CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 24 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: July 6, 1981

SUBJECT: Petition to Amend the Tolerance for Bromoxynil and Conditionally Register the Mixed Ester Formulations: Dragon Broadleaf Herbicide and Dragon Mate Broadleaf Herbicide (PP#0F2346; Acc. Nos. 099394 and 099392) CASWELL #119 & 557C

FROM: George W. Robinson, D.V.M. 7/6/81
Toxicology Branch/HED (TS-769)

TO: Robert J. Taylor, Product Manager
Team No. 25/RD (TS-767)

THRU: Chris Chaisson, Acting Chief
Toxicology Branch/HED (TS-769)

R. Bruce Jaeger 7/14/81

Petitioner: Union Carbide Agricultural
Products Co., Inc.
Ambler, PA 19002

Currently there are two formulations of bromoxynil registered with EPA for agronomic weed control. The products are Brominal Broadleaf Herbicide (EPA reg. No. 264-204) containing the octanoic acid ester of bromoxynil as the active ingredient and Brominal Plus (EPA Reg. No. 264-239) containing the octanoic ester of bromoxynil and the butoxyethanol ester of MCPA

I. Action Requested

A. A tolerance amendment to change 40 CFR 180.24 and permit application of bromoxynil as either the currently approved octanoic acid ester or the butyric acid ester.

B. Conditional registration of the mixed ester formulations: Dragon Broadleaf Herbicide and Dragon Mate Broadleaf Herbicide.

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II. Conclusions

1. A tolerance of 0.1 ppm is established for negligible residues of bromoxynil from the application of its octanoic acid ester in or on grain, green forage and straw of barley, oats, rye and wheat, flaxseed and flaxstraw (40 CFR 180.324). Tolerances established for MCPA on the same crops appear in 40 CFR 180.339.

2. The actual use rate of Dragon and Dragon Mate will result in the same amount of bromoxynil equivalents as the presently registered uses of the formulations Brominal® and Brominal Plus®.

3. Residue Chemistry Branch (PP#0F2346, memo of 12/24/80, K.H. Arne) does not expect the metabolism of the octanoic and butyric acid esters of bromoxynil to differ to any significant extent from one ester to the other, except that hydrolysis will produce different acids. Since the butyrate will produce butyric acid (commonly found in nature) in small amounts they conclude that the residue of concern (bromoxynil) is the same for either ester. RCB further concluded "The proposed use of the butyric acid ester will not cause the existing tolerances for bromoxynil to be exceeded."

III. Recommendations:

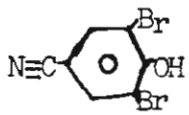
1. Toxicology Branch has no objection to the conditional registration of Dragon Broadleaf Herbicide and Dragon Mate Broadleaf Herbicide. However, acute oral and inhalation toxicity studies should be repeated and resubmitted for consideration of any proposed registration of bromoxynil butyrate.

2. The proposed change in the existing tolerance for bromoxynil is recommended.

IV. Information on the technical chemicals:

A. Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile)

Structure:



MCPA/
Bromoxynil toxicology review

Page _____ is not included in this copy.

Pages 3 through 4 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

X Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

VI. Toxicology Review

A. Previously submitted toxicity data .

The following summary of toxicity data from PP#7F0536 was reviewed by Dr. J.B. Brouwer dated 9/8/67.

1. Bromoxynil, Technical (PP#7F0536)

a. Acute oral, rat, LD_{50} = 270 (225-320) mg/kg

b. Acute oral, rabbit, LD_{50} = 335 (225-505) mg/kg

c. Acute oral, pheasant chicks, LC_{50} = 4400 ppm

d. Acute oral, dog, LD_{50} < 150 mg/kg

e. Acute oral, rat, LD_{50} = 250 (205-305) mg/kg

f. 4-Week feeding, dog, NOEL < 30 mg/kg/day

g. 13-Week feeding, dog - Moderately depressed weight gain @ 25 mg/kg/day

h. 13-Week feeding, rat - Slightly growth inhibition @ 781 ppm; profoundly toxic effect on growth @ 1953 ppm

i. Acute inhalation, rat, LCT 50 = 51 mg/L/hr

2. Bromoxynil Octanoate (PP#7F0536)

a. Acute oral, pheasant chicks, LC_{50} = 5000 ppm

b. Acute oral, rat, LD_{50} = 255 mg/kg

c. Acute oral, mouse, LD_{50} = 245 mg/kg

d. Acute dermal, rabbit, LD_{50} = 2 g/kg

e. Subacute dermal (21 days), rabbit, mildly irritative to intact and abraded skin @ 366 mg/kg; NOEL = 73 mg/kg

f. Acute inhalation, rat, LC_{50} = 38 mg/L/hr

g. Acute oral, rat, LD_{50} = 90 mg/kg

h. Acute oral, mouse, LD_{50} = 160 mg/kg

The following summary of toxicity data from PP#9F0761 was reviewed by Robert D. Coberly dated 6/24/71.

1. MCPA (2-methyl-4-chlorophenoxyacetic acid)

   a. Acute oral, rat, LD_{50} = 700-800 mg/kg

   b. Acute oral, rat (69.1% alkanoline salts of MCP), LD_{50} = 1090-1410 mg/kg

   c. Acute oral, rat (amine salts of MCP), LD_{50} = 1200 mg/kg

   d. Acute oral, guinea pig (69.1% alkanoline salts of MCP), LD_{50} = 550 mg/kg

   e. Acute oral, rabbit (69.1% alkanoline salts of MCP), LD_{50} = 810 mg/kg

   f. Acute oral, chicken (69.1% alkanoline salts of MCP), LD_{50} = 940 mg/kg

   g. Acute oral, bovine, LD_{50} = > 5.0 ppm

   g. Acute eye irritation (69.1% alkanoline salts of MCP), undiluted material produced severe eye irritation which persisted beyond 7 days in both irrigated and non-irrigated eyes.

   i. Acute dermal toxicity (69.1% alkanoline salts of MCP), LD_{50} = 5000 mg/kg

   j. 4-Day oral, bovine, NOEL = > 50 ppm

   k. 21-Day oral, bovine, NOEL = > 30 mg/kg

   l. 3-Week oral, rat, NOEL = 60 mg/kg

   m. 90-Day feeding, rat, NOEL = 8 mg/kg/day

   n. 13-Week oral, dog, NOEL = 160 ppm

   o. 7-Month feeding, rat, NOEL = < 100 ppm

   p. Teratogenicity, mice, no effects at 100 mg/kg (highest dose tested).

B. New submitted toxicity data

1. Bromoxynil Butyrate - Technical, 16378

a. Acute oral toxicity - Albino Rat; Technical Bromoxynil Butyrate, FOOD @ DRUG RESEARCH LABORATORIES, INC., October 20, 1977; by J. Griffiths & J.G. Babish.

The acute (single dose) oral toxicity was determined in rats employing a modified procedure in "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics", published by the Association of Food and Drug Officials of the U.S. (1959).

Adult albino rats, BLU:(LE) BR Long-Evans derived, fasted 18 hours prior to dosage. Groups of 5 males and 5 females were then intubated with the test material at: .01, .05, .10, .50, .75 and 2.00 gm/kg. Test animals were supplied with food and water ad libitum after dosage. Animals were observed daily for 14 days following administration of the test material, and deaths were recorded.

Results :

An approximate acute oral LD_{50} of 0.13 ± 0.01 gm/kg of body weight was estimated by interpolation from the probit response curve by the testing laboratory.

Conclusions:

1. The estimated LD_{50} of 0.13 gm/kg is incorrect; 7 of 10 rats died a the dose level of 0.10 gm/kg. Graphich interpolation from a dose-response curve determined the LD_{50} to be $\simeq$ 0.08 gm/kg.

2. A summary of symptoms and necropsy findings at each test group dose level in general terms is inadequate.

3. No quantification by sex was presented.

4. Testing data should be reported in much more detail such that an adequate toxicological evaluation can be accomplished. The petitioner is referred to subsection 163.80-4, 43 FR 37354 and subsection 163.81-1, 43 FR 3755 for proper testing procedure and reporting of data.

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Classification - Core, Supplementary Study.

Submission of data from this testing to allow adequate toxicological evaluation might possibly place the study in Core-Minimum Data or above. Otherwise, the conduct of another acute oral toxicity study and subsequent submission of all data, information, and analysis required by the "Data reporting and evaluation" paragraphs of the above cited subsections should be accomplished.

b. Acute Inhalation Toxicity - Albino Rat; Technical Bromoxynil Butyrate, FOOD & DRUG RESEARCH LABORATORIES, INC., December 9, 1977; by T.A. Re.

The purposes of the study was to determine the toxicity of the test material when administered as a mist under atmospheric conditions.

Procedure:

Ten young adult albino rats (BLU:(SD) BR Sprague-Dawley descent rats purchased from Blue Spruce Farms, Inc., Altamont, New York) weighing between 145 and 190 grams comprised the test group of 5 males and 5 females.

The group of 10 rats was then placed in a 75 liter chamber equipped with an air supply of 10 liters per minute. After the animals became accustomed to the chamber conditions, the test material was atomized into the air supply as an ethyl acetate solution at a rate calculated to yield the required concentration of 200 mg/L for one hour.

After exposure, the animals were individually housed in mesh-botton cages with food and water supplied ad libitum . They were observed daily for a period of 14 days, and their appearance and behavior noted.

Results:

Following the 1 hour inhalation period, all animals exhibited bloody nasal discharge, salivation, urinary incontinence and decreased activity. Eight of the 10 animals died during the observation period: 6 on day 1, 1 on day 2, and 1 on day 4. The 2 surviving animals showed signs of bloody nasal discharge for four days. Signs of urinary incontinence, salivation and hyperactivity were noted for 10 days.

Conclusions:

1. Although technical bromoxynil butyrate, the test material, caused significant toxicity at a concentration of 200 mg/liter, an LC_{50} cannot be calculated from this one shot high dose level; the actual concentration was not measured.

2. An ethyl acetate solution (vehicle) control group should have been tested.

Classification: Core-Supplementary Study.

Another acute inhalation toxicity study is necessary to support this registration.

c. Primary Skin Irritation Study with Albino Rabbits; Technical Bromoxynil Butyrate, FOOD & DRUG RESEARCH LABORATORIES, INC., September 19, 1977; by J. Griffiths & J.G. Babish.

Procedure:

The acute skin irritation test was conducted on 6 adult albino rabbits selected from healthy, acclimated animals. The method employed was patterned after the Draize procedure as described in 16 CFR 1500.41. The back of each animal was shaved free of hair; intact skin was exposed on the left half of the shaved area, and abraded on the right half. The material (0.5 ml or 0.5 g) was introduced under a square patch of surgical gauze measuring 1 inch x 1 inch. Patches were removed after 24 hours and observations recorded. Observations were gain made after 72 hours.

Results:

Scoring of the effects produced by the test material was according to Draize. Erythema was observed in the intact and abraded skin of all six animals. Edema was observed in the intact skin of five animals, and abraded skin of six of three animals during the 72-hour observation period. Erythema persisted throughout this period of all six animals.

Primary skin irritation scores were 3.50, 3.50, 2.00, 2.50, 2.75 and 2.00, respectively, for the individual animals, resulting in an average score of 2.54/8.0.

Conclusion:

On the basis of the above primary irritation scores and Draize scoring criteria, technical bromoxynil butyrate is a moderate irritant to the rabbit skin.

Classification: Core-Minimum Data; Category III

d. Primary Eye Irritation in Albino Rabbits; Technical Bromoxynil Butyrate, FOOD & DRUG RESEARCH LABORATORIES, INC., May 20, 1977 by J.T. Griffiths and J.B. Babish.

Procedure:

This study was conducted on 6 young adult albino rabbits selected from healthy, acclimated animals according to the procedure decribed in 16 FR 1500.42 (Test for eye irritants; Chapter II - Consumer Product Safety Commission). "(a)(1) Six albino rabbits are used for each test substance..... Both eyes of each animal in the test group shall be examined before testing and only those animals without eye defects or irritation shall be used.... The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animals is released. The other eye, remaining untreated, serves as a control."

The ocular reactions were observed and recorded at 24, 48, and 72 hours, and again at 7 days after installation of the test material.

Results:

Conjunctival redness was observed in all six animals. This effect cleared in two rabbits by the 7th day of observation. There was no evidence of corneal opacity or iritis.

Conclusion :

On the basis of the data presented, technical bromoxynil butyrate appears to be a mild irritant to the eyes of rabbits.

Classification: Core-Minimum Data; Category III.

Petitioner is referred to subsection 163.81-4, 43 FR 37359 for the proper conduct of a primary eye irritation study.

2. Formulated Material - Dragon Broadleaf Herbicide (formerly identified as AXF-1050)

In all tests, animals were individually housed in wire botton cages in temperature controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Food and water were avialable ad libitum except as noted.

a. Acute Oral Toxicity Study in Rats. AXF-1050 Emulsifiable Concentrate Bromoxynil by D.R. Damske, F.J. Meckler & R.J. Weir, Litton Bionetics, Inc., LBI Project No. 21142-01(0), January 1980.

The test material was dissolved in deionized water at a concentration of 60 mg/ml; the density was estimated to be 1200 mg/ml. The dose levels were expressed in terms of the material as received.

Experiment Design: Young adult rats (weighing 179 to 222 g, and 57 to 67 days of age at the time of first dose, October 31, 1979) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles Breeding Laboratories, Inc., Portage, Michigan. The animals were acclimated to laboratory conditions for 7 days.

Prior to determining the LD_{50}, a trial test was performed on 5 animals of each sex at the 5000 mg/kg level; and in a range finding study, 2 rats of each sex were dosed at 1200 and 120 mg/kg. Based on the response of these animals, single graded doses of the test material were administered by oral gavage to 8 rats of each sex at each of 4 dose levels: 681, 464, 316, and 215 mg/kg.

The rats were observed frequently on the day of treatment and twice daily thereafter. The animals were weighed on the day of treatment, and on days 7 and 14 following treatment. Necropsies were performed on all animals that died during the study and on the surviving animals that were killed 14 days after treatement.

Results:

Based on the deaths occurring in the 14 days after treatment and using the Litchfield Wilcoxon Method (J. Pharm and Exp Ther, 96:99-113, 1949) LD_{50} values of 545 (95% confidence limits: 456-651) and 428 mg/kg (95% confidence limits: 345-530) for male and female rats, respectively, were calculated.

Signs of toxicity included reduced motor activity and general weakness in some males and females at 681 and 464 mg/kg and a male at 316 mg/kg. Other observations consisted of one male and three females (316 mg/kg) with wetness around the perianal region; one female (464 mg/kg) with eyes partially closed and labored breathing; two males (464 mg/kg) with swollen eyelids;

one male (464 mg/kg) and one female (316 mg/kg) with black crust around one eye; and two females (681 mg/kg) with liquid stool and one male (681 mg/kg) with wetness around the mouth.

Necropsy findings among surviving animals consisted of one male (464 mg/kg) with mottled lungs; three males (215 mg/kg) with small shriveled stomachs, two of these with red small intestines and one with yellow fluid in the stomach.

Among rats that died, necropsy findings included a hard heart, white material in the stomach and mottled or hemorrhagic lungs in some or all rats of dose levels where animals died. Mottled kidneys were seen in one male and one female (464 mg/kg), and mottled liver in one male (464 mg/kg) and a female (316 mg/kg).

Conclusion: Acute oral LD_{50} values were 545 mg/kg for males and 428 mg/kg for females.

Classification: Core-Minimum Data; Category III.

b. Acute Dermal Toxicity Study in Rabbits: AXF-1050 Emulsifiable Concentrate Bromoxynil by D.R. Damske, F.J. Meckler and R.P. Beliles, Litton Bionetics, Inc., LBI Project No. 21141-01(D), October 1979.

Experimental Design: Five male and five female New Zealand White (albino) rabbits (weighing 2.2 to 2.9 kg, and 11 to 13 weeks of age at the time of first dose, August 14, 1979) were used. The animals were acclimated to laboratory conditions for 8 days. Each rabbit was prepared by clipping the skin of the trunk of free of hair 24 hours prior to dosing. Three male and two female rabbits were further prepared by making epidermal abrasions every 2-3 cm/longitudinally over the areas to be exposed. The abrasions were sufficiently deep to penetrate the stratum corneum, but not to disturb the derma. A single 2.0 g/kg dose of the test material was applied to the skin of the rabbits. The test material was applied undiluted and the density was determined to be 1.2 g/ml.

After application, the site was covered with gauze and occlusive wrap, and the rabbits were placed in restraining collars for 24 hours. At the end of 24 hours, the wrap and collars were removed. The animals were observed daily for gross signs of poisoning and skin reactions for two weeks after dosing. Body weights were obtained initially and at 7 and 14 days after testing. All animals surviving 14 days after treatment were necropsied and any changes in the internal organs were noted.

No deaths occurred during the 14 days post-treatment. Mild to moderate skin irritation was observed at the 2 g/kg level in both the abraded and intact skin of test rabbits from day 1 through day 14 after treatment. Erythema was observed on all rabbits through day 7; moderate erythema was seen in three rabbits each with abraded skin and intact skin; respectively. Edema was absent from all of the treated rabbits. Flaky skin was observed on all rabbits and small scabs appeared on the backs of three rabbits.

No abnormal necropsy finding was observed in any of the treated rabbits.

Conclusion:

No deaths or signs of systemic toxicity were observed in treated animals; therefore, the dermal LD_{50} was greater than 2.0 g/kg. The dermal response of the skin of the rabbits to this dose of the test material suggests a moderate potential for skin irritation.

Classification: Core-Minimum Data; Category III.

c. Acute Inhalation Toxicity Study in Rats: AXF-1050 Emulsifiable Concentrate Bromoxynil by P.J. Knapinski, F.J. Meckler and R.J. Weir, LBI Project No. 21142-0(I), Litton Bionetics, Inc., January 1980.

The test material was suspended in air at nominal concentrations of 2.8 and 14.8 mg/liter. The test atmosphere was generated using a DeVilbliss Nebulizer and a pump-driven reservior.

Experimental Design: Young adult rats (weighing 212 to 384 g, and 8 to 13 weeks of age at the time of first exposure, September 17, 1979 and November 7, 1979) of the Charles River CD strain [CFR: COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. The animals were acclimated to laboratory conditions for 7 to 40 days.

Five animals of each sex were exposed in a 30-liter glass cylindrical chamber. The animals were exposed to the atmosphere for 4 hours. The total air flow through the chambers was 6 liters per minute at the low level and 12 liters per minute at the high level. Gravimetric analysis of the chamber atmospheres during the exposures yielded a time-weighted average concentration of 0.5 mg/liter for the low level chamber and 3.8 mg/liter for the high level chamber. The time-weighted average temperature was 22.1°C in the low level exposure chamber and was 22.4°C in the high level exposure chamber.

The nominal concentrations for these chambers were 2.8 mg/liter and 14.8 mg/liter, respectively. In addition, one liter samples of the chamber atmospheres were collected in 10 ml of methanol and sent to the sponsor for analysis.

A large difference existed between the nominal concentrations and the gravimetric samples. This was due to some deposition of the test material on the chamber walls but mostly due to vaporization of portions of the test aerosol. The gravimetric sampling captured only that portion of the test material which did not vaporize.

The rats were observed frequently on the day of exposure, and twice daily thereafter. The animals were weighed on the day of exposure an on days 2, 3, 4, 7 and 14 following exposure. Necropsies were performed on all animals that died during the study and on the surviving animals that were killed 14 days after exposure.

Results: LD_{50} values were estimated to be 10.3 mg/liter and 8.8 mg/liter for males and females, respectively.

Signs of toxicity included abnormal breathing sounds in all rats at the high level and in one male at the low level. All rats at the high level exhibited signs of labored breathing during the day of exposure. In the low level rats one male developed runny stool after exposure. All animals of both levels showed various signs of staining on the nose, mouth or forepaws.

Necropsy findings among surviving animals consisted of mottled lungs in the single high level survivor and in two of the five low level males, and in four of the five low level females. Among rats that died, necropsy findings included mottling of the lungs and darkened liver which may have occurred after death. In three of the males and in three of the females, the heart was hardened.

Conclusion: Acute inhalation LD_{50} values were 10.3 and 8.8 mg/liter, respectively.

Classification: Core-Minimum Data; Category III

d. Primary Eye Irritation and Corrosiveness Study in Rats: AXF-1050 Emulsifiable Concentrate Bromoxynil by D.R. Damske, F.J. Meckler, and R.P. Beliles, LBI Project No. 21142-01(E), Litton Bionetics, Inc., September 1979.

Experimental Design: Nine females New Zealand White rabbits were obtained from B&H Rabbitry, Rockville, Maryland. The animals were acclimated for 24 hours. The rabbits weighed 2.2 to 2.5 kg at the time of treatment and were 9 to 11 weeks of age.

Rabbits were randomly divided into two groups as follows:

Group Numnber	Animal Numbers	Eye Washed After Treatment
1	2258-2563	No
2	2564-2566	Yes

Group 2 rabbits had the treated eye flushed with lukewarm water for 60 seconds, 30 seconds after the test material installation. The eyes of all rabbits were washed with sodium chloride solution after the 24-hour observation.

The eyes of the rabbits were examined with the aid of 2% sodium fluorescein before testing to ensure that the eyes were without defects or irritation. The animals were firmly held and 0.1ml of the test material was instilled into one eye of each rabbit. The eyes were examined and graded according to the Draize method; scores were recorded at 1, 24, 48, and 72 hours, and at 7 days.

Results:

Changes in the treated eye of Group 1 rabbits consisted of corneal opacity in four animals, chemosis in five animals, and redness in all rabbits. In the Group 2 animals where the treated eyes were flushed 30 seconds after instillation, only redness in two and chemosis in all three rabbits were observed. This indicated that flushing the eye with water was a significant aid in reducing irritation. A white discharge and yellow crust around the treated eye was observed in 8 of 9 rabbits, but the duration of the sign was reduced in the rabbits which had the eye flushed 30 seconds after instillation.

Corneal opacity, observed through 72 hours, was absent on day 7.

Conclusion: Based on the evaluation up to 7 days after treatment, the test material, AXF-1050 was judged to be severe irritant to the eye of rabbits.

Classification: Core-Minimum Data; Category II.

e. Primary Skin Irritation Study In Rabbits: AXF-1050 Emulsifiable Concentrate Bromoxynil by D.R. Damske, F.J. Meckler and R.P. Beliles, LBI Project No. 21142-01(S), Litton Bionetics, Inc., September 1979.

Experimental Design: Six female New Zealand White rabbits were obtained from B&H Rabbitry, Rockville, Maryland. The animals were acclimated for 24 hours.

The hair was clipped from the backs of the rabbits 24 hours prior to the administration of the test material. On one side, was abraded by making minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma. The undiluted test material was introduced under four 1-inch patches: two 0.5 ml doses on the intact skin and two 0.5 ml doses on the abraded skin.

The animals then had their entire trunks wrapped with occlusive dressing and placed in restraining collars. After 24 hours of exposure in this manner, the patches were removed and the resulting reactions were evaluated and scored.

Readings were made daily for 7 days and the reading on each was recorded. The reading for each incident in each rabbit, for example, edema formation in the intact skin, was averaged. The evaluation was based on readings at 24 and 72 hours after application. This produced 16 values. The 16 values were divided by 8 to produce a primary irritation index.

Results:

The primary irritation score was 1.27, suggesting that the test material was a mild irritant. Other signs of dermal injury included flaky skin on five animals at days 6 and 7, slightly stiff skin and yellow material adhering to the fur of the back on one animal each, and thin yellow lines along the abrasions of two rabbits. The degree of irritation seen indicated that abrasions did not potentiate the dermal response to the test material. No signs suggestive of systemic toxicity were observed during the course of the study.

Conclusion: The test material, AXF-1050, was judged to be a mild irritant when applied to either intact or abraded skin of rabbits.

Classification: Core-Minimum Data; Category IV.

3. Formulated Material - Dragon Mate Broadleaf Herbicide (AXF-1053): Mixed Ester Formulation of Octanoic Acid and Butyric Esters of Bromoxynil and the Butoxyethyl Ester of MCPA.

In all studies, animals were individually housed in wire botton cages in temperature controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Food and water were available ad libitum except as noted.

a. Acute Oral Toxicity Study in Rats: AXF-1053 Emulsifiable Concentrate Bromoxynil MCPA by D.R. Damske, F.J. Meckler & R.J. Weir, Litton Bionetics, Inc., LBI Project No. 21142-02(0), January 1980.

Experimental Design: Young adult rats (weighing 181.5 to 219.8 g, and 9 to 10 weeks of age) of the Charles River CD strain [CRL:CD(SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. The animals were acclimated to laboratory conditions for 7 days. Food was removed from the cages the night before treatment and then provided freely following treatment.

A range finding study of 2 rats of each sex dosed at 1200 and 120 mg/kg was performed. Based on the response of these animals, single graded doses of the test material were administered by oral gavage to 8 rats of each sex at each of 4 dose levels: 681, 464, 316, and 215 mg/kg.

The rats were observed frequently on the day of treatment and twice daily thereafter. They were weighed on the day of treatment, and on days 7 and 14 following treatment. Necropsies were performed on all animals that died during the study and on the surviving animals that were killed 14 days after treatment.

Results: Based on the deaths occurring in the 14 days after treatment, and using the Litchfield Wilcoxon method (J. Pharm. and Exp. Ther., 96:99-113, 1949) LD_{50} values of 530 (95% confidence limits: 427.4-657.2) and 470 mg/kg (95% confidence limits: 385.2-573.4) for male and female rats, respectively, were calculated.

Signs of toxicity included reduced motor activity in some animals of both sexes at 681 and 464 mg/kg and males at 215 mg/kg; and abnormal respiratory sounds in one female at 464 mg/kg.

Necropsy findings among surviving animals consisted of 1 male at 316 mg/kg with mottled lungs. Among rats that died, necropsy findings included a hard heart, mottled or hemorrhagic lungs, and white fluid in the stomach in some animals of both sexes; red spotted livers and kidneys in two males at 464 mg/kg; and, a black liver in 1 female at 464 mg/kg.

Conclusion: Acute oral LD_{50} for male rats was 530 mg/kg, and for females, 470 mg/kg.

Classification: Core-Minimum Data; Category III.

b. Acute Dermal Toxicity Study in Rabbits: AXF-1053 Emulsifiable Concentrate Bromoxynil MCPA by D.R. Damske, F.J. Meckler & R.P. Beliles, Litton Bionetics, Inc., LBI Project No. 21142-02(D), October 1979.

Experimental Design: Five male and five female New Zealand White (albino) rabbits (weighing 2.3 to 2.8 kg, and 2 to 3 months of age) were obtained from B and H Rabbitry, Rockville, Maryland. The animals were acclimated to laboratory conditions for 7 days.

The 10 rabbits were prepared by clipping the skin of the trunk free of hair 24 hours prior to dosing. Five rabbits (3 male and 2 female) were further prepared by making epidermal abrasions every 2-3 cm longitudinally over the areas to be exposed. The abrasions were sufficiently deep to penetrate the stratum corneum, but not to disturb the derma. A single 2.0 g/kg dose of the test material was applied to the skin of the rabbits.

After application, the site was covered with gauze and plastic wrap, and the rabbits were placed in restraining collars for 24 hours. At the end of 24 hours, the wrap and collars were removed. The rabbits were cleaned by thorough wiping and the amount of

unabsorbed material was determined. The animals were observed daily for gross signs of poisoning and skin reactions for two weeks after dosing. All animals surviving 14 days after treatment were necropsied and any changes in the internal organs noted.

Results: One death occurred in the 14 days following treatment; therefore, the LD_{50} was greater than 2 g/kg. The dead animal did not eat or drink the day prior to its death and at necropsy mottled lungs were observed.

Rabbits with abraded and intact skin showed mild evidence of skin irritation from days 1 to 7 after treatment. Well defined erythema was observed on all animals at days 1 and 2 after application and at least slight erythema was noted on all rabbits through day 6. On two rabbits the erythema persisted until day 7.

No signs of systemic toxicity or lesions attributable to the test material upon necropsy of surviving animals.

Conclusion: The acute dermal LD_{50} was greater than 2 g/kg of the test material; no signs of systemic toxicity were observed.

Classification: Core-Minimum Data; Category III.

c. Acute Inhalation Toxicity Study in Rats: AXF-1053 Emulsifiable Concentrate Bromoxynil MCPA by P.J. Knapinski, F.J. Meckler and R.J. Weir, Litton Bionetics, Inc., LBI Project No. 21142-02, January 1980.

Experimental Design: Young adult rats (weighing 215 to 409 g, and 8 to 13 weeks of age) of the Charles River CD strain [CRL: COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. The animals were acclimated to laboratory conditions for 7 to 34 days.

The test material was suspended in air at nominal concentrations of 3.3 and 16.6 mg/liter. The test atmosphere was generated using a DeVilbliss Nebulizer and a pump-driven reservoir; particle size of the suspended material was determined using an optical analyzer.

Five animals of each sex were exposed in a 30-liter cylindrical glass exposure chamber to nominal concentrations of 3.32 and 16.6 mg/liter for 4 hours. Total chamber airflow was 6 liters/minute for the low level and 12 liters/minute for the high level.

The rats were observed frequently on the day of exposure and twice daily thereafter. Animals were weighed on the day of exposure, and on days 2, 3, 4, 7 and 14 post-exposure. Necropsies were performed on all animals that died during the study and on the surviving animals that were killed 14 days after exposure.

Results: Mortality was 3/5 deaths among males and 5/5 deaths among females in the 16.6 mg/liter exposure level; no deaths occurred in rats of either sex at the 3.3 mg/liter exposure level. Using a graph, nominal LD_{50} values of 14.4 and 10.0 mg/liter nominal concentrations for male and female rats, respectively, were estimated.

Signs of toxicity included labored breathing and abnormal respiratory sounds in all rats of the high exposure level and abnormal respiratory sounds in four of five females at the low exposure level.

Necropsy findings among surviving animals consisted of mottled lungs and kidneys in 2 males which survived the high exposure level and mottled lungs in 3/5 males and 2/5 females of the low exposure level. Among rats that died, necropsy findings included mottled lungs, mottled kidneys, dark areas on the spleen, and white patches on the liver.

Conclusion: Acute inhalation LC_{50} values of 14.4 and 10.0 mg/liter were estimated for male and female rats, respectively.

Classification: Core-Minimum Data; Category III.

d. Primary Eye Irritation and Corrosiveness Study in Rabbits: AXF-1053 Emulsifiable Concentrate Bromoxynil MCPA by D.R. Damske, F.J.Meckler and R.P. Beliles, Litton Bionetics, Inc., LBI Project No. 21142-02(E), September 1979.

Experimental Design: Nine female New Zealand White (albino) rabbits were obtained from B and H Rabbitry, Rockville, Maryland and acclimated for 1 day in the laboratory. The rabbits weighed 2.2 to 2.5 kg and were 10 to 11 weeks of age. The animals were randomly divided into two groups: Group 1 - six rabbits, and Group 2 - three rabbits.

The eyes of the rabbits were examined with the aid of 2% sodium fluorescein before testing to ensure that the eyes were without defects or irritation. The animals were firmly held and 0.1 ml of the test material was instilled into one eye of each rabbit.

Group 2 rabbits had the treated eye flushed with lukewarm water for 60 seconds, 30 seconds after test material installation. The eyes of all rabbits were washed with sodium chloride after 24-hour observation. The eyes were examined and scores recorded at 1, 24, 48, and 72 hours, and at 7 days.

Results: All rabbits of Group 1 and one of Group 2 exhibited a discharge from the treated eye. Positive signs of irritation consisted of one Group 1 and two Group 2 animals with a Grade 1 cornea, and one Group 1 animal with a Grade 2 cornea; three Group 1 and one Group 2 animals with a Grade 1 iris; one Group 1 rabbit with a Grade 2 conjunctival redness; and a Grade 2 chemosis of one Group 1 and all the Group 2 rabbits. While only three of six rabbits in Group (unwashed) had a positive score for irritation, three of three in Group 2 (washed) had a positive score.

Conclusion: Because of the observed corneal opacity and iritis, the test material is judged to be a moderate irritant when placed into the eyes of rabbits. Corneal opacity and other irritation were reversible within 7 days.

Classification: Core-Minimum Data; Category II.

e. Primary Skin Irritation Study in Rabbits: AXF-1053 Emulsifiable Concentrate Bromoxynil MCPA by D.R. Damske, F.J. Meckler and R.P. Beliles, Litton Bionetics, Inc., IBT Project No. 21142-02(S), September 1979.

Experimental Design: Six female New Zealand White (albino) rabbits were obtained from B and H Rabbitry, Rockville, Maryland and acclimated for one day in the laboratory. The rabbits weighed 2.2 to 2.6 kg at the time of treatment and were 10 to 11 weeks of age.

The hair was clipped from the backs of the rabbits 24 hours prior to administration of the test material, and on one side, two 1-inch squares were abraded by making minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma. The undiluted test material was introduced under four 1-inch patches: two 0.5 ml doses on the intact skin and two 0.5 ml doses on the abraded skin. The rabbits then had their entire trunks wrapped with occlusive wrap and were placed in restraining collars.

The patches were removed 24 hours after exposure. Readings were made and recorded daily for 7 days on each rabbit.

Results: The primary irritation score at 24 hours after treatment was 2.0/8.0 for both intact and abraded skin. At 72 hours, the primary irritation scores were 1.17/8.0 for intact skin and 1.25/8.0 for abraded skin. There was no edema formation and no signs suggestive of systemic toxicity.

Conclusion: The test material produced mild irritation at 72 hours with an average primary irritation score of 1.2/8.0.

Classification: Core-Minimum Data; Category IV.

VIII. Additional Toxicology Data in Support of: Brominal (EPA 264-204). Brominal Plus (EPA 264-239); and, Nu-Lawn Weeder (EPA 264-250).

On May 8, 1980 EPA requested specific citations for chronic toxicology and environmental chemistry data to support the petition. Union Carbide submitted additional toxicology data in support of Brominal (264-204), Brominal Plus (264-239), and Nu-Lawn Weeder (264-250) registrations, dated March 10, 1980, Acc.#242060. A review of these studies follows:

1. Thirty-Day Dose Range Finding Study in with Bromoxynil in Albino Mice by D.E. Bailey and M.A. Gallo, Food & Drug Research Laboratories, Inc., Lab. No. 5092, July 16, 1976 (EPA Accession No. 242060).

   The objective of this study was to evaluate the pharmacotoxic effects of bromoxinil in the diets of albino mice and to provide data for the selection of dosage levels to be used in a chronic feeding study.

   The sample used in this study consisting of a tan powdered material was received at the Waverly Research Center of Food and Drug Laboratories, Inc., and was identified as "TECHNICAL BROMOXYNIL".

   Experimental Design: Sixty male and 60 female albino mice were selected at random and assigned to six groups of 10 males and 10 females each. The test material at levels of 0, 10, 30, 100, 300, and 1,000 ppm, respectively, was administered as a dietary admixture for 30 consecutive days. The mice were housed in groups of 5 per sex in mesh botton cages in temperature and humidity controlled quarters with food and tap water ad libitum.

Gross observations were recorded daily noting appetite and elimination, general appearance and behavior, gross signs of pharmacotoxic effects and mortality. Body weights and food consumption were recorded weekly during the study.

Gross necropsies were conducted on all animals that died and all survivors at the 30-day termination, and all major organs were fixed in 10% neutral buffered formalin and retained for reference. Organ weights and organ-to-body weight ratios were recorded for: thyroids, liver, spleen, kidneys, adrenals, testes and ovaries.

Results: All animals survived the 30 days of the study. Male mice had a slight reduction in food consumption with diets containing 100 and 300 ppm of bromoxynil, which was not observed in the females of these groups. This reduction was not observed after the first week. The high dose containing 1,000 ppm produced a marked decrease in food consumption in both males and females during the first week of study and this was reflected by a weight loss. Food consumption of the females was normal throughout the remainder of the study and weight gain was normal. In males the food consumption remained slightly depressed through week 3 and was comparable to controls in week 4 but body weight remained depressed. A dose related increase in liver weights and relative liver weight rations were comparable to controls.

Conclusion: There was no mortality from bromoxynil fed in the diet of adult albino mice for 30 consecutive days. At dosages of 300 and 1,000 ppm there was at some time depression of food consumption, accompanying decreased weight gain and dose related increase in liver weight.

Classification: Core-Minimum Data; Category III.

2. Teratologic Evaluation of Bromoxynil in Rats by J.G. Babish and K.R. Stevens, Waverly Research Center, Food and Drug Research Laboratories, Inc., Laboratory No. 5097, January 3, 1977.

The sample used in this study consisting of a tan powdered material was received at the Waverly Research Center of Food and Drug Research Laboratories, Inc., and was marked "TECHNICAL BROMOXYNIL".

Procedures - Virgin, adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature controlled quarters. Each rat had free access to food and fresh tap water. They were mated with young adult males and detection of the vaginal sperm plug was considered to be day 0 of gestation. Beginning on day 6 and continuing daily through day of 15 gestation, each female received the appropriate quantity of Bromoxynil to achieve a final dose of 0, 1.5, 5.0, or 15.0 mg/kg/day. The gavage vehicle was oil. The study was set up using both positive (aspirin) and negative control groups as tabulated below:

	Groups (mg/kg				
	Control 0	Aspirin 250	Aspirin 1.5	Bromoxynil 5.0	Bromoxynil 15.0
Females bred	23	25	24	24	24
No. pregnancies	22	21	22	21	24

Body weights were recorded on days 0, 6, 11, 15 and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects.

On day 20 of gestation, each dam was sacrificed by chloroform overdose and subjected to Cesarean section. The uterine contents were examined and the number of implantation sites for each unterine horn were recorded along with the number of live pups and the number of corpora lutea were recorded. The urogenital tract was examined for signs of gross abnormality.

One third of the pups in each litter were randomly chosen and fixed in Bouin's solution; these animals were then examined for soft tissue abnormalities using the Wilson freehand slicing technique. All pups showing any gross abnormality were included in this preparation.

The remaining pups were fixed in 70% isopropyl alcohol and arranged in ice cube trays to maintain their identities as to location in the uterus. They were then cleared in KOH, stained

with Alizarin Red S, and individually stored in glycerin and then subjected to a detailed skeletal examination under low power magnification. All observations of skeletal abnormalities were recorded. All animals were evaluated according to normally accepted degrees of development for a 20 day fetus.

Statistical Methodology - Rates of appearance of abnormalities were compared statistically by computation of exact probability or by means of interpolation of graphs of confidence belts as presented in Steel, R.C.D. & Torrie, J.H.: Principles and Procedures of Statistic, McGraw-Hill Book Co., New York 1960.

Regression analyses were performed on percent data using the arcsine transformation for normalization as described in Snedecor, G.W. & Cochran, W.G.: Statistical Methods, The Iowa State University Press, Ames 1971. For regression calculations, the log dose was considered the independent variable and the transformed percent the dependent variable. Determinations of slope, intercept and correlation coefficients were as described in Snedecor & Cochran.

Results: Individual reproduction data for all rats in each of the five test groups were supplied in the appendix. In addition the following were provided in tabular form: (a) average litter reproduction data; (b) summary of skeletal findings of pups; (c) summary of soft tissue abnormalities of pups; and, (d) average body weights of dams during gestation.

Neither aspirin (250 mg/kg) nor Bromoxynil (1.5, 5.0, or 15.0 mg/kg) interfered with pregnancy; live litters were born to dams in all treatment groups. Bromoxynil did not significantly affect the number of implantation sites, resorptions or live fetuses per dam as compared to controls. Aspirin, on the other hand, induced a significant number of resorptions and dead fetuses, as expected.

There was no significant production of soft tissue anomalies associated with Bromoxynil treatment.

Conclusions:

a. Wavy ribs and ribs in excess of 13 are not considered teratologic entities.

b. Although aspirin at 250 mg/kg, the positive control, induced a significant number of resorptions and dead fetuses, there were no signs of teratologic pathology in the fetuses of dams so treated.

c. Bromoxynil, Technical at 15 mg/kg neither affected reproductive performance nor induced any teratologic pathology in the fetuses of dams so treated; this dosage, the highest dose tested, was much too low.

d. It was not specified as to whether a vehicle control group or a separate control group receiving a sham treatment was used as the negative control.

e. Dosage levels should be employed as specified in subsection 163.83-3(b)(7) of the Proposed Guidelines in the August 22, 1978 Federal Register (43 FR, Vol. 163, page 37383). The study should be repeated using higher doses because it provides no findings for an adequate toxicological evaluation of teratological effects of bromoxynil.

Classification: Core-Supplementary Study.

3. Microbial Mutagen Assays with Technical Bromoxynil by Michael A. Gallo, Food & Drug Research Laboratories, Inc., Laboratory No. 5633, September 12, 1977.

The Ames Salmonella/Microsome Mutagen Assay was conducted to determine if Bromoxynil (Tech) was mutagenic at the his-locus in the Salmonella tryphimurium tester strains of Ames. The organisms tested were 24-hour trypticase broth cultures from frozen stock of S. tryphimurium strains: TA98, TA100, TA1535, TA1537, TA1538.

Procedures:

a. Minimum Inhibitory Concentration (MIC): 2-fold serial dilutions were performed in trypticase soy broth to determine the MIC in ppm; growth was observed after 48 hours at 35C and the test dose was chosen from the inhibitory screen.

b. Test Method: Ames spot test (disc) technique with and without activation with rat liver induced S-9 mix.

(1) 20 ml. of Vogel-Bonner Minimal Medium (VBMM) was poured and allowed to harden.

(2) To this was added an overlay containing

	Not activated	Activated
Soft agar	2.0	2.0
Culture	0.1	0.1
S-9 Mix	---	0.5
	2.1 ml	2.6 ml

(3) The overlay was gently mixed to form a confluent layer over the base layer and the disc (containing 16 ppm, of Technical Bromoxynil dissolved in 50% ethanol) was placed on top. Discs for positive and negative controls contained, respectively:

(1) MNN-nitrosoguanidine (20 ug) - mutagen for TA100 and TA1535 without inactivation; and,

(2) Ethanol Solvent

(4) The numbers of revertant colonies were counted on the treated plates (T) and on the control plates (C) after incubation for 48 hours at 35C.

(5) A T/C ratio of 2.0 or more was taken to indicate a possible mutagenic effect.

Results: Laboratory data was submitted for tests in all strains of S. typhimurium, treated and control plates.

a. Minimum Inhibitory Concentration (MIC): MIC (ppm) was 16 ppm for each of the five S. typhimurium tester strains used in this study.

b. Ames Salmonella/Microsome Assay - Technical Bromoxynil was not mutagenic for Ames Salmonella typhimurium tester strains (TA98, TA100, TA1535, TA1537, TA1538) when tested at 100 ug with and without rat liver activation.

(1) Test Compound - Technical Bromoxynil

Organism	T/C Ratio*	Result
TA98	0.9	Inactive
TA100	0.5	Inactive
TA1535	0.8	Inactive
TA1537	0.8	Inactive
TA1538	1.1	Inactive

*activated

(2) Mutagen Control - MNN-nitrosoquanidine 20 ug

Organism	T/C Ratio	Result
TA100	> 100	Active
TA1535	> 100	Active

(3) Solvent Control - 50% Ethanol

T/C Ratio = < 2.0 in each of the five S. typhimurium tester strains used in this study.

Conclusions:

The results indicate that Technical Bromoxynil was non-mutagenic to the bacterial tester strains commonly used to determine point mutations. S. typhimurium strains [Histidine deficient mutants (HIS^-)] TA98 and TA1538 bear the same frame-shift mutation responsible for their phenotype, HIS^-, TA1535 and TA100 possess a base pair substitution responsible for their HIS^- auxotrophy. Neither a frame-shift nor a base pair substitution was induced in either an activated (S-9 liver fraction) or non-activated system by this compound.

4. Evaluation of the Effects of Bromoxynil on the Reproductive Performance of FDRL Wistar Rats through Three Successive Generations by K.R. Stevens and J.G. Babish, Waverly Research Center, Food and Drug Research Laboratories, Inc., Laboratory No. 5096, September 20, 1978.

The sample used in this study, a tan material, was received at the Waverly Research Laboratory of Food and Drug Research Laboratories, Inc. (FDRL), on May 13, 1976 and was identified as "Technical Bromoxynil". This material was used in a dose range finding study (Study No. 5092) as well as the reproduction test described in this report.

Procedures: One hundred twenty weanling albino FDRL Wistar rats were obtained from the FDRL breeding colony for this study. Animals were caged individually in suspended wire mesh cages and were given free access to feed (Charles River RHM meal) and clean tap water.

The feed was admixed with technical bromoxynil at levels of 0, 30, 100, or 300 ppm.

Feed was weighed before being put into the feeders and weighed back at the end of each weekly feeding period; weekly feed consumption was represented by the difference of these feed weights.

Food consumption and body weights of all animals were determined weekly for each generation except during mating, gestation and lactation. However, body weights of females were recorded on days 0, 8, 15, 20, 28, 35, and 42 after observation of the copulatory plug.

Reproduction was begun, approximately 100 days after weaning of each generation. Two females were assigned to each male. Females were placed into the male's cage until breeding was indicated by a copulatory plug. If either female was not bred within one week, she was assigned to another male within the same treatment group. Each bred female was returned to her cage. On the 20th day of gestation, each bred female was transferred to a plastic "littering" cage containing approximately 1" of sterilized corn cob chips.

At parturition, the number of live and still births were recorded. Litter weight, by sex, was recorded at 24-hours, and at 4, 7, and 21 days after birth. The first litter of any dam was killed at weaning, and each animal examined grossly for evidence of pathology. At weaning of the second litter, however, the offspring were separated by sex and one male and two females from each litter were randomly assigned to the reproductive phase of the next generation. Enough weanlings were randomly assigned to obtain 10 males and 20 females for the next reproductive phase. In each generative phase, sibling matings were avoided. Offspring not assigned to the reproductive phase were killed and examined grossly for abnormalities.

Following successful breeding or weaning of a second litter, the males and females, respectively, were killed and subjected to gross autopsy. Preservation of the following tissues in 10% neutral formalin was carried out on the F_{2B} fetuses:

brain	small intestines	spleen
eye	urinary bladder	adrenals
lung	bone	pancreas
liver	pituitary	large intestine
kidneys	thyroid	gonads
stomach	heart	bone marrow (sternum)

and any unusual lesions.

Statistical Methods - Data were analyzed by analysis of variance by sex and data collection period. If a treatment effect was detected at a probability level of < 0.05, a least significant difference test was performed to compare each treatment mean with the control. Differences in means were declared significant when their calculated rate of occurrence, due to chance alone, was less than 5%.

Discrete data were analyzed by computation of exact probabilities. Each treatment mean was compared with that of the control. Differences were declared significant as above.

Statistical procedures are described in Steel, R.G.D. and Torrie, J.H.: Principles and Procedures of Statistics, McGraw-Hill Book Co., Inc., New York, 1960.

Results - In general, there were no untoward treatment-related observations during the course of this study. The indices of fertility and growth did not decline with progressing generations.

a. Fertility Index - There was no dose-related or generation-related depression in fertility of the rats. The fertility overall was good -- beyond 85% in all generations and all doses.

b. Gestation Index - Gestation Index was not affected.

c. Length of Gestation - The average length of gestation was not significantly affected by bromoxynil treatment at any generation period.

d. Mean Litter Size - Litter size was not appreciably affected at any generation period by dose levels of up to 300 ppm bromoxynil except the F_{3A} period when litter size in the control group was 12.4 versus 9.7 (pups/litter) in the 300 ppm bromoxynil group. This chance occurrence is probably of little or no significance since litter sizes of the F_{3B} generation control and high level treatment groups were 11.9 and 11.3 pups/litters, respectively.

e. Mean Body Weight at Birth - There were no significant differences in the weight of offspring at birth in any generation except the F_{2A} at the level of 300 ppm dietary bromoxynil. Again, this is probably a chance occurrence since no birth weight differences were observed between control and bromoxynil treatment groups in any other litters at any other generations.

f. Survival - There was no significant effect of bromoxynil treatment on viability indices at any generation periods; these indices were measures of survival of offspring from birth to day 4 and were calculated two ways: (1) percent of pups born alive that survived 4 days, and (2) percent of offspring/dam that survived from birth to day 4.

When the viability indices are calculated from day 4 to weaning (day 21) they are termed indices of lactation.

There were no differences in survival among groups at any generation period regardless of the method by which the index was calculated.

g. Mean Body Weight - At 7 days of age, three groups of offspring treated with 300 ppm bromoxynil in the F_{2A}, F_{2B}, and F_{3A} generations had significantly lower weights than controls. The differences seen in the F_{3A} generation did not extend to 21 days. However, at weaning (21 days), in four of the six generation periods (F_{1B}, F_{2A}, F_{2B}, and F_{3B}), the mean body weight of offspring receiving 300 ppm bromoxynil was lower than that of controls.

Mean weekly body weights were presented for the period subsequent to weaning. Males of F_0 generation grew at similar rats regardless of treatment. However, during the F_{1B} generation, males receiving 100 and 300 ppm bromoxynil had significantly lower mean body weights than control and 30 ppm groups until the 5th week post-weaning; afterwards, they grew at rates which were not dissimilar. In males of the F_{2B} generation treated with 100 ppm, this lag in growth extended through the 15th week post-weaning, after which mean weights were not statistically dissimilar.

Among generations of females, treatment-related weight differences were not observed, except in the F_{2B} generation where significantly lower mean body weight occurred in groups receiving 100 and 300 ppm bromoxynil through the 10th week post-weaning.

h. Food Consumption - In males food consumption did not differ among treatment groups in F_0 or F_{1B} generations. In F_{2B} generation males, consumption of feed by controls exceeded that of all test groups during post-weaning weeks 3, 4, and 6. Thereafter, the food consumption of the 30 and 100 ppm groups of males was significantly lower than the controls while the 300 ppm group was not .

In females, there were sporadic differences in food consumption between control and treated groups, but these differences did not occur consistently throughout any generation.

i. Gross Pathology - The rats were sacrificed and underwent autopsy following the weaning of the B litters of each generation. There was no grossly abnormal finding in any generation which appeared to be treatment related. The most frequent findings of hollow, fluid-filled kidneys, mottled livers and granular appearance of spleens occurred with approximately the same frequency in both control and bromoxynil-treated groups.

Conclusions - There were no observable adverse effects on fertility, gestation, litter size, survival of offspring, and gross pathology in rats which underwent a 3-generation reproduction study during which bromoxynil was incorporated into their diets of 0, 30, 100 and 300 ppm. In treated litters of several generations, mean body weights were less than that of controls during lactation and up to 10 weeks post-weaning. This lesser body weight coincided directly with less food consumption by the so affected animals. This reduced body weight was most likely due to decreased palatability because of the presence of 100 and 300 ppm bromoxynil. The NOEL on reproductive performance is 300 ppm or greater.

Classification: Core-Minimum Data.

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