats in each treatment group are plotted in Figures 1-4. The daily weights of the rats in each group were first averaged. The growth curves were then smoothed by the method of moving averages (Feller, 1957) using the formula $X^{(n)} = (Y_n + Y_{n+1})/2$. Subsequently, each fourth consecutive datum became a point on the final plot.

[3] Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.
[4] General Biochemicals, Chagrin Falls, Ohio 44022; average iodine content 0.05-0.07 ppm
[5] Paltone, Pitman-Moore Co., Indianapolis, Indiana
[6] Iodotope, Squibb Sodium Radio-Iodide ($I^{131}$) solution U.S.P., E. R. Squibb and Sons, New York; 0.218 mc/ml; the solution for injection was diluted to $0.6 \times 10^{-3}$ mc/ml

## SECTION III.
# Results

Growth curves for the 16 treatment groups of rats are shown in Figures 1, 2, 3, and 4. Significant differences ($p<0.05$) among various combinations of mean weights of the treatment groups are tabulated in Table II.

Groups 1 and 2 were fed the commercial complete laboratory ration[2] to produce the maximal growth rate for comparison with groups 3 and 4, respectively, which were on the iodine-deficient Remington diet. In each case the complete ration produced greater weight gains than the iodine deficient diet.

Examination of Figures 2, 3, 4, and Table II reveals that no significant weight differences developed among any of the treatment groups of *intact* rats (groups 3, 5, 7, 9, 11, 13, 15). The single exception was a brief period (17 days) in the case of group 3 versus group 15. This result is interpreted as spurious and probably would not have been evident if all rats of the same sex had been used.

The significant differences were observed among the various treatment groups of rats which had been thyroidectomized (even-numbered treatment groups). Potassium iodide (0.4 mmole/kg) promoted significant weight gains in the absence of the thyroid, although the gains were signifi-

TABLE II

SIGNIFICANCE OF MEAN WEIGHT DIFFERENCES AMONG THE TREATMENT GROUPS

| The mean weight[a] of this group[b] . . . | | | | | | | 1 | | 2 | 3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| differed significantly[c] from this group[b] . . . | | | | | | | 2 | 3 | 4 | 4 | 5 |
| during this period[d]. | | | | | | | 56-60 | 3-32 | 3-60 | 17-60 | NS[e] |
| 3 | | | | | 4 | | | | | | 5 |
| 7 | 9 | 11 | 13 | 15 | 6 | 8 | 10 | 12 | 14 | 16 | 6 |
| NS | NS | NS | NS | 25-41 | 10-60 | 16-60 | NS | 5-60 | 19-60 | NS | 47-60 |
| 5 | | | 6 | | | | | 7 | | | 8 |
| 7 | 13 | 15 | 8 | 10 | 12 | 14 | 16 | 8 | 9 | 11 | 10 |
| NS | NS | NS | NS | 14-60 | 5-60 | NS | 9-60 | 43-60 | NS | NS | 21-60 |
| | 9 | 10 | | 11 | | 12 | 13 | | 14 | 15 | |
| | 10 | 12 | 14 | 12 | 15 | 16 | 14 | 15 | 16 | 16 | |
| | 16-60 | 46-60 | 23-60 | 14-60 | NS | 20-60 | NS | NS | 19-60 | 31-60 | |

[a] Weight in grams
[b] See Table I
[c] $p<0.05$
[d] Days
[e] Not significant at any time

cantly lower than those of the intact rats. Sodium fluoride had no effect on weight gains. Sodium fluoride together with $T_4$ produced the same results as $T_4$ alone. Treatment with AMOX significantly retarded the growth of thyroidectomized rats. Treatment with $T_4$, however, had approximately the same growth-promoting effect in the AMOX-treated groups (12 and 16) as in the groups treated with $T_4$ without AMOX (groups 4 and 6).

Results of the determination of $^{131}$I-uptake in the intact rats are shown in Figure 5. Arithmetic means with standard deviations are given. Rats on the low-iodine Remington diet (groups 3 and 5) had significantly higher uptakes than the other groups. Uptake was not significantly affected by thyroxine administration. A highly significant and marked depression of $^{131}$I-uptake was evident in group 7 which was treated with the high level of potassium iodide. Groups 1, 9, 11, 13, and 15 did not differ from each other but differed from the untreated rats on the Remington diet (group 3).

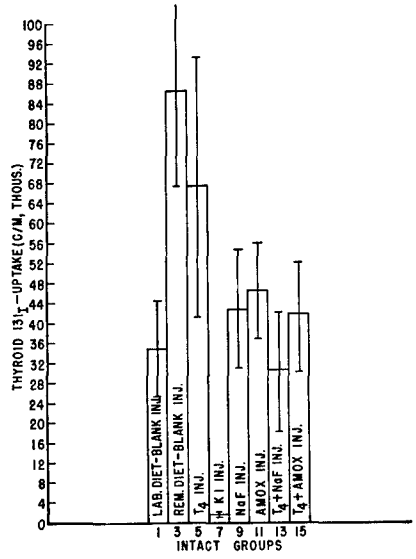


**Figure 5. Thyroid $^{131}$I-uptake of the intact rats. Bars represent the arithmetic means for the groups with plus and minus 1 standard deviation. Each rat received 0.6 microcurie of $^{131}$I six hours prior to euthanasia.**

The mean $^{131}$I-uptakes of the thyroidectomized rats ranged from 0.07% to 0.47% of the injected dose. Certain individual rats had uptakes suggesting that tiny amounts of residual thyroid tissue were present which were not considered significant in this experiment. Three thyroidectomized rats had significant $^{131}$I-uptakes. The data derived from the latter individuals were not used.

The mean combined adrenal weights plus or minus one standard deviation for the intact rats are shown in Figure 6. No significant differences existed among the groups on the Remington diet regardless of treatment.

The mean combined adrenal weights with standard deviations for the thyroidectomized rats are shown in Figure 7. In general thyroxine and potassium iodide promoted normal or nearly normal attainment of adrenal weight; whereas AMOX and sodium fluoride had no effect on adrenal weight.

A cursory *post mortem* gross examination was performed on each rat at the end of the experiment during the removal of the thyroid and adrenal tissues. The only remarkable lesion observed was a severe fibrinous peritonitis which was present in each rat injected with AMOX.

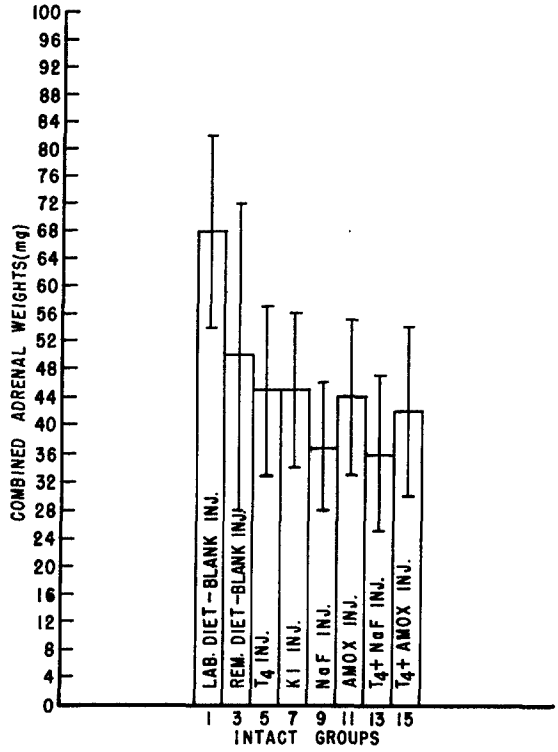


**Figure 6. Combined adrenal weights of the intact rats. Bars represent the arithmetic means for the groups with plus and minus 1 standard deviation.**

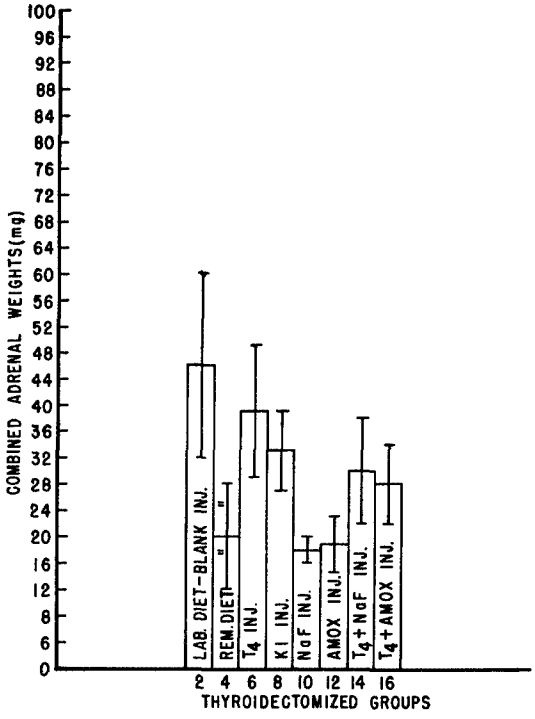


**Figure 7. Combined adrenal weights of the thyroidectomized rats. Bars represent the arithmetic means with plus and minus 1 standard deviation.**

## SECTION IV.
# Discussion

The metabolism of $NF_3O$ is unknown. The products of its apparent degradation in aqueous solution are likewise unknown (Lee, 1966). Lee has suggested that part of the overall toxic effect of AMOX could be the result of the formation of HF. A large part of the effect is apparently attributable to the reaction of the oxidizer with the milieu of easily reduced biological substances, notably proteins, following intraperitoneal injection. The result is a massive peritonitis with its sequelae.

The relationship of inorganic fluoride to the thyroid gland has received much attention with conflicting results. Hein *et al.* (1956) suggested that the thyroid preferentially concentrates fluoride ($^{18}F^-$). Similar findings were reported by Demole *et al.* (1959) using chemical methods. However, in studies using $^{18}F$, Wallace-Durbin (1954) found no concentration by the thyroid. Chen *et al.* (1961) conclude that no soft tissue stores fluoride.

The effect of fluoride on thyroid function, regardless of whether or not it is concentrated by the gland, is equally controversial. Saka *et al.* (1965) list nine references either confirming or denying that fluoride affects thyroid function. They found that fluoride did not affect I-uptake in rats. Similar findings have been reported by other workers (Henning *et al.*, 1961; Leone *et al.*, 1964).

Iodide, while usually necessary in small quantities for normal thyroid function, has been shown to be thyrotoxic in higher concentrations (Wolff and Chaikoff, 1948; Serif and Kirkwood, 1956; Paris *et al.*, 1960; Frey, 1964; Posner and Pimentel, 1966). Similar thyrotoxic levels of iodide have also been shown to promote nearly normal growth and skeletal maturation in thyroidectomized rats (Evans *et al.*, 1960; Asling and Evans, 1963; Evans *et al.*, 1964). For this experiment, a thyrotoxic dose (65 mg/kg/day) was given to both intact and thyroidectomized rats. In the former, $^{131}I$-uptake was severely inhibited; in the latter, nearly normal growth was promoted. The dosages of sodium fluoride and AMOX chosen were the molar equivalent (in fluoride) to the thyrotoxic iodide dosage.

The results of the present study indicate that neither fluoride nor AMOX has any effect on thyroid function in the rat as measured by growth, $^{131}I$-uptake, and adrenal weight. The growth retardation evident in the AMOX-treated rats appears to be independent of thyroid function since thyroxine causes an approximately equivalent growth promotion in both the AMOX-treated and untreated thyroidectomized rats. The fact that growth retardation was slight or absent in the intact rats treated with AMOX suggests a sparing effect of the thyroid on AMOX toxicity. The mechanism of this effect is purely speculative. However, the higher metabolic rate of euthyroid rats compared to hypothyroid rats might affect the rate of detoxification and elimination of AMOX and its metabolic products, which could explain an apparent sparing effect.

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13. ABSTRACT

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| Toxicology | | | | | | |
| Thyroid Function | | | | | | |
| Rodents | | | | | | |