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IBT Validation Report—Simazine

Carcinogenicity Evaluation with Simazine Technical in Albino Mice (IBT No. 8580-08907)

Submitted by:
Dynamac Corporation
Enviro Control Division
The Dynamac Building
11140 Rockville Pike
Rockville, MD 20852

IBT Validation Report--Simazine

Carcinogenicity Evaluation with Simazine Technical in Albino Mice

(IBT No. 8580-08907)

Submitted to:

United States Environmental Protection Agency
Office of Pesticide Programs
Hazard Evaluation Division
Toxicology Branch

Thomas Roetzel, Project Officer

Under:

Contract No. 68-01-5824

Dynamac Corporation
Enviro Control Division
The Dynamac Building
11140 Rockville Pike
Rockville, MD 20852

John R. Strange, Project Director

August 20, 1982

DYNAMAC
CORPORATION

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August 20, 1982

Mr. Tom Roetzel
U.S. Environmental Protection Agency
Toxicology Branch (TS-769)
Room 816-C; CM #2
1921 Jefferson Davis Highway
Arlington, Virginia 22202

Dear Tom:

In reference to the IBT Carcinogenicity Evaluation Study with Simazine Technical in Albino Mice (IBT No. 8580-08907) sponsored by CIBA-GEIGY Corporation, to the best of our knowledge, no conflict of interest exists with regard to review of this study's data and any previous work done by Dynamac Corporation.

Sincerely,

DYNAMAC CORPORATION
ENVIRO CONTROL DIVISION

William L. McLellan

for Richard E. Tucker, Ph.D.
Vice President

IBT VALIDATION REPORT

(1) CHEMICAL: Simazine.

(2) TYPE OF FORMULATION: Technical.

(3) CITATION: IBT No. 8580-08907. Carcinogenicity Evaluation with Simazine Technical in Albino Mice. April 14, 1981.

(4) SPONSOR: CIBA-GEIGY Corporation.

(5) EPA ACCESSION NUMBER and/or Pesticide Petition No. and/or Registration No. for this IBT Report: None provided.

(6) VALIDATION PERFORMED BY:

John R. Strange, Ph.D.
Department Director
Dynamac Corporation
Signature: William L. McLellan
Date: Aug. 19, 1982

Cipriano Cueto, Ph.D.
Program Manager
Dynamac Corporation
Signature: Cipriano Cueto
Date: 19 August 1982

(7) Based upon findings listed in this Dynamac Corporation validation report (which included examination of the microfiched raw data, the sponsor validation report, the final test report, and the SPRD preliminary report when available), I concur with this validity determination.

Toxicologist
Toxicology Branch
HED, EPA
Signature: Mary J Burin
Date: 8/25/82

Section Head
Toxicology Branch
HED, EPA
Signature: W. Teeters for L. Chitlik
Date: 8/25/82

(8) TOPIC: This study has information pertinent to the discipline of toxicology; topic, oncogenicity. It relates to the Proposed Guidelines data requirement 163.83-2.

(9) VALIDATION REPORT CONCLUSION:

____ VALID

____ SUPPLEMENTARY

_X_ INVALID

SUMMARY

The sponsor validation report noted that corn oil was given to the control animals instead of the acetone used as a vehicle for the test animals (Deficiency 4). It also noted that the intervals between diet preparations were often long (Deficiency 1) and that checks for "tumor formation" were not done before 10/27/77 (Deficiency 7). The sponsor's validator reported that some of the animals were dusted with Rotenone (Deficiency 6) and that the animals were moved during the study (Deficiency 5). The sponsor's validator found "this study...sufficient to evaluate the carcinogenic potential of simazine technical."

This review determines the study to be invalid because the absence of diet preparation records prevents verification that the test material was administered at the required dietary levels during the first 13 months of the study. Also, autolysis and missing tissues among control and experimental animals resulted in an extensive reduction in the number of animals and tissues for which histopathologic data were available. In addition, high mortality resulted in early termination of the study, the corn oil control group was inappropriate for this study, the animals were moved to another test site during the study, and examination of animals for tissue masses was not conducted for the first 16 months of the study.

DEFICIENCIES AND DISCREPANCIES NOTED DURING COMPARISONS OF THE FINAL REPORT, PROTOCOL, AND RAW DATA

1. Verification of proper dosing of experimental animals during the first 13 months of the study was not possible. Three deficiencies were noted with respect to dosing of animals during this period.

   a. As indicated in the sponsor validation report (arrow on Reference 1), no records of diet preparation were found in the raw data for the first 13 months of the study. The only indication of diet preparation is a listing of dates in a letter from IBT to CIBA-GEIGY (arrow on Reference 2, page 1) that indicated four actual dates and five "estimated dates" (arrow on Reference 2, page 2) of preparation during this period of the study. The first record of diet formulation in the raw data was dated 7/5/77 (arrow on Reference 3), 13 months after initiation of the study. Although diet formulation calculations indicated the preparation of sufficient test diet for a 4-5 week period, there remained at least a 21-week period during the first 13 months of the study for which no actual dates of preparation were given.

   b. A limited number of dietary analyses were performed during the first 13 months of the study. Diet analysis data for this period indicated that the concentration of Simazine in the T-III diet at 3 months was 27% below the required level (arrow A on Reference 4). However, because diet analysis data were available for only 0-, 3-, 6-, and 12-month samples, the actual dietary levels of test material administered during most of the first 13 months could not be determined.

   c. Although records of weekly feed changes were present on daily observation pages from 10/26/77 to 4/5/78 (e.g., arrow A on Reference 5) and on a weekly feed change form from 4/5/78 to the end of the study (Reference 6), there were no records of compound administration present between 11/17/76 (the start of the study) and 10/26/77 (11 months after initiation of the study).

2. Although the protocol indicated that microscopic examinations were to be conducted on tissues and organs from all surviving mice in the control and T-III groups at final sacrifice as well as from all mice in these groups that died during the study (arrow on Reference 7, page 2), and Amendment No. 1 to the protocol, dated March 30, 1978, indicated that microscopic examinations were to be conducted upon tissues and organs from all surviving mice at the 24-month sacrifice as well as from all mice that died during the study and from all mice that were sacrificed <u>in extremis</u> (arrow on Reference 7, page 7), the number of tissues actually examined histopathologically was considerably less than the number specified by the protocol.

a. The histopathologic report by Experimental Pathology Laboratories, Inc. (EPL) acknowledged that "most tissues from mice that died prior to sacrifice had varying degrees of autolysis" (arrow A on Reference 8, pages 1 and 2). It was further stated that in some of these tissues "autolysis was of such severity that critical evaluation or even identificaton of tissues was difficult" (arrow B on Reference 8, page 2). The degree of autolysis reported by IBT on the gross pathology sheets was checked for the control and T-III female animals. According to the gross pathology performed at IBT, the degree of autolysis of some animals was rather severe, as indicated by an autolysis grading of 3 or higher (on a scale of 1 to 4) on the gross pathology sheets (e.g., arrow on Reference 9) for 38% of the female animals in the control and T-III groups. Attachment B provides documentation of the degree of autolysis given on the pathology forms for the control and T-III female animals. A tissue inventory (Attachment A, pages 1 and 3) indicated that autolysis was most frequently reported for the eye and the gastrointestinal tract. However, significant autolysis was reported for the urinary bladder, liver, and spleen (12, 10, and 10%, respectively) in the female control group, and for the urinary bladder (13%) in the T-III female group.

b. A review of the control and T-III histopathology data indicated there were a considerable number of tissues, not designated as autolyzed, for which no histopathologic data were present. Major tissues in the female control group for which considerable data were missing included: mammary gland (65%), mesenteric lymph node (35%), cervical lymph node (22%), urinary bladder (20%), and ovary (18%). Other tissues with large percentages of data missing in the female control group included: thymus, mediastinal lymph node, inguinal lymph node, lumbar lymph node, renal lymph node, sternum, mesentery, and parathyroid. In the female T-III group, the following major tissues had large percentages of data missing: mammary gland (55%), mesenteric lymph node (37%), urinary bladder (29%), cervical lymph node (26%), ovary (18%), and uterus (14%). Other tissues in the female T-III group with large amounts of missing data included: mesentery, thymus, sternum, renal lymph node, mediastinal lymph node, inguinal lymph node, lumbar lymph node, and parathyroid.

Major tissues for which substantial percentages of the total number of tissues were not examined, due to autolysis or a lack of histopathologic examination, are shown on Attachment A, pages 1-4 and included: mammary gland, mesenteric lymph node, urinary bladder, cervical lymph node, and ovary for the female control group (65, 38, 32, 25, and 23%, respectively). Other tissues in the female control group with large percentages of tissues not examined included: thymus, mediastinal lymph node, inguinal lymph node, lumbar lymph node, renal lymph node, sternum, mesentery, and parathyroid (100, 100, 100, 98, 98, 98, 98, and 73%, respectively).

In the female T-III group, the following major tissues had substantial percentages of the total number of tissues not examined: mammary gland, urinary bladder, mesenteric lymph node, cervical lymph node, ovary, spleen, liver, uterus, and kidney (55, 42, 42, 28, 25, 18, 17, 17, and 15%, respectively). Other tissues in the female T-III group with large percentages of tissues not examined included: mesentery, thymus, sternum, mediastinal lymph node, inguinal lymph node, lumbar lymph node, renal lymph node, and parathyroid (100, 98, 98, 98, 98, 98, 98, and 55%, respectively). In summary, the limited numbers of animals and tissues examined histopathologically may have precluded the detection of neoplastic compound-related effects.

3. Poor animal survival was reported among control and experimental groups and resulted in early termination of the study. Although the protocol called for a 24-month study to be conducted (arrow A on Reference 7, page 1), female T-III animals were sacrificed on 11/29/77 (month 17, arrows A and B on Reference 10) due to high mortality. The female T-I, and T-II groups were sacrificed on 4/28/78 (arrows A and B on References 11 and 12), 22 months after initiation of the study. The female control animals were sacrificed on 4/26/78 (arrows A and B on Reference 13), 22 months after initiation of the study. High mortality among male control animals resulted in early termination of all male animals (arrows A and B on Reference 14). All surviving male animals were sacrificed on 3/14/78 (21 months after initiation of the study, arrows A and B on References 15, 16, 17, and 18). Attachment C contains graphs of the number of survivors versus months on study for male and female control and T-III groups. The graphs indicate a high mortality between the 13th and 17th months. By 17 months, the male control group was reduced to nearly 50% and the male T-III group had less than a 50% survival rate; by 20 months, only 24 (40%) and 18 (30%) of the initial 60 animals in each of the male control and T-III groups remained and the survivors were terminated after 21 months.

   The female control group had a much higher survival rate, with 48/60 (80%) surviving at 20 months. However, the female T-III group had an extremely high mortality, with only 15/60 (25%) of the animals remaining during the 17th month. The T-III female group was sacrificed during the 17th month due to high mortality.

4. The control animals used in this experiment were shared with a carcinogenicity study of Atrazine (arrow A on Reference 19). The shared control was an inappropriate control for the Simazine experiment because the vehicle used to dissolve the test material was different in the two experiments. Corn oil was used as the vehicle in the Atrazine study and was incorporated into the diet of the concurrent control group, whereas acetone was used as a vehicle in the preparation of diets in the Simazine study (arrows A on Reference 20, pages 1 and 2).

5. Although there was no documentation present in the raw data, the animals were moved to another test site during the study. As indicated in the IBT final report, the move involved a distance of over 5 miles (arrow on Reference 21) and took place 9 months after initiation of the study. The sponsor validator acknowledged that some groups exhibited a slight weight depression at month 10," but it was noted that "no unusual mortality was observed during or immediately following this period" (arrow A on Reference 22). The sponsor validator concluded that the oncogenic evaluation was not adversely influenced by the move (arrow B on Reference 22).

6. Although, it was not mentioned in the final report procedures, the raw data indicated that some of the animals were dusted with Rotenone (arrow B on Reference 5) for "hair loss."

7. Although the protocol required that animals be examined weekly for signs of "tumor formation" (arrow B on Reference 7 page 1), there was no recording of examinations for tissue masses until 10/24/77 (arrow B on Reference 19); i.e., for the first 16 months of the study.

TOXICOLOGY STUDY PROCEDURES
AS STATED IN THE FINAL REPORT

1. Compound Name and Number: Simazine (F1-761262).

2. Sponsor: CIBA-GEIGY Corporation.

3. IBT Project No.: 8580-08907.

4. Title of Study: Carcinogenicity Evaluation with Simazine Technical in Albino Mice.

5. Laboratory: Wedge's Creek Research Farm.

6. Final Report Date: September 30, 1980.

7. Species: Mouse.

8. Strain: Charles River CD-1/Swiss White.

9. No. of Animals: 480.

10. Sex: Male 240 Female 240.

11. Source of Animals: Charles River Breeding Laboratories.

12. Age/Weight of Animals at Beginning of Study: The animals were 5-7 weeks old at the start of the study. Body weights were present in the final report for day 0, but were considered unsatisfactory by the sponsor and were not used in computations.

13. Route of Administration (Method of Preparation): The test material was administered by incorporation in the diet. A premix was prepared by mixing test material with acetone (2.5 g/10 ml). This premix was then mixed with the appropriate amounts of stock feed to yield the required concentrations of test material in the diet. The vehicle control diet was prepared by adding corn oil to the stock feed in a 1:500 (V/W) ratio instead of acetone, since the controls were shared with another study in which corn oil was used as a vehicle for the test material.

14. Experimental Design: (Group Designation):

Group	No. of Animals Male	Female	Dietary Level of Test Material (ppm)
Control	60	60	0
T-I	60	60	15
T-II	60	60	1,000
T-III	60	60	3,000

15. Clinical Observations Schedule: "Pharmacotoxic observations" were made daily, except for weekends, during the first 16 months of the study. More detailed observations plus palpation for tissue masses were initiated on a daily basis on 10/24/77 (month 16) and then on a weekly basis after 1/26/78 (month 19).

16. Body Weight Measurement Schedule: All test animals were weighed initially and then monthly for the duration of the study.

17. Food Consumption Measurement Schedule: Not required by the protocol.

18. Clinical Studies Schedule: Not required by the protocol.

19. Sacrifice Schedule (Method of Sacrifice): All surviving male mice were sacrificed on 3/14/78 (month 21). Control females were sacrificed on 4/26/78 (month 22), T-I and T-II females were sacrificed on 4/28/78 (month 22), and T-III females were sacrificed on 11/29/77 (month 17). The method of sacrifice was not stated in the final report.

20. Animals Necropsied: Postmortem examinations were conducted on all animals that died on study, were sacrificed in extremis, or were sacrificed at the termination of the study.

21. Tissues Examined and/or Preserved: Tissues taken at necropsy and preserved are those specified in item 24 and included other tissues with gross lesions.

22. Organ Weights Recorded: Not required by the protocol.

23. Animals Whose Tissues Were Examined Microscopically: Tissues were examined from all animals that died during the study, were sacrificed in extremis, or were sacrificed at the termination of the study. The tissues were examined by EPL.

24. Tissues Examined Microscopically: Microscopic examination of the following tissues was performed by EPL: brain, spinal cord, eye, optic nerve, adrenal, pituitary, thyroid, parathyroid, trachea, esophagus, heart, salivary gland, lymph nodes (cervical and mesenteric), spleen, lung, aorta, kidney, liver, stomach, nerve, and bone marrow. Other tissues showing gross abnormalities were also examined microscopically. This information was obtained from the EPL report (Appendix J of the CIBA-GEIGY final report).

25. Other Procedural Information:

a. Transfer of Animals: The animals were transferred from the animal laboratory facility at the main premises to the Globe animal laboratory facility (5.6 miles) approximately 9 months after initiation of the study.

b. Statistical Analysis: Mean body weight data were statistically analyzed by using a one-way analysis of variance. Tukey's multiple range test and Scheffe's multiple comparison were used to analyze the differences between means of all test groups. Mean body weight gain data were analyzed by one-way analysis of variance with Duncan's multiple range test. Gross pathologic findings were analyzed by contingency chi-square analysis.

RESULTS OF RAW DATA REVIEW

I. Administrative Information

A. Protocol (Design and/or Addenda): A CIBA-GEIGY protocol was present and dated May 1976 (Reference 7, pages 1-3). A revised protocol from the sponsor, dated July 1977, was also present (Reference 7, pages 4-6). The two protocols were essentially the same, except the revised protocol contained additional statements not found in the initial protocol regarding frequency of diet preparation, determination of stability of test material in the diet, and statistical analysis of data. Amendment No. 1 to the protocol, dated March 30, 1978, was also present and differed from the original and revised protocols in that it called for microscopic examination of tissues of all surviving mice in all groups as well as from all mice that died during the study and from all mice sacrificed in extremis. The original and revised protocols called for the examination of all tissues from surviving mice from the control and T-III groups as well as from all mice that died during the study in these two groups. The protocol and final report procedures were essentially the same with three exceptions. The protocol required a 24-month study; the actual length of the study was 21 months for the male animals, 17 months for the T-III females, and 22 months for the control, T-I, and T-II females. The final report procedures stated that the vehicle control group was shared with another study and that the vehicle used to prepare the control diet was different from the vehicle used to prepare the test diets (arrow A on Reference 19); this was not mentioned in the protocol but was documented in the raw data (arrow A on Reference 20, pages 1 and 2). Although the protocol required weekly examinations for "signs of tumor formation" (arrow B on Reference 7, page 1), the final report procedures stated that palpations for the presence of tissue masses were initiated on 10/24/77 (month 16 of the study) (arrow B on Reference 19).

B. Rationale for Dose Administration Levels: A 28-day range-finding experiment was conducted to establish dietary feeding levels for this 24-month carcinogenicity study. Also, a page of interoffice correspondence outlined the data used for selecting the carcinogenicity test dose levels (Reference 23).

C. Letter of Authorization: A letter of authorization was present from J.W. Barnett of CIBA-GEIGY to G.L. Kennedy of IBT. It was dated 5/25/76 and stamped received on 5/27/76 at IBT (Reference 24).

II. Compound

A. Identification (Chemical Name, Chemical Number, Lot Numbers): The compound was identified as Simazine Technical, Batch Nos. FL-761262 and FL-751090 on diet formulation pages (arrows B on Reference 20, pages 1 and 2). These diet records indicate that formulations were made with Batch FL-761262 until 2/14/78. From 2/21/78 until the end of the study, Batch FL-751090 was used for diet formulations.

B. Shipping Receipts: A shipping invoice, dated 5/17/76, was present from CIBA-GEIGY to IBT for 5 liters of Simazine Technical Batch No. FL 761262 (arrow on Reference 25). A second shipping invoice, dated 2/7/78, was also present from CIBA-GEIGY to IBT for 9 pounds of herbicide (Reference 26, pages 1 and 2). The IBT study number for this study was written on the form (arrow on Reference 26, page 1), and apparently this order form was for additional Simazine.

C. Stability/Special Handling: No information was present in the raw data.

D. Storage: No information was present in the raw data.

E. Analysis: Samples of the test compound were sent to the sponsor for analysis (arrow on Reference 27), but no results were present in the raw data.

F. Purity: The compound was noted as 99% pure on a diet formulation sheet (arrow C on Reference 20).

III. Compound Preparation/Administration

A. Methods of Compound Preparation: A diet formulation sheet was present showing that the test material was mixed with acetone to a concentration of 250 mg/ml to form a "premix." This "premix" was then added in appropriate amounts to the stock feed to produce the desired concentration of test material in the diet (arrow D on Reference 20, page 1). The control diet consisted of corn oil combined with the stock feed (arrow A on Reference 20, page 1).

B. Frequency of Compound Preparation: Data were present on diet preparation sheets showing that test diets were prepared weekly after 7/5/77 (week 56). Previous to this date, six dates of diet preparation were present, with the longest gap between preparations being 26 weeks. Attachment D documents the dates recorded for diet preparation.

C. Calculations for Compound Preparation: Calculations were present on an interoffice correspondence sheet (Reference 28). These calculations were checked and if followed would have provided the correct amount of test material in each diet.

D. Storage of Prepared Compound: No information was present in the raw data.

E. Analysis of Prepared Compound: Samples of the prepared compound were collected by IBT and sent to CIBA-GEIGY for analysis (e.g., arrow on Reference 27). Results of CIBA-GEIGY analysis were present in the raw data (e.g., Reference 29), and analysis results were reported for 0, 3, 6, 9, 12, 15, 18, 21, and 22 months. Considering the early termination of the study (Deficiency 3), these intervals of analysis agreed with the protocol that called for submission of samples at 0, 3, 6, 12, 18, and 24 months. Similar results of test diet analysis were also found in the sponsor validation report (Reference 4). However, the sponsor validation report contained additional analysis data for months 16, 17, 19, 20, and 22 that were not found in the raw data (Reference 4). Numerous inconsistent levels of test material in the diet ranging from 56% to 460% of that required were indicated (e.g., arrows B and C on Reference 4). The diet was underformulated at 3 months (73% of that required) and at 22 months (56%) in the T-III group (arrows A and B on Reference 4, respectively). Overformulations were reported at 22 months in T-I (460% of that required), and at 16 and 21 months in the T-III group (128 and 123%, respectively) (arrows D and E on Reference 4).

F. Administration: Records of feed changes were present on daily observation pages from 10/26/77 (week 72) to 4/5/78 (week 95) (e.g., arrow A on Reference 5) and on a weekly feed change form from 4/5/78 to the end of the study (Reference 6). The recorded data indicated that food was changed weekly during this time. No records of compound administration were present between 6/10/76 (the start of the study) and 10/26/77 (week 72). There were also no actual dates of diet preparation given during this 72-week period (Reference 2, page 1).

G. Start of Compound Administration: The starting date of test diet administration was given as 6/10/76 on a page of daily observations (arrow A on Reference 30).

H. Termination of Compound Administration: The last weekly feed change was recorded as 4/26/78 (arrow on Reference 6), indicating that the animals received the test feed until the end of the study (4/28/78).

IV. Animal Information

A. Date of Order: The date present on the order acknowledgment form was 5/13/76 (arrow A on Reference 31).

B. Date of Receipt: A pretest observation page recorded that animals were received on 5/20/76 (arrow B on Reference 30).

C. Total Number of Animals Ordered: A total of 980 animals (490 males and 490 females) were noted on an order acknowledgment form from Charles River Breeding Laboratories to Wedge's Creek Research Farm (Reference 31).

D. Species/Strain: The animals were noted as CD-1 mice on the order acknowledgment form (arrow B on Reference 31).

E. Source: Charles River Breeding Labs., Inc. was noted as the source of the animals on the order acknowledgment form (arrow C on Reference 31).

F. Age/Weight: The mice were 21 days old on 5/17/76 as noted on the order acknowledgment form (arrows D and E on Reference 31). Thus they were 6-7 weeks old at the start of the study on 6/10/76. Body weight data were present for the T-I, T-II, and T-III animals that showed that initially males weighed between 38.3 and 54.0 g, with an average of 47 g. Females weighed between 38.4 and 58.3 g, with an average of 43 g. However, these weights were 10-15 g higher than the animals' weights at 2 months, and were considered unreliable by the sponsor (arrows on Reference 32).

G. Duration of Quarantine: A page of pretest observations was present showing that the mice were observed for 21 days (5/20/76 to 6/10/76) before test feed was administered (Reference 30).

H. Start of Animal Phase: The animals were fed the test feed starting on 6/10/76 (arrow A on Reference 30).

I. Termination of Animal Phase: Different groups were sacrificed on different days. The T-III females were sacrificed on 11/29/77 (month 17, arrows A and B on Reference 10). The control, T-I, T-II, and T-III males were sacrificed 4 months later on 3/14/78 (arrows A and B on References 15, 16, 17, and 18). The control females were sacrificed on 4/26/78 (month 22, arrows A and B on Reference 13). The surviving T-I and T-II female animals were sacrificed on 4/28/78 (month 22, arrows A and B on References 11 and 12).

V. Environmental Conditions

A. Room Identification: The room was identified as room No. 2 on the sheets used to record temperature and humidity (e.g., arrow on Reference 33). These sheets were present from 7/27/77 to 4/28/78 (the end of the study).

B. Caging Information: The animals were gang housed initially (arrow C on Reference 30). A note was present on a page of daily observations for 11/8/77 recording that females were put in individual cages (arrow on Reference 34). A letter from IBT to CIBA-GEIGY noted this and stated that "an internal decision was made to individually house all mice" (arrow on Reference 35). However, individual caging of males could not be verified in the raw data.

C. Temperature: Temperature records were present for 5 days each week from 7/27/77 to 8/5/77 and daily from 8/8/77 to 4/28/78 (the end of the study) (e.g., Reference 33).

D. Humidity: Humidity records were present for 5 days each week from 7/27/77 to 8/5/77 and daily from 8/8/77 to 4/28/78 (the end of the study) (e.g., Reference 33).

E. Air Change: No information was present in the raw data.

F. Lighting: No information was present in the raw data.

VI. Biological Parameters

A. Body Weights: Individual body weight data were present for all animals in the T-I, T-II, and T-III groups initially and then monthly for the duration of the study. However, the values recorded for the initial and month 1 weighings were at least 10 g higher than those recorded for the following months (Reference 32). The accuracy of these initial weights were questioned by the sponsor (Reference 32). Individual body weight data were present for the control group from month 13 until the end of the study. The data were present on computer printout forms, with some data present on handwritten forms (e.g., Reference 36 pages 1 and 2). Attachment E documents the presence of body weight data for control and T-III female animals. Individual body weight values for the control and T-III females for months 13, 15, 17, and 22 were compared with the final report; no discrepancies were found (Attachment F).

B. Food Consumption Data: Not required by the protocol.

C. Clinical Observation/Mortality: No individual animal clinical observations were recorded in the raw data. Clinical observations and mortality data were present on a group basis for the controls, T-I, T-II, and T-III groups for the length of the study. Observations were present for 5 days per week initially and then daily after 2/13/78 (e.g., Reference 37). Mortality data were recorded twice daily after 3/8/78.

The study was terminated early due to poor survival of the test animals. For males, 41/180 (23%) survived to final sacrifice (21 months). The survival-to-sacrifice data of males by dose groups was as follows: control, 13/60 (22%); T-I, 18/60 (30%); T-II, 14/60 (23%); and T-III, 9/60 (15%). For females, 48/180 (27%) survived to sacrifice (22 months). The survival-to-sacrifice data of females by dose groups was as follows: control 27/60, (45%); T-I, 26/60 (43%); T-II, 16/60 (27%); and T-III, 6/60 (10%). Attachment B documents the disposition of the control and T-III female groups.

Mice were observed for skin lesions and palpated for tissue masses weekly from 10/26/77 to the end of the study. These data were listed weekly on clinical observation forms from 10/26/77 to 1/23/77 and weekly on lesion/tissue mass tables from 1/24/78 to the conclusion of the study (e.g., Reference 38). No data were present prior to 10/26/77 (month 16).

D. Clinical Studies: Not required by the protocol.

E. Organ Weights: Not required by the protocol.

F. Gross Pathology/Necropsy: Data were present on individual gross pathology forms (e.g., References 10, 11, 12, 13, 16, 17, and 18) for all animals given the test material (groups T-I, T-II, and T-III). Data were present for the control animals on the individual gross pathology forms for the Atrazine study (e.g., References 13 and 15). Attachment B documents the presence of gross pathology observations for the control and T-III female groups and also documents the degree of autolysis if listed on the pathology sheet as 3 or higher on a scale of 1 to 4. A sample consisting of the gross pathology raw data for the control and T-III female groups was compared with the final report records for gross pathology (Attachment G); no discrepancies were found.

G. Histopathology: The histopathology was performed by EPL, and the results were reported in Appendix J of the final report.

H. Inventory of Examined Tissues: A tissue inventory is presented in Attachment A for the female control and T-III groups. This inventory showed that 53 and 56% of the tissues to be examined were either autolyzed or not present for the female control and

T-III groups, respectively. Major tissues in the female control group for which substantial percentages of the total number of tissues were not examined included mammary, mesenteric lymph node, urinary bladder, cervical lymph node, and ovary (65, 38, 32, 25 and 23%, respectively). Other tissues in the female control group with large percentages of tissues not examined included: thymus, mediastinal lymph nodes, inguinal lymph node, lumbar lymph node, renal lymph node, sternum, mesentery, and parathyroid (100, 100, 100, 98, 98, 98, 98, and 73%, respectively). In the female T-III group, the following major tissues had substantial percentages of the total number of tissues not examined: mammary gland, urinary bladder, mesenteric lymph node, cervical lymph node, ovary, spleen, liver, uterus, and kidney (55, 42, 42, 28, 25, 18, 17, 17, and 15%, respectively). Other tissues in the female T-III group with large percentages of tissues not examined included: mesentery, renal lymph node, and parathyroid (100, 98, 98, 98, 98, 98, 98, and 55%, respectively). Therefore, the number of tissues not examined was considerable and may have precluded the detection of compound-related tumors.

I. Tumor Incidence: Tumor incidence data were present and reported by EPL in Appendix J of the final report.

J. Autolysis: A degree of autolysis of 3 or higher on a scale of 1 to 4 was recorded by IBT on the pathology sheets for approximately 38% of the female control and T-III animals (e.g., arrow A on Reference 8; Attachment B, autolysis column). The EPL report stated that most tissues from animals that died prior to sacrifice had some degree of autolysis. In some cases, the autolysis was so severe that critical evaluation or even identification of the affected tissues was difficult (arrows A and B on Reference 8, page 2). A review of the EPL results (Attachment A) indicated that autolysis was reported most frequently for the eyes and the gastrointestinal tract (Tissue Inventory Sheets, Attachment A) for the female control and T-III groups. However, relatively high incidences of autolysis were also reported for the urinary bladder (12%), liver (10%), and spleen (10%) in the female control group, and for the urinary bladder (13%) in the T-III female group.