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DIETARY SUBACUTE TOXICITY OF ETHYLENEBISISOTHIOCYANATE SULFIDE IN THE LABORATORY RAT

By

Ralph I. Freudenthal
BATTELLE
Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201

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Project Officer

Ronald L. Baron
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, N.C. 27711

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, n.c. 27711

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Studies of the metabolic fate of toxic chemicals give the Agency further insight into the significance of these agents in the environment. The metabolism of toxicants generally results in formation of chemicals of unknown toxicological properties. Chemical identification and toxicological evaluation of these chemicals and their metabolites continues to be an integral part of the environmental assessment necessary for continued safe use of chemicals.

John H. Knelson, M.D.
Director
Health Effects Research Laboratory

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INTRODUCTION

The zinc and manganese salts of ethylenebisdithiocarbamic acid (EBDC) are extensively used as crop fungicides. Studies investigating the degradation of the EBDC fungicides have demonstrated the presence of ethylene thiourea (ETU), ethylenediamine, ethylenebisisothiocyanate sulfide (EBIS, DITT [5, 6-dehydro-3H-imidazo-1, 2, 4-dithiozole-3-thione], formerly called ETM), carbon disulfide and inorganic metallic salts (1, 2, 3, 4, 5). Early studies evaluating the toxicity of the EBDC fungicides reported a major effect to be thyroid hyperplasia and neoplasia (6, 7). The toxicity of ETU has also been studied and found to be essentially similar to that of the parent fungicide with the exception that ETU produces thyroid neoplasia in rats (8, 9) and liver tumors in mice (10). The toxicity of EBIS, which has been demonstrated to occur as an autooxidation product of EBDC's and may exist as a residue in and on food crops, has not previously been evaluated.

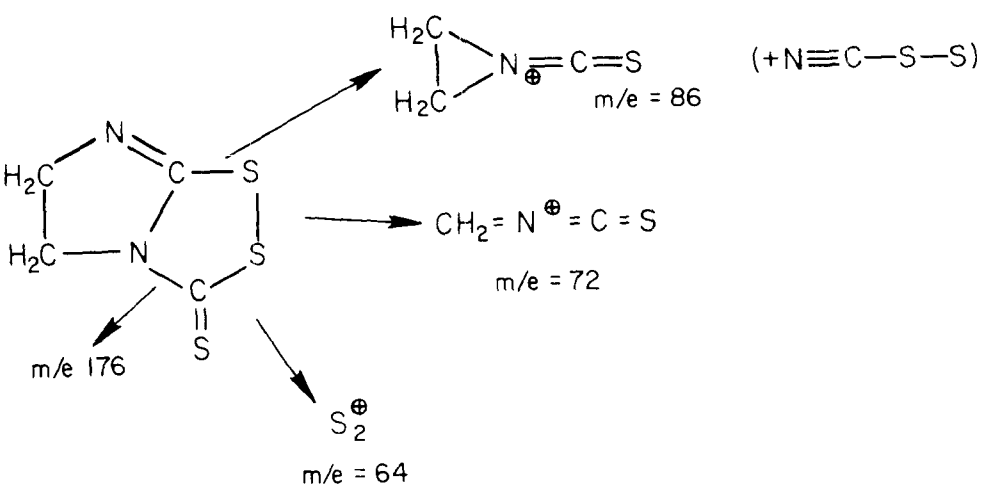
EXPERIMENTAL

Materials

Sprague Dawley derived rats were purchased from Charles River. EBIS was obtained from Trans World Chemicals Inc. (Silver Spring, Md.), and methimazole and amitrole from Sigma Chemical Co. (St. Louis, Mo.). Measurement of serum triiodothyroxine (T-3) and thyroxine (T-4) was performed with the aid of clinical kits purchased from Nuclear Medical Corp. (Dallas, Texas). ^{125}I used in iodine uptake studies was obtained from New England Nuclear (Boston, Mass.). ^{125}I was used in place of ^{131}I because of its longer half life relative to the more commonly used isotope ^{131}I.

EBIS Structure Confirmation and Purity Analysis

EBIS was analyzed by mass spectroscopy and the spectra were compared to an EBIS reference standard supplied by the Environmental Protection Agency (Health Effects Research Laboratory, Research Triangle Park, N.C.). Using direct probe high resolution mass spectrometry, a strong parent ion was detected at m/e 176 and fragmenta at m/e 86, m/e 72, m/e 64, and m/e 60. These fragments were rationalized based on the currently accepted structure for EBIS and are shown below.



The purity, based on analysis using high pressure liquid chromatography and thin layer chromatography, was considered to be greater than 98 percent based on the analytical standard.

Thyroid Hormone Function

Thyroid hormone is biosynthesized in the thyroid gland by the iodination of tyrosine. Iodotyrosines are coupled to form T-3 and T-4. These active hormones are released into the bloodstream and are distributed throughout the body where they regulate metabolic processes.

An increased body burden of thyroid hormone (either T-3 or T-4; both have essentially identical hormone action) causes an increase in cellular oxygen consumption. There is an increase in the rate of metabolism of carbohydrates, fats and proteins, a rise in cardiac output and an increased irritability of the nervous system. A decrease in thyroid hormone levels results in decreased cellular metabolism.

The thyroid and its feedback control system are designed to provide a constant supply of thyroid hormone to peripheral tissues. Chemical substrates that impair the synthesis or release of thyroid hormone from the gland cause thyroid hyperplasia. Continued exposure to antithyroid chemicals such as propylthiouracil or methimazole results in the malignant transformation of the benign hyperplastic thyroid tissue. It is now well documented that chemicals affecting thyroid function can initiate thyroid neoplasia (8, 9).

The clinical test kits used in this study measure the amount of total T-4 in the serum and the amount T-3 bound to thyroid binding globulin, the carrier protein for thyroid hormone. We also report the Free Thyroxine Index (FTI) for each test group. The FTI is a measurement of free serum T-4. While the T-4 level, measured by the T-4 radioimmunosassy kit, is influenced by thyroid binding globulin (TBG) concentrations, the FTI is a measurement of the amount of T-4 free in serum, independent of the serum TBG concentration.

Evaluation of Thyroid Function Test Kits

To show that chemically induced thyroid function alterations in rats could be measured with the available techniques, two chemicals (methimazole and amitrole) known to affect thyroid were administered to rats. Groups of six male and six female rats each received 0.6 mg/kg methimazole twice daily for four days or amitrole daily at a dose of 4 g/kg, for four days. Both chemicals were administered by oral intubation, dissolved in distilled water. Control rats received distilled water. These dosage levels have been previously shown to cause measureable thyroid alterations (11, 12).

After four treatment days, blood was taken by heart puncture from one-half of each treatment group, to be used for T-3 and T-4 evaluations. The remaining rats were used for ^{125}I uptake studies.

The T-4 diagnostic kit quantitatively determines total serum thyroxine by radioimmunoassay. Ten microliters of serum is mixed with 200 microliters of an acidic reagent, which completely inactivates endogenous thyroxine binding proteins. Radioactive T-4-^{125}I is then added to the test tube and thoroughly mixed with the endogenous unbound T-4. Anti-T-4 serum is added and mixed well. The mixture is incubated for 45 minutes at room temperature to allow formation of antibody complexes. The endogenous T-4 competes with the isotopically labeled T-4 for the binding sites on the antibody. The labeled and unlabeled T-4 are each bound in proportion to their relative concentration in the mixture. Since the antibody will bind both T-4-^{125}I and serum T-4 equally well, the amount of radioactive T-4 recovered will reflect the concentration of T-4 in the serum sample.

After the 45 minute incubation, the antibody-bound T-4 is precipitated by the addition of an ammonium sulfate solution to each tube. Following centrifugation, the free fraction of T-4 in the supernatant fluid is discarded by decantation and the antibody-bound fraction is counted. Serum T-4 levels are then determined from a standard curve which is prepared from a series of four serum standards assayed simultaneously with the experimental serum samples.

The T-3 kit used in this study actually measured the unsaturated TBG binding capacity. Radiolabeled T-3, added to a serum sample, competes with endogenous T-3 for TBG binding sites. Serum is added to the T-3-^{125}I reagent and upon mixing, the labeled T-3 binds to serum TBG. Separation of the free fraction is accomplished by adding a silicate tablet which is allowed to stand for 10 minutes prior to centrifugation. After centrifugation, the supernatant containing the bound fraction of T-3 is decanted. The radioactivity on the silicate is then compared to a standard curve which was assayed simultaneously with the test samples.

EBIS Study

EBIS dissolved in corn oil was incorporated into powdered Purina rodent feed, with a final corn oil concentration of 1 percent. The test diets were prepared fresh weekly. The dietary levels of EBIS were 1000, 100, 10, 1, and 0 ppm. The control group received powdered diet containing 1 percent corn oil. Five dosage groups each containing 60 male and 60 female rats, and a control group of 30 male and 30 female rats, were placed on study as outlined in Table 1. At 30-day intervals, ten rats of each sex (five of each sex of the control group) were used for the T-3 and T-4 thyroid function tests, for hematology and for necropsy. The remaining rats were used for thyroid ^{125}I uptake studies. Food consumption and body weight were determined weekly for each test animal. Each rat was individually caged throughout the study.

The 40 tissues removed at necropsy for histologic evaluation are listed in Table 2. Those tissues with asterick were also weighed.

Tissues taken at necropsy were fixed in 10 percent buffered formalin, sectioned at 5 micron and stained with Hematoxylin-Eosin. Blocks containing tongue and skin from the animals on the 100 ppm/30 days feeding study and

TABLE 1. EBIS PROTOCOL DESIGN

EBIS (ppm)	Sex	Number of Rats/Treatment Period		
		30-Days	60-Days	90-Days
1000	M	20	20	20
1000	F	20	20	20
100	M	20	20	20
100	F	20	20	20
10	M	20	20	20
10	F	20	20	20
1	M	20	20	20
1	F	20	20	20
0	M	10	10	10
0	F	10	10	10

TABLE 2. TISSUES REMOVED FOR HISTOLOGIC EVALUATION

Mammary Gland	Uterus	Liver*
Trachea	Testicles*	Thyroid*
Lung	Epididymus	Parathyroid*
Abdominal Aorta	Prostate	Adrenal*
Heart*	Seminal Vesicle	Eye
Mesenteric Lymph Node	Salivary Gland	Ear
Mandibular Lymph Node	Tongue	Sciatic Nerve
Tracheobronchial Lymph Node	Esophagus	Rib
Spleen*	Stomach	Femur
Thymus	Duodenum	Diaphragm
Kidneys*	Jejunum	Pituitary*
Ureter	Ileum	Brain*
Urinary Bladder	Colon	Spinal Cord
Ovary*	Pancreas	

Several selected control blocks were stained with toluidine blue metachromatic stain for mast cells. Brain and cord sections from the animals on a diet level of 1000 ppm, which exhibited posterior paralysis, were stained with luxol fast blue.

Hematology analysis was carried out from blood smears prepared by tail tip cuts on the day prior to sacrifice. Polymorphonuclear leukocytes, eosinophils, lymphocytes and monocytes were counted.

RESULTS

Preliminary Study

Since it has been previously demonstrated that both amitrole and methimazole treatment for 4 days results in a substantial alteration of thyroid function, the rats receiving these chemicals were sacrificed after four daily treatments and T-3, T-4 and ^{125}I were determined. The results of the thyroid function assays are shown in Table 3.

TABLE 3. RAT THYROID FUNCTION EVALUATION AFTER PRETREATMENT WITH AMITROLE AND METHIMAZOLE

Group	T-3(% Uptake)	T-4 (ug %)	^{125}I (% Uptake)
Control	68.9 ± 1.50	6.65 ± 0.74	2.84 ± 0.52
Amitrole	68.8 ± 1.22	4.28 ± 1.03	0.73 ± 0.14
Methimazole	65.1 ± 2.07	1.95 ± 0.84	9.79 ± 0.89

Neither amitrole nor methimazole have an effect on thyroxine binding globulin (TBG), as indicated by the T-3 values. In contrast, a significant difference is observed in the total serum thyroxine (T-4) and iodide uptake. It is interesting to note that while both amitrole and methimazole cause marked thyroid hyperplasia and a decrease in free thyroxine, their effect on iodide uptake differs. Amitrole exerts an inhibitory effect on thyroid iodide uptake while methimazole markedly stimulates iodide uptake.

This preliminary study shows that the commercially available T-3 and T-4 kits can be used to measure chemically induced alterations in thyroid function in the laboratory rat.

EBIS Toxicity Evaluation

Many of the rats receiving 1000 ppm EBIS showed marked toxicity by the enc of the first week. Partial to complete paralysis of the hind legs and lower back, shaggy hair coat, extreme loss of weight and unresponsive disposition were observed. A substantial number of rats receiving the highest dose died after having developed a posterior paralysis. EBIS ingestion ranged from 1.4 to 11.3 mg/day. A high percentage of the female rats died 7 to 10 days after initiating the experiment, whereas the majority of the male rats died 11 to 14 days after the initial feeding, suggesting that the female rat may be more susceptible than the male rat to the acute toxicity of EBIS.

Daily observation of the rats receiving diets of 100, 10, or 1 ppm EBIS or control diets showed no observable toxic effects.

Because of the acute toxicity of EBIS at 1000 ppm, several rats were used for thyroid function assays 7 days after being placed on the 1000 ppm test diet. The results from these assays are shown in Table 4.

TABLE 4. RAT THYROID FUNCTION AFTER 7-DAY INGESTION OF 1000 PPM EBIS IN THE DIET

Group (n)	T-3 (% Uptake)	T-4 (ug %)	^{125}I (%)	FTI
Control (10)	59.9 $\pm$ 0.76	4.88 $\pm$ 0.67	3.73 $\pm$ 1.17	2.74
EBIS (10)	60.8 $\pm$ 1.13	1.13 $\pm$ 0.68	2.13 $\pm$ 1.16	0.73

The results of the T-3 assay suggest that EBIS pretreatment at 1000 ppm for 7 days has no effect on thyroxine binding globulin. The total capacity for thyroid hormone binding is unchanged. A significant difference is seen in the total serum T-4. EBIS pretreatment resulted in a very significant decrease in serum T-4 and the FTI, which measures free serum T-4. EBIS pre-treatment at 1000 ppm also substantially decreased iodide uptake.

In contrast to the limited data for rats at the 1000 ppm dietary level, EBIS ingestion at 100, 10 or 1 ppm for 30, 60 or 90 days, had no measurable effect on thyroid function. The T-3, T-4, ^{125}I and FTI measurements for the rats receiving 100, 10 or 1 ppm EBIS were not significantly different from the values obtained with control rats. These results are presented in Table 5.

The amount of test diet consumed per week is presented in Table 6. With the exception of those rats receiving 1000 ppm EBIS, there was no significant difference in feed consumption in any other group of rats. A reduced food consumption was observed at 1000 ppm.

TABLE 5. RAT THYROID FUNCTION - EBIS DIETARY STUDY[a,b]

EBIS (ppm)	Days on Study	Sex	T-3 (percent uptake)	T-4 (µg percent)	^{125}I (percent uptake)	FTI[c]
100	30	F	61.9 ± 1.1	3.8 ± 0.7	3.4 ± 1.7	2.34
100	30	M	65.3 ± 1.4	5.1 ± 0.6	2.7 ± 0.6	3.32
100	60	F	63.4 ± 1.5	4.2 ± 0.8	3.4 ± 1.6	2.69
100	60	M	65.1 ± 1.2	6.2 ± 0.8	2.8 ± 0.7	4.02
100	90	F	62.4 ± 1.1	3.2 ± 0.5	2.7 ± 1.1	2.61
100	90	M	65.3 ± 1.1	4.9 ± 0.9	2.8 ± 0.8	3.21
10	30	F	65.2 ± 1.5	3.2 ± 0.6	3.8 ± 1.4	2.11
10	30	M	67.6 ± 1.9	5.6 ± 2.0	3.6 ± 0.9	3.77
10	60	F	59.4 ± 1.6	3.8 ± 0.7	2.8 ± 1.1	2.28
10	60	M	62.5 ± 0.9	5.7 ± 0.8	3.2 ± 1.2	3.56
10	90	F	57.6 ± 2.3	3.6 ± 0.4	2.4 ± 0.6	2.11
10	90	M	59.8 ± 1.2	5.2 ± 0.9	2.7 ± 0.5	3.10
1	30	F	61.4 ± 1.8	4.1 ± 0.8	3.1 ± 0.9	2.52
1	30	M	64.3 ± 1.0	4.9 ± 1.1	3.3 ± 0.6	3.20
1	60	F	69.0 ± 1.2	2.3 ± 0.9	2.3 ± 0.6	1.43
1	60	M	70.1 ± 1.2	3.4 ± 0.7	2.0 ± 0.9	2.46
1	90	F	60.3 ± 1.2	3.2 ± 1.5	2.4 ± 0.4	1.89
1	90	M	62.5 ± 0.9	5.4 ± 2.1	2.9 ± 0.7	3.38
0	30	F	62.3 ± 1.4	4.5 ± 0.2	3.5 ± 1.6	2.89
0	30	M	64.6 ± 1.2	5.2 ± 0.6	2.8 ± 0.2	3.37
0	60	F	65.2 ± 1.9	3.9 ± 1.5	1.9 ± 0.2	2.57
0	60	M	68.4 ± 0.3	4.2 ± 0.6	2.0 ± 0.3	3.26
0	90	F	60.8 ± 1.6	4.3 ± 0.6	2.5 ± 0.3	2.57
0	90	M	63.7 ± 0.9	5.1 ± 0.8	2.5 ± 0.5	3.86

[a] Expressed as the mean ± S.D.

[b] Student's t test was used to make comparisons between the control and treated animals. No significant differences were found between the control and corresponding experimental group.

[c] See Table 3.

TABLE 6. WEEKLY FEED CONSUMPTION*

EBIS (ppm)	Sex	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	Week No. 12
1000	F	27 ± 22	34 ± 7										
1000	M	59 ± 7	46 ± 18										
100	F	126 ± 26	143 ± 37	147 ± 39	149 ± 31	142 ± 33	156 ± 53	153 ± 49	114 ± 32	72 ± 7	96 ± 29	81 ± 8	94 ± 22
100	M	171 ± 18	177 ± 27	195 ± 39	198 ± 39	199 ± 39	195 ± 64	196 ± 55	150 ± 32	145 ± 20	154 ± 46	140 ± 11	148 ± 14
10	F	126 ± 22	142 ± 32	144 ± 30	142 ± 43	157 ± 44	168 ± 46	148 ± 42	124 ± 62	149 ± 42	138 ± 27	120 ± 19	111 ± 21
10	M	167 ± 20	173 ± 20	176 ± 22	182 ± 30	180 ± 36	186 ± 46	165 ± 50	164 ± 32	179 ± 34	162 ± 26	157 ± 22	148 ± 17
1	F	105 ± 35	112 ± 26	119 ± 28	118 ± 22	105 ± 31	107 ± 27	106 ± 27	119 ± 21	123 ± 21	116 ± 26	101 ± 23	128 ± 21
1	M	150 ± 36	158 ± 25	171 ± 31	173 ± 29	151 ± 36	163 ± 38	147 ± 24	167 ± 31	151 ± 29	160 ± 25	141 ± 34	133 ± 26
0	F	115 ± 23	132 ± 22	118 ± 42	137 ± 32	138 ± 34	129 ± 69	118 ± 20	103 ± 18	102 ± 16	79 ± 5	77 ± 8	114 ± 19
0	M	158 ± 33	165 ± 20	166 ± 38	181 ± 30	166 ± 39	164 ± 38	130 ± 16	137 ± 26	141 ± 10	128 ± 34	137 ± 8	137 ± 29

*Data presented in grams; mean ± S. D.

Table 7 and Figure 1 depict the amount of EBIS consumed. In the 1000 ppm group ingestion of EBIS ranged from 1.4 to 11.3 mg per day for the first week of study while the rats receiving 100 ppm EBIS consumed from o.6 mg to 3.2 mg per day during the same period.

The rats given 1000 ppm of EBIS in their diet for 4 days began eleciting kyphosis (humped backs) and progressive weakness of the posterior limbs that culminated in posterior paralysis. The most severely affected animals showed loss of weight due to malnutrition and dehydration. One spontaneous death occurred on day 6, three on day 7, and one on day 11 of the feeding experiment. Groups of four animals were euthanized on days 9, 10 and 16, having exhibited weight loss and posterior paralysis.

To determine whether the posterior paralysis, observed in rats after feeding EBIS at 1000 ppm for about 8 days, was reversible, a separate group of rats were placed on the 1000 ppm EBIS test diet until they developed paralysis. They were then removed from the test diet and allowed access to control diet. The clinical signs of paralysis disappeared within four days. When these animals were placed back on diets containing 1000 ppm EBIS, paralysis again occurred in about 8 days. Again, removel from the test diet resulted in alleviation of the clinical signs establishing that the paralysis is reversible.

During the course of the experiment, one animal in the 1 ppm/60 days dosage group and three animals in the 0 ppm/60 days dosage group developed skin irritation of the neck and shoulders which resulted in self multillation due to scratching. This resulted in a denuded, ulcerated area that was resolved by scab formation and overgrowth of new skin. No EBIS related clinical signs could be discerned within any of the remaining dosage groups (0-100 ppm for 30-90 days). All animals, except those 4 described above, appeared normal and in good condition when presented for necropsy.

Individual body weight data were recorded weekly throughout the study. Table 8 summarizes the body weight data (group mean $\pm$ S.D.) for the 12-week test period. Only those rats which received the 1000 ppm EBIS diet lost weight. No difference in body weight gain was observed for those rats receiving 100, 10, or 1 ppm EBIS or the control diet. This is shown in Figure 2, the cumulative body weight gain for 12 weeks.

Hematology values are shown in Table 9. No significant differences were measured for the rats receiving 100, 10, 1, or 0 ppm EBIS.

At the conclusion of the study gross necropsy including organ weight data and evaluation of thyroid function was performed. The number of animals examined is described in Table 10. Table 11 lists the organ weights obtained from the animals on study for 30, 60, and 90 days. There was no significant difference in organ weights between those rats on the EBIS diets and the control rats. Organ weight to body weight ratios for the test animals were calculated and are presented in Tables 12 and 13. No significant difference exists between groups. Organ weight to brain weight ratios were calculated and are presented in Tables 14 and 15. There was no significant difference in brain weight ratios between groups.

TABLE 7. WEEKLY EBIS CONSUMPTION*

EBIS (ppm)	Sex	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	Week No. 12
1000	F	27.63	34.34										
1000	M	59.34	46.52										
100	F	12.58	14.31	14.71	14.94	14.22	15.61	15.25	11.40	7.17	9.64	8.11	9.40
100	M	17.12	17.67	19.48	19.75	19.88	19.47	19.56	15.04	14.58	15.38	13.98	14.81
10	F	1.26	1.42	1.44	1.42	1.57	1.68	1.48	1.24	1.49	1.38	1.20	1.11
10	M	1.67	1.73	1.76	1.82	1.80	1.86	1.65	1.64	1.79	1.62	1.57	1.48
1	F	0.11	0.11	0.12	0.11	0.11	0.11	0.10	0.12	0.12	0.12	0.10	0.13
1	M	0.15	0.16	0.17	0.17	0.15	0.16	0.14	0.16	0.15	0.16	0.14	0.13
0	F	0	0	0	0	0	0	0	0	0	0	0	0
0	M	0	0	0	0	0	0	0	0	0	0	0	0

* Presented as the mean for each group, expressed as mg EBIS per week.

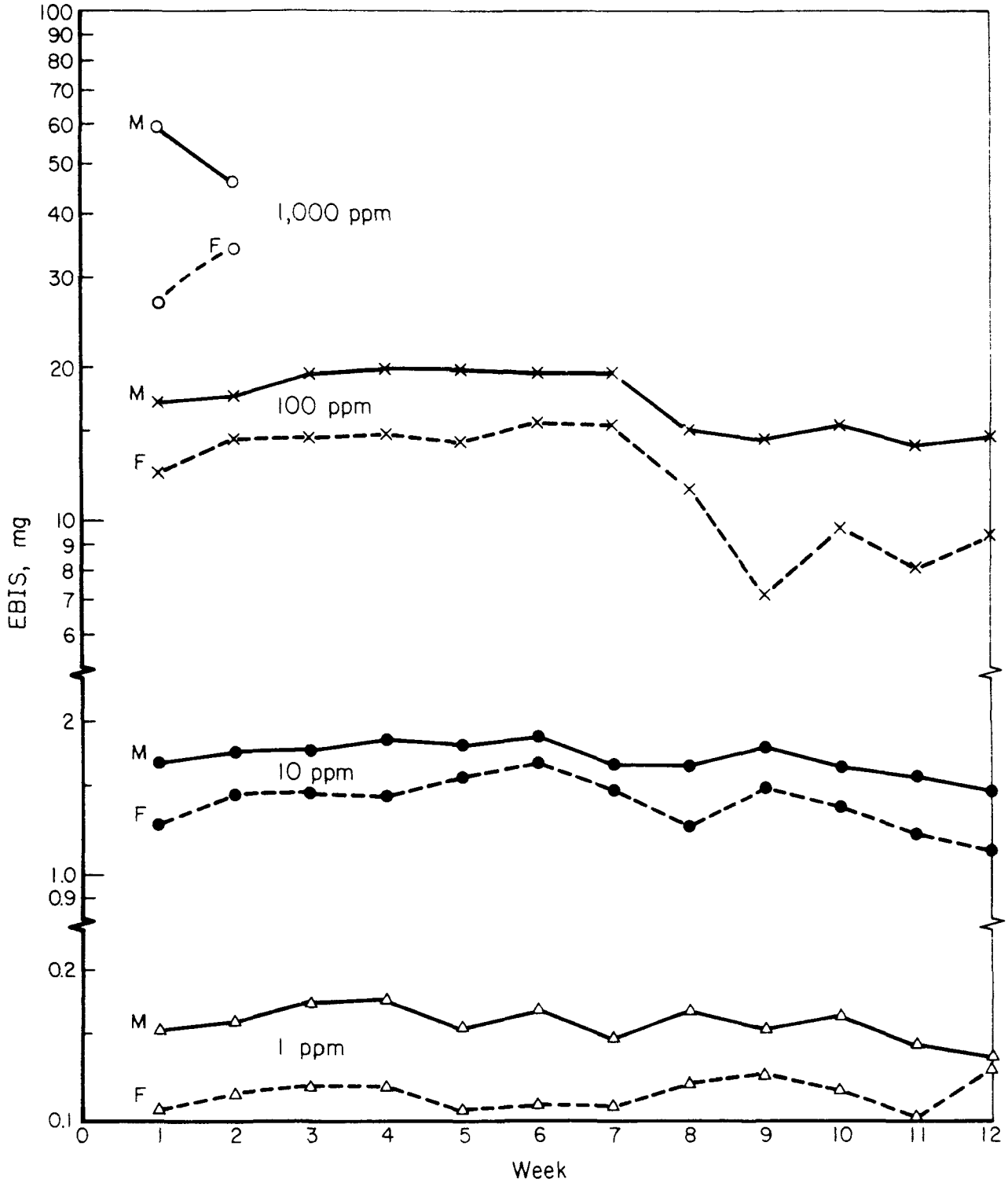


FIGURE 1. EBIS CONSUMED PER WEEK (mg)

TABLE 8. WEEKLY BODY WEIGHTS*

EBIS (ppm)	Sex	Starting Weight	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	Week No. 12
1000	F	153 ± 14	139 ± 28	106 ± 15	All animals receiving 1000 ppm EBIS died by the middle of the third week.									
1000	M	148 ± 13	133 ± 21	124 ± 40										
100	F	174 ± 18	191 ± 29	213 ± 21	223 ± 36	243 ± 21	248 ± 20	259 ± 25	264 ± 25	272 ± 3	275 ± 29	276 ± 33	275 ± 47	287 ± 29
100	M	201 ± 27	255 ± 28	297 ± 23	340 ± 31	367 ± 52	393 ± 29	428 ± 37	442 ± 38	460 ± 41	486 ± 49	498 ± 49	515 ± 54	527 ± 56
10	F	176 ± 14	200 ± 14	217 ± 22	228 ± 27	244 ± 21	250 ± 20	264 ± 19	271 ± 21	282 ± 27	235 ± 32	289 ± 23	298 ± 25	300 ± 34
10	M	216 ± 21	270 ± 22	310 ± 36	351 ± 26	381 ± 29	397 ± 32	420 ± 33	442 ± 39	462 ± 42	463 ± 76	498 ± 37	514 ± 43	524 ± 49
1	F	163 ± 13	190 ± 20	210 ± 23	227 ± 24	240 ± 24	252 ± 26	263 ± 27	272 ± 27	278 ± 28	280 ± 41	287 ± 37	293 ± 41	294 ± 39
1	M	191 ± 29	254 ± 28	312 ± 47	349 ± 30	377 ± 38	399 ± 72	424 ± 54	449 ± 35	466 ± 40	473 ± 49	488 ± 64	486 ± 75	504 ± 69
0	F	160 ± 31	189 ± 20	210 ± 19	229 ± 31	244 ± 20	251 ± 21	265 ± 22	273 ± 24	278 ± 24	273 ± 27	289 ± 42	274 ± 30	284 ± 29
0	M	190 ± 34	254 ± 34	290 ± 31	335 ± 34	362 ± 23	383 ± 20	407 ± 27	422 ± 33	434 ± 38	451 ± 31	468 ± 31	462 ± 55	466 ± 66

*Mean, ± S.D.; presented in grams.

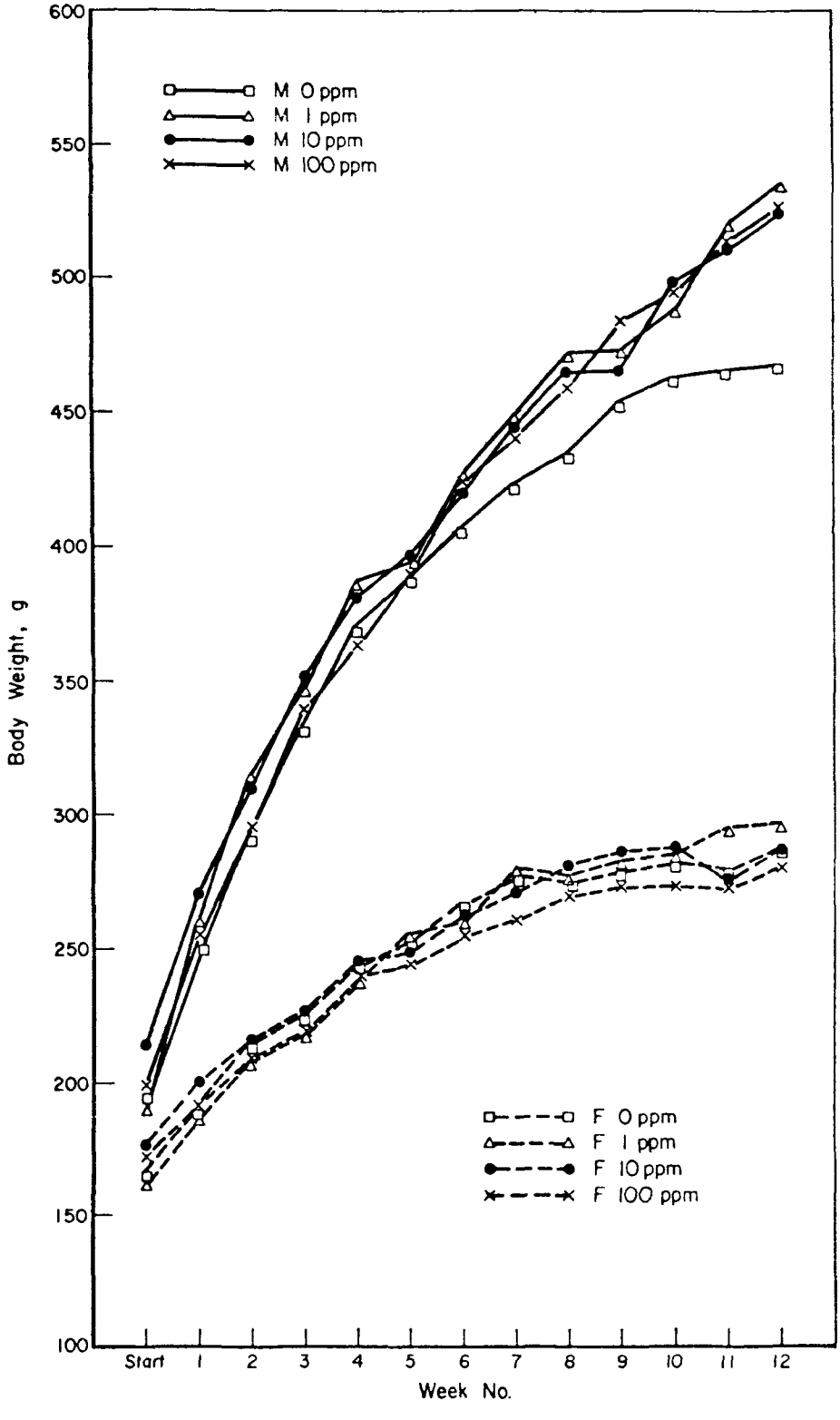


FIGURE 2. BODY WEIGHT CHANGE

TALBE 9. HEMATOLOGY VALUES

EBIS (ppm)	Days on Study	Sex	Polymorphonuclear leukocytes	Eosinophils	Lymphocytes	Monocytes
1000	13	F	17.3 ± 7.0	0.6 ± 1.1	76.0 ± 10.4	5.0 ± 3.1
1000	13	M	17.3 ± 7.0	0.6 ± 1.1	75.7 ± 15.1	5.7 ± 2.5
100	30	F	13.4 ± 4.3	2.9 ± 2.1	74.8 ± 6.6	8.9 ± 3.6
100	30	M	18.0 ± 6.2	1.8 ± 1.3	68.7 ± 8.8	11.5 ± 3.9
100	60	F	15.6 ± 7.2	4.2 ± 3.9	76.5 ± 9.0	3.5 ± 1.5
100	60	M	15.2 ± 4.5	2.1 ± 1.5	80.3 ± 4.3	2.2 ± 1.6
100	90	F	16.9 ± 8.1	2.3 ± 1.3	78.6 ± 8.6	1.9 ± 1.8
100	90	M	14.0 ± 6.3	2.2 ± 1.4	80.9 ± 7.3	3.2 ± 1.6
10	30	F	15.3 ± 7.4	2.4 ± 2.7	80.3 ± 7.9	1.9 ± 1.9
10	30	M	19.3 ± 8.4	1.6 ± 1.4	75.3 ± 9.9	2.8 ± 1.6
10	60	F	20.1 ± 4.8	2.5 ± 2.1	74.7 ± 6.0	2.5 ± 1.8
10	60	M	19.0 ± 11.6	2.0 ± 1.3	76.7 ± 12.5	2.3 ± 1.3
10	90	F	15.6 ± 6.8	2.8 ± 2.0	78.9 ± 9.0	2.0 ± 1.1
10	90	M	18.9 ± 7.2	2.0 ± 1.6	77.4 ± 8.5	1.5 ± 1.2
1	30	F	17.4 ± 7.9	1.9 ± 1.1	73.9 ± 13.5	3.5 ± 1.4
1	30	M	20.4 ± 5.2	1.5 ± 1.1	74.6 ± 5.4	3.4 ± 2.4
1	60	F	20.1 ± 8.2	3.1 ± 5.1	76.0 ± 6.6	5.8 ± 2.1
1	60	M	21.2 ± 9.3	2.2 ± 1.2	69.1 ± 10.9	5.5 ± 2.6
1	90	F	19.1 ± 6.5	2.8 ± 1.2	73.9 ± 7.4	4.4 ± 1.7
1	90	M	15.7 ± 6.3	1.8 ± 1.1	78.8 ± 6.2	3.7 ± 1.3
0	30	F	18.2 ± 5.6	1.6 ± 1.1	65.8 ± 7.5	3.8 ± 2.9
0	30	M	12.8 ± 3.4	2.2 ± 1.3	74.2 ± 3.1	- - - -
0	60	F	13.0 ± 6.8	3.3 ± 2.6	80.8 ± 9.2	2.8 ± 2.1
0	60	M	18.0 ± 10.8	- - - -	66.7 ± 11.6	3.6 ± 0.6
0	90	F	18.7 ± 5.7	3.5 ± 0.6	75.0 ± 6.1	3.0 ± 0.0
0	90	M	18.3 ± 9.7	1.8 ± 1.5	77.8 ± 9.1	2.3 ± 2.1

TABLE 10. GROSS NECROPSIES AND HISTOLOGIC EVALUATIONS

EBIS (ppm)	Days on Study	Number of Animals Subjected to Gross Necropsy	Number of Animals Subjected to Histologic Review
1000	Varied	17	17
100	90	20	10
100	60	20	10
100	30	20	10
10	90	19	0
10	60	20	0
10	30	19	0
1	90	20	0
1	60	20	0
1	30	20	0
0	90	8	8
0	60	11	11
0	30	9	9

TABLE 11. ORGAN WEIGHTS OBTAINED AT NECROPSY

EBIS (ppm)	Days on Study	Sex	Heart (g)	Spleen (g)	Rt. Kidney (g)	Left Kidney (g)	Ovary (paired) (g)	Testicle (paired) (g)	Liver (g)	Pituitary (mg)	Thyroid (paired) (mg)	Adrenal (paired) (mg)	Brain (g)
100	90	F	0.89 ± 0.11	0.48 ± 0.07	0.93 ± 0.13	0.89 ± 0.17	0.11 ± 0.02		9.44 ± 2.00	17.0 ± 4.8	23.1 ± 6.3	59.8 ± 10.7	1.89 ± 0.06
100	90	M	1.48 ± 0.11	0.82 ± 0.13	1.62 ± 0.15	1.60 ± 0.67		5.52 ± 0.79	17.97 ± 1.05	13.2 ± 3.4	32.6 ± 14.6	50.1 ± 14.6	2.17 ± 0.05
10	90	F	0.92 ± 0.13	0.51 ± 0.08	0.97 ± 0.09	0.95 ± 0.12	0.12 ± 0.01		9.90 ± 1.10	20.7 ± 6.3	30.1 ± 9.6	71.7 ± 6.1	1.96 ± 0.12
10	90	M	1.61 ± 0.28	0.87 ± 0.14	1.70 ± 0.20	1.63 ± 0.27		5.35 ± 1.7	18.05 ± 2.14	13.4 ± 2.6	37.3 ± 6.5	55.4 ± 12.3	2.15 ± 0.07
1	90	F	1.01 ± 0.14	0.53 ± 0.07	1.00 ± 0.14	0.95 ± 0.12	0.11 ± 0.01		9.84 ± 1.47	19.4 ± 5.2	25.9 ± 6.1	71.7 ± 14.0	1.93 ± 0.07
1	90	M	1.53 ± 0.12	0.84 ± 0.24	1.59 ± 0.22	1.58 ± 0.20		5.74 ± 0.54	17.05 ± 2.14	14.2 ± 2.9	33.2 ± 7.5	52.1 ± 12.7	2.16 ± 0.09
0	90	F	0.90 ± 0.13	0.47 ± 0.03	0.89 ± 0.17	0.87 ± 0.15	0.11 ± 0.02		9.82 ± 1.17	15.6 ± 1.8	26.5 ± 4.2	61.8 ± 5.2	1.88 ± 0.07
0	90	M	1.41 ± 0.14	0.78 ± 0.06	1.57 ± 0.06	1.59 ± 0.05		5.44 ± 0.73	16.56 ± 1.60	11.2 ± 1.0	38.3 ± 6.0	49.3 ± 4.9	2.10 ± 0.07
100	60	F	0.97 ± 0.14	0.55 ± 0.09	0.97 ± 0.06	0.92 ± 0.05	0.11 ± 0.03		10.62 ± 1.35	21.8 ± 5.3	24.9 ± 5.9	68.9 ± 8.8	1.89 ± 0.06
100	60	M	1.34 ± 0.10	0.80 ± 0.08	1.50 ± 0.17	1.47 ± 0.17		4.98 ± 0.39	17.58 ± 2.36	13.3 ± 1.8	39.6 ± 7.4	60.0 ± 13.4	2.06 ± 0.07
10	60	F	0.92 ± 0.10	0.57 ± 0.08	0.94 ± 0.10	0.91 ± 0.10	0.09 ± 0.02		10.25 ± 0.97	16.3 ± 2.8	20.8 ± 4.9	66.3 ± 10.0	1.89 ± 0.07
10	60	M	1.36 ± 0.08	0.71 ± 0.13	1.33 ± 0.13	1.29 ± 0.09		5.15 ± 0.26	13.96 ± 3.64	11.3 ± 3.8	32.1 ± 8.1	47.2 ± 8.6	2.10 ± 0.05
1	60	F	0.98 ± 0.08	0.53 ± 0.04	0.99 ± 0.05	0.96 ± 0.05	0.15 ± 0.02		10.58 ± 1.09	14.2 ± 3.0	21.6 ± 5.5	81.6 ± 8.1	1.89 ± 0.07
1	60	M	1.52 ± 0.19	0.81 ± 0.24	1.69 ± 0.19	1.66 ± 0.17		5.22 ± 0.39	17.69 ± 1.45	12.8 ± 1.7	32.1 ± 9.5	59.9 ± 9.6	2.06 ± 0.18
0	60	F	0.94 ± 0.06	0.64 ± 0.07	1.03 ± 0.04	1.02 ± 0.06	0.14 ± 0.02		11.72 ± 1.34	11.2 ± 4.2	27.3 ± 6.9	77.3 ± 9.5	1.99 ± 0.07
0	60	M	1.39 ± 0.13	0.84 ± 0.11	1.57 ± 0.08	1.60 ± 0.11		5.40 ± 0.43	18.36 ± 2.45	13.7 ± 2.8	30.3 ± 2.5	65.3 ± 5.7	2.21 ± 0.08
100	30	F	1.05 ± 0.29	0.55 ± 0.07	1.00 ± 0.10	0.99 ± 0.09	0.13 ± 0.02		11.85 ± 0.98	15.2 ± 2.7	17.9 ± 4.0	78.1 ± 8.7	1.88 ± 0.11
100	30	M	1.23 ± 0.11	0.72 ± 0.12	1.45 ± 0.13	1.45 ± 0.11		4.76 ± 0.39	16.45 ± 1.55	18.8 ± 9.9	28.9 ± 1.5	68.1 ± 9.9	2.04 ± 0.13
10	30	F	0.81 ± 0.11	0.80 ± 0.11	0.90 ± 0.13	0.88 ± 0.13	0.13 ± 0.05		9.62 ± 2.10	15.0 ± 5.2	29.9 ± 5.0	58.1 ± 12.1	1.85 ± 0.09
10	30	M	1.39 ± 0.13	0.84 ± 0.10	1.52 ± 0.15	1.42 ± 0.15		5.25 ± 0.33	16.38 ± 1.17	13.6 ± 4.3	33.3 ± 8.4	59.8 ± 9.9	2.08 ± 0.08
1	30	F	0.92 ± 0.10	0.59 ± 0.07	0.93 ± 0.10	0.91 ± 0.09	0.11 ± 0.03		10.15 ± 1.50	16.2 ± 3.1	22.7 ± 5.9	73.9 ± 11.7	1.89 ± 0.06
1	30	M	1.30 ± 0.12	0.73 ± 0.11	1.44 ± 0.06	1.41 ± 0.09		4.68 ± 0.54	15.43 ± 1.96	11.6 ± 1.5	29.4 ± 7.8	57.1 ± 8.3	2.06 ± 0.09
0	30	F	0.85 ± 0.06	0.54 ± 0.02	0.96 ± 0.06	0.92 ± 0.10	0.11 ± 0.03		11.07 ± 1.04	14.6 ± 4.0	28.2 ± 4.4	87.2 ± 14.1	1.99 ± 0.09
0	30	M	1.30 ± 0.08	0.83 ± 0.08	1.42 ± 0.18	1.43 ± 0.15		4.75 ± 0.39	18.02 ± 1.83	14.5 ± 6.0	28.8 ± 8.8	63.0 ± 12.7	2.04 ± 0.08

TABLE 12. ORGAN WEIGHT:BODY WEIGHT RATIO

EBIS (ppm)	Days on Study	Sex	Heart (g)	Spleen (g)	Rt. Kidney (g)	Left Kidney (g)	Ovary (paired)(g)	Testicle (paired)(g)
100	90	F	3.31×10^{-3}	1.78×10^{-3}	3.46×10^{-3}	3.31×10^{-3}	3.94×10^{-4}	
100	90	M	2.89×10^{-3}	1.65×10^{-3}	3.27×10^{-3}	3.22×10^{-3}		1.11×10^{-2}
10	90	F	2.99×10^{-3}	1.68×10^{-3}	3.15×10^{-3}	3.08×10^{-3}	3.73×10^{-4}	
10	90	M	3.06×10^{-3}	1.66×10^{-3}	2.23×10^{-3}	3.10×10^{-3}		1.02×10^{-2}
1	90	F	3.51×10^{-3}	1.87×10^{-3}	3.47×10^{-3}	3.31×10^{-3}	3.68×10^{-4}	
1	90	M	3.03×10^{-3}	1.67×10^{-3}	3.14×10^{-3}	3.12×10^{-3}		1.14×10^{-2}
0	90	F	3.13×10^{-3}	2.16×10^{-3}	3.43×10^{-3}	3.40×10^{-3}	4.57×10^{-4}	
0	90	M	2.81×10^{-3}	1.71×10^{-3}	3.18×10^{-3}	3.24×10^{-3}		1.09×10^{-2}
100	60	F	3.46×10^{-3}	1.99×10^{-3}	3.46×10^{-3}	3.28×10^{-3}	4.00×10^{-4}	
100	60	M	2.92×10^{-3}	1.75×10^{-3}	3.27×10^{-3}	3.20×10^{-3}		1.08×10^{-2}
10	60	F	3.32×10^{-3}	2.08×10^{-3}	3.39×10^{-3}	3.28×10^{-3}	3.36×10^{-4}	
10	60	M	3.11×10^{-3}	1.64×10^{-3}	3.05×10^{-3}	2.95×10^{-3}		1.18×10^{-2}
1	60	F	3.44×10^{-3}	1.88×10^{-3}	3.47×10^{-3}	3.38×10^{-3}	5.23×10^{-4}	
1	60	M	3.19×10^{-3}	1.70×10^{-3}	3.54×10^{-3}	3.48×10^{-3}		1.09×10^{-2}
0	60	F	3.14×10^{-3}	1.67×10^{-3}	3.15×10^{-3}	3.06×10^{-3}	3.68×10^{-4}	
0	60	M	2.73×10^{-3}	1.52×10^{-3}	3.04×10^{-3}	3.08×10^{-3}		1.05×10^{-2}
100	30	F	4.06×10^{-3}	2.13×10^{-3}	3.86×10^{-3}	3.83×10^{-3}	5.18×10^{-4}	
100	30	M	3.22×10^{-3}	1.88×10^{-3}	3.79×10^{-3}	3.79×10^{-3}		1.25×10^{-2}
10	30	F	3.37×10^{-3}	2.07×10^{-3}	3.79×10^{-3}	3.71×10^{-3}	5.69×10^{-4}	
10	30	M	3.32×10^{-3}	2.00×10^{-3}	3.63×10^{-3}	3.56×10^{-3}		1.25×10^{-2}
1	30	F	3.83×10^{-3}	2.44×10^{-3}	3.88×10^{-3}	3.79×10^{-3}	4.79×10^{-4}	
1	30	M	3.38×10^{-3}	1.79×10^{-3}	3.52×10^{-3}	3.47×10^{-3}		1.14×10^{-2}
0	30	F	3.44×10^{-3}	2.15×10^{-3}	3.89×10^{-3}	3.72×10^{-3}	4.48×10^{-4}	
0	30	M	3.21×10^{-3}	2.08×10^{-3}	3.51×10^{-3}	3.55×10^{-3}		1.17×10^{-2}

TABLE 13. ORGAN WEIGHT:BODY WEIGHT RATIO

EBIS (ppm)	Days on Study	Sex	Liver (g)	Pituitary (g)	Thyroid (paired)(g)	Adrenal (Paired)(mg)	Brain (g)
100	90	F	3.51×10^{-2}	6.32×10^{-5}	8.58×10^{-5}	2.22×10^{-4}	7.02×10^{-3}
100	90	M	3.62×10^{-2}	2.66×10^{-5}	6.57×10^{-5}	1.01×10^{-4}	4.38×10^{-3}
10	90	F	3.21×10^{-2}	6.72×10^{-5}	9.77×10^{-5}	2.33×10^{-4}	6.36×10^{-3}
10	90	M	3.43×10^{-2}	2.55×10^{-5}	7.08×10^{-5}	1.05×10^{-4}	4.08×10^{-3}
1	90	F	3.42×10^{-2}	6.74×10^{-5}	8.90×10^{-5}	2.49×10^{-4}	6.70×10^{-3}
1	90	M	3.37×10^{-2}	2.81×10^{-5}	6.57×10^{-5}	1.03×10^{-4}	4.27×10^{-3}
0	90	F	3.91×10^{-2}	3.75×10^{-5}	9.10×10^{-5}	2.57×10^{-4}	6.63×10^{-3}
0	90	M	3.72×10^{-2}	2.77×10^{-5}	6.13×10^{-5}	1.32×10^{-4}	4.47×10^{-3}
100	60	F	3.79×10^{-2}	7.78×10^{-5}	8.89×10^{-5}	2.46×10^{-4}	6.75×10^{-3}
100	60	M	3.83×10^{-2}	2.90×10^{-5}	8.63×10^{-5}	1.31×10^{-4}	4.49×10^{-3}
10	60	F	3.70×10^{-2}	5.88×10^{-5}	7.50×10^{-5}	2.39×10^{-4}	6.81×10^{-3}
10	60	M	3.20×10^{-2}	2.59×10^{-5}	7.35×10^{-5}	1.08×10^{-4}	4.81×10^{-3}
1	60	F	3.71×10^{-2}	4.98×10^{-5}	7.58×10^{-5}	2.86×10^{-4}	6.63×10^{-3}
1	60	M	3.71×10^{-2}	2.68×10^{-5}	6.73×10^{-5}	1.26×10^{-4}	4.32×10^{-3}
0	60	F	3.45×10^{-2}	5.26×10^{-5}	9.30×10^{-5}	2.17×10^{-4}	6.60×10^{-3}
0	60	M	3.21×10^{-2}	2.18×10^{-5}	7.42×10^{-5}	1.56×10^{-4}	4.07×10^{-3}
100	30	F	4.58×10^{-2}	5.58×10^{-5}	6.92×10^{-5}	3.02×10^{-4}	7.26×10^{-3}
100	30	M	4.30×10^{-2}	4.92×10^{-5}	7.56×10^{-5}	1.78×10^{-4}	5.34×10^{-3}
10	30	F	4.05×10^{-2}	6.32×10^{-5}	8.38×10^{-5}	2.45×10^{-4}	7.79×10^{-3}
10	30	M	3.91×10^{-2}	3.25×10^{-5}	7.96×10^{-5}	1.43×10^{-4}	4.97×10^{-3}
1	30	F	4.23×10^{-2}	6.75×10^{-5}	9.46×10^{-5}	3.08×10^{-4}	7.88×10^{-3}
1	30	M	3.77×10^{-2}	2.84×10^{-5}	7.19×10^{-5}	1.36×10^{-4}	5.04×10^{-3}
0	30	F	4.47×10^{-2}	5.89×10^{-5}	1.14×10^{-4}	3.52×10^{-4}	8.03×10^{-3}
0	30	M	4.45×10^{-2}	3.61×10^{-5}	7.12×10^{-5}	1.56×10^{-4}	5.04×10^{-3}

TABLE 14. ORGAN WEIGHT: BRAIN WEIGHT RATIO

EBIS (ppm)	Days on Study	Sex	Heart (g)	Spleen (g)	Rt. Kidney (g)	Left Kidney (g)	Liver (g)
100	90	F	4.71×10^{-1}	2.54×10^{-1}	4.92×10^{-1}	4.71×10^{-1}	4.99
100	90	M	7.83×10^{-1}	3.78×10^{-1}	7.47×10^{-1}	7.37×10^{-1}	8.28
10	90	F	4.69×10^{-1}	2.63×10^{-1}	4.95×10^{-1}	4.85×10^{-1}	5.10
10	90	M	7.49×10^{-1}	4.06×10^{-1}	7.91×10^{-1}	7.58×10^{-1}	8.40
1	90	F	5.23×10^{-1}	2.79×10^{-1}	5.18×10^{-1}	4.94×10^{-1}	5.10
1	90	M	7.08×10^{-1}	3.91×10^{-1}	7.36×10^{-1}	7.31×10^{-1}	7.89
0	90	F	4.71×10^{-1}	3.26×10^{-1}	5.18×10^{-1}	5.13×10^{-1}	5.89
0	90	M	6.29×10^{-1}	3.83×10^{-1}	7.10×10^{-1}	7.24×10^{-1}	8.31
100	60	F	5.13×10^{-1}	2.95×10^{-1}	5.13×10^{-1}	4.87×10^{-1}	5.62
100	60	M	6.50×10^{-1}	3.96×10^{-1}	7.28×10^{-1}	7.14×10^{-1}	8.53
10	60	F	4.87×10^{-1}	3.05×10^{-1}	4.97×10^{-1}	4.81×10^{-1}	5.42
10	60	M	6.48×10^{-1}	3.41×10^{-1}	6.33×10^{-1}	6.14×10^{-1}	6.65
1	60	F	5.18×10^{-1}	2.84×10^{-1}	5.23×10^{-1}	5.10×10^{-1}	5.60
1	60	M	7.38×10^{-1}	3.94×10^{-1}	8.20×10^{-1}	8.06×10^{-1}	8.59
0	60	F	4.76×10^{-1}	2.53×10^{-1}	4.77×10^{-1}	4.63×10^{-1}	5.22
0	60	M	6.71×10^{-1}	3.73×10^{-1}	7.48×10^{-1}	7.57×10^{-1}	7.89
100	30	F	5.61×10^{-1}	2.93×10^{-1}	5.32×10^{-1}	5.27×10^{-1}	6.30
100	30	M	6.03×10^{-1}	3.52×10^{-1}	7.11×10^{-1}	7.11×10^{-1}	8.06
10	30	F	4.32×10^{-1}	2.66×10^{-1}	4.86×10^{-1}	4.76×10^{-1}	5.20
10	30	M	6.68×10^{-1}	4.02×10^{-1}	7.31×10^{-1}	7.16×10^{-1}	7.86
1	30	F	4.87×10^{-1}	3.10×10^{-1}	4.92×10^{-1}	4.81×10^{-1}	5.37
1	30	M	6.65×10^{-1}	3.56×10^{-1}	6.99×10^{-1}	6.89×10^{-1}	7.49
0	30	F	4.29×10^{-1}	2.68×10^{-1}	4.84×10^{-1}	4.64×10^{-1}	5.56
0	30	M	6.37×10^{-1}	4.13×10^{-1}	6.97×10^{-1}	7.04×10^{-1}	8.83

TABLE 15. ORGAN WEIGHT: BRAIN WEIGHT RATIO

EBIS (ppm)	Days on Study	Sex	Ovary (paired)(g)	Testicle (paired)(g)	Pituitary (mg)	Thyroid (paired)(g)	Adrenal (paired)(mg)
100	90	F	5.61×10^{-2}		8.99×10^{-3}	1.22×10^{-2}	3.16×10^{-2}
100	90	M		2.54	6.08×10^{-3}	1.50×10^{-2}	2.31×10^{-2}
10	90	F	5.88×10^{-2}		10.60×10^{-3}	1.54×10^{-2}	3.66×10^{-2}
10	90	M		2.49	6.23×10^{-3}	1.73×10^{-2}	2.58×10^{-2}
1	90	F	5.49×10^{-2}		10.10×10^{-3}	1.34×10^{-2}	3.72×10^{-2}
1	90	M		2.66	6.57×10^{-3}	1.54×10^{-2}	2.41×10^{-2}
0	90	F	6.88×10^{-2}		5.65×10^{-3}	1.37×10^{-2}	3.88×10^{-2}
0	90	M		2.44	6.19×10^{-3}	1.37×10^{-2}	2.95×10^{-2}
100	60	F	5.93×10^{-2}		11.51×10^{-3}	1.32×10^{-2}	3.65×10^{-2}
100	60	M		2.42	6.46×10^{-3}	1.92×10^{-2}	2.91×10^{-2}
10	60	F	4.93×10^{-1}		8.62×10^{-3}	1.10×10^{-2}	3.51×10^{-2}
10	60	M		2.45	5.38×10^{-3}	1.53×10^{-2}	2.25×10^{-2}
1	60	F	7.88×10^{-2}		7.51×10^{-3}	1.14×10^{-2}	4.32×10^{-2}
1	60	M		2.53	6.21×10^{-3}	1.56×10^{-2}	2.91×10^{-2}
0	60	F	5.59×10^{-2}		7.98×10^{-3}	1.41×10^{-2}	3.29×10^{-2}
0	60	M		2.59	5.38×10^{-3}	1.82×10^{-2}	2.35×10^{-2}
100	30	F	7.13×10^{-2}		8.09×10^{-3}	0.95×10^{-2}	4.15×10^{-2}
100	30	M		2.33	9.25×10^{-3}	1.42×10^{-2}	3.34×10^{-2}
10	30	F	7.30×10^{-2}		8.11×10^{-3}	1.08×10^{-2}	3.14×10^{-2}
10	30	M		2.52	6.55×10^{-3}	1.60×10^{-2}	2.88×10^{-2}
1	30	F	6.08×10^{-2}		8.57×10^{-3}	1.20×10^{-2}	3.91×10^{-2}
1	30	M		2.27	5.63×10^{-3}	1.43×10^{-2}	2.71×10^{-2}
0	30	F	5.58×10^{-2}		7.34×10^{-3}	1.42×10^{-2}	4.38×10^{-2}
0	30	M		2.33	7.16×10^{-3}	1.41×10^{-2}	3.09×10^{-2}

Tissues were taken for histologic examination from the animals receiving EBIS at 1000 and 100 ppm and also from control rats. Histopathologic observations for all experimental groups presented for evaluation are described in Table 16. Tissues examined from the 1000 ppm animals that died spontaneously primarily exhibited mild congestion of the pulmonary aveolar capillaries and pulmonary veins coupled with mild to moderate nonsuppurative interstitial pneumonitis, and bronchitis. Mild congestion was also observed in liver sinusoids and in the pyramidal zone of the kidney. One of these animals that died on day 6 had a marked suppurative interstitial nephritis and another, which died on day 11, had dissecting hemorrhage in skeletal muscles of the rear legs, abdominal wall and vertebral area. The remaining 12 animals which were sacrificed exhibited similar pulmonary, hepatic and nephritic lesions, but in a smaller percentage than the five animals that died spontaneously.

The only histopathologically observable changes in the animals feed 100 ppm was the presence of mast cell granules in the connective tissue of the tongue and elsewhere. This occurred only in the animals feed 100 ppm EBIS for 30 days. This observation was extremely prominent when tissues were stained with H & E but was not apparent with toluidine blue stained sections. The only unique non EBIS-related lesion was the suppurative folliculitis and ulcerative dermatitis that appeared in three control animals and one rat feed 1 ppm EBIS for 60 days. Histopathologic changes such as inflammatory cell infiltration and congestion of the lungs, heart, kidneys and liver, and random lesions of the lymph nodes, adrenals, stomach, brain, and prostate appeared with similar frequency among both control and exposed groups.

The pulmonary lesions combined with otitis media observed in several of the rats which received 1000 ppm EBIS are suggestive of lesions noted with chronic murine pneumonia and are apparently not related to EBIS. The advanced nephritic syndrome or hemorrhagic conditions which were found respectively in two animals which died spontaneously were not encountered in any other animals of this group. No histologic lesion could be identified in either H & E or luxol fast blue-stained sections of brain, spinal cord, or peripheral nerves from paralyzed animals.

The etiology of the dermatitis, observed in a small number of 0 to 100 ppm diet fed animals, remains undetermined after repeated dry mount preparations, skin scrappings, culturing, and histologic examination of numerous sections of affected skin and surrounding tissue. One might speculate that the small number of animals involved may indicate an individual variation in sensitivity to either a cleaning agent or non EBIS diet component associated with the feed cup, as the lesions were all at the shoulder level and cephalad.

The nature of the changes was in general very mild and found in all dosage levels and in controls. The incidence and degree of pulmonary involvement exhibited in rats which received 1000 ppm EBIS may be relatable to non lethal viral infections that are often seen in laboratory rats, but no attempt was made to verify their presence. The similarity of occurrence, or low incidence of other changes noted are interpreted as being of a non EBIS-induced nature. The only deviation from this interpretation is the 90 percent

TABLE 16. HISTOPATHOLOGIC REVIEW, EBIS STUDY
(Reported as ncidence and Percent Incidence)

	Dose, ppm / Days on Diet Animals per Group													
	1000 / 30 17		0 / 30 9		100 / 30 10		0 / 60 11		100 / 60 10		0 / 90 8		100 / 90 10	
	Incidence	Percent Incidence	Incidence	Percent Incidence	Incidence	Percent Incidence	Incidence	Percent Incidence	Incidence	Percent Incidence	Incidence	Percent Incidence	Incidence	Percent Incidence
Skin, ulcerative dermatitis, focal suppurative, moderate	-	-	-	-	-	-	3	.27	-	-	-	-	-	-
Connective tissue, tongue, skin, etc., prominently stained mast cells	-	-	-	-	9	.90	-	-	-	-	-	-	1	.10
Lymph nodes														
Submandibular, congestion or hemorrhage, medullary sinuses, mild	-	-	1	.11	3	.30	-	-	-	-	-	-	-	-
Trachobronchial, medullary sinuses, lymphocytic depletion, mild	-	-	1	.11	-	-	4	.36	-	-	3	.36	3	.30
Trachobronchial, cortical sinuses, histiocytic macrophages, mild	-	-	1	.11	-	-	-	-	-	-	-	-	-	-
Mesenteric, medullary sinuses, lymphocytic depletion, mild	-	-	-	-	3	.30	2	.18	-	-	-	-	-	-
Submandibular, medullary sinuses, lymphocytic depletion, mild	-	-	-	-	1	.10	-	-	-	-	-	-	-	-
Thymus														
Medulla, congestion and extravasated RBC's, mild	1	.05	1	.11	2	.20	-	-	-	-	-	-	-	-
Cortex, lymphocytic depletion, mild	2	.11	-	-	-	-	-	-	-	-	-	-	-	-
Heart, Myocarditis, primarily endocardial, focal, nonsuppurative, mild	1	.05	2	.22	1	.10	-	-	2	.20	1	.12	1	.10
Lung														
Para bronchial lymphoid nodules, mild	-	-	-	-	6	.60	3	.27	3	.30	3	.36	7	.70
Para bronchial lymphoid nodules, moderate	-	-	2	.22	2	.20	1	.009	-	-	2	.24	-	-

incidence of an increase in affinity for the hematoxylin eosin stain, of mast cell granules in the 100 ppm/30 days diet group. Comparative sections from this and other groups stained with toluidine blue stain for metachromasia exhibited no discernable change in granule size, color, or number. No variation in the handling of these tissues throughout their processing has been uncovered.

The data presented show no significant measurable effects of dietary EBIS at dosage levels of 100 ppm and below. Severe toxic responses were observed at 1000 ppm within one week of feeding.

SUMMARY

Ethylenebisisothiocyanate sulfide (EBIS) was fed to groups of rats at 0, 1, 10, 100, and 1000 ppm for up to 90 days. Only those rats receiving EBIS at 1000 ppm demonstrated a toxic response to the test chemical reflected as a reversible paralysis of the hind legs noted within 8 to 14 days. If left on the 1000 ppm diet, the animals soon died. When removed from the diet, the animals recovered, only to become ataxic on further dietary exposure at the high level. No histologic lesion could be identified in either H & E or luxol fast blue stained sections of brain, spinal cord, or peripheral nerves from the paralyzed animals. The ability to reverse the paralysis by removing the animals from the test diet coupled with the lack of histologically observable lesions adds credence to a purposed biochemical lesion.

Ingestion of 1000 ppm EBIS for 7 days also resulted in measurable changes in thyroid function. Total serum thyroxine levels were markedly decreased as was iodide uptake by the thyroid.

Dietary levels of 100, 10, 1 and 0 ppm EBIS for 90 days produced no observable toxicity. Growth as seen by body weight increases, diet consumption and thyroid function were normal. No EBIS-related lesions were detected during the histopathologic evaluation of the 42 tissues taken from rats which received 100 ppm EBIS. A no effect level for this 90 day dietary study for EBIS is 100 ppm in the diet, equivalent to an average intake ranging from 67 mg/kg body weight at week 1 to 31 mg/kg body weight at week 12.

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