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REPORT TO

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

TERATOGENIC STUDIES WITH FROZEN, THERMALLY PROCESSED, COBALT IRRADIATED, AND ELECTRON IRRADIATED BEEF IN ALBINO MICE, ALBINO RATS, GOLDEN SYRIAN HAMSTERS, AND ALBINO RABBITS

CONTRACT NO. DADA 17-71-C-1030

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#43

Industrial **BIO-TEST** *Laboratories, Inc.*
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September 11, 1975

Col. Roger W. Baker
U. S. Army Medical Research
and Development Command
Forrestall Building
Washington, D. C. 20314

Dear Col. Baker:

Re: IBT Nos. 651-01785 and 622-01786 - Teratogenic Studies with Frozen, Thermally Processed, Cobalt Irradiated, and Electron Irradiated Beef in Albino Mice, Albino Rats, Golden Syrian Hamsters, and Albino Rabbits - Contract No. DADA 17-71-C-1030

We are submitting herewith our laboratory report prepared in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:bp

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REPORT TO

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CONTRACT NO. DADA 17-71-C-1030

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I. Introduction

An investigation was initiated, at the request of the U. S. Army Medical Research and Development Command, to determine the teratogenic potential of frozen, thermally processed, cobalt irradiated, and electron irradiated beef in albino mice, albino rats, golden Syrian hamsters, and albino rabbits. This report presents the results of the investigation.

II. Abstract

An investigation was conducted to determine the teratogenic potential of frozen, thermally processed, cobalt irradiated, and electron irradiated beef. Diets containing beef were fed to groups of pregnant albino mice, albino rats, golden Syrian hamsters, and albino rabbits during the period of fetal organogenesis. Each type of beef was fed at dietary concentrations of either 35 or 70 percent beef on a dry matter basis. Each beef diet was fed to 2 separate groups of pregnant animals. The experimental design included 2 control groups fed the appropriate standard stock ration. The experimental design for the experiments with albino mice, albino rats, and golden Syrian hamsters included 2 additional control groups fed the semi-purified diet which comprised the remaining portion of each of the beef diets in these 3 experiments. The parameters recorded included maternal body weights, food consumption, reproductive effects, and fetal body weight, external development, skeletal development, and internal development. The 24-hour survival of the young was also observed in the experiment with albino rabbits.

The groups of albino mice, albino rats, and golden Syrian hamsters fed diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef exhibited no effects in maternal body weights or reproductive findings. The groups of these animals fed beef diets consumed more food than that consumed by the appropriate control groups fed the standard stock ration. The groups of albino rabbits fed diets containing beef exhibited either body weight losses or reductions of body weight gains during Gestation

Days 6 through 18 when the animals were fed the test diets. The albino rabbits fed beef diets exhibited normal overall body weight gains during Gestation Days 0 through 29. The albino rabbits fed beef diets consumed less food than that consumed by the control groups fed the standard stock ration. The food consumption reductions observed in the groups of albino rabbits fed diets containing beef were expected, as beef does not comprise part of the normal diet of these animals. Reproductive parameters measured in the beef fed rabbits were not significantly different than those of the controls.

An increase in fetal malformations was not observed in any species fed either of the 4 beef diets. No effects were noted in the body weights, external development, skeletal development, or internal development of the fetuses obtained from the albino mice, albino rats, golden Syrian hamsters, and albino rabbits which could be attributed to prenatal exposure to diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef. The 24-hour survival of the young obtained from the albino rabbits fed diets containing beef was comparable to that of the young obtained from the control groups fed the standard stock ration.

Feeding diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef to groups of pregnant albino mice, albino rats,

golden Syrian hamsters and albino rabbits during the period of fetal organogenesis did not induce teratogenic responses in these test systems.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Diet Preparation

The proportions of dietary constituents for the diets fed to the albino mice, albino rats, and golden Syrian hamsters are listed in Table I. The proportions of the dietary constituents for the diets fed to the albino rabbits are listed in Table II. The formulation of the complete semipurified diet is presented in Tables III-A, III-B, and III-C. The frozen beef was removed from the freezer 3 days prior to anticipated use and was placed in a refrigerator to thaw. The frozen, thermally processed, and cobalt irradiated beef cans were all checked with a vacuum gauge prior to being opened to ensure that a vacuum of at least 1 inch existed. The electron irradiated beef packets were all inspected for structural integrity and proper sealing before being used.

The entire contents of the containers were placed in covered stainless steel pans and were heated in a preheated convection oven at 400°F for 15 minutes immediately after the containers were opened and the contents were weighed. The covers were removed from the pans during the last 3 minutes of cooking. Immediately after cooking, the appropriate amount of beef and either semipurified diet or standard stock ration (in the experiment with albino rabbits only) for each diet was placed in a high speed blender and mixed into a thick paste. Following mixing, each diet was immediately fed or was stored up to 48 hours before use.

TABLE I

TEST MATERIAL: Irradiated Beef

Teratogenic Studies: Albino Mice, Albino Rats, and Golden Syrian Hamsters

Proportions of Dietary Constituents

Group	Diet	Beef* (percent)	Dietary Constituents Complete Semipurified Diet (percent)	Standard Stock Ration (percent)
Control I	Purina Chow	0	0	100
Control II	Purina Chow	0	0	100
CGN I	Complete Semipurified Diet	0	100	0
CGN II	Complete Semipurified Diet	0	100	0
CGF I	Frozen Beef	35	65	0
CGF II	Frozen Beef	35	65	0
CGF I	Frozen Beef	70	30	0
CGF II	Frozen Beef	70	30	0
CGT I	Thermally Processed Beef	35	65	0
CGT II	Thermally Processed Beef	35	65	0
CGT I	Thermally Processed Beef	70	30	0
CGT II	Thermally Processed Beef	70	30	0
TGCO I	Cobalt Irradiated Beef	35	65	0
TGCO II	Cobalt Irradiated Beef	35	65	0
TGCO I	Cobalt Irradiated Beef	70	30	0
TGCO II	Cobalt Irradiated Beef	70	30	0
TGEL I	Electron Irradiated Beef	35	65	0
TGEL II	Electron Irradiated Beef	35	65	0
TGEL I	Electron Irradiated Beef	70	30	0
TGEL II	Electron Irradiated Beef	70	30	0

* Beef levels are expressed on a dry matter basis.

TABLE II

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Proportions of Dietary Constituents

Group	Diet	Dietary Constituents: Beef* (percent)	Dietary Constituents: Standard Stock Ration (percent)
Control I	Purina Chow	0	100
Control II	Purina Chow	0	100
CGF I	Frozen Beef	35	65
CGF II	Frozen Beef	35	65
CGF I	Frozen Beef	70	30
CGF II	Frozen Beef	70	30
CGT I	Thermally Processed Beef	35	65
CGT II	Thermally Processed Beef	35	65
CGT I	Thermally Processed Beef	70	30
CGT II	Thermally Processed Beef	70	30
TGCO I	Cobalt Irradiated Beef	35	65
TGCO II	Cobalt Irradiated Beef	35	65
TGCO I	Cobalt Irradiated Beef	70	30
TGCO II	Cobalt Irradiated Beef	70	30
TGEL I	Electron Irradiated Beef	35	65
TGEL II	Electron Irradiated Beef	35	65
TGEL I	Electron Irradiated Beef	70	30
TGEL II	Electron Irradiated Beef	70	30

* Beef levels are expressed on a dry matter basis.

TABLE III-A

TEST MATERIAL: Irradiated Beef

Teratogenic Studies - Albino Mice, Albino Rats and Golden Syrian Hamsters

Formulation of the Complete Semipurified Diet

Diet Constituent	%	Lb	Kg
Corn Starch	26.0	100.0	45.4
Glucose	26.0	100.0	45.4
Casein	22.8	87.7	39.9
Fat, Hydrogenated	10.0	38.5	17.5
Corn Oil	5.0	19.2	8.8
Cellulose	4.0	15.4	7.0
Bernhart Tommerilli Salt Mixture*	4.0	15.4	7.0
Vitamin Diet Fortification Mixture**	2.0	7.7	3.5
L-Cystine	0.2	0.8	0.35
Total	100.0	384.7	174.9

* The constituents of the salt mixture are presented in Table III-A.
** The constituents of the vitamin mixture are presented in Table III-B.

TABLE III-B

TEST MATERIAL: Irradiated Beef

Teratogenic Studies - Albino Mice, Albino Rats and Golden Syrian Hamsters

Formulation of the Complete Semipurified Diet

Bernhart Tommerilli Salt Mixture

Salt Mixture Constituents	Percent
Calcium Carbonate	2.100
Calcium Phosphate	73.500
Cupric Citrate	0.046
Ferric Citrate	0.558
Magnesium Oxide	2.500
Manganese Citrate	0.835
Potassium Iodide	0.0007
Potassium Sulfate	6.800
Potassium Phosphate Dibasic	8.100
Sodium Chloride	3.060
Sodium Phosphate	2.140
Zinc Citrate	0.133
Citric Acid	0.117

TABLE III-C

TEST MATERIAL: Irradiated Beef

Teratogenic Studies - Albino Mice, Albino Rats and Golden Syrian Hamsters

Formulation of the Complete Semipurified Diet

Vitamin Diet Fortification Mixture

Vitamin Mixture Constituents	mg of Constituent per 100 g of Semipurified Diet
Vitamin A	0.390
Vitamin D_3	0.003
Vitamin E	11.0
Vitamin K	0.1
Thiamine - HCl	1.0
Riboflavin	1.0
Pyridoxine - HCl	1.0
Niacin	6.0
Calcium-D-pantothenate	4.0
Folic Acid	0.5
Biotin	0.1
Vitamin B_{12}	0.005
Choline bitartrate	180.0
Ascorbic Acid	7.5
Starch to 2 g	

IV. Statistical Evaluations

A. Maternal Body Weights

Statistical analyses were conducted upon maternal body weights. First a One-Way Analysis of Variance was conducted upon the body weights, and significant effects disclosed by that treatment were further studied by the Student's "t"-Test.

B. Reproductive Data

Statistical analyses were conducted upon the data related to reproduction (number of resorption sites, number of animals showing resorption sites, and number of viable fetuses). A Chi-square analysis in a 2 x 2 contingency table with a correction factor for continuity was conducted upon the data related to reproduction.

V. Experiment I - Albino Mice

A. Summary

Diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef were fed to groups of pregnant albino mice during the period of fetal organogenesis. Each type of beef was fed at dietary concentrations of either 35 or 70 percent beef (on a dry matter basis). Each beef diet was fed to 2 groups of pregnant animals. The experimental design included 2 control groups fed a standard stock ration and 2 groups fed the semipurified diet which comprised the remaining portion of each of the beef diets.

Maternal body weight, reproductive effects (including early and late resorption sites, the number of viable fetuses, and the number of females with 1 or more resorption sites), fetal body weight, and fetal development (external, skeletal, and internal) findings are summarized in Table IV. The only group with consistent findings differing from those normally observed for pregnant albino mice of this strain in this laboratory was 1 group fed the diet containing 35 percent electron irradiated beef [TGEL (35%) I]. This group exhibited a reduction in maternal body weight and weight gain, an increase in early resorption sites, a decrease in the number of viable fetuses, an increase in the number of females with 1 or more resorption sites, and an increase in the number of fetuses with incidental skeletal findings. The increase in early resorption sites in this group was due to 1 female with 5 early resorptions. The increase in the number of fetuses with incidental skeletal findings in this group was due to an increase in the incidence of

incompletely ossified and/or non-ossified sternum sections which are indicative of the extent of skeletal calcification at the time of sacrifice. The findings in the TGEL (35%) I group were not confirmed by the results obtained either with the other group fed a diet containing 35 percent electron irradiated beef or with the groups fed a diet containing 70 percent electron irradiated beef. Other findings noted in Table IV as being other than that normally expected were not confirmed as being significant either by the results obtained with the other group fed the same diet or by the results obtained with groups fed the same beef at a higher concentration.

The ingestion of diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef resulted in no maternal body weight changes which could be attributed to exposure to the test diets. The groups fed beef diets consumed 7 to 46 percent more food than that consumed by the control groups. There were no changes noted in the data related to reproduction which could be attributed to the ingestion of diets containing beef. No deaths or abnormal behavioral reactions were noted among any of the animals in this phase of the investigation. No effects on fetal body weights were observed that could be attributed to prenatal exposure to diets containing beef. Fetal external, skeletal, and internal development was comparable in all groups fed either beef diets or control diets. Feeding diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef to pregnant albino mice during the period of fetal organogenesis did not induce a teratogenic response in this test system.

TABLE IV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Summary of Findings

Group	(Beef Content)		Reproductive Effects: Maternal Body Weight	Resorption Sites: Early	Resorption Sites: Late	Number of Viable Fetuses	Number of Females with 1 or More Resorption Sites	Fetal Body Weight	Fetal Development: External	Fetal Development: Skeletal	Fetal Development: Internal
Control	(None)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGN	(None)	I	-	-	-	-	-	-	-	-	-
		II	-	+(I)	-	-	-	-	-	-	-
CGF	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGF	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	+(I)	-	-	-	-
CGT	(35%)	I	-	-	-	-	-	-	-	+	-
		II	-	-	-	-	-	-	-	-	-
CGT	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGCO	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	+(I)	-	-	-	-	-	-	-
TGCO	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	+	-
TGEL	(35%)	I	+(D)	+(I)	-	+(D)	+(I)	-	-	+	-
		II	-	-	-	-	-	-	+	-	-
TGEL	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-

- = Normal findings
+ = Finding other than normal
(I) = increase
(D) = decrease

B. Procedure

1. Experimental Animals

The animals employed were Charles River strain* Swiss Webster mice. The mice were bred at Charles River Breeding Laboratories and copulation was confirmed by sperm-positive results of vaginal examinations. Day zero is defined as the day of insemination. The animals were then shipped directly to this laboratory on Day 3 of gestation.

2. Organization of Groups

A structural outline of this portion of the investigation is given in Table V.

* Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

TABLE V

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Outline of Experiment

Group	Number of Pregnant Animals	Diet	% Beef*
Control I	15	Purina Chow	None
Control II	12	Purina Chow	None
CGN I	13	Complete Semipurified Diet	None
CGN II	12	Complete Semipurified Diet	None
CGF I	16	Frozen Beef	35
CGF II	15	Frozen Beef	35
CGF I	10	Frozen Beef	70
CGF II	19	Frozen Beef	70
CGT I	16	Thermally Processed Beef	35
CGT II	13	Thermally Processed Beef	35
CGT I	13	Thermally Processed Beef	70
CGT II	18	Thermally Processed Beef	70
TGCO I	14	Cobalt Irradiated Beef	35
TGCO II	20	Cobalt Irradiated Beef	35
TGCO I	18	Cobalt Irradiated Beef	70
TGCO II	17	Cobalt Irradiated Beef	70
TGEL I	8	Electron Irradiated Beef	35
TGEL II	13	Electron Irradiated Beef	35
TGEL I	12	Electron Irradiated Beef	70
TGEL II	14	Electron Irradiated Beef	70

* Beef levels are expressed on a dry matter basis.

3. Exposure and Feeding

All diets were prepared in the manner outlined in section III of this report. Animals in control groups I and II were offered the standard stock ration* utilized at this laboratory, as were dams of the test groups prior to and following the treatment period. Dams of each group were offered their respective diets fresh daily from the 6th day of their gestation period to the 15th day inclusive (a total of 10 days of exposure). Daily records of food consumption were recorded. All animals were allowed food and water ad libitum.

4. Body Weight Data

Reported body weight data include the mean group body weights on Days 6 (initial exposure day), 9, 12, and 15 (final day of exposure) of gestation, and sacrifice (Gestation Day 17).

5. Mortality and Reactions

Daily records of mortality and untoward behavioral reactions were maintained throughout the investigation.

6. Reproductive Effects

All females were sacrificed by carbon dioxide asphyxiation on the 17th day of gestation. An incision was made in the abdominal wall and the full extent of both uterine horns was exposed immediately. Fetal swellings and implantation sites were counted, special attention being paid to resorption sites or any other uterine abnormalities.

The number of viable fetuses present in the uterus was determined, spontaneous movement and a more ruddy color distinguishing live from dead animals.

* Purina Rat Chow, Ralston Purina Company, St. Louis, Missouri.

7. Fetal Development

a. Body Weight

All fetuses were removed from the chorion after cutting the umbilical cord. Blotting paper was then used to remove excess amniotic fluid and the fetuses were sexed and weighed.

b. External Examination

An external examination of the fetuses was conducted with special attention paid to detection of the following abnormalities: hydrocephaly, exencephaly, meningoencephalocele, simple meningocele, anophthalmia, microphthalmia, cleft lip, oblique facial cleft, micrognathia, external ear abnormalities in size, shape or position, unusual size or position of the limbs, number and disposition of the digits, umbilical hernia, gastroschisis, myelomeningocele, spina bifida and scoliosis.

c. Fetal Skeletal and Internal Development

All of the fetuses which were obtained were examined for either skeletal or internal development. When possible, equal numbers of fetuses of each sex from each litter were examined by each method. Evaluation of skeletal development was conducted using Hurley's* method of Alizarin staining. Internal development was evaluated using the free-hand razor blade section technique of Wilson and Warkany**.

* Hurley, Lucille S., "Demonstration A-Alizarin Staining of Bone," (revised) Supplement to Teratology Workshop Manual, Berkeley, California, January 25-30, 1965, pp. 121-122.

** Wilson, James G. and Warkany, Josef, Teratology Principles and Techniques, University of Chicago Press, Chicago, Illinois, 1965, pp. 271-277.

C. Results

1. Body Weights

Mean body weight data are presented in Table VI. A slight reduction in body weight gains was observed in the TGEL (35%) I group. The TGEL (35%) II group and both the TGEL (70%) groups exhibited body weight gains comparable to those observed for other groups fed diets containing beef and for the CGN groups fed the semipurified diet. There were no changes in body weights or body weight gains which could be attributed to the ingestion of the test diets.

TABLE VI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Body Weight Data

Summary of Results

Group	(Beef Content)		Mean Body Weight (g) Day of Gestation: 6	9	12	15	17	Total Weight Gain (g)
Control	(None)	I	32	33	35	41	49	17
		II	30	32	35	40	48	18
CGN	(None)	I	33*	34	39**	44*	47	14
		II	30	33	36	41	45*	15
CGF	(35%)	I	30	34*	37*	44*	45*	15
		II	29	35**	39**	45**	46	17
CGF	(70%)	I	30	35*	38*	45**	45*	15
		II	29*	35**	39**	44**	44**	14
CGT	(35%)	I	30	34	36	45**	45*	15
		II	32	36**	38**	48**	51	19
CGT	(70%)	I	32	36**	38*	48**	49	17
		II	30	34	37**	45*	46	16
TGCO	(35%)	I	31	36*	40**	47**	49	18
		II	31	34	39**	46**	48	17
TGCO	(70%)	I	31	36**	39**	45**	49	18
		II	30	34	39**	44**	47	17
TGEL	(35%)	I	31	32	38	44	42**	11
		II	30	32	36	43	43**	13
TGEL	(70%)	I	31	33	37	44**	45*	14
		II	30	33	37	44**	45*	14

* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

2. Food Consumption

Results of the daily food consumption measurements are presented in Table VII. The groups fed diets containing beef consumed 7 to 46 percent more food than that consumed by the control groups fed the standard stock ration. The animals fed diets containing 70 percent beef consumed more food than that consumed by the animals fed diets containing 35 percent beef. The animals fed the semipurified diet (CGN) containing no beef consumed 12 percent more food than that consumed by the control groups.

TABLE VII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Daily Food Consumption Data

Summary of Results

Group	(Beef Content)		Mean Daily Food Consumption (g/mouse) Day of Gestation: 6	7	8	9	10	11	12	13	14	15	Total Food Consumed Days 6-15 (g/mouse)
Control	(None)	I	6.1	8.2	8.1	9.0	8.1	8.4	6.1	7.8	9.7	7.0	78.5
		II	10.0	8.1	9.2	8.3	8.6	7.9	6.5	7.7	8.1	8.2	82.6
CGN	(None)	I	3.8	10.9	9.1	7.9	9.8	9.0	8.5	9.1	11.1	11.7	90.9
		II	2.5	7.6	10.0	8.4	10.7	8.2	8.0	9.4	12.1	13.5	90.4
CGF	(35%)	I	7.4	7.8	6.1	8.6	10.2	10.2	10.1	12.3	10.3	12.0	95.0
		II	4.9	9.2	6.5	9.3	10.7	10.5	11.6	9.7	9.8	12.3	94.5
CGF	(70%)	I	7.9	8.8	7.9	11.1	13.2	11.5	12.5	11.7	11.1	14.0	109.7
		II	6.7	9.2	8.2	10.3	13.4	12.4	12.3	11.1	12.2	13.2	109.0
CGT	(35%)	I	4.2	6.7	10.0	11.1	9.7	11.4	11.6	9.5	12.4	11.4	98.0
		II	5.0	8.4	7.3	10.6	9.6	9.5	10.9	9.0	10.2	10.5	91.0
CGT	(70%)	I	10.2	12.1	11.0	10.3	10.8	12.6	12.0	11.5	13.2	14.2	117.9
		II	8.7	13.3	10.6	13.0	11.4	10.7	12.3	10.7	12.3	13.0	116.0
TGCO	(35%)	I	6.1	10.8	11.5	11.3	10.4	9.1	11.3	8.3	13.0	8.6	100.4
		II	6.6	10.1	10.3	10.2	6.1	8.3	10.5	8.5	12.7	8.7	92.0
TGCO	(70%)	I	6.8	11.1	10.8	12.6	11.8	10.2	12.1	9.9	13.3	10.6	109.2
		II	4.5	8.9	11.6	13.2	10.8	11.0	12.8	11.0	12.4	10.4	106.6
TGEL	(35%)	I	6.5	9.9	6.9	10.2	8.1	11.4	9.6	10.6	12.1	10.0	95.3
		II	5.4	9.1	7.0	10.5	7.2	9.8	7.7	8.4	10.8	10.4	86.3
TGEL	(70%)	I	8.9	9.8	8.9	11.6	8.1	11.1	9.0	8.7	12.9	11.4	100.4
		II	6.6	12.4	9.4	12.7	8.8	12.1	10.0	9.7	11.9	12.7	106.3

3. Mortality and Reactions

There were no deaths among test or control animals during the investigation. No untoward behavioral reactions were observed among test or control animals.

4. Reproductive Effects

The data related to reproduction which were collected at the time of sacrifice on Gestation Day 17 are summarized in Table VIII. These data include the mean numbers of corpora lutea, implantation sites, resorption sites, and viable fetuses per female. The CGN (None) II, TGCO (35%) II, and TGEL (35%) I groups exhibited increases in the mean number of early resorption sites per female. In each group, 1 female accounted for 5 or more early resorption sites. The CGN (None) I, TGCO (35%) I, and TGEL (35%) II groups, which were paired with the groups showing increases in resorption sites, did not exhibit similar effects. The CGF (70%) II and TGEL (35%) I groups exhibited increases in the percent of females with 1 or more resorption sites. The CGF (70%) I and TGEL (35%) II groups did not exhibit increases in the percent of females with 1 or more resorption sites. The above observations demonstrate the normal variability of results expected with albino mice. There were no reproductive effects noted which could be related to the ingestion of the test diets.

TABLE VIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Reproductive Effects

Summary of Results

Group	(Beef Content)		Number of Pregnant Females Examined	Autopsy Findings (Mean/Female) Corpora Lutea	Implantation Sites	Resorption Sites[a] (e)	(l)	Viable Fetuses	Females With 1 or More Resorption Sites Total	Percent
Control	(None)	I	15	11.4	11.0	0.9	0.4	9.7	8	53.3
		II	12	11.4	11.2	0.8	0.3	10.1	8	66.7
CGN	(None)	I	13	11.9	11.8	0.9	0.3	10.6	10	76.9
		II	12	12.7	12.2	1.3[b]	0.0	10.9	8	66.7
CGF	(35%)	I	16	12.0	11.6	0.4*	0.1	11.1	6	37.5
		II	15	12.2	11.9	0.5	0.3	11.0	7	46.7
CGF	(70%)	I	10	12.3	11.4	0.7	0.3	10.4	4	40.0
		II	19	11.3	11.0	1.0	0.5	9.5	17	89.5
CGT	(35%)	I	16	11.8	11.4	0.8	0.0	10.6	10	62.5
		II	13	12.7	12.3	0.5*	0.0	11.8	4	30.8
CGT	(70%)	I	13	12.9	12.0	0.2**	0.0	11.8	3	23.1
		II	18	10.9	10.4	1.0	0.0	9.4	12	66.7
TGCO	(35%)	I	14	12.6	12.1	1.1	0.0	11.0	7	50.0
		II	20	12.4	11.7	1.6[b]	0.1	10.0	15	75.0
TGCO	(70%)	I	18	12.3	11.9	0.6	0.1	11.2	7	38.9
		II	17	10.9	10.5	0.9	0.5	9.1	12	70.5
TGEL	(35%)	I	8	11.7	10.4	1.8**[b]	0.7	7.9	8	100.0
		II	13	12.3	11.9	0.5	0.1	11.3	7	53.8
TGEL	(70%)	I	12	11.8	11.6	0.7	0.1	10.8	7	58.3
		II	14	11.7	11.6	0.4	0.2	11.0	8	57.1

* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.
[a] (e) = Early resorption site; (l) = late resorption site
[b] One female accounted for 5 or more early resorption sites.

5. Fetal Development

a. Body Weight

Fetal body weight data are presented in Table IX. The body weights of the fetuses obtained from some of the groups receiving diets containing beef were slightly reduced. The body weight reductions were minimal and were not dose-related. There were no significant differences in fetal body weight noted between groups fed diets containing irradiated beefs and those fed diets containing non-irradiated beefs. No effects on fetal body weights were observed that could be attributed to prenatal exposure to diets containing beef.

TABLE IX

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Body Weight Data

Summary of Results

Group	(Beef Content)		Number of Males	Mean Body Weight (g)	Number of Females	Mean Body Weight (g)	Number of Males per 100 Fetuses
Control	(None)	I	79	1.0	66	1.1	54.5
		II	70	0.9	51	0.9	57.8
CGN	(None)	I	67	1.0	71	0.9	48.6
		II	70	1.0	61	0.9	53.4
CGF	(35%)	I	90	0.8	87	0.7	50.8
		II	92	0.7	73	0.7	55.8
CGF	(70%)	I	51	0.7	53	0.7	49.0
		II	90	0.8	90	0.7	50.0
CGT	(35%)	I	90	0.8	80	0.8	52.9
		II	88	0.9	66	0.9	57.1
CGT	(70%)	I	86	0.9	68	0.9	55.8
		II	91	0.9	78	0.8	53.8
TGCO	(35%)	I	80	0.7	75	0.7	56.0
		II	112	0.7	88	0.7	56.0
TGCO	(70%)	I	108	0.8	94	0.7	53.5
		II	87	0.7	67	0.7	56.5
TGEL	(35%)	I	28	0.8	35	0.8	44.4
		II	74	0.8	73	0.8	50.3
TGEL	(70%)	I	70	0.8	60	0.8	53.8
		II	73	0.8	80	0.8	47.7

b. External Development

The results of the examinations for fetal external development are presented in Table X. One (1) TGEL (35%) II female accounted for 11 of the 13 runt fetuses noted in this group. This observation was not attributed to the ingestion of the diet for this group. There were no external abnormalities noted in the TGEL (35%) I group which received the same diet. There were no changes in the type or incidence of external abnormalities which could be attributed to the ingestion of the test diets.

TABLE X

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

External Fetal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	145	Hematoma	3	2.1
			Runt	1	0.7
			Total number of fetuses with findings	3	2.1
			Total number of normal fetuses	142	97.9
	II	121	Hematoma	3	2.5
			Runt	2	1.7
			Total number of fetuses with findings	5	4.1
			Total number of normal fetuses	116	95.9
CGN	(None) I	132	Runt	2	1.5
			Gastroschisis	1	0.8
			Total number of fetuses with findings	2	1.5
			Total number of normal fetuses	130	98.5
	II	131	Hematoma	1	0.8
			Total number of fetuses with findings	1	0.8
			Total number of normal fetuses	130	99.2

TABLE X continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

External Fetal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%) I	177	Hematoma	1	0.6
			Runt	1	0.6
			Cranioschisis	1	0.6
			Total number of fetuses with findings	2	1.1
			Total number of normal fetuses	175	98.9
	II	165	No external malformations noted	-	-
CGF	(70%) I	104	Hematoma	1	1.0
			Total number of fetuses with findings	1	1.0
			Total number of normal fetuses	103	99.0
	II	180	Hematoma	1	0.6
			Total number of fetuses with findings	1	0.6
			Total number of normal fetuses	179	99.4
CGT	(35%) I	170	Hematoma	1	0.6
			Total number of fetuses with findings	1	0.6
			Total number of normal fetuses	169	99.4
	II	154	No external malformations noted	-	-

TABLE X continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

External Fetal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(70%) I	154	No external malformations noted	-	-
	II	169	No external malformations noted	-	-
TGCO	(35%) I	155	Cranioschisis	1	0.6
			Total number of fetuses with findings	1	0.6
			Total number of normal fetuses	154	99.4
	II	200	No external malformations noted	-	-
TGCO	(70%) I	202	Hematoma	1	0.5
			Runt	1	0.5
			Total number of fetuses with findings	2	1.0
			Total number of normal fetuses	200	99.0
	II	63	No external malformations noted	-	-

TABLE X continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

External Fetal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%) I	63	No external malformations noted	-	-
	II	147	Runt	13*	8.8
			Total number of fetuses with findings	13	8.8
			Total number of normal fetuses	134	91.2
TGEL	(70%) I	130	Shortened tail	1	0.8
			Total number of fetuses with findings	1	0.8
			Total number of normal fetuses	129	99.2
	II	153	No external malformations noted	-	-

* Eleven (11) fetuses were obtained from 1 litter.

c. Skeletal Development

A summary and interpretation of skeletal findings is presented in Table XI. Observations of fetal skeletal development at caesarian section are presented in Table XII.

Those fetuses having sternum sections that were only partially stained with the calcium positive Alizarin dye were classified as incompletely ossified sections. Sternum sections that did not retain any of the dye were classified as non-ossified. These classifications are measures of the relative extent of fetal skeletal calcification at the time of sacrifice. Incompletely ossified sternum sections, non-ossified sternum sections, and supernumerary ribs are classified as incidental skeletal findings. These incidental skeletal findings are normally observed in fetuses obtained from untreated control does.

A few groups [CGT (35%) I, TGCO (70%) II, TGEL (35%) I] exhibited increases in the percentage of fetuses with incidental skeletal findings. The incidences of incidental skeletal findings in these groups exhibiting increases were within the ranges normally observed for fetuses obtained from albino mice used in similar teratogenic studies conducted in this laboratory. The incidences of incidental skeletal findings were considered normal in all groups. There were no changes in the occurrence of skeletal abnormalities which could be attributed to prenatal exposure to the test diets.

TABLE XI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Summary and Interpretation of Findings

Group	(Beef Content)		Number of Fetuses	Number of Fetuses with Skeletal Findings (Abnormalities + Incidental Findings)	Number of Fetuses with Skeletal Abnormalities	Number of Fetuses with Incidental Findings Only	Number of Fetuses with no Findings	Total Fetuses Unaffected by Treatment
Control	(None)	I	72	43	1	42	29	71
			Percent:	59.7	1.4	58.3	40.3	98.6
		II	59	41	2	39	18	57
			Percent:	69.5	3.4	66.1	30.5	96.6
CGN	(None)	I	66	38	0	38	28	66
			Percent:	57.6	0.0	57.6	42.4	100.0
		II	64	36	1	35	28	63
			Percent:	56.2	1.6	54.7	43.8	98.4
CGF	(35%)	I	89	68	1	67	21	88
			Percent:	76.4	1.1	75.3	23.6	98.9
		II	82	58	1	57	24	81
			Percent:	70.7	1.2	69.5	29.3	98.8
CGF	(70%)	I	57	38	1	37	19	56
			Percent:	66.7	1.8	64.9	33.3	98.2
		II	92	54	3	51	38	89
			Percent:	58.7	3.3	55.4	41.3	96.7
CGT	(35%)	I	87	72	0	72	15	87
			Percent:	82.8	0.0	82.8	17.2	100.0
		II	80	46	0	46	34	80
			Percent:	57.5	0.0	57.5	42.5	100.0
CGT	(70%)	I	80	52	0	52	28	80
			Percent:	65.0	0.0	65.0	35.0	100.0
		II	88	69	0	69	19	88
			Percent:	78.4	0.0	78.4	21.6	100.0
TGCO	(35%)	I	76	55	0	55	21	76
			Percent:	72.4	0.0	72.4	27.6	100.0
		II	100	65	0	65	35	100
			Percent:	65.0	0.0	65.0	35.0	100.0
TGCO	(70%)	I	99	72	0	72	27	99
			Percent:	72.7	0.0	72.7	27.3	100.0
		II	77	63	0	63	14	77
			Percent:	81.8	0.0	81.8	18.1	100.0
TGEL	(35%)	I	31	28	0	28	3	31
			Percent:	90.3	0.0	90.3	9.7	100.0
		II	72	54	1	53	18	71
			Percent:	75.0	1.4	73.6	25.0	98.6
TGEL	(70%)	I	65	33	0	33	32	65
			Percent:	50.8	0.0	50.8	49.2	100.0
		II	77	59	2	57	18	75
			Percent:	76.6	2.6	74.0	23.4	97.4

TABLE XII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	72	Incompletely ossified sternum section(s)	29	40.3
			Non-ossified sternum section(s)	21	29.2
			Sternocostal asymmetry	1	1.4
			Supernumerary ribs	5	6.9
			Total number of fetuses with findings	43	59.7
			Total number of normal fetuses	29	40.3
	II	59	Incompletely ossified sternum section(s)	16	27.1
			Non-ossified sternum section(s)	30	50.8
			Dual ossification of sternum sections	1	1.7
			Supernumerary ribs	9	15.2
			Angulated ribs	1	1.7
			Total number of fetuses with findings	41	69.5
			Total number of normal fetuses	18	30.5

TABLE XII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGN	(None) I	66	Incompletely ossified sternum section(s)	15	22.7
			Non-ossified sternum section(s)	27	40.7
			Total number of fetuses with findings	38	57.6
			Total number of normal fetuses	28	42.4
	II	64	Incompletely ossified sternum section(s)	13	20.3
			Non-ossified sternum section(s)	23	35.9
			Sternocostal asymmetry	1	1.6
			Supernumerary ribs	4	6.2
			Total number of fetuses with findings	36	56.2
			Total number of normal fetuses	28	43.8
CGF	(35%) I	89	Incompletely ossified sternum section(s)	34	38.2
			Non-ossified sternum section(s)	34	38.2
			Sternocostal asymmetry	1	1.1
			Supernumerary ribs	17	19.1
			Total number of fetuses with findings	68	76.4
			Total number of normal fetuses	21	23.6

TABLE XII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%) II	82	Incompletely ossified sternum section(s)	36	43.9
			Non-ossified sternum section(s)	28	34.1
			Sternocostal asymmetry	1	1.2
			Supernumerary ribs	12	14.6
			Total number of fetuses with findings	58	70.7
			Total number of normal fetuses	24	29.3
CGF	(70%) I	57	Incompletely ossified sternum section(s)	29	50.9
			Non-ossified sternum section(s)	21	36.8
			Extra sternum section	1	1.8
			Supernumerary ribs	6	10.5
			Total number of fetuses with findings	38	66.7
			Total number of normal fetuses	19	33.3
	II	92	Incompletely ossified sternum section(s)	24	26.1
			Non-ossified sternum section(s)	30	32.6
			Sternocostal asymmetry	2	2.2
			Extra sternum section	1	1.1
			Supernumerary ribs	11	12.0
			Total number of fetuses with findings	54	58.7
			Total number of normal fetuses	38	41.3

TABLE XII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%) I	87	Incompletely ossified sternum section(s)	37	42.5
			Non-ossified sternum section(s)	43	49.4
			Supernumerary ribs	11	12.6
			Total number of fetuses with findings	72	82.8
			Total number of normal fetuses	15	17.2
	II	80	Incompletely ossified sternum section(s)	20	25.0
			Non-ossified sternum section(s)	16	20.0
			Supernumerary ribs	15	18.8
			Total number of fetuses with findings	46	57.5
			Total number of normal fetuses	34	42.5
CGT	(70%) I	80	Incompletely ossified sternum section(s)	30	37.5
			Non-ossified sternum section(s)	36	45.0
			Supernumerary ribs	11	13.8
			Total number of fetuses with findings	52	65.0
			Total number of normal fetuses	28	35.0
	II	88	Incompletely ossified sternum section(s)	30	34.1
			Non-ossified sternum section (s)	46	52.3
			Supernumerary ribs	11	12.5
			Total number of fetuses with findings	69	78.4
			Total number of normal fetuses	19	21.6

TABLE XII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%) I	76	Incompletely ossified sternum section(s)	33	43.4
			Non-ossified sternum section(s)	31	40.8
			Supernumerary ribs	10	13.2
			Total number of fetuses with findings	55	72.4
			Total number of normal fetuses	21	27.6
	II	100	Incompletely ossified sternum section(s)	25	25.0
			Non-ossified sternum section(s)	45	45.0
			Supernumerary ribs	4	4.0
			Total number of fetuses with findings	65	65.0
			Total number of normal fetuses	35	35.0
TGCO	(70%) I	99	Incompletely ossified sternum section(s)	43	43.4
			Non-ossified sternum section(s)	37	37.4
			Supernumerary ribs	15	15.2
			Total number of fetuses with findings	72	72.7
			Total number of normal fetuses	27	27.3
	II	77	Incompletely ossified sternum section(s)	40	51.9
			Non-ossified sternum section(s)	41	53.2
			Supernumerary ribs	5	6.5
			Total number of fetuses with findings	63	81.8
			Total number of normal fetuses	14	18.1

TABLE XII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%) I	31	Incompletely ossified sternum section(s)	19	61.3
			Non-ossified sternum section(s)	18	58.1
			Supernumerary ribs	4	12.9
			Total number of fetuses with findings	28	90.3
			Total number of normal fetuses	3	9.7
	II	72	Incompletely ossified sternum section(s)	27	37.5
			Non-ossified sternum section(s)	37	51.4
			Supernumerary ribs	9	12.5
			Dome-shaped frontal and parietal bones	1	1.4
			Total number of fetuses with findings	54	75.0
			Total number of normal fetuses	18	25.0

TABLE XII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(70%) I	65	Incompletely ossified sternum section(s)	20	30.8
			Non-ossified sternum section(s)	16	24.6
			Supernumerary ribs	5	7.7
			Total number of fetuses with findings	33	50.8
			Total number of normal fetuses	32	49.2
	II	77	Incompletely ossified sternum section(s)	34	44.1
			Non-ossified sternum section(s)	40	51.9
			Supernumerary ribs	10	13.0
			Malformed (curved) femur and tibula-fibula	1	1.3
			Extra digit	1	1.3
			Total number of fetuses with findings	59	76.6
			Total number of normal fetuses	18	23.4

d. Internal Development

The results of the evaluation of fetal internal development are presented in Table XIII. Large and small atria noted in this table are considered to be incidental findings. Slight variations in the size of the atrium are considered to occur as a result of fixation and the extent of fetal viability prior to sacrifice. There were no changes in the types or incidences of internal findings in any of the groups which could be a attributed to prenatal exposure to the test diets.

TABLE XIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	73	Small atria	5	6.8
			Large atria	3	4.1
			Total number of fetuses with findings	8	11.0
			Total number of normal fetuses	65	89.0
	II	62	Large atria	1	1.6
			Cleft palate	1	1.6
			Total number of fetuses with findings	2	3.2
			Total number of normal fetuses	60	96.8

TABLE XIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGN	(None) I	72	Large atria	2	2.8
			Cleft palate	1	1.4
			Gastroschisis	1	1.4
			Total number of fetuses with findings	4	5.6
			Total number of normal fetuses	68	94.4
	II	67	Large atria	1	1.5
			Total number of fetuses with findings	1	1.5
			Total number of normal fetuses	66	98.5

TABLE XIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%) I	88	Large atria	9	10.2
			Total number of fetuses with findings	9	10.2
			Total number of normal fetuses	79	89.8
	II	83	Small atria	1	1.2
			Large atria	15	18.1
			Total number of fetuses with findings	16	19.3
			Total number of normal fetuses	67	80.7
CGF	(70%) I	47	Large atria	7	14.9
			Total number of fetuses with findings	7	14.9
			Total number of normal fetuses	40	85.1
	II	88	Large atria	10	11.4
			Total number of fetuses with findings	10	11.4
			Total number of normal fetuses	78	88.6

TABLE XIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%) I	83	Small atria	1	1.2
			Large atria	2	2.4
			Total number of fetuses with findings	3	3.6
			Total number of normal fetuses	80	96.4
	II	74	Large atria	11	14.9
			Total number of fetuses with findings	11	14.9
			Total number of normal fetuses	63	85.1
CGT	(70%) I	74	Large atria	5	6.8
			Cleft palate	1	1.4
			Total number of fetuses with findings	6	8.1
			Total number of normal fetuses	68	91.9
	II	81	Small atria	1	1.2
			Large atria	3	3.7
			Total number of fetuses with findings	4	4.9
			Total number of normal fetuses	77	95.1

TABLE XIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%) I	79	Large atria	5	6.3
			Cleft palate	3	3.8
			Total number of fetuses with findings	8	10.1
			Total number of normal fetuses	71	89.9
	II	100	Large atria	3	3.0
			Total number of fetuses with findings	3	3.0
			Total number of normal fetuses	97	97.0
TGCO	(70%) I	103	Large atria	8	7.8
			Total number of fetuses with findings	8	7.8
			Total number of normal fetuses	95	92.2
	II	77	Large atria	4	5.2
			Total number of fetuses with findings	4	5.2
			Total number of normal fetuses	73	94.8

TABLE XIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Mice

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%) I	32	Large atria	5	15.6
			Total number of fetuses with findings	5	15.6
			Total number of normal fetuses	27	84.4
	II	75	Large atria	5	6.7
			Cleft palate	1	1.3
			Total number of fetuses with findings	6	8.0
			Total number of normal fetuses	69	92.0
TGEL	(70%) I	65	Large atria	6	9.2
			Cleft palate	1	1.5
			Total number of fetuses with findings	7	10.8
			Total number of normal fetuses	58	89.2
	II	76	Large atria	6	7.9
			Cleft palate	1	1.3
			Total number of fetuses with findings	7	9.2
			Total number of normal fetuses	69	90.8

VI. Experiment II - Albino Rats

A. Summary

Diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef were fed to groups of pregnant albino rats during the period of fetal organogenesis. Each type of beef was fed at dietary concentrations of either 35 or 70 percent beef (on a dry matter basis). Each beef diet was fed to 2 groups of pregnant animals. The experimental design included 2 control groups fed a standard stock ration and 2 groups fed the semipurified diet which comprised the remaining portion of each of the beef diets.

Maternal body weight, reproductive effects (including early and late resorption sites, the number of viable fetuses, and the number of females with 1 or more resorption sites), fetal body weight, and fetal development (external, skeletal, and internal) findings are summarized in Table XIV. None of the groups had consistent findings differing from those normally observed for pregnant albino rats of this strain in this laboratory. The findings noted in Table XIV as being other than that normally expected were not confirmed as being significant by either the results obtained with the other group fed the same diet or by the results obtained with groups fed the same beef at a higher concentration.

The ingestion of diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef resulted in no maternal body weight changes which could be attributed to the test diets. The groups fed beef

diets generally consumed more food than that consumed by the control groups. There were no changes noted in the data related to reproduction which could be attributed to the ingestion of diets containing beef. No deaths or abnormal behavioral reactions were noted among any of the animals in this phase of the investigation. The body weights of fetuses obtained from females fed diets containing beef were comparable to those of fetuses obtained from females fed control diets (standard stock ration or semipurified diet). Examinations for external, skeletal, and internal development revealed no findings which could be attributed to prenatal exposure to the test diets. Feeding diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef to pregnant albino rats during the period of fetal organogenesis did not induce a teratogenic response in this test system.

TABLE XIV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Summary of Findings

Group	(Beef Content)		Reproductive Effects: Maternal Body Weight	Resorption Sites: Early	Resorption Sites: Late	Number of Viable Fetuses	Number of Females with 1 or More Resorption Sites	Fetal Body Weight	Fetal Development: External	Fetal Development: Skeletal	Fetal Development: Internal
Control	(None)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGN	(None)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGF	(35%)	I	-	+(I)	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGF	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGT	(35%)	I	-	+(I)	-	-	-	-	-	-	+
		II	-	-	-	-	-	-	-	-	+
CGT	(70%)	I	-	-	-	-	-	-	-	+	-
		II	-	-	-	-	-	-	-	-	-
TGCO	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGCO	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGEL	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGEL	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-

- = Normal findings
+ = Finding other than normal
(I) = Increase

B. Procedure

1. Experimental Animals

The animals employed were Charles River strain* albino rats. The rats were bred at Charles River Breeding Laboratories and copulation was confirmed by sperm-positive results of vaginal examinations. Day zero is defined as the day of insemination. The animals were then shipped directly to this laboratory on Day 1 of gestation.

2. Organization of Groups

A structural outline of this portion of the investigation is given in Table XV.

* Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

TABLE XV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Outline of Experiment

Group	Number of Pregnant Animals	Diet	% Beef*
Control I	19	Purina Chow	None
Control II	21	Purina Chow	None
CGN I	19	Complete Semipurified Diet	None
CGN II	18	Complete Semipurified Diet	None
CGF I	18	Frozen Beef	35
CGF II	16	Frozen Beef	35
CGF I	17	Frozen Beef	70
CGF II	17	Frozen Beef	70
CGT I	16	Thermally Processed Beef	35
CGT II	19	Thermally Processed Beef	35
CGT I	16	Thermally Processed Beef	70
CGT II	14	Thermally Processed Beef	70
TGCO I	19	Cobalt Irradiated Beef	35
TGCO II	20	Cobalt Irradiated Beef	35
TGCO I	18	Cobalt Irradiated Beef	70
TGCO II	19	Cobalt Irradiated Beef	70
TGEL I	14	Electron Irradiated Beef	35
TGEL II	20	Electron Irradiated Beef	35
TGEL I	15	Electron Irradiated Beef	70
TGEL II	20	Electron Irradiated Beef	70

* Beef levels are expressed on a dry matter basis.

3. Exposure and Feeding

All diets were prepared in the manner outlined in section III of this report. Animals in control groups I and II were offered the standard stock ration* utilized at this laboratory, as were dams of the test groups prior to and following the treatment period. Dams of each group were offered their respective test diets fresh daily from the 6th day of their gestation period to the 15th day inclusive (a total of 10 days of exposure). Daily records of food consumption were recorded. All animals were allowed food and water ad libitum.

4. Body Weight Data

Body weight data reported include the mean group body weights on Days 6 (initial exposure day), 9, 12, and 15 (final day of exposure) of gestation, and sacrifice (Gestation Day 20).

5. Mortality and Reactions

Daily records of mortality and untoward behavioral reactions were maintained throughout the investigation.

6. Reproductive Effects

All females were sacrificed by carbon dioxide asphyxiation on the 20th day of gestation. An incision was made in the abdominal wall and the full extent of both uterine horns was exposed immediately. Fetal swellings and implantation sites were counted, special attention being paid to resorption sites or any other uterine abnormalities.

The number of viable fetuses present in the uterus was determined, spontaneous movement and a more ruddy color distinguishing live from dead animals.

* Purina Rat Chow, Ralston Purina Company, St. Louis, Missouri.

7. Fetal Development

a. Body Weight

All fetuses were removed from the chorion after cutting the umbilical cord. Blotting paper was then used to remove excess amniotic fluid and the fetuses were sexed and weighed.

b. External Examination

An external examination of the fetuses was conducted with special attention paid to detection of the following abnormalities: hydrocephaly, exencephaly, meningoencephalocele, simple meningocele, anophthalmia, microphthalmia, cleft lip, oblique facial cleft, micrognathia, external ear abnormalities in size, shape or position, unusual size or position of the limbs, number and disposition of the digits, umbilical hernia, gastroschisis, myelomeningocele, spina bifida and scoliosis.

c. Fetal Skeletal and Internal Development

All of the fetuses which were obtained were examined for either skeletal or internal development. When possible, equal numbers of fetuses of each sex from each litter were examined by each method. Evaluation of skeletal development was conducted using Hurley's* method of Alizarin staining. Internal development was evaluated using the free-hand razor blade section technique of Wilson and Warkany**.

* Hurley, Lucille S., "Demonstration A-Alizarin Staining of Bone," (revised) Supplement to Teratology Workshop Manual, Berkeley, California, January 25-30, 1965, pp. 121-122.

** Wilson, James G. and Warkany, Josef, Teratology Principles and Techniques, University of Chicago Press, Chicago, Illinois, 1965, pp. 271-277.

C. Results

1. Body Weight Data

Mean body weight data are presented in Table XVI. The groups fed diets containing 35 percent frozen beef, and either 35 or 70 percent thermally processed and electron irradiated beef exhibited body weight gains which were slightly lower than those of either the control groups or the groups fed the semipurified diet. The other groups fed beef diets exhibited weight gains that were comparable to those of the control groups and to the groups fed the semipurified diet. There were no changes in body weights or body weight gains which could be attributed to the ingestion of the test diets.

TABLE XVI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Body Weight Data

Summary of Results

Group	(Beef Content)		Mean Body Weight (g) Day of Gestation: 6	9	12	15	20	Total Weight Gain (g)
Control	(None)	I	206	223	249	269	333	127
		II	204	224	252	274	340	136
CGN	(None)	I	196*	214*	239*	264	317*	121
		II	203	228	255	282	343	140
CGF	(35%)	I	194**	215	238*	260*	309**	115
		II	192**	211*	233**	259	311**	119
CGF	(70%)	I	199	221	246	272	335	136
		II	190**	216	240	264	310**	120
CGT	(35%)	I	178**	200**	220**	248**	290**	112
		II	172**	189**	214**	240**	283**	111
CGT	(70%)	I	175**	205**	222**	246**	282**	107
		II	171**	191**	218**	245**	280**	109
TGCO	(35%)	I	199	220	247	273	328	129
		II	192**	210**	237**	264	315*	123
TGCO	(70%)	I	191**	211*	236*	263	327	136
		II	190**	210**	235**	260	310**	120
TGEL	(35%)	I	206	213	236*	260	321	115
		II	207	216	244	270	324	117
TGEL	(70%)	I	204	217	241	268	321	117
		II	203	212*	241	273	318*	115

* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

2. Food Consumption

Results of the daily food consumption measurements are presented in Table XVII. The groups fed diets containing 35 percent thermally processed beef consumed slightly less food than that consumed by either the control groups or by the groups fed the semipurified diet. The groups fed diets containing 35 percent frozen beef consumed amounts of food comparable to that consumed by the control groups. All other groups fed diets containing beef consumed 4 to 47 percent more food than that consumed by the control groups fed the standard stock ration. The animals fed diets containing 70 percent beef consumed more food than that consumed by the animals fed diets containing 35 percent beef. The animals fed the semipurified diet (CGN) containing no beef consumed 3 percent more food than that consumed by the control groups.

TABLE XVII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Daily Food Consumption Data

Summary of Results

Group	(Beef Content)		Mean Daily Food Consumption (g/rat) Day of Gestation: 6	7	8	9	10	11	12	13	14	15	Total Food Consumed Days 6-15 (g/rat)
Control	(None)	I	22.3	20.8	18.0	23.3	28.3	31.2	29.4	27.6	27.8	28.2	256.9
		II	22.6	21.8	25.3	26.0	34.7	31.1	30.5	24.5	28.4	29.1	274.0
CGN	(None)	I	19.7	22.8	26.0	23.9	32.6	34.5	29.4	27.0	26.9	27.3	270.1
		II	20.9	23.3	21.5	26.5	31.3	34.8	29.4	31.0	28.9	29.1	276.7
CGF	(35%)	I	13.6	14.2	28.6	25.2	26.0	29.9	37.1	19.6	26.9	37.9	259.0
		II	13.2	13.0	24.1	26.9	27.7	27.7	47.7	20.9	33.6	36.8	271.6
CGF	(70%)	I	23.6	29.3	33.3	33.4	36.3	43.0	43.9	48.6	48.4	50.7	390.5
		II	29.8	31.4	32.2	26.5	33.5	42.3	44.8	45.6	49.1	46.6	381.8
CGT	(35%)	I	5.7	11.3	20.4	25.8	30.6	30.4	33.7	33.1	33.4	29.9	254.3
		II	4.8	8.9	21.5	22.6	30.0	17.2	33.6	34.1	34.6	31.3	238.6
CGT	(70%)	I	9.5	20.9	26.7	32.4	33.1	33.9	35.8	36.8	40.6	33.6	303.3
		II	4.4	10.0	20.1	25.3	33.4	35.6	37.7	36.3	39.2	34.1	276.1
TGCO	(35%)	I	24.5	28.9	31.2	30.1	31.7	35.7	35.9	37.1	36.9	36.4	328.4
		II	22.0	27.2	33.0	28.8	30.6	31.0	35.4	36.0	36.9	35.2	316.1
TGCO	(70%)	I	23.1	28.0	30.0	27.3	31.5	33.7	35.2	35.7	37.2	32.9	314.6
		II	23.4	31.3	31.5	27.9	35.1	37.0	36.9	37.7	37.7	36.5	335.0
TGEL	(35%)	I	30.5	26.8	32.1	31.3	33.1	35.2	32.8	33.1	34.3	38.3	327.5
		II	59.1	30.2	32.5	33.5	35.6	36.2	32.1	35.0	34.7	39.9	368.8
TGEL	(70%)	I	32.0	33.1	36.4	35.3	37.5	40.5	33.6	36.3	37.6	38.5	360.8
		II	32.3	35.0	34.5	36.4	38.8	42.2	36.6	37.5	39.6	39.0	371.9

3. Mortality and Reactions

There were no deaths among test or control animals during the investigation. No untoward behavioral reactions were observed among test or control animals.

4. Reproductive Effects

The data related to reproduction which were collected at the time of sacrifice on Gestation Day 20 are summarized in Table XVIII. These data include the mean numbers of corpora lutea, implantation sites, resorption sites, and viable fetuses per female. The CGF (35%) I, and CGT (70%) I groups exhibited increases in the mean number of early resorption sites per female. In each group, 2 females each accounted for 4 or more early resorption sites. The CGF (35%) II and CGT (70%) II groups, which were paired with the groups showing increases in resorption sites, did not exhibit similar affects. The groups fed diets containing beef exhibited a slightly lower mean number of viable fetuses per female. This was due to a slightly lower number of corpora lutea and thus a slightly lower number of implantation sites in these animals. The above observations demonstrate the normal variability of results expected with albino rats. There were no reproductive effects noted which could be related to the ingestion of the test diets.

TABLE XVIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Reproductive Effects

Summary of Results

Group	(Beef Content)		Number of Pregnant Females Examined	Autopsy Findings (Mean/Female) Corpora Lutea	Implantation Sites	Resorption Sites[a] (e)	(l)	Viable Fetuses	Females with 1 or More Resorption Sites Total	Percent
Control	(None)	I	19	12.0	10.3	0.3	0.0	10.0	5	26.3
		II	21	13.0	10.5	0.2	0.0	10.3	4	19.0
CGN	(None)	I	19	10.8	9.1	0.6*	0.0	8.5	7	36.8
		II	18	13.4	9.9	0.4	0.0	9.5	6	33.3
CGF	(35%)	I	18	11.4	9.9	1.1**[b]	0.0	8.8	9	50.0
		II	16	10.6	8.2	0.3	0.0	7.9	3	18.8
CGF	(70%)	I	17	12.1	10.4	0.4	0.0	10.0	6	35.3
		II	17	12.0	9.5	0.4	0.0	9.1	3	17.6
CGT	(35%)	I	16	11.1	9.8	0.7*	0.0	9.1	8	50.0
		II	19	11.2	10.1	0.7*	0.0	9.4	8	42.1
CGT	(70%)	I	16	12.1	10.0	1.2**[c]	0.0	8.8	7	43.8
		II	14	10.8	9.8	0.5	0.0	9.3	4	28.6
TGCO	(35%)	I	19	11.4	9.3	0.6*	0.0	8.7	8	42.1
		II	20	11.3	9.7	0.6*	0.0	9.1	9	47.4
TGCO	(70%)	I	18	10.8	9.8	0.4	0.0	9.4	6	31.6
		II	19	10.9	9.2	0.5	0.0	8.7	7	36.8
TGEL	(35%)	I	14	11.6	10.1	0.2	0.0	9.9	3	21.4
		II	20	11.1	9.6	0.6	0.0	9.0	9	45.0
TGEL	(70%)	I	15	11.6	9.5	0.4	0.1	9.0	4	26.7
		II	20	11.0	9.6	0.4	0.0	9.2	6	30.0

[a] (e) = early resorption site; (l) = late resorption site.
[b] Two females each accounted for 4 or more early resorption sites.
[c] Two females each accounted for 5 resorption sites.
* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

5. Fetal Development

a. Body Weight

Fetal body weight data are presented in Table XIX. The body weights of the fetuses obtained from some of the groups receiving diets containing beef were slightly reduced. The body weight reductions were minimal and were not dose-related. There were no significant differences in fetal body weight noted between groups fed diets containing irradiated beefs and those fed diets containing non-irradiated beefs. The body weights of fetuses obtained from females fed diets containing beef were comparable to those of fetuses obtained from females fed control diets (standard stock ration or semipurified diet).

TABLE XIX

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Body Weight Data

Summary of Results

Group	(Beef Content)		Number of Males	Mean Body Weight (g)	Number of Females	Mean Body Weight (g)	Number of Males per 100 Fetuses
Control	(None)	I	90	4.4	100	4.1	47.4
		II	112	4.5	104	4.4	51.8
CGN	(None)	I	79	4.5	82	4.2	49.1
		II	88	5.0	83	5.1	51.5
CGF	(35%)	I	77	4.1	73	3.9	51.3
		II	57	4.1	70	4.0	44.9
CGF	(70%)	I	71	4.3	98	4.0	42.0
		II	68	4.4	87	4.1	43.9
CGT	(35%)	I	61	3.7	85	3.5	41.8
		II	89	3.6	89	3.5	50.0
CGT	(70%)	I	77	3.7	64	3.7	54.6
		II	71	4.1	59	3.8	54.1
TGCO	(35%)	I	86	4.3	80	4.0	51.8
		II	82	4.1	99	3.8	45.3
TGCO	(70%)	I	85	4.1	85	4.0	50.0
		II	86	4.9	79	4.5	52.1
TGEL	(35%)	I	70	3.8	69	3.6	50.4
		II	80	4.0	100	3.9	43.8
TGEL	(70%)	I	58	4.4	77	4.2	43.0
		II	101	4.4	83	4.1	54.9

b. External Development

The examinations for fetal external development revealed 2 fetuses with hematomas. One fetus with a hematoma was obtained from a Control (None) I group dam and the other was obtained from a TGCO (70%) I group dam. All other fetuses obtained during this phase of the investigation were judged to be free of gross external abnormalities.

c. Skeletal Development

A summary and interpretation of skeletal findings is presented in Table XX. Observations of fetal skeletal development at caesarian section are presented in Table XXI.

Those fetuses having sternum sections that were only partially stained with the calcium positive Alizarin dye were classified as incompletely ossified sections. Sternum sections that did not retain any of the dye were classified as non-ossified. These classifications are measures of the relative extent of fetal skeletal calcification at the time of sacrifice. Incompletely ossified sternum sections, non-ossified sternum sections, and supernumerary ribs are classified as incidental skeletal findings. These incidental skeletal findings are normally observed in fetuses obtained from untreated control does.

The CGT (70%) I group exhibited an increase in the percentage of fetuses with incidental skeletal findings. The incidences of incidental skeletal findings in the CGT (70%) I group were within the ranges normally observed for fetuses obtained from albino mice used in similar teratogenic studies conducted in this laboratory. The incidences of incidental skeletal findings were considered normal in all groups. There were no changes in the occurrence of skeletal abnormalities which could be attributed to prenatal exposure to the test diets. Observations of fetal skeletal development disclosed no effects which could be attributed to prenatal exposure to the test diets.

TABLE XX

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Summary and Interpretation of Findings

Group	(Beef Content)		Number of Fetuses	Number of Fetuses with Skeletal Findings (Abnormalities + Incidental Findings)	Number of Fetuses with Skeletal Abnormalities	Number of Fetuses with Incidental Findings Only	Number of Fetuses with no Findings	Total Fetuses Unaffected by Treatment
Control	(None)	I	93	30	0	30	63	93
			Percent:	32.2	0.0	32.2	67.8	100.0
		II	104	31	0	31	73	104
			Percent:	29.8	0.0	29.8	70.2	100.0
CGN	(None)	I	77	26	0	26	51	77
			Percent:	33.8	0.0	33.8	66.2	100.0
		II	84	13	0	13	71	84
			Percent:	15.5	0.0	15.5	84.5	100.0
CGF	(35%)	I	75	18	0	18	57	75
			Percent:	24.0	0.0	24.0	76.0	100.0
		II	63	20	0	20	43	63
			Percent:	31.7	0.0	31.7	68.3	100.0
CGF	(70%)	I	84	25	0	25	59	84
			Percent:	29.8	0.0	29.8	70.2	100.0
		II	73	22	1	21	51	72
			Percent:	30.1	1.4	28.8	69.9	98.6
CGT	(35%)	I	71	23	0	23	48	71
			Percent:	32.4	0.0	32.4	67.6	100.0
		II	88	25	0	25	63	88
			Percent:	28.4	0.0	28.4	71.6	100.0
CGT	(70%)	I	70	38	0	38	32	70
			Percent:	54.3	0.0	54.3	45.7	100.0
		II	65	21	0	21	44	65
			Percent:	32.3	0.0	32.3	67.7	100.0
TGCO	(35%)	I	77	21	0	21	56	77
			Percent:	27.3	0.0	27.3	72.7	100.0
		II	85	23	0	23	62	85
			Percent:	27.1	0.0	27.1	72.9	100.0
TGCO	(70%)	I	81	14	0	14	67	81
			Percent:	17.3	0.0	17.3	82.7	100.0
		II	76	10	0	10	66	76
			Percent:	13.2	0.0	13.2	86.8	100.0
TGEL	(35%)	I	69	15	0	15	54	69
			Percent:	21.7	0.0	21.7	78.3	100.0
		II	84	16	0	16	68	84
			Percent:	19.0	0.0	19.0	81.0	100.0
TGEL	(70%)	I	62	9	0	9	53	62
			Percent:	14.5	0.0	14.5	85.5	100.0
		II	87	6	0	6	81	87
			Percent:	6.9	0.0	6.9	93.1	100.0

TABLE XXI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	93	Incompletely ossified sternum section(s)	21	22.6
			Non-ossified sternum section(s)	12	12.9
			Total number of fetuses with findings	30	32.2
			Total number of normal fetuses	63	67.8
	II	104	Incompletely ossified sternum section(s)	25	24.0
			Non-ossified sternum section(s)	4	3.8
			Supernumerary ribs	4	3.8
			Total number of fetuses with findings	31	29.8
			Total number of normal fetuses	73	70.2

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGN	(None) I	77	Incompletely ossified sternum section(s)	18	23.4
			Non-ossified sternum section(s)	4	5.2
			Supernumerary ribs	7	9.1
			Total number of fetuses with findings	26	33.8
			Total number of normal fetuses	51	66.2
	II	84	Incompletely ossified sternum section(s)	5	6.0
			Non-ossified sternum section(s)	2	2.4
			Supernumerary ribs	6	7.1
			Total number of fetuses with findings	13	15.5
			Total number of normal fetuses	71	84.5

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%)	I	75	Incompletely ossified sternum section(s)	16	21.3
				Non-ossified sternum section(s)	3	4.0
				Supernumerary ribs	2	2.7
				Total number of fetuses with findings	18	24.0
				Total number of normal fetuses	57	76.0
		II	63	Incompletely ossified sternum section(s)	11	17.5
				Non-ossified sternum sections	8	12.7
				Supernumerary ribs	2	3.2
				Total number of fetuses with findings	20	31.7
				Total number of normal fetuses	43	68.3

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(70%)	I	84	Incompletely ossified sternum section(s)	13	15.5
				Non-ossified sternum section(s)	5	6.0
				Supernumerary ribs	8	9.5
				Total number of fetuses with findings	25	29.8
				Total number of normal fetuses	59	70.2
		II	73	Incompletely ossified sternum section(s)	12	16.4
				Non-ossified sternum section(s)	10	13.7
				Supernumerary ribs	4	5.5
				Spiraled cauda	1	1.4
				Total number of fetuses with findings	22	30.1
				Total number of normal fetuses	51	69.9

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(70%)	I	70	Incompletely ossified sternum section(s)	27	38.6
				Non-ossified sternum section(s)	15	21.4
				Supernumerary ribs	2	2.9
				Total number of fetuses with findings	38	54.3
				Total number of normal fetuses	32	45.7
		II	65	Incompletely ossified sternum section(s)	15	23.1
				Non-ossified sternum section(s)	4	6.2
				Supernumerary ribs	3	4.6
				Total number of fetuses with findings	21	32.3
				Total number of normal fetuses	44	67.7

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%)	I	77	Incompletely ossified sternum section(s)	15	19.5
				Non-ossified sternum section(s)	1	1.3
				Supernumerary ribs	6	7.8
				Total number of fetuses with findings	21	27.3
				Total number of normal fetuses	56	72.7
		II	85	Incompletely ossified sternum section(s)	14	16.5
				Non-ossified sternum section(s)	5	5.9
				Supernumerary ribs	7	8.2
				Total number of fetuses with findings	23	27.1
				Total number of normal fetuses	62	72.9

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(70%)	I	81	Incompletely ossified sternum section(s)	7	8.6
				Non-ossified sternum section(s)	2	2.5
				Supernumerary ribs	7	8.6
				Total number of fetuses with findings	14	17.3
				Total number of normal fetuses	67	82.7
		II	76	Incompletely ossified sternum section(s)	9	11.8
				Supernumerary ribs	1	1.3
				Total number of fetuses with findings	10	13.2
				Total number of normal fetuses	66	86.8

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%)	I	69	Incompletely ossified sternum section(s)	8	11.6
				Non-ossified sternum section(s)	2	2.9
				Supernumerary ribs	6	8.7
				Total number of fetuses with findings	15	21.7
				Total number of normal fetuses	54	78.3
		II	84	Incompletely ossified sternum section(s)	11	13.1
				Non-ossified sternum section(s)	3	3.6
				Supernumerary ribs	2	2.4
				Total number of fetuses with findings	16	19.0
				Total number of normal fetuses	68	81.0

TABLE XXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(70%)	I	62	Incompletely ossified sternum section(s)	6	9.7
				Non-ossified sternum section(s)	2	3.2
				Supernumerary ribs	1	1.6
				Total number of fetuses with findings	9	14.5
				Total number of normal fetuses	53	85.5
		II	87	Incompletely ossified sternum section(s)	4	4.6
				Non-ossified sternum section(s)	1	1.1
				Supernumerary ribs		
				Total number of fetuses with findings	6	6.9
				Total number of normal fetuses	81	93.1

d. Internal Development

The results of the evaluation of fetal internal development are presented in Table XXII. Large and small atria noted in this table are considered to be incidental findings. Slight variations in the size of the atrium are considered to occur as a result of fixation and the extent of fetal viability prior to sacrifice. Three fetuses, 2 from the CGT (35%) I group and 1 from the CGT (35%) II group were observed with small right kidneys. This finding was not observed among any of the fetuses from the CGT (70%) I and II groups. There were no changes in the types or incidences of internal findings in any of the groups which could be attributed to prenatal exposure to the test diets.

TABLE XXII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	97	Small atria	4	4.1
			Large atria	33	34.0
			Edema	1	1.0
			Total number of fetuses with findings	38	39.2
			Total number of normal fetuses	59	60.8
	II	112	Small atria	5	4.5
			Large atria	22	19.6
			Total number of fetuses with findings	27	24.1
			Total number of normal fetuses	85	75.9

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGN	(None) I	84	Small atria	6	7.1
			Large atria	23	27.4
			Edema	1	1.2
			Total number of fetuses with findings	29	34.5
			Total number of normal fetuses	55	65.5
	II	87	Small atria	11	12.6
			Large atria	7	8.0
			Total number of fetuses with findings	18	20.7
			Total number of normal fetuses	69	79.3

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%)	I	75	Small atria	18	24.0
				Large atria	2	2.7
				Total number of fetuses with findings	20	26.7
				Total number of normal fetuses	55	73.3
		II	64	Small atria	10	15.6
				Large atria	3	4.7
				Total number of fetuses with findings	13	20.3
				Total number of normal fetuses	51	79.7

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(70%)	I	85	Small atria	12	14.1
				Large atria	3	3.5
				Total number of fetuses with findings	15	17.6
				Total number of normal fetuses	70	82.4
		II	82	Small atria	4	4.9
				Large atria	3	3.7
				Total number of fetuses with findings	7	8.5
				Total number of normal fetuses	75	91.5

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%)	I	75	Small atria	11	14.7
				Large atria	1	1.3
				Small kidney (right)	2	2.7
				Total number of fetuses with findings	14	18.7
				Total number of normal fetuses	61	81.3
		II	90	Small atria	19	21.1
				Large atria	1	1.1
				Caudal renal ectopia	2	2.2
				Small kidney (right)	1	1.1
				Total number of fetuses with findings	22	24.4
				Total number of normal fetuses	68	75.6

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(70%)	I	71	Small atria	23	32.4
				Large atria	2	2.8
				Total number of fetuses with findings	25	35.2
				Total number of normal fetuses	46	64.8
		II	65	Small atria	7	10.8
				Large atria	3	4.6
				Enlarged bladder	2	3.1
				Total number of fetuses with findings	11	16.9
				Total number of normal fetuses	54	83.1

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%)	I	89	Small atria	7	7.9
				Large atria	3	3.4
				Total number of fetuses with findings	10	11.2
				Total number of normal fetuses	79	88.8
		II	96	Small atria	4	4.2
				Large atria	2	2.1
				Total number of fetuses with findings	6	6.2
				Total number of normal fetuses	90	93.8

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(70%)	I	89	Small atria	8	9.0
				Large atria	5	5.6
				Total number of fetuses with findings	13	14.6
				Total number of normal fetuses	76	85.4
		II	89	Small atria	3	3.4
				Large atria	7	7.9
				Total number of fetuses with findings	10	11.2
				Total number of normal fetuses	79	88.8

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%)	I	70	Small atria	7	10.0
				Total number of fetuses with findings	7	10.0
				Total number of normal fetuses	63	90.0
		II	96	Small atria	5	5.2
				Large atria	3	3.1
				Total number of fetuses with findings	8	8.3
				Total number of normal fetuses	88	91.7

TABLE XXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rats

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(70%)	I	73	Small atria	1	1.4
				Large atria	6	8.2
				Total number of fetuses with findings	7	9.6
				Total number of normal fetuses	66	90.4
		II	97	Small atria	2	2.1
				Large atria	5	5.2
				Total number of fetuses with findings	7	7.2
				Total number of normal fetuses	90	92.8

VII. Experiment III - Golden Syrian Hamsters

A. Summary

Diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef were fed to groups of pregnant golden Syrian hamsters during the period of fetal organogenesis. Each type of beef was fed at dietary concentrations of either 35 or 70 percent beef (on a dry matter basis). Each beef diet was fed to 2 groups of pregnant animals. The experimental design included 2 control groups fed a standard stock ration and 2 groups fed the semipurified diet which comprised the remaining portion of each of the beef diets.

Maternal body weight, reproductive effects (including early and late resorption sites, the number of viable fetuses, and the number of females with 1 or more resorption sites), fetal body weight, and fetal development (external, skeletal, and internal) findings are summarized in Table XXIII. The only groups with consistent findings differing from those normally observed for pregnant Syrian hamsters of this strain in this laboratory were the control groups fed the semipurified diet [CGN (None) I and II]. These groups exhibited reductions in maternal body weights and weight gains, reductions in the amounts of food consumed, increases in the number of runt fetuses (external development), and increases in the number of fetuses with incidental skeletal findings. Seventy-two (72) percent of the runt fetuses were from 4 females (2 females from each group). The increase in the number of fetuses with

incidental skeletal findings in these groups was due to an increase in the incidence of non-ossified sternum sections, which is indicative of the extent of skeletal calcification at the time of sacrifice. Other findings noted in Table XXIII as being other than that normally expected were not confirmed as being significant by the results obtained with groups fed the same beef at a higher concentration.

The ingestion of diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef resulted in no body weight changes which could be attributed to the test diets. The groups fed beef diets consumed 33 to 267 percent more food than that consumed by the control groups. There were no changes noted in the data related to reproduction which could be attributed to the ingestion of diets containing beef. No deaths or abnormal behavioral reactions were noted among any of the animals in this phase of the investigation. The body weights of fetuses obtained from females fed diets containing beef were comparable to those of fetuses obtained from females fed control diets (standard stock ration or semipurified diet). Fetal external, skeletal, and internal development was comparable in all groups fed either beef diets or the control diet. Feeding diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef to pregnant golden Syrian hamsters during the period of fetal organogenesis did not induce a teratogenic response in this test system.

TABLE XXIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Summary of Findings

Group	(Beef Content)		Reproductive Effects: Maternal Body Weight	Resorption Sites: Early	Resorption Sites: Late	Number of Viable Fetuses	Number of Females with 1 or More Resorption Sites	Fetal Body Weight	Fetal Development: External	Fetal Development: Skeletal	Fetal Development: Internal
Control	(None)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGN	(None)	I	+(D)	-	-	-	-	-	+	+	-
		II	+(D)	-	-	-	-	-	+	+	-
CGF	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGF	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGT	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
CGT	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGCO	(35%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGCO	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-
TGEL	(35%)	I	-	-	-	-	-	-	-	+	-
		II	-	-	-	-	-	-	-	+	-
TGEL	(70%)	I	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-

- = Normal finding (D) = Decrease
+ = Finding other than normal

B. Procedure

1. Experimental Animals

The animals employed were golden Syrian hamsters. The hamsters were bred at Lakeview Hamster Colony* and copulation was confirmed by sperm-positive results of vaginal examinations. Day zero is defined as the day of insemination. The animals were shipped directly to this laboratory on Day 3 of gestation.

2. Organization of Groups

A structural outline of this portion of the investigation is given in Table XXIV.

* Lakeview Hamster Colony, Newfield, New Jersey.

TABLE XXIV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Outline of Experiment

Group	Number of Pregnant Animals	Diet	% Beef*
Control I	24	Purina Chow	None
Control II	25	Purina Chow	None
CGN I	23	Complete Semipurified Diet	None
CGN II	23	Complete Semipurified Diet	None
CGF I	21	Frozen Beef	35
CGF II	15	Frozen Beef	35
CGF I	22	Frozen Beef	70
CGF II	11	Frozen Beef	70
CGT I	23	Thermally Processed Beef	35
CGT II	22	Thermally Processed Beef	35
CGT I	20	Thermally Processed Beef	70
CGT II	21	Thermally Processed Beef	70
TGCO I	19	Cobalt Irradiated Beef	35
TGCO II	18	Cobalt Irradiated Beef	35
TGCO I	18	Cobalt Irradiated Beef	70
TGCO II	24	Cobalt Irradiated Beef	70
TGEL I	23	Electron Irradiated Beef	35
TGEL II	19	Electron Irradiated Beef	35
TGEL I	22	Electron Irradiated Beef	70
TGEL II	22	Electron Irradiated Beef	70

* Beef levels are expressed on a dry matter basis.

3. Exposure and Feeding

All diets were prepared in the manner outlined in section III of this report. Animals in control groups I and II were offered the standard stock ration* utilized at this laboratory, as were dams of the test groups prior to the treatment period. Dams of each group were offered their respective diets fresh daily from the 6th day of their gestation period to the 15th day inclusive (a total of 10 days of exposure). Daily records of food consumption were recorded from Day 6 through 14 inclusive. All animals were allowed food and water ad libitum.

4. Body Weight Data

Body weight data reported include the mean group body weights on Days 6 (initial exposure day), 9, 12, and 15 (final day of exposure and day of sacrifice).

5. Mortality and Reactions

Daily records of mortality and untoward behavioral reactions were maintained throughout the investigation.

6. Reproductive Effects

All females were sacrificed by carbon dioxide asphyxiation on the 15th day of gestation. An incision was made in the abdominal wall and the full extent of both uterine horns was exposed immediately. Fetal swellings and implantation sites were counted, special attention being paid to resorption sites or any other uterine abnormalities.

The number of viable fetuses present in the uterus was determined, spontaneous movement and a more ruddy color distinguishing live from dead animals.

* Purina Rat Chow, Ralston Purina Company, St. Louis, Missouri.

7. Fetal Development

a. Body Weight

All fetuses were removed from the chorion after cutting the umbilical cord. Blotting paper was then used to remove excess amniotic fluid and the fetuses were sexed and weighed.

b. External Examination

An external examination of the fetuses was conducted with special attention paid to detection of the following abnormalities: hydrocephaly, exencephaly, meningoencephalocele, simple meningocele, anophthalmia, microphthalmia, cleft lip, oblique facial cleft, micrognathia, external ear abnormalities in size, shape or position, unusual size or position of the limbs, number and disposition of the digits, umbilical hernia, gastroschisis, myelomeningocele, spina bifida and scoliosis.

c. Fetal Skeletal and Internal Development

All of the fetuses which were obtained were examined for either skeletal or internal development. When possible, equal numbers of fetuses of each sex from each litter were examined by each method. Evaluation of skeletal development was conducted using Hurley's* method of Alizarin staining. Internal development was evaluated using the free-hand razor blade section technique of Wilson and Warkany**.

* Hurley, Lucille S., "Demonstration A-Alizarin Staining of Bone," (revised) Supplement to Teratology Workshop Manual, Berkeley, California, January 25-30, 1965, pp. 121-122.

** Wilson, James G. and Warkany, Josef, Teratology Principles and Techniques, University of Chicago Press, Chicago, Illinois, 1965, pp. 271-277.

C. Results

1. Body Weight Data

Mean body weight data are presented in Table XXV. The animals fed the semipurified diet (CGN (None) I and II) exhibited a 56 percent reduction of body weight gain during the treatment period (Days 6-15). The animals fed test diets containing beef exhibited body weight gains comparable to those of the control animals. There were no changes in body weights or body weight gains which could be attributed to the ingestion of the test diets containing beef.

TABLE XXV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Body Weight Data

Summary of Results

Group	(Beef Content)		Mean Body Weight (g) Day of Gestation: 6	9	12	15	Total Weight Gain (g)
Control	(None)	I	138	146	161	166	28
		II	140	152	163	168	28
CGN	(None)	I	124**	133**	138**	138**	14
		II	127**	131**	134**	137**	10
CGF	(35%)	I	127**	134**	145**	153*	26
		II	124**	130**	145**	153*	29
CGF	(70%)	I	127**	135**	153*	163	36
		II	128**	137*	155	160	32
CGT	(35%)	I	134	142	154	160	26
		II	138	144	160	168	30
CGT	(70%)	I	136	143	162	166	30
		II	138	147	166	175	37
TGCO	(35%)	I	134	136**	153	159	25
		II	142	144	158	162	20
TGCO	(70%)	I	133	140*	159	164	31
		II	133	137**	155	158	25
TGEL	(35%)	I	142	147	161	166	24
		II	135	140*	154	157	22
TGEL	(70%)	I	138	149	163	168	30
		II	138	150	164	175	37

* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

2. Food Consumption

Results of the daily food consumption measurements are presented in Table XXVI. The groups fed diets containing beef consumed 33 to 267 percent more food than that consumed by the control groups fed the standard stock ration. The animals fed diets containing 70 percent beef generally consumed more food than that consumed by the animals fed diets containing 35 percent beef. The animals fed the semipurified diet (CGN) containing no beef consumed 49 percent less food than that consumed by the control groups.

TABLE XXVI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Daily Food Consumption Data

Summary of Results

Group	(Beef Content)		Mean Daily Food Consumption (g/hamster) Day of Gestation: 6	7	8	9	10	11	12	13	14	Total Food Consumed Days 6-14 (g/hamster)
Control	(None)	I	5.6	9.8	12.8	11.7	10.5	14.1	12.0	12.4	12.7	101.6
		II	4.4	11.3	12.4	12.5	10.0	13.9	12.3	13.4	13.3	103.5
CGN	(None)	I	4.7	5.8	4.4	4.8	4.9	6.3	4.6	7.1	8.6	51.2
		II	5.0	5.7	4.6	4.3	5.3	7.6	5.9	7.2	8.3	53.9
CGF	(35%)	I	6.1	9.8	16.1	19.6	23.6	29.5	33.4	33.4	45.9	217.4
		II	10.3	9.3	19.2	19.7	26.9	27.3	31.2	33.3	42.3	219.5
CGF	(70%)	I	10.2	24.1	23.4	23.8	34.5	32.4	35.1	40.4	50.1	274.0
		II	7.6	18.4	16.0	17.9	24.7	23.9	24.9	36.2	33.2	202.8
CGT	(35%)	I	15.1	13.3	14.3	11.6	14.3	26.4	20.3	28.0	37.0	180.3
		II	15.4	14.4	14.8	12.8	16.4	27.0	21.2	22.4	30.2	174.6
CGT	(70%)	I	13.8	18.3	17.4	13.2	18.7	27.0	23.0	23.0	31.2	185.6
		II	14.8	18.4	16.1	15.0	19.4	28.9	22.7	26.8	31.0	193.1
TGCO	(35%)	I	10.9	13.5	15.7	17.3	14.5	20.6	22.6	29.8	32.1	177.0
		II	12.4	16.5	19.3	23.9	23.8	29.4	30.4	32.4	39.5	227.6
TGCO	(70%)	I	9.7	16.4	15.8	17.8	17.8	21.4	26.2	33.4	35.7	194.2
		II	11.2	16.3	18.7	23.0	21.9	23.7	25.6	29.3	26.0	195.7
TGEL	(35%)	I	11.7	14.2	17.8	13.4	12.2	20.0	18.6	20.7	26.5	155.1
		II	11.4	12.1	17.4	10.9	11.8	14.8	17.6	21.1	19.5	136.6
TGEL	(70%)	I	12.5	17.2	19.1	15.4	12.2	17.3	22.3	20.9	20.6	157.5
		II	11.2	17.9	19.9	14.6	12.5	19.9	19.4	20.4	21.0	156.8

3. Mortality and Reactions

There were no deaths among test or control animals during the investigation. No untoward behavioral reactions were observed among test or control animals.

4. Reproductive Effects

The data related to reproduction which were collected at the time of sacrifice on Gestation Day 17 are summarized in Table XXVII. These data include the mean numbers of corpora lutea, implantation sites, resorption sites, and viable fetuses per female. The mean numbers of corpora lutea, implantation sites, resorption sites and viable fetuses per female were comparable in all groups. There were no reproductive effects noted which could be related to the ingestion of the test diets.

TABLE XXVII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Reproductive Effects

Summary of Results

Group	(Beef Content)		Number of Pregnant Females Examined	Autopsy Findings (Mean/Female) Corpora Lutea	Implantation Sites	Resorption Sites[a] (e)	(l)	Viable Fetuses	Number of Females with 1 or More Resorption Sites Total	Percent
Control	(None)	I	24	14.1	13.2	0.3	0.2	12.7	9	37.5
		II	25	13.5	12.5	1.0	0.2	11.3	15	60.0
CGN	(None)	I	23	13.4	12.7	1.2	0.1	11.4	11	45.8
		II	23	13.7	12.6	1.8**	0.8**	10.0	16	72.7
CGF	(35%)	I	21	13.1	12.2	1.0	0.2	11.0	13.	62.0
		II	15	14.5	13.1	0.7	0.1	12.3	8	53.0
CGT	(70%)	I	22	13.9	12.8	0.6	<0.1	12.2	7	31.8
		II	11	13.2	12.3	0.9	0.0	11.4	3	27.2
CGT	(35%)	I	23	14.2	13.9	0.9	<0.1	13.0	11	47.8
		II	22	13.5	12.7	0.4	0.0	12.3	8	36.4
CGT	(70%)	I	20	14.0	13.4	0.6	<0.1	12.8	6	30.0
		II	21	14.1	13.5	0.4	0.0	13.1	6	28.6
TGCO	(35%)	I	19	13.8	12.9	0.9	0.0	12.0	7	36.8
		II	18	14.1	13.0	0.6	<0.1	12.4	7	38.9
TGCO	(70%)	I	18	14.8	13.3	0.9	0.0	12.4	11	61.1
		II	24	14.4	13.3	1.7**	0.2	11.4	20*	83.3
TGEL	(35%)	I	23	14.2	13.2	0.9	0.1	12.2	12	52.1
		II	19	12.3	13.1	1.3	0.0	11.8	12	63.2
TGEL	(70%)	I	22	13.8	12.6	0.5	<0.1	12.1	7	31.8
		II	22	15.0	13.7	0.7	<0.1	13.0	10	45.2

[a] (e) = Early resorption site; (l) = Late resorption site.
* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

5. Fetal Development

a. Body Weight

Fetal body weight data are presented in Table XXVIII. The body weights of the fetuses obtained from animals receiving diets containing beef were comparable to those of fetuses obtained from control animals.

TABLE XXVIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Body Weight Data

Summary of Results

Group	(Beef Content)		Number of Males	Mean Body Weight (g)	Number of Females	Mean Body Weight (g)	Number of Males per 100 Fetuses
Control	(None)	I	144	1.8	161	1.7	47.2
		II	151	1.8	135	1.8	52.8
CGN	(None)	I	127	1.5	138	1.5	47.9
		II	106	1.6	125	1.5	45.9
CGF	(35%)	I	121	2.0	111	1.9	37.5
		II	101	2.0	84	2.1	54.6
CGF	(70%)	I	128	2.1	140	2.0	47.8
		II	51	2.0	74	2.0	41.8
CGT	(35%)	I	126	1.7	171	1.7	42.4
		II	117	1.9	154	1.8	43.1
CGT	(70%)	I	107	1.8	149	1.8	41.8
		II	115	1.9	162	1.9	41.5
TGCO	(35%)	I	110	1.6	119	1.5	48.0
		II	97	1.6	126	1.5	43.5
TGCO	(70%)	I	107	1.7	117	1.4	47.8
		II	111	1.7	163	1.7	40.5
TGEL	(35%)	I	158	1.7	122	1.6	56.4
		II	130	1.7	94	1.7	58.0
TGEL	(70%)	I	124	1.9	141	1.9	46.8
		II	141	1.9	145	1.9	49.3

b. External Development

The results of the examinations for fetal external development are presented in Table XXIX. Two CGN (None) I females accounted for 22 of the 32 runt fetuses observed in this group. Two CGN (None) II females accounted for 23 of the 30 runt fetuses observed in this group. There were no changes in the type or incidence of external abnormalities which could be attributed to ingestion of the test diets containing beef.

TABLE XXIX

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

External Fetal Development

Summary of Results

Group	(Beef Content)		Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None)	I	305	Runt	1	0.3
				Total number of fetuses with findings	1	0.3
				Total number of normal fetuses	304	99.7
		II	286	Hematoma	2	0.7
				Runt	7	2.4
				Total number of fetuses with findings	8	2.8
				Total number of normal fetuses	278	97.2
CGN	(None)	I	265	Hematoma	4	1.5
				Runt	32*	12.1
				Extended lower jaw	1	0.4
				Total number of fetuses with findings	37	14.0
				Total number of normal fetuses	228	86.0
		II	231	Hematoma	2	0.9
				Runt	30**	13.0
				Protruding snout	1	0.4
				Assymmetrical facial features	1	0.4
				Total number of fetuses with findings	34	14.7
				Total number of normal fetuses	197	85.3
CGF	(35%)	I	232	Runt	5	2.2
				Anurous condition	1	0.4
				Slightly edematous	6	2.6
				Total number of fetuses with findings	11	4.7
				Total number of normal fetuses	221	95.3
		II	185	Hematoma	1	0.5
				Runt	4	2.2
				Total number of fetuses with findings	4	2.2
				Total number of normal fetuses	181	97.8
CGF	(70%)	I	268	Runt	1	0.4
				Total number of fetuses with findings	1	0.4
				Total number of normal fetuses	267	99.6
		II	125	Runt	4	3.2
				Total number of fetuses with findings	4	3.2
				Total number of normal fetuses	121	96.8
CGT	(35%)	I	297	Hematoma	1	0.3
				Runt	3	1.0
				Umbilical hernia	1	0.3
				Unusual curvature of spinal column	1	0.3
				Small area of spinal column (posterior) exposed	1	0.3
				Total number of fetuses with findings	7	2.4
				Total number of normal fetuses	290	97.6
		II	271	Runt	2	0.7
				Total number of fetuses with findings	2	0.7
				Total number of normal fetuses	269	99.3
CGT	(70%)	I	256	Cranioschisis	1	0.4
				Slightly edematous	1	0.4
				Malformation of lower jaw	1	0.4
				Cleft palate	1	0.4
				Total number of fetuses with findings	2	0.8
				Total number of normal fetuses	254	99.2
		II	277	Hematoma	1	0.4
				Runt	3	1.1
				Gastroschisis	1	0.4
				Cranioschisis	1	0.4
				Total number of fetuses with findings	3	1.1
				Total number of normal fetuses	274	98.9

TABLE XXIX continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

External Fetal Development

Summary of Results

Group	(Beef Content)		Number of Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%)	I	229	Hematoma	2	0.9
				Total number of fetuses with findings	2	0.9
				Total number of normal fetuses	227	99.1
		II	223	Gastroschisis	1	0.4
				Total number of fetuses with findings	1	0.4
				Total number of normal fetuses	222	99.6
TGCO	(70%)	I	224	Hematoma	2	0.9
				Runt	1	0.4
				Total number of fetuses with findings	3	1.3
				Total number of normal fetuses	221	98.7
		II	274	Hematoma	1	0.4
				Runt	2	0.7
				Hydrocephalus	1	0.4
				Total number of fetuses with findings	3	1.1
				Total number of normal fetuses	271	98.9
TGEL	(35%)	I	280	Hematoma	1	0.4
				Runt	1	0.4
				Total number of fetuses with findings	2	0.7
				Total number of normal fetuses	278	99.3
		II	224	No external malformations noted		
TGEL	(70%)	I	265	Rear limbs malformed	1	0.4
				Total number of fetuses with findings	1	0.4
				Total number of normal fetuses	264	99.6
		II	286	Hematoma	1	0.4
				Runt	1	0.4
				Total number of fetuses with findings	1	0.4
				Total number of normal fetuses	285	99.6

* Twenty-two (22) fetuses were obtained from 2 litters.
** Twenty-three (23) fetuses were obtained from 2 litters.

c. Skeletal Development

A summary and interpretation of skeletal findings is presented in Table XXX. Observations of fetal skeletal development at caesarian section are presented in Table XXXI.

Those fetuses having sternum sections that were only partially stained with the calcium positive Alizarin dye were classified as incompletely ossified sections. Sternum sections that did not retain any of the dye were classified as non-ossified. These classifications are measures of the relative extent of fetal skeletal calcification at the time of sacrifice. Incompletely ossified sternum sections, non-ossified sternum sections, and supernumerary ribs are classified as incidental skeletal findings. These incidental skeletal findings are normally observed in fetuses obtained from untreated control does.

The fetuses obtained from the groups fed the semipurified diet [CGN (None) I and II] exhibited an increase in the number of fetuses with incidental skeletal findings. This increase was due to an increase in the number of fetuses with non-ossified sternum sections. Two groups fed diets containing 35 percent electron irradiated beef [TGEL (35%) I and II] exhibited increases in the percentage of fetuses with findings. The TGEL (35%) I and II groups exhibited increases in incompletely ossified sternum sections that accounted for the increases in the percentage of fetuses with incidental skeletal findings in the groups fed diets containing 70 percent of the same beef [TGEL (70%) I and II]. The incidences of

incidental skeletal findings were considered normal in all groups fed diets containing beef. There were no changes in the occurrence of skeletal abnormalities which could be attributed to prenatal exposure either to the semipurified diet or to diets containing frozen, thermally processed, cobalt irradiated beef, or electron irradiated beef. Observations of fetal skeletal development disclosed no effects which could be attributed to prenatal exposure to diets containing beef.

TABLE XXX

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Summary and Interpretation of Findings

Group	(Beef Content)		Number of Fetuses	Number of Fetuses with Skeletal Findings (Abnormalities + Incidental Findings)	Number of Fetuses with Skeletal Abnormalities	Number of Fetuses with Incidental Findings Only	Number of Fetuses with no Findings	Total Fetuses Unaffected by Treatment
Control	(None)	I	152	105	2	103	47	150
			Percent:	69.1	1.3	67.8	30.9	98.7
		II	139	99	13	86	40	126
			Percent:	71.2	9.4	61.9	28.8	90.6
CGN	(None)	I	134	112	12	100	22	122
			Percent:	83.6	9.0	74.6	16.4	91.0
		II	114	93	2	91	21	112
			Percent:	81.6	1.8	79.8	18.4	98.2
CGF	(35%)	I	111	76	1	75	35	110
			Percent:	68.5	0.9	67.6	31.5	99.1
		II	93	46	4	42	47	89
			Percent:	49.5	4.3	45.2	50.5	95.7
CGF	(70%)	I	132	68	0	68	64	132
			Percent:	51.5	0.0	51.5	48.5	100.0
		II	59	31	2	29	28	57
			Percent:	52.5	3.4	49.2	47.5	96.6
CGT	(35%)	I	144	107	2	105	37	142
			Percent:	74.3	1.4	72.9	25.7	98.6
		II	136	76	2	74	60	134
			Percent:	55.9	1.5	54.4	44.1	98.5
CGT	(70%)	I	124	66	4	62	58	120
			Percent:	53.2	3.2	50.0	46.8	96.8
		II	130	70	3	67	60	127
			Percent:	53.8	2.3	51.5	46.2	97.7
TGCO	(35%)	I	109	75	4	71	34	105
			Percent:	68.8	3.7	65.1	31.2	96.3
		II	104	73	1	72	31	103
			Percent:	70.2	1.0	69.2	29.8	99.0
TGCO	(70%)	I	108	53	1	52	55	107
			Percent:	49.1	0.9	48.1	50.9	99.1
		II	131	63	5	58	68	126
			Percent:	48.1	3.8	44.3	51.9	96.2
TGEL	(35%)	I	135	109	7	102	26	128
			Percent:	80.7	5.2	75.6	19.3	94.8
		II	111	84	2	82	27	109
			Percent:	75.7	1.8	73.9	24.3	98.2
TGEL	(70%)	I	127	83	2	81	44	125
			Percent:	65.4	1.6	63.8	34.6	98.4
		II	134	67	1	66	67	133
			Percent:	50.0	0.7	49.2	50.0	99.3

TABLE XXXI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	152	Incompletely ossified sternum section(s)	82	53.9
			Non-ossified sternum section(s)	39	25.7
			Asymmetry of sternum section(s)	1	0.6
			Bifurcated sternum	1	0.6
			Supernumerary ribs	23	15.1
			Total number of fetuses with findings	105	69.1
			Total number of normal fetuses	47	30.9
	II	139	Incompletely ossified sternum section(s)	59	42.4
			Non-ossified sternum section(s)	41	29.5
			Asymmetry of sternum section(s)	4	2.9
			Bifurcated sternum	3	2.2
			Supernumerary ribs	29	20.9
			Fused ribs	3	2.2
			Forked ribs	2	1.4
			Angulated ribs	1	0.7
			Total number of fetuses with findings	99	71.2
			Total number of normal fetuses	40	28.8

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGN	(None) I	134	Incompletely ossified sternum section(s)	57	42.5
			Non-ossified sternum section(s)	92	68.6
			Dual ossification of sternum section(s)	1	0.7
			Asymmetry of sternum section(s)	1	0.7
			Fused sternum section(s)	1	0.7
			Bifurcated sternum	2	1.5
			Supernumerary ribs	12	9.0
			Fused ribs	3	2.2
			Shortened rib(s)	1	0.7
			Spina bifida	1	0.7
			Mandible extended-nasal curves upward	2	1.5
			Total number of fetuses with findings	112	83.6
			Total number of normal fetuses	22	16.4
	II	114	Incompletely ossified sternum section(s)	59	51.8
			Non-ossified sternum section(s)	59	51.8
			Dual ossification of sternum section(s)	2	1.8
			Supernumerary ribs	14	12.3
			Total number of fetuses with findings	93	81.6
			Total number of normal fetuses	21	18.4

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%)	I	111	Incompletely ossified sternum section(s)	42	37.8
				Non-ossified sternum section(s)	38	34.2
				Asymmetry of sternum section(s)	1	0.9
				Supernumerary ribs	11	9.9
				Total number of fetuses with findings	76	68.5
				Total number of normal fetuses	35	31.5
		II	93	Incompletely ossified sternum section(s)	19	20.4
				Non-ossified sternum section(s)	22	23.6
				Asymmetry of sternum sections	4	4.3
				Supernumerary ribs	10	10.8
				Total number of fetuses with findings	46	49.5
				Total number of normal fetuses	47	50.5

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(70%)	I	132	Incompletely ossified sternum section(s)	52	39.4
				Non-ossified sternum section(s)	21	15.9
				Supernumerary ribs	19	14.4
				Total number of fetuses with findings	68	51.5
				Total number of normal fetuses	64	48.5
		II	59	Incompletely ossified sternum section(s)	23	39.0
				Non-ossified sternum section(s)	19	32.2
				Supernumerary ribs	6	10.2
				Fused ribs	2	3.4
				Total number of fetuses with findings	31	52.5
				Total number of normal fetuses	28	47.5

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%)	I	144	Incompletely ossified sternum section(s)	76	52.8
				Non-ossified sternum section(s)	45	31.2
				Dual ossification of sternum sections	2	1.4
				Supernumerary ribs	28	19.4
				Total number of fetuses with findings	107	74.3
				Total number of normal fetuses	37	25.7
		II	136	Incompletely ossified sternum section(s)	59	43.4
				Non-ossified sternum section(s)	24	17.6
				Supernumerary ribs	14	10.3
				Fissure in mandible	2	1.5
				Total number of fetuses with findings	76	55.9
				Total number of normal fetuses	60	44.1

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(70%)	I	124	Incompletely ossified sternum section(s)	44	35.5
				Non-ossified sternum section(s)	27	21.8
				Supernumerary ribs	23	18.5
				Fused ribs	2	1.6
				Angulated ribs	1	0.8
				Mandible retarded	1	0.8
				Total number of fetuses with findings	66	53.2
				Total number of normal fetuses	58	46.8
		II	130	Incompletely ossified sternum section(s)	51	39.2
				Non-ossified sternum section(s)	21	16.1
				Dual ossification of sternum sections	2	1.5
				Supernumerary ribs	13	10.0
				Fused ribs	1	0.8
				Total number of fetuses with findings	70	53.8
				Total number of normal fetuses	60	46.2

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%)	I	109	Incompletely ossified sternum section(s)	63	57.8
				Non-ossified sternum section(s)	30	27.5
				Asymmetry of sternum sections	1	0.9
				Supernumerary ribs	17	15.6
				Fused ribs	3	2.8
				Total number of fetuses with findings	75	68.8
				Total number of normal fetuses	34	31.2
		II	104	Incompletely ossified sternum section(s)	47	45.2
				Non-ossified sternum section(s)	50	48.1
				Supernumerary ribs	6	5.8
				Fused ribs	1	1.0
				Total number of fetuses with findings	73	70.2
				Total number of normal fetuses	31	29.8

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(70%)	I	108	Incompletely ossified sternum section(s)	46	42.6
				Non-ossified sternum section(s)	17	15.7
				Supernumerary ribs	4	3.7
				Fused ribs	1	0.9
				Total number of fetuses with findings	53	49.1
				Total number of normal fetuses	55	50.9
		II	131	Incompletely ossified sternum section(s)	49	37.4
				Non-ossified sternum section(s)	14	10.7
				Asymmetry of sternum sections	1	0.8
				Supernumerary ribs	10	7.6
				Fused ribs	3	2.3
				Mandible extended	1	0.8
				Total number of fetuses with findings	63	48.1
				Total number of normal fetuses	68	51.9

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%)	I	135	Incompletely ossified sternum section(s)	89	65.9
				Non-ossified sternum section(s)	55	40.7
				Asymmetry of sternum sections	2	1.5
				Bifurcated spinal column	1	0.7
				Supernumerary ribs	12	8.9
				Fused ribs	1	0.7
				Angulated ribs	1	0.7
				Humerus and radius shortened	1	0.7
				Ulna missing	1	0.7
				Total number of fetuses with findings	109	80.7
				Total number of normal fetuses	26	19.3
		II	111	Incompletely ossified sternum section(s)	74	66.7
				Non-ossified sternum section(s)	27	24.3
				Dual ossification of sternum sections	1	0.9
				Asymmetry of sternum sections	1	0.9
				Supernumerary ribs	17	15.3
				Total number of fetuses with findings	84	75.7
				Total number of normal fetuses	27	24.3

TABLE XXXI continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(70%)	I	127	Incompletely ossified sternum section(s)	72	57.5
				Non-ossified sternum section(s)	26	20.5
				Dual ossification of sternum sections	1	0.8
				Supernumerary ribs	4	3.1
				Fused ribs	1	0.8
				Total number of fetuses with findings	83	65.4
				Total number of normal fetuses	44	34.6
		II	134	Incompletely ossified sternum section(s)	56	41.8
				Non-ossified sternum section(s)	9	6.7
				Supernumerary ribs	17	12.7
				Shortened rib(s)	1	0.7
				Total number of fetuses with findings	67	50.0
				Total number of normal fetuses	67	50.0

d. Internal Development

The results of the evaluation of fetal internal development are presented in Table XXXII. Large and small atria noted in this table are considered to be incidental findings. Slight variations in the size of the atrium are considered to occur as a result of fixation and the extent of fetal viability prior to sacrifice. There were no changes in the types or incidences of internal findings in any of the groups which could be attributed to prenatal exposure to the test diets.

TABLE XXXII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	153	Small atria	11	7.2
			Large atria	11	7.2
			Total number of fetuses with findings	22	14.4
			Total number of normal fetuses	131	85.6
	II	147	Small atria	10	6.8
			Large atria	6	4.1
			Total number of fetuses with findings	16	10.9
			Total number of normal fetuses	131	89.1

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGN	(None) I	131	Small atria	8	6.1
			Large atria	12	9.2
			Total number of fetuses with findings	20	15.3
			Total number of normal fetuses	111	84.7
	II	117	Small atria	8	6.8
			Large atria	4	3.4
			Cleft palate	1	0.8
			Hole in heart	1	0.8
			Total number of fetuses with findings	14	12.0
			Total number of normal fetuses	103	88.0

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%)	I	121	Small atria	4	3.3
				Large atria	5	4.1
				Small kidneys	1	1.0
				Undescended testes	2	1.7
				Anurous condition	1	1.0
				Total number of fetuses with findings	12	9.9
				Total number of normal fetuses	109	90.1
		II	92	Small atria	3	3.3
				Large atria	8	8.7
				Total number of fetuses with findings	11	12.0
				Total number of normal fetuses	81	88.0

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(70%)	I	136	Small atria	13	9.6
				Large atria	2	1.5
				Total number of fetuses with findings	15	11.0
				Total number of normal fetuses	121	89.0
		II	66	Small atria	6	9.1
				Total number of fetuses with findings	6	9.1
				Total number of normal fetuses	60	90.9

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%)	I	153	Small atria	4	2.6
				Large atria	4	2.6
				Moderate internal hydrocephalus	1	0.6
				Partially fibroid heart	1	0.6
				Total number of fetuses with findings	9	5.9
				Total number of normal fetuses	144	94.1
		II	135	Small atria	5	3.7
				Large atria	7	5.2
				Cleft palate	1	0.7
				Total number of fetuses with findings	13	9.6
				Total number of normal fetuses	122	90.4

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(70%)	I	132	Small atria	6	4.5
				Large atria	2	1.5
				Total number of fetuses with findings	8	6.1
				Total number of normal fetuses	124	93.9
		II	147	Small atria	4	2.7
				Large atria	7	4.8
				Severe internal hydrocephalus	1	0.7
				Total number of fetuses with findings	12	8.2
				Total number of normal fetuses	135	91.8

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%)	I	120	Small atria	3	2.5
				Large atria	2	1.7
				Total number of fetuses with findings	5	4.2
				Total number of normal fetuses	115	95.8
		II	119	Small atria	6	5.0
				Large atria	9	7.6
				Gastroschisis	1	0.8
				Total number of fetuses with findings	16	13.4
				Total number of normal fetuses	113	86.6

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(70%)	I	116	Small atria	8	6.9
				Large atria	3	2.6
				Total number of fetuses with findings	11	9.5
				Total number of normal fetuses	105	90.5
		II	143	Small atria	2	1.4
				Large atria	5	3.5
				Cleft palate	3	2.1
				Undescended testes	2	1.4
				Total number of fetuses with findings	12	8.4
				Total number of normal fetuses	131	91.6

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%)	I	145	Small atria	1	0.7
				Large atria	10	6.9
				Small kidney (right)	1	0.7
				Total number of fetuses with findings	12	8.3
				Total number of normal fetuses	133	91.7
		II	113	Small atria	3	2.7
				Large atria	9	8.0
				Undescended testes	1	0.9
				Total number of fetuses with findings	13	11.5
				Total number of normal fetuses	100	88.5

TABLE XXXII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Golden Syrian Hamsters

Fetal Internal Development

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(70%)	I	138	Small atria	11	8.0
				Large atria	7	5.1
				Total number of fetuses with findings	18	13.0
				Total number of normal fetuses	120	87.0
		II	152	Small atria	4	2.6
				Large atria	8	5.3
				Fibroid heart	2	1.3
				Total number of fetuses with findings	13	8.6
				Total number of normal fetuses	139	91.4

VIII. Experiment IV - Albino Rabbits

A. Summary

Diets containing either frozen, thermally processed, cobalt irradiated, or electron irradiated beef were fed to groups of pregnant albino rabbits during the period of fetal organogenesis. Each type of beef was fed at dietary concentrations of either 35 or 70 percent beef (on a dry matter basis). Each beef diet was fed to 2 groups of pregnant animals. The experimental design included 2 control groups fed a standard stock ration.

Maternal body weight, reproductive effects (including early and late resorption sites, the number of viable fetuses, and the number of females with 1 or more resorption sites), fetal body weight, 24-hour survival of the young, and fetal development (external, skeletal, and internal) findings are summarized in Table XXXIII. The groups fed frozen, thermally processed, cobalt irradiated, or electron irradiated beef exhibited either body weight losses or reductions of body weight gains during Gestation Days 6 through 18 when the animals were on the test diets. These groups exhibited normal overall body weight gains during Gestation Days 0 through 29. The groups fed beef diets consumed 4 to 25 percent less food than that consumed by the control groups. The food consumption reductions observed in the groups of albino rabbits fed diets containing beef were expected, as beef does not comprise part of the normal diet of these animals. Three (2.1 percent) of the CGF (35%) I group fetuses

were observed with external abnormalities. These 3 fetuses were all from the same CGF (35%) I group doe. This observation was not attributed to the ingestion of the diet for this group. There were no external abnormalities noted in the CGF (35%) II group which received the same diet, and there were no external abnormalities observed which could be attributed to the ingestion of the test diet in the groups fed the diet containing 70 percent of the same beef. The CGF (35%) I, CGF (70%) I and II, and the CGT (35%) I group fetuses exhibited a slight increase in the percent of fetuses with skeletal abnormalities. The increase in skeletal abnormalities in these groups was due to an increase in the number of fetuses with incompletely ossified frontal and/or parietal bones (a finding observed among fetuses obtained from control New Zealand albino rabbits used in similar teratogenic studies conducted in this laboratory). Thirty to 100 percent of the fetuses exhibiting incompletely ossified frontal and/or parietal bones from each of these groups, were obtained from the same litter. No other findings differing from those normally observed for pregnant albino rabbits of this strain in this laboratory were noted.

The ingestion of diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef resulted in no overall body weight changes which could be attributed to the test diets. The groups fed beef diets consumed 4 to 25 percent less food than that consumed by the control groups. The animals fed diets containing 70 percent beef generally consumed amounts of food comparable to those consumed by the animals fed

diets containing 35 percent beef. There were no changes in the data related to reproduction which could be attributed to the ingestion of diets containing beef. No deaths or abnormal behavioral reactions were noted among any of the animals in this phase of the investigation. The body weights of fetuses obtained from females fed diets containing beef were comparable to those of fetuses obtained from females fed the control diet (standard stock ration). Fetal external, skeletal, and internal development was considered normal in all groups fed either beef diets or control diets. Feeding diets containing frozen, thermally processed, cobalt irradiated, or electron irradiated beef to pregnant albino rabbits during the period of fetal organogenesis did not induce a teratogenic response in this test system.

TABLE XXXIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Summary of Findings

Group	(Beef Content)		Maternal Body Weights Gestation Days: 6-18	0-29	Reproductive Effects: Resorption Sites (Early)	(Late)	Number of Viable Fetuses	Number of Females With 1 or More Resorption Sites	Fetal Body Weight	24-Hour Survival of the Young	Fetal Development: External	Skeletal	Internal
Control	(None)	I	-	-	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-	-	-
CGF	(35%)	I	+ (D)	-	-	-	-	-	-	-	+	+	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-
CGF	(70%)	I	+ (D)	-	-	-	-	-	-	-	-	+	-
		II	+ (D)	-	-	-	-	-	-	-	-	+	-
CGT	(35%)	I	+ (D)	-	-	-	-	-	-	-	-	+	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-
CGT	(70%)	I	+ (D)	-	-	-	-	-	-	-	-	-	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-
TGCO	(35%)	I	+ (D)	-	-	-	-	-	-	-	-	-	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-
TGCO	(70%)	I	+ (D)	-	-	-	-	-	-	-	-	-	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-
TGEL	(35%)	I	+ (D)	-	-	-	-	-	-	-	-	-	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-
TGEL	(70%)	I	+ (D)	-	-	-	-	-	-	-	-	-	-
		II	+ (D)	-	-	-	-	-	-	-	-	-	-

Key
- = normal findings
+ = findings other than normal
(D) = decrease

B. Procedure

1. Experimental Animals

The animals employed in the study were New Zealand albino rabbits. Each doe was housed individually for 18 days prior to being employed in the investigation.

2. Organization of Groups

A structural outline of the experiment is provided in Table XXXIV.

TABLE XXXIV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Outline of Experiment

Group	Number of Pregnant Animals	Diet	% Beef*
Control I	17	None	-
Control II	20	None	-
CGF I	18	Frozen Beef	35
CGF II	20	Frozen Beef	35
CGF I	20	Frozen Beef	70
CGF II	20	Frozen Beef	70
CGT I	20	Thermally Processed Beef	35
CGT II	18	Thermally Processed Beef	35
CGT I	15	Thermally Processed Beef	70
CGT II	15	Thermally Processed Beef	70
TGCO I	16	Cobalt Irradiated Beef	35
TGCO II	18	Cobalt Irradiated Beef	35
TGCO I	15	Cobalt Irradiated Beef	70
TGCO II	20	Cobalt Irradiated Beef	70
TGEL I	19	Electron Irradiated Beef	35
TGEL II	19	Electron Irradiated Beef	35
TGEL I	16	Electron Irradiated Beef	70
TGEL II	16	Electron Irradiated Beef	70

* Dietary levels are based on dry matter weight.

3. Breeding

Gestation Day 0 was the day of insemination. Each female received an intravenous injection containing 2.0 mg of pituitary luteinizing hormone per kg of body weight and was inseminated with 0.7 ml of diluted, pooled semen from proven bucks. Bred does were housed individually in hutches for the remainder of the investigation.

4. Exposure and Feeding

All diets were prepared in the manner outlined in section III of this report. Animals in control groups I and II were offered the standard stock rabbit ration utilized at this laboratory, as were the test group does prior to and following the treatment period. The does of each group were offered their respective diets fresh daily from the 6th day of their gestation period through the 18th day (a total of 13 days of exposure). Individual food consumption was measured and recorded daily during the treatment period (Gestation Days 6-18). All animals were allowed food and water ad libitum.

5. Body Weight Data

The body weight data reported include the mean body weights for each group on Days 6 (initial exposure day), 9, 12, 15, and 18 (final day of exposure) of gestation, and sacrifice (Gestation Day 29).

6. Mortality and Reactions

Daily records of mortality and untoward behavioral reactions were maintained throughout the investigation.

7. Reproductive Effects

All females were sacrificed by cervical dislocation on the 29th day of gestation. An incision was made in the abdominal wall and the full extent of both uterine horns was exposed immediately. Fetal swellings and implantation sites were counted, special attention being paid to resorption sites or any other uterine abnormalities.

The number of viable fetuses present in the uterus was determined, spontaneous movement and a more ruddy color distinguishing live from dead animals.

8. Fetal Development

a. Body Weight

All fetuses were removed from the chorion after cutting the umbilical cord and then weighed.

b. External Examination

An external examination of the fetuses was conducted with special attention paid to detection of the following abnormalities: hydrocephaly, exencephaly, meningoencephalocele, simple meningocele, anophthalmia, microphthalmia, cleft lip, oblique facial cleft, micrognathia, external ear abnormalities in size, shape or position, unusual size or position of the limbs, number and disposition of the digits, umbilical hernia, gastroschisis, myelomeningocele, spina bifida and scoliosis.

c. Observation of Viable Young During Incubation

Immediately after external examination, the viable young were placed in an incubator at 37°C. Observations for viability, as indicated by respiratory and paw movements, were made hourly for 7 hours and again after 24 hours.

d. Fetal Internal and Skeletal Development

All young were examined by careful dissection. Particular attention was paid to any differences in size, shape, and orientation of the major organs and blood vessels. An examination of skeletal tissue was then performed employing a modified method for the demonstration of skeletal tissues in embryos as described by Hurley*.

* Hurley, Lucille, S., "Demonstration A-Alizarin Staining of Bone," (revised) Supplement to Teratology Workshop Manual, Berkeley, California, January 25-30, 1965, pp. 121-122.

C. Results

1. Body Weight Data

The mean body weight data are summarized in Table XXXV. The mean body weight gain data are presented in Table XXXVI. The groups fed frozen (CGF), thermally processed (CGT), cobalt irradiated (TGCO), or electron irradiated (TGEL) beef exhibited either body weight losses or reductions of body weight gains during treatment (Gestation Days 6-18). However, the animals from the groups fed diets containing beef exhibited overall (Gestation Days 0-29) body weight gains that were comparable to those normally observed for control New Zealand albino rabbits used in similar teratogenic studies conducted in this laboratory.

TABLE XXXV

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Mean Body Weight Data

Group	(Beef Content)		Number of Does	Mean Body Weights (kg) Day Number: 0	6	9	12	15	18	29
Control	(None)	I	17	3.76	3.76	3.84	3.89	3.98	4.01	4.16
		II	20	3.62	3.62	3.72	3.76	3.83	3.92	3.96
CGF	(35%)	I	18	3.73	3.73	3.66	3.67*	3.77	3.76**	4.02
		II	20	3.72	3.70	3.67	3.69*	3.78*	3.78**	4.06
CGF	(70%)	I	20	3.67	3.71	3.67	3.65*	3.72*	3.73**	3.98
		II	20	3.63	3.68	3.65	3.64**	3.76*	3.75**	3.99
CGT	(35%)	I	20	3.82*	3.89**	3.83	3.91	4.00	4.02	4.21
		II	18	3.85*	3.92**	3.86	3.90	3.97	4.02	4.17
CGT	(70%)	I	15	3.81	3.84	3.74	3.80	3.91	3.91	4.15
		II	15	3.75	3.78	3.75	3.74	3.81	3.85	4.10
TGCO	(35%)	I	16	3.81	3.80	3.75	3.75	3.82	3.82*	4.00
		II	18	3.72	3.74	3.68	3.67*	3.75*	3.75**	3.93
TGCO	(70%)	I	15	3.61	3.63	3.58*	3.59**	3.72*	3.70**	3.89
		II	20	3.93**	3.98**	3.89	3.86	3.94	3.90	4.16
TGEL	(35%)	I	19	3.50*	3.57	3.53**	3.54**	3.63**	3.65**	3.88
		II	19	3.54	3.65	3.60*	3.64*	3.72*	3.77**	3.96
TGEL	(70%)	I	16	3.89*	3.91**	3.83	3.81	3.90	3.90	4.14
		II	16	3.71	3.76	3.65	3.65*	3.74*	3.73**	3.99

* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

TABLE XXXVI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Mean Body Weight Gain Data

Group	(Beef Content)		Mean Body Weight Gains (g) Days 6-18	Days 0-29
Control	(None)	I	250	400
		II	300	340
CGF	(35%)	I	30	290
		II	80	340
CGF	(70%)	I	20	310
		II	70	360
CGT	(35%)	I	130	390
		II	100	320
CGT	(70%)	I	70	340
		II	70	350
TGCO	(35%)	I	20	190
		II	10	210
TGCO	(70%)	I	70	280
		II	-80	230
TGEL	(35%)	I	80	380
		II	120	420
TGEL	(70%)	I	-10	250
		II	-30	280

2. Food Consumption

Results of the daily food consumption measurements are presented in Table XXXVII. The groups fed diets containing beef consumed 4 to 25 percent less food than that consumed by the control groups. The animals fed diets containing cobalt irradiated beef (TGCO groups) consumed slightly less food than that consumed by the animals fed diets containing frozen, thermally processed, or electron irradiated beef. The animals fed diets containing 70 percent beef generally consumed amounts of food comparable to those consumed by the animals fed diets containing 35 percent beef.

TABLE XXXVII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Mean Food Consumption Data

Group	(Beef Content)		Food Consumption (g/rabbit/day) Gestation Day: 6	7	8	9	10	11	12	13	14	15	16	17	18	Total Food Consumed Days 6-18 (g/rabbit)
Control	(None)	I	170	170	170	167	159	153	167	157	159	170	170	170	168	2,150
		II	170	170	170	168	160	155	168	162	161	168	168	169	168	2,157
CGF	(35%)	I	113	136	138	150	150	151	141	152	153	152	160	150	146	1,892
		II	124	138	151	154	156	157	152	154	158	148	160	152	156	1,960
CGF	(70%)	I	79	136	143	150	149	144	145	152	152	156	164	163	156	1,889
		II	71	123	142	160	158	156	166	157	155	136	164	162	163	1,913
CGT	(35%)	I	143	149	148	158	160	158	164	153	162	163	164	167	165	2,054
		II	136	146	141	150	140	144	154	162	164	157	167	162	159	1,982
CGT	(70%)	I	132	131	132	154	156	147	160	167	161	156	161	151	149	1,957
		II	143	130	138	163	165	164	167	169	153	147	156	151	160	2,006
TGCO	(35%)	I	109	107	109	112	116	120	111	144	135	131	131	148	152	1,625
		II	127	145	138	139	132	134	122	144	140	154	144	145	158	1,822
TGCO	(70%)	I	110	134	132	141	136	105	117	130	134	147	145	162	154	1,747
		II	110	133	136	132	128	134	133	146	143	143	148	152	158	1,796
TGEL	(35%)	I	153	159	147	152	159	162	160	155	164	165	162	159	165	2,062
		II	143	150	143	133	150	156	159	159	162	163	169	166	165	2,018
TGEL	(70%)	I	130	149	150	156	147	140	151	148	159	158	163	168	158	1,977
		II	132	129	127	148	131	126	132	152	155	162	155	166	161	1,876

3. Mortality and Reactions

There were no deaths among test or control animals during the investigation. No untoward behavioral reactions were observed among test or control animals.

4. Reproductive Effects

The data related to reproduction which were collected at the time of sacrifice on Gestation Day 29 are summarized in Table XXXVIII. These data include total implantation sites, number of resorption sites, number of live young, number of abortions, and numbers of resorption sites and of live young per 100 implantation sites. The data related to reproduction which were collected at the time of sacrifice revealed no effects which could be related to the ingestion of the test diets.

TABLE XXXVIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Reproductive Effects

Summary of Results

Group	(Beef Content)		Number of Pregnant Does	Number of Implantation Sites	Number of Resorption Sites Early	Number of Resorption Sites Late	Number of Does Showing Resorptions	Number of Live Young	Number of Resorption Sites per 100 IS	Number of Live Young per 100 IS	Number of Fetuses Aborted	Number of Dose Showing Abortions
Control	(None)	I	17	156	7	7	9	142	9.0	91.0	0	0
		II	20	195	19	21	15	155	20.5	79.5	0	0
CGF	(35%)	I	18	161	9	12	11	140	13.0	87.0	0	0
		II	20	182	4	9	8	169	7.1**	92.9	0	0
CGF	(70%)	I	20	166	13	12	12	141	15.1	84.9	0	0
		II	20	162	13	16	15	133	17.9	82.1	0	0
CGT	(35%)	I	20	205	10	15	13	180	12.2	87.8	0	0
		II	18	179	3	8	8	168	6.1**	93.9	0	0
CGT	(70%)	I	15	154	21	21	13	113	26.6**	73.4	0	0
		II	15	125	18	12	9	95	24.0**	76.0	0	0
TGCO	(35%)	I	16	122	16	5	8	101	17.2	82.8	0	0
		II	18	132	3	5	7	124	6.1**	93.9	0	0
TGCO	(70%)	I	15	114	4	21	9	89	21.9	78.1	0	0
		II	20	171	35	13	11	123	28.1**	71.9	0	0
TGEL	(35%)	I	19	153	28	3	11	122	20.3	79.7	0	0
		II	19	176	9	22	14	137	17.6	77.8	8	1
TGEL	(70%)	I	16	133	16	2	7	95	15.9	84.1	0	0
		II	16	124	10	9	11	105	15.3	84.7	0	0

IS = Implantation Sites

* Statistically significant intergroup difference at the 95 percent confidence level.
** Statistically significant intergroup difference at the 99 percent confidence level.

5. Fetal Development

a. Body Weight

Fetal body weight data are presented in Table XXXIX. The body weights of the fetuses from all groups were considered normal for fetuses obtained from control New Zealand albino rabbits used in similar teratogenic studies conducted in this laboratory.

TABLE XXXIX

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Summary of Progeny Body Weights

Group	(Beef Content)		Number of Rabbit Progeny	Mean Body Weights (g)
Control	(None)	I	142	37.3
		II	155	36.4
CGF	(35%)	I	140	35.2
		II	169	36.0
CGF	(70%)	I	141	35.3
		II	133	35.2
CGT	(35%)	I	180	33.1
		II	168	32.6
CGT	(70%)	I	113	36.4
		II	95	37.6
TGCO	(35%)	I	101	37.3
		II	124	38.4
TGCO	(70%)	I	89	38.6
		II	123	35.3
TGEL	(35%)	I	122	36.3
		II	137	34.9
TGEL	(70%)	I	95	37.7
		II	105	39.3

b. Fetal External Development

The results of the examinations for fetal external development are presented in Table XL. Three (2.1 percent) of the CGF (35%) I group fetuses were observed with external abnormalities. These 3 fetuses were all from the same CGF (35%) I group doe. This observation was not attributed to the ingestion of the diet for this group. There were no external abnormalities noted in the CGF (35%) II group which received the same diet. The types and incidences of external abnormalities observed in the other groups have been observed among fetuses obtained from control New Zealand albino rabbits used in similar teratogenic studies conducted in this laboratory . Examinations for fetal external development revealed no effects which could be attributed to the ingestion of the test diets.

TABLE XL

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Gross Fetal Abnormalities

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None)	I	142	No external findings noted	-	-
		II	155	No external findings noted	-	-
CGF	(35%)	I	140	Cervical elongation, enlarged neck (diameter), and bilateral talipes varus	1	0.7
				Cervical elongation, enlarged neck (diameter), and unilateral talipomanus	1	0.7
				Cervical elongation and enlarged neck (diameter)	1	0.7
				Total number of fetuses with findings	3*	2.1
				Total number of normal fetuses	137	97.9
		II	169	No external findings noted	-	-
CGF	(70%)	I	141	Acrania, bilateral talipomanus, bilateral ectrodactyly (forefeet), and unilateral talipes varus	1	0.7
				Total number of fetuses with findings	1	0.7
				Total number of normal fetuses	140	99.3
		II	133	No external findings noted	-	-

TABLE XL continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Gross Fetal Abnormalities

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%)	I	180	Unilateral talipomanus	1	0.6
				Total number of fetuses with findings	1	0.6
				Total number of normal fetuses	179	99.4
		II	168	Acrania	1	0.6
				Total number of fetuses with findings	1	0.6
				Total number of normal fetuses	167	99.4
CGT	(70%)	I	113	No external findings noted	-	-
		II				
		II	95	Hemimelia	1	1.0
				Total number of fetuses with findings	1	1.0
				Total number of normal fetuses	94	99.0
TGCO	(35%)	I	101	No external findings noted	-	-
		II	124	Edema and ascites	1	0.8
				Total number of fetuses with findings	1	0.8
				Total number of normal fetuses	123	99.2
TGCO	(70%)	I	89	No external findings noted	-	-
		II	123	No external findings noted	-	-

TABLE XL continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Gross Fetal Abnormalities

Summary of Results

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%)	I	122	No external findings noted	-	-
		II	137	No external findings noted	-	-
TGEL	(70%)	I	95	No external findings noted	-	-
		II	105	Umbilical hernia	1	1.0
				Total number of fetuses with findings	1	1.0
				Total number of normal fetuses	104	99.0

* The 3 abnormal CGF (35%) I group fetuses were all from the same doe.

c. Observation of Viable Young During Incubation

The numbers of young (both normal and deformed) surviving the 24-hour incubation period are presented in Table XLI. Prenatal exposure to the test diets did not affect the 24-hour survival of the young.

TABLE XLI

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Summary of Observations of Viability of Young

24-Hour Incubation Period

Group	(Beef Content)		Number of Viable Young Hour: 0	1	2	3	4	5	6	7	24	24-Hour Viability Index*
Control	(None)	I	142	138	138	138	138	138	138	135	131	92.2
		II	155	151	151	151	151	151	151	148	145	93.5
CGF	(35%)	I	140	140	135	135	135	135	135	133	122	87.1
		II	169	165	163	163	163	163	163	162	151	89.3
CGF	(70%)	I	141	132	129	126	126	126	126	126	126	89.4
		II	133	130	129	129	129	129	129	129	129	97.0
CGT	(35%)	I	180	171	170	166	166	166	166	166	161	89.4
		II	168	161	159	159	159	159	159	159	154	91.7
CGT	(70%)	I	113	111	111	111	111	111	111	110	99	87.6
		II	95	87	86	86	86	86	86	85	83	87.4
TGCO	(35%)	I	101	100	95	93	93	93	93	93	93	92.1
		II	124	121	120	120	120	120	120	120	117	94.4
TGCO	(70%)	I	89	88	87	86	86	86	86	86	86	96.6
		II	123	114	110	110	108	108	108	106	106	86.2
TGEL	(35%)	I	122	117	117	112	112	112	112	111	111	91.0
		II	137	122	118	117	117	117	117	117	117	85.4
TGEL	(70%)	I	95	93	91	91	91	91	91	89	87	91.6
		II	105	104	102	102	101	101	100	98	98	93.3

$$* \frac{\text{Number of Viable Young at 24 Hours}}{\text{Number of Viable Young at Birth}} \times 100$$

d. Skeletal Development

A summary and interpretation of skeletal findings is presented in Table XLII. Observations of fetal skeletal development at caesarian section are presented in Table XLIII.

Those fetuses having sternum sections that were only partially stained with the calcium positive Alizarin dye were classified as incompletely ossified sections. Sternum sections that did not retain any of the dye were classified as non-ossified. These classifications are measures of the relative extent of fetal skeletal calcification at the time of sacrifice. Incompletely ossified sternum sections, non-ossified sternum sections, supernumerary ribs, and thickened ribs are classified as incidental skeletal findings. These incidental skeletal findings are normally observed in fetuses obtained from untreated control does. The incidences of incidental skeletal findings observed for the fetuses obtained from the TGCO (35%) I and II group does, from the TGEL (35%) I and II group does, and from the TGEL (70%) I and II group does were slightly increased. However, the incidences of incidental skeletal findings in these 6 groups were within the range observed for fetuses obtained from control New Zealand albino rabbits used in similar teratogenic studies conducted in this laboratory. The incidences of incidental skeletal findings observed in the other groups all compared favorably with those observed in the control groups.

The CGF (35%) I, CGF (70%) I and II, and the CGT (35%) I group fetuses exhibited a slight increase in the percent of fetuses with

skeletal abnormalities. The increase in skeletal abnormalities in these groups was due to an increase in the number of fetuses with incompletely ossified frontal and/or parietal bones (a finding observed among fetuses obtained from control New Zealand albino rabbits used in similar teratogenic studies conducted in this laboratory). Thirty to 100 percent of the fetuses exhibiting incompletely ossified frontal and/or parietal bones, from each of these groups, were obtained from the same litter. The types and incidences of the other skeletal abnormalities observed in any group either were considered normal for fetuses obtained from rabbits of this strain in this laboratory or were associated with a gross external abnormality. Observations of fetal skeletal development disclosed no effects which could be attributed to prenatal exposure to the test diets.

TABLE XLII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Summary and Interpretation of Findings

Group	(Beef Content)		Number of Fetuses	Number of Fetuses with Skeletal Findings (Abnormalities + Incidental Findings)	Number of Fetuses with Skeletal Abnormalities	Number of Fetuses with Incidental Findings Only	Number of Fetuses with no Findings	Total Fetuses Unaffected by Treatment
Control	(None)	I	142	90	5	85	52	137
			Percent:	63.4	3.5	59.8	36.6	96.5
		II	155	98	7	91	57	148
			Percent:	63.2	4.5	58.7	36.8	95.5
CGF	(35%)	I	140	71	8	63	69	132
			Percent:	50.7	5.7	45.0	49.3	94.3
		II	169	84	1	83	85	168
			Percent:	49.7	0.6	49.1	50.3	99.4
CGF	(70%)	I	141	81	8	73	60	133
			Percent:	57.4	5.7	51.8	42.6	94.3
		II	133	76	7	69	57	126
			Percent:	57.1	5.3	51.9	42.9	94.7
CGT	(35%)	I	180	87	9	78	93	171
			Percent:	48.3	5.0	43.3	51.7	95.0
		II	168	95	6	89	73	162
			Percent:	56.5	3.6	53.0	43.6	96.4
CGT	(70%)	I	113	67	2	65	46	111
			Percent:	59.3	1.8	57.5	40.7	98.2
		II	95	55	2	53	40	93
			Percent:	57.9	2.1	55.8	42.1	97.9
TGCO	(35%)	I	101	67	0	67	34	101
			Percent:	66.3	0.0	66.3	33.7	100.0
		II	124	90	1	89	34	123
			Percent:	72.5	0.8	71.8	27.4	99.2
TGCO	(70%)	I	89	49	0	49	40	89
			Percent:	55.0	0.0	55.0	45.0	100.0
		II	123	63	2	61	60	121
			Percent:	51.2	1.6	49.6	48.8	98.4
TGEL	(35%)	I	122	85	5	80	37	117
			Percent:	69.7	4.1	65.6	30.3	95.9
		II	137	91	3	88	46	134
			Percent:	66.4	2.2	64.2	33.6	97.8
TGEL	(70%)	I	95	71	0	71	24	95
			Percent:	74.7	0.0	74.7	25.3	100.0
		II	105	74	0	74	31	105
			Percent:	70.5	0.0	70.5	29.5	100.0

TABLE XLIII

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
Control	(None) I	142	Incompletely ossified sternum section(s)	10	7.0
			Non-ossified sternum section(s)	20	14.1
			Supernumerary ribs	66	46.5
			Thickened rib(s)	8	5.6
			Forked ribs	1	0.7
			Asymmetric sternum section(s)	1	0.7
			Cleft sternum section	1	0.7
			Split sternum section	1	0.7
			Supernumerary sternum section	1	0.7
			Total number of fetuses with findings	90	63.4
			Total number of fetuses with no findings	52	36.6
	II	155	Incompletely ossified sternum section(s)	18	11.6
			Non-ossified sternum section(s)	17	11.0
			Supernumerary ribs	59	38.1
			Thickened rib(s)	18	11.6
			Fused ribs	1	0.6
			Asymmetric sternum section(s)	1	0.6
			Fused sternum sections	2	1.3
			Cleft sternum section	1	0.6
			Split sternum section	3	1.9
			Total number of fetuses with findings	98	63.2
			Total number of fetuses with no findings	57	36.8

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(35%)	I	140	Incompletely ossified sternum section(s)	10	7.1
				Non-ossified sternum section(s)	35	25.0
				Supernumerary ribs	27	19.3
				Thickened rib(s)	7	5.0
				Fused ribs	1	0.7
				Shortened rib	1	0.7
				Cleft sternum section	2	1.4
				Split sternum section	2	1.4
				Incompletely ossified frontal and parietal bones	3[a]	2.1
				Total number of fetuses with findings	71	50.7
				Total number of fetuses with no findings	69	49.3
		II	169	Incompletely ossified sternum section(s)	11	6.5
				Non-ossified sternum section(s)	10	5.9
				Supernumerary ribs	60	35.5
				Thickened rib(s)	9	5.3
				Split sternum section	1	0.6
				Total number of fetuses with findings	84	49.7
				Total number of fetuses with no findings	85	50.3

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(70%) I	141	Incompletely ossified sternum section(s)	13	9.2
			Non-ossified sternum section(s)	13	9.2
			Supernumerary ribs	57	40.4
			Thickened rib(s)	5	3.5
			Shortened rib	1	0.7
			Dual ossification of sternum section(s)	1	0.7
			Fused sternum sections	2	1.4
			Split sternum section	2	1.4
			Incompletely ossified frontal and parietal bones	3	2.1
			Acrania, bilateral ectrodactyly (forefeet), and incompletely ossified cervical vertebrae	1	0.7
			Total number of fetuses with findings	81	57.4
			Total number of fetuses with no findings	60	42.6

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGF	(70%)	II	133	Incompletely ossified sternum section(s)	12	9.0
				Non-ossified sternum section(s)	16	12.0
				Supernumerary ribs	52	39.1
				Thickened rib(s)	10	7.5
				Angulated rib(s)	1	0.8
				Dual ossification of sternum section(s)	1	0.8
				Asymmetric sternum section(s)	1	0.8
				Split sternum section	1	0.8
				Incompletely ossified frontal and/or parietal bones	3[b]	2.2
				Total number of fetuses with findings	76	57.1
				Total number of fetuses with no findings	57	42.9

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%) I	180	Incompletely ossified sternum section(s)	14	7.8
			Non-ossified sternum section(s)	13	7.2
			Supernumerary ribs	63	35.0
			Thickened rib(s)	2	1.1
			Dual ossification of sternum section(s)	2	1.1
			Incompletely ossified frontal and/or parietal bones	7[c]	3.9
			Total number of fetuses with findings	87	48.3
			Total number of fetuses with no findings	93	51.7

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(35%) II	168	Incompletely ossified sternum section(s)	17	10.1
			Non-ossified sternum section(s)	9	5.4
			Supernumerary ribs	75	44.6
			Thickened rib(s)	5	3.0
			Angulated rib(s)	1	0.6
			Dual ossification of sternum section(s)	1	0.6
			Split sternum section	1	0.6
			Incompletely ossified frontal and parietal bones	2	1.2
			Acrania	1	0.6
			Incompletely ossified nasal bone	1	0.6
			Total number of fetuses with findings	95	56.5
			Total number of fetuses with no findings	73	43.6

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
CGT	(70%)	I	113	Incompletely ossified sternum section(s)	20	17.7
				Non-ossified sternum section(s)	21	18.6
				Supernumerary ribs	37	32.7
				Thickened rib(s)	2	1.8
				Angulated rib(s)	1	0.9
				Incompletely ossified frontal and parietal bones	1	0.9
				Total number of fetuses with findings	67	59.3
				Total number of fetuses with no findings	46	40.7
		II	95	Incompletely ossified sternum section(s)	7	7.4
				Non-ossified sternum section(s)	10	10.5
				Supernumerary ribs	40	42.1
				Dual ossification of sternum section(s)	1	1.0
				Hemimelia (foreleg)	1	1.0
				Total number of fetuses with findings	55	57.9
				Total number of fetuses with no findings	40	42.1

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(35%)	I	101	Incompletely ossified sternum section(s)	28	27.7
				Non-ossified sternum section(s)	10	9.9
				Supernumerary ribs	48	47.5
				Total number of fetuses with findings	67	66.3
				Total number of fetuses with no findings	34	33.7
		II	124	Incompletely ossified sternum section(s)	29	23.4
				Non-ossified sternum section(s)	8	6.4
				Supernumerary ribs	62	50.0
				Thickened rib(s)	1	0.8
				Angulated rib(s)	1	0.8
				Supernumerary sternum section	1	0.8
				Total number of fetuses with findings	90	72.6
				Total number of fetuses with no findings	34	27.4

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)	Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGCO	(70%) I	89	Incompletely ossified sternum section(s)	17	19.1
			Non-ossified sternum section(s)	9	10.1
			Supernumerary ribs	26	29.2
			Total number of fetuses with findings	49	55.0
			Total number of fetuses with no findings	40	45.0
	II	123	Incompletely ossified sternum section(s)	19	15.4
			Non-ossified sternum section(s)	8	6.5
			Supernumerary ribs	52	42.3
			Shortened rib	1	0.8
			Incompletely ossified frontal and parietal bones	1	0.8
			Total number of fetuses with findings	63	51.2
			Total number of fetuses with no findings	60	48.8

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(35%)	I	122	Incompletely ossified sternum section(s)	38	31.1
				Non-ossified sternum section(s)	14	11.5
				Supernumerary ribs	56	45.9
				Thickened rib(s)	2	1.6
				Asymmetric sternum section(s)	3	2.4
				Cleft sternum section	1	0.8
				Incompletely ossified frontal bones	1	0.8
				Total number of fetuses with findings	85	69.7
				Total number of fetuses with no findings	37	30.3
		II	137	Incompletely ossified sternum section(s)	42	30.6
				Non-ossified sternum section(s)	8	5.8
				Supernumerary ribs	59	43.1
				Thickened rib(s)	1	0.7
				Fused sternum sections	1	0.7
				Supernumerary sternum section	1	0.7
				Incompletely ossified frontal and parietal bones	1	0.7
				Total number of fetuses with findings	91	66.4
				Total number of fetuses with no findings	46	33.6

TABLE XLIII continued

TEST MATERIAL: Irradiated Beef

Teratogenic Study - Albino Rabbits

Fetal Skeletal Development

Skeletal Findings

Group	(Beef Content)		Fetuses Examined	Findings	Incidence	Percent of Total Examined
TGEL	(70%)	I	95	Incompletely ossified sternum section(s)	46	48.4
				Non-ossified sternum section(s)	20	21.0
				Supernumerary ribs	37	38.9
				Total number of fetuses with findings	71	74.7
				Total number of fetuses with no findings	24	25.3
		II	105	Incompletely ossified sternum section(s)	34	32.4
				Non-ossified sternum section(s)	10	9.5
				Supernumerary ribs	49	46.7
				Total number of fetuses with findings	74	70.5
				Total number of fetuses with no findings	31	29.5

a = The 3 pups with incompletely ossified frontal and/or parietal bones were from 1 litter.
b = Two of the 3 pups with incompletely ossified frontal and/or parietal bones were from 1 litter.
c = Two of the 7 pups with incompletely ossified frontal and/or parietal bones were from 1 litter.

e. Internal Development

Dissection of the young obtained from does fed frozen, thermally processed, cobalt irradiated, or electron irradiated beef revealed no gross internal abnormalities.