008345

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

APR 23 1991

APR 23 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Developmental Toxicity Study in Rats with BROMACIL.

TO: Jay Ellenberger/Mario Fiol, PM-50
Registration Division, H7505C

FROM: David S. Liem, Ph.D David S. Liem 3/25/91
Section II, Toxicology Branch II/HED, H7509C

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 3/26/91
Section II, Toxicology Branch II, HED, H7509C
and
Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 4/16/91
Toxicology Branch II, Health Effects Division, H7509C

EPA ID Nos.: MRID No.: 409848-02 ID No.: 012301
Caswell No.: 111 HED Project No.: 1-0745

ACTION REQUESTED: To review a developmental Toxicity Study in Rats with Technical BROMACIL (INN-976).

CONCLUSIONS

Four groups of 25 mated female Crl:CD BR rats were given oral administration of 0, 20, 75, 200, 500 mg/kg/day of Bromacil from days 7 to 16 of gestation.

The maternal toxicity NOEL is determined to be 20 mg/kg. The maternal toxicity LOEL is 75 mg/kg based on the decreased body weight gain and the decreased of food intake during the first two days of dosing. The absolute and relative liver weights were significantly increased in the highest dose tested (500 mg/kg).

The developmental toxicity NOEL is determined to be 75 mg/kg. The developmental toxicity LOEL is 200 mg/kg, based on increased incidences of rudimentary lumbar ribs and of an extra thoracic vertebra. Significant increases of skeletal developmental variations due to retarded development, namely the retarded or partial ossification of the axial skeleton (interparietal, parietal, and supraoccipital of the skull bones; bipartite and dumbbelled centrum of the vertebrae; sternum; and hyoid) and the partial ossification of the appendicular skeleton (pubis and ischium) were observed in the highest dose tested (500 mg/kg).

CLASSIFICATION: Core-minimum. This study satisfies the guideline requirements (83-3) for a Developmental Toxicity study in rat.

001

Printed on Recycled Paper

008345

Primary Reviewer: David S. Liem, Ph.D. David Liem 3/15/91
Section II, Toxicology Branch II/HED
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 3/15/91
Section II, Toxicology Branch II/HED

DATA EVALUATION RECORD

Study Type: Teratology • Developmental Toxicity GUIDELINE: 83-3

Test Animal: Crl:CD BR Rat

EPA ID Nos.: MRID No.: 409848-02 ID No.: 012301
Caswell No.: 111 HED Project No.: 1-0745

Test Material: Bromacil (5-Bromo-3-sec-butyl-6-methyluracil) with a purity of 95.1%

Synonym: 2,4(1H,3H)-Pyridinedione, 5-bromo-6-methyl-3-(1-methylpropyl); INN 976; CAS# 314-40-9

Dosages: 0, 20, 75, 200, 500 mg/kg

Sponsor: Agricultural Products Department, E.I. DuPont de Nemours and Co., Inc., Wilmington, DE 19898

Study Number: 16473; MR-7977-001

Testing Facility: Haskell Laboratory for Toxicology and Industrial Medicine, E.I. DuPont de Nemours and Co., Inc., Newark, DE 19714

Title of Report: Teratogenicity Study in INN-976 in Rats

Author: Louis Alvarez

Report Issued: April 6, 1988

CONCLUSIONS

Four groups of 25 mated female Crl:CD BR rats were given oral administration of 0, 20, 75, 200, 500 mg/kg/day of Bromacil from days 7 to 16 of gestation.

The maternal toxicity NOEL is determined to be 20 mg/kg. The maternal toxicity LOEL is 75 mg/kg based on the decreased body weight gain and the decreased of food intake during the first two days of dosing. It is noted that the absolute and relative liver weights were significantly increased in the highest dose tested (500 mg/kg).

The developmental toxicity NOEL is determined to be 75 mg/kg. The developmental toxicity LOEL is 200 mg/kg, based on increased incidences of rudimentary lumbar ribs and of an extra thoracic vertebra. Significant increases of skeletal developmental variations due to retarded development, namely the retarded or partial ossification of the axial skeleton (interparietal, parietal, and supraoccipital of the skull bones; bipartite and dumbbelled centrum of the vertebrae; sternum; and hyoid) and the partial ossification of the appendicular skeleton (pubis and ischium) were observed in the highest dose tested (500 mg/kg).

CLASSIFICATION: Core-minimum. This study satisfies the guideline requirements (83-3) for a developmental toxicity study in rat.

Study Title: Teratogenicity Study in INN-976 in Rats

Author: Louis Alvarez Report Date: April 6, 1988

Study No.: 16473; MR-7977-001

Study Period: April 13 to May 15, 1987 (In-life phase)

Testing Facility: Haskell Laboratory for Toxicology and Industrial Medicine, E.I. DuPont de Nemours and Co., Inc., Newark, DE 19714

Test Material: Bromacil (5-Bromo-3-sec-butyl-6-methyluracil) with a purity of 95.1%

Test Animal: Crl:CD BR Rat

A. OBJECTIVE

The objective of this study was to assess the developmental toxicity of Bromacil following oral administration to pregnant rats during the period of major organogenesis (gestation days 7-16).

B. MATERIALS AND METHODS

Test Compound: A tan solid substance with a purity of 95.1%
Lot no.: 180-806 3T Batch 31; N.B. 5103-146
Storage: Not noted in the study report

Vehicle: A 0.5% aqueous suspension of methylcellulose (4000 centipoise) obtained from Fischer Scientific, Fair Lawn, N.J. (lot # 852077).

Test Animals: Species: Crl:CD BR Rat
Source: Charles River Breeding Lab., Kingston, N.Y.
Acclimation period: About 9 days before mating
Age: Females- 63 days, and males- 84 days on arrival
Body Weight: Females = 174.0 to 218.9 gms on arrival
Males = 310.9 to 382.5 gms on arrival
Caging: In individual suspended wire cages
Feed: Purina Certified Rodent Chow #5002 and water ad libitum

Environmental Parameter: Air temperature = 21°C - 25°C; Relative Humidity = 40-60%; Photoperiod: 12 hrs dark/light cycle.

Study Design

This study was designed to assess the developmental toxicity potential of Bromacil when administered by gavage to female rats on gestation days 7 through 16, inclusive.

Dose Selection:

Dose levels for the study were based on a pilot study conducted to determine the MTD in groups of 6 female rats dosed on days 7-16 of gestation at daily dose levels of 0, 300, 500, 700, 900 mg/kg of body weight. One 900 mg/kg dam died. Feed consumption was significantly reduced in all treated groups, but equivocal effects on body weights effects were noted. Abnormal clinical signs were observed in the 700 and 900 mg/kg dose groups. Liver weights were increased for the 500, 700 and 900 mg/kg dose groups. Litter size in 900 mg/kg dose was reduced. Based on this data, dose levels of 20, 75, 200, and 500 mg/kg were selected for this study.

Group Arrangement:

Animals selected for the study were ranked by their body weights and then they were assigned to the study groups by random sampling from strata established in the ranked list, as follows:

Group	Dose Group	Dose Level (mg/kg)	Number Assigned
1	Vehicle Control	0	25
2	Low Dose	20	25
3	Low-mid Dose	75	25
4	High-mid Dose	200	25
5	High Dose	500	25

Mating:

Female rats were mated with males (1:1) until copulation was confirmed by the presence of a vaginal plug. The day of copulation was designated as day 1 of gestation.

Dose Preparations and Analyses:

One day prior to dosing, appropriate amounts of INN-976 were suspended in 0.5% aqueous methyl cellulose to produce suspensions of 2.0, 7.5, 20, and 50 mg/ml for the low, low-mid, high-mid, and high dose, respectively. Duplicate samples of each test suspension were taken at the beginning, once during, and at the end of the treatment period. Samples were also taken, one immediately after preparation and another, 5 hours thereafter. The stability and concentration of the dosing suspensions were analyzed.

Dosing:

Dose suspensions were administered by gavage and dosages were based on the most recently recorded body weights.

Clinical Observations

The animals were checked daily for mortality, moribundity and signs of toxicity. On day 22 all surviving females were sacrificed.

Maternal Body Weights:

Individual body weights (BW) were taken on the day after arrival, before mating, and on days 1, 7-17, and 22 post coitum. Net terminal body weights were calculated by subtracting the gravid uterine weight from the terminal body weight. The net body weight gain from day 0 is the corrected BW minus day 0 body weight.

Maternal Food Consumption Data

Individual food consumption was determined on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 22 of gestation.

Postmortem data

On day 22 post coitum, all surviving females were sacrificed, and internal organ abnormalities were evaluated macroscopically. The reproductive organs were evaluated for gross abnormalities. The uterus and ovaries were examined and the number of corpora lutea, implantation sites, resorptions, and live and dead fetuses were recorded. The liver as well as empty and gravid uteri were weighed. The uterus of apparently "no-pregnant" rats was opened and stained with ammonium sulfide to detect very early resorptions.

Each fetus was subjected to external macroscopic examination, sexed, and weighed. Every other live fetus in each litter was decapitated, and visceral tissues were examined macroscopically. The heads were fixed in Bouin's solution, for future examinations. All fetuses were eviscerated, fixed in 70% ethanol, and macerated in 1% aqueous potassium hydroxide. They were then stained with alizarin red S for skeletal alteration evaluations.

For each litter, the maximum stunted weight (MSW) was calculated by substracting the lightest weight from the total weight, dividing by the remaining number of fetuses and multiplying by 0.66. A fetus weighing less than the MSW was considered stunted, and its weight was excluded from mean litter weight calculations.

Statistical analysis

The statistical methods are appended as Appendix A.

Compliance

- o A signed Statement of Confidentiality Claim was provided.
- o A signed Statement of compliance with EPA GLP's was provided
- o A signed Quality Assurance Statement was provided.

C. RESULTS AND DISCUSSIONS

a. Dose Suspension Analysis

The nominal concentrations of the test material were 2.0, 7.5, 20.0, and 50.0 mg/ml, representing the low, low-mid, high-mid and high dose groups. Samples of dose suspension frozen after preparation showed concentration between 95% to 110% of nominal, while samples frozen after 5 hours at room temperature gave values between 93% and 112% of the nominal concentration.

b. Maternal Mortality

All dams survived to scheduled sacrifice on Day 22 of gestation.

c. Maternal Clinical Observations

Clinical signs observed are summarized in Appendix B. As seen from this Appendix, during the dosing period (days 7-16 of gestation), increased incidences of perinasal, periocular, and perioral stains and alopecia were observed in the high dose group. Perinasal and periocular stains were also observed in the other treated groups during this dosing period. It is noted that alopecia was also significantly increased in the low and high dose groups, during the post-dosing period (days 17-22 of gestation). The toxicological meaning of these findings is not clear. No other significant or treatment-related clinical observations were noted.

d. Maternal Body Weight Data

The summary mean maternal body weights and body weight gains are presented in Appendices C and D. The mean maternal body weights for the predosing period (days 1 and 7) were generally comparable among the groups. During the first two days of dosing, body weights for the high-mid and high dose groups were drastically decreased (weight loss occurred) as compared to the control. The body weight values increased after day 11 of gestation and were comparable with the control, at terminal sacrifice (see attached Appendix C). During the first two days of the dosing period (days 7-9), statistically significant maternal body weight gain reductions were observed in the low-mid, high-mid, and high dose groups as compared to the control. Only the high-mid and high dose groups' maternal body weight gains were statistically decreased during the entire dosing period, as compared to the controls. It is noted that during the post-dosing period (days 17-22 of gestation), the body weight gains were increased for the low-mid (statistically significant), high-mid, and the high dose groups as compared to the control. The net body weight gains in the high-mid and high dose groups were lower than the control. The observed body weight gain reductions in the low-mid, high-mid, and high dose groups are considered to be related to treatment.

e. Food Consumption Values

Group mean food consumption values are presented in the attached Appendix E. As seen from this Appendix, statistical significant food consumption reductions were observed in the low-mid, high-mid and high dose groups during the first two days (days 7-9 of gestation) of the dosing period as compared to the control. Statistically significant food consumption reductions were also observed in the nigh-mid and high dose groups during days 7-11 of gestation; food consumption values for these two dose groups were also statistically reduced throughout the dosing period, as compared to the control. These food consumption reductions are judged to be related to treatment. It is noted that statistically significant food consumption increase was observed in the low-mid dose group during the pre- and post-dosing periods.

f. Maternal Gross Pathological Observations

Only the following significant maternal gross pathological findings were noted: excess fluid in the amniotic sac found in one high-mid dose group, kidney hydronephrosis found in one high-mid and in one high dose group. There is no evidence that these findings are related to treatment.

The absolute and relative liver weights were significantly increased in the high dose group as compared to the control (see attached Appendix F). This liver weight increase in the high dose group is considered to be related to treatment.

g. Pregnancy Rates

The pregnancy rates were 96% for control and high dose groups, and 92% for the low, low-mid, and high-mid dose groups.

h. Caesarean Section Observations

Caesarean section data are presented in the attached Appendix G. As seen from this Appendix, caesarian section data are comparable among the groups. These included numbers of corpora lutea, numbers of implantation sites (nidation), numbers of resorptions, numbers of live and dead fetuses, and the numbers of stunted fetuses. The number of female fetuses per litter was statistically increased in the low-mid and the high-mid dose groups as compared to the control, but since no clear dose-related trends were evident, this finding is not considered to be related to treatment.

i. Fetal External Observation

Imperforate anus, vestigial tail, and skin hemorrhaging were observed in three different low dose fetuses. Skin edema was observed in three fetuses of the high dose group (see attached Appendices H and I). These findings are not related to treatment.

j. Fetal Visceral and Skeletal Malformations (see Appendix H)

None of the visceral and skeletal malformations observed in the treated groups were statistically different from those of the controls and no clear dose-related trends were evident. Thus these differences are not considered compound-related effects.

k. Fetal Visceral and Skeletal Variations (see attached Appendix I)

As seen from Appendix I, no fetal visceral variations observed in the treated groups were statistically different from the control and no dose-related trends were evident. Significant increases of developmental skeletal variations as well as skeletal variations due to retarded development were observed; significant trends accompanied some of these changes. Significant increases of skeletal developmental variations included the rudimentary lumbar ribs and the presence of an extra thoracic vertebra in the high-mid and the high dose groups as well as the unilateral caudal shift of the ilium in the high dose group. Significant increases of skeletal developmental variations due to retarded development observed in the high-dose group included the retarded or partial ossification of the axial skeleton (interparietal, parietal, and supraoccipital of the skull bones; bipartite and dumbbelled centrum of the vertebrae; sternum; and hyoid) and the partial ossification of the appendicular skeleton (pubis and ischium). All of the noted variations are judged to be related to treatment.

CONCLUSIONS

Four groups of 25 mated female Crl:CD BR rats were given oral administration of 0, 20, 75, 200, 500 mg/kg/day of Bromacil from days 7 to 16 of gestation.

The maternal toxicity NOEL is determined to be 20 mg/kg. The maternal toxicity LOEL is 75 mg/kg based on the decreased body weight gain and food intake during the first two days of dosing. It is noted that the absolute and relative liver weights were significantly increased in the highest dose tested (500 mg/kg). The developmental toxicity NOEL is determined to be 75 mg/kg. The developmental toxicity LOEL is 200 mg/kg, based on increased incidences of rudimentary lumbar ribs and of an extra thoracic vertebra. Significant increases of skeletal developmental variations due to retarded development, namely the retarded or partial ossification of the axial skeleton (interparietal, parietal, and supraoccipital of the skull bones; bipartite and dumbbelled centrum of the vertebrae; sternum; and hyoid) and the partial ossification of the appendicular skeleton (pubis and ischium) were observed in the highest dose tested (500 mg/kg).

CLASSIFICATION: Core-minimum. This study satisfies the guideline requirements (83-3) for a developmental toxicity study in rat.

008345

APPENDICES

APPENDIX A: Statistical Methods Employed (copied from p. 14-15 of the study report)

APPENDIX B: Summary Clinical Sign Observations (copied from p. 23 of the study report)

APPENDIX C: Summary Body Weights Data (mg) (copied from p. 55-59 of the study report)

APPENDIX D: Summary Body Weights Gain Data (copied from p. 21 of the study report)

APPENDIX E: Summary Food Consumption Data (mg) (copied from p. 60-64 of the study report)

APPENDIX F: Summary Mean Absolute and Relative Liver Weights (mg) (copied from p. 24 of the study report)

APPENDIX G: Summary Maternal Reproductive Data (copied from p. 25 of the study report)

APPENDIX H: Summary Incidence of Fetal Malformations (copied from p. 26-27 of the study report)

APPENDIX I: Summary Incidence of Fetal Variations (copied from p. 28-31 of the study report)

Bromacil RIN: 1607-93

Page ____ is not included in this copy.

Pages 11 through 22 are not included.

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 quality control procedures.

____ Identity of the source of product ingredients.

____ 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.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/SACB/TOX
ONELINERS

TOXCHEM NO.: 111 (Bromacil; 95.1% purity)

CITATION	MATERIAL	ACCESSION/ MRID NUMBER	RESULTS	TOX. CAT.	CORE GRADE/ DOCUMENT #
Guideline 83-3 Developmental Toxicity Species: Crl:CD BR Rat E.I. DuPont de Nemours No. 16473; MR-7977-001 Date: April 6, 1988	Bromacil Technical Purity = 95.1% Lot #:180-906 3T and N.B. 5103-146	409848-02	Dosage: 0, 20, 75, 200, 500 mg/kg via oral gavage Maternal toxicity NOEL = 20 mg/kg. Maternal toxicity LOEL = 75 mg/kg, based on the decreased body weight gain and food intake during the dosing period. Significant maternal liver weight increase was observed in the 500 mg/kg dose group. Developmental toxicity NOEL = 75 mg/kg. Developmental toxicity LOEL = 200 mg/kg, based on increased incidences of rudimentary lumbar ribs and of an extra thoracic vertebra. Significant increases of skeletal developmental variations due to retarded development, namely the retarded or partial ossification of the axial skeleton (interparietal, supraoccipital and parietal of the skull bones; bipartite and dumbbelled centrum of the vertebrae; sternum; and hyoid) and the partial ossification of the appendicular skeleton (pubis and ischium) were observed in the highest dose tested at 500 mg/kg.		Minimum

008345

008345

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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Bromacil Teratology Study in Rabbits

TO: Mario Fiol
SRRD (H7508W)

FROM: Karen E. Whitby, Ph.D. 4/4/91
Section, II
Toxicology Branch II/(HED) (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 4/15/91
Section Head
Toxicology Branch II/(HED) (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 4/16/91
Chief, Toxicology Branch II/(HED) (H7509C)

EPA MRID No. 409848-01
HED Project No. 1-0745

The Data Evaluation Report for the subject developmental toxicity study is attached.

Action Requested

Please review a rabbit teratology study on Bromacil.

Study Title and Conclusions

Title of Report: Teratogenicity Study of INN-976 in Rabbits EPA MRID No. 409848-01

Conclusions:

Bromacil was administered to presumed pregnant New Zealand White Rabbits by gavage at 0, 30, 100, 300, and 500 mg/kg. Maternal toxicity was observed at 300 and 500 mg/kg as evidenced by decreased bodyweight gain and food consumption. Developmental

toxicity was evidenced by an increased incidence of resorptions at these levels.

Core Classification: Core Minimum Data.

Maternal NOEL = 100 mg/kg
Maternal LOEL = 300 mg/kg
Developmental Toxicity NOEL = 100 mg/kg
Developmental Toxicity LOEL = 300 mg/kg

008345

008345

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. 4/4/91
Toxicologist, Review Section II, Toxicology Branch II/HED
(H7509C)

Secondary Review by: K. Clark Swentzel K. Clark Swentzel 4/15/91
Section Head, Review Section II, Toxicology Branch II/HED
(H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
Guideline: 83-3

EPA Identification No.s:

EPA MRID (Accession) No.: 409848-01
Caswell No.: 111
HED Project No.: 1-0745

Test Material:

2,4(1H,3H)-Pyrimidinedione, 5-bromo-6-methyl-3-(1-methylpropyl)-; also written as

5-bromo-6-methyl-3-(1-methylpropyl)-2,4(1H,3H)-pyrimidinedione

Synonyms: Bromacil
5-Bromo-3-sec-butyl-6-methyluracil
INN-976

Sponsor: Agricultural Products Department
E.I du Pont de Nemours and Company, Inc.
Wilmington, DE 19898

Study Number(s): Medical Research No.: 8187-001
Haskell Lab. Report No.: 527-87

Testing Facility:

E.I. du Pont de Nemours and Co., Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714

Title of Report: Teratogenicity Study of INN-976 in Rabbits

Author(s): John E. Zellers

Report Issued: December 18, 1987

008345

Study Dates: Live Phase - Initiated: August 2, 1987
Live Phase - Terminated: August 28, 1987

Bibliographic Citation: Zellers, J.E. (1987) Teratogenicity Study of INN-976 in Rabbits. Medical Research No. 8187-001; Haskell Laboratory Report No. 527-87. (Testing Facility: E.I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Delaware 19714; Sponsor: Agricultural Products Department, E.I du Pont de Nemours and Company, Inc.)

Conclusions:

Bromacil was administered to presumed pregnant New Zealand White Rabbits by gavage at 0, 30, 100, 300, and 500 mg/kg. Maternal toxicity was observed at 300 and 500 mg/kg as evidenced by decreased bodyweight gain and food consumption. Developmental toxicity was evidenced by an increased incidence of resorptions at these levels.

Core Classification: Core Minimum Data.

Maternal NOEL = 100 mg/kg
Maternal LOEL = 300 mg/kg
Developmental Toxicity NOEL = 100 mg/kg
Developmental Toxicity LOEL = 300 mg/kg

008345

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 95.1%
Description: tan solid with a melting point of 150°C and a molecular weight of 261.1
Lot No.: 180-806 3T Batch 31
Contaminants: composition of contaminants was not identified.

Vehicle(s): The vehicle was a 0.5% aqueous suspension of methyl cellulose (4000 centipoise). The vehicle (CAS No.9004-67-5, lot 852077) was obtained from Fisher Scientific, Fair Lawn, New Jersey.

Test Animal(s):

Species:	Rabbit (nulliparous)
Strain:	Hra:(NZW)SPF
Source:	Hazelton, Research Products, Inc., Denver, PA.
Age:	approx. 21 weeks upon receipt approx. 26 weeks at start of study
Weight:	upon receipt mean = 3581.5±279.93 g range = 2928 to 4177 g

B. Study Design

This study was designed to assess the developmental toxicity potential of INN-976 when administered by gavage to presumed pregnant rabbits on gestation days 7 through 19, inclusive.

Mating

There were five groups of twenty animals/treatment group. Semen was collected from 7 proven fertile male rabbits of the same strain, from the same supplier for artificial insemination of the females. Females were inseminated with semen collected that day. Semen was diluted with normal saline.

Nineteen days prior to the insemination of the first group, females were injected (via ear vein) with 50 U.S.P. units of chorionic gonadotropin (A.P.L.®, Ayerst Laboratories, Inc., New York, N.Y.). On the morning of insemination females were injected with 100 U.S.P. units of chorionic gonadotropin in the same manner, prior to insemination. On each of five consecutive days one group of females was artificially inseminated.

Group Arrangement:

Test Group	Treatment	Dose Level (mg/kg)	Number Assigned
I	Vehicle	0	20
II	INN-976	30	20
III	INN-976	100	20
IV	INN-976	300	20
V	INN-976	500	20

Selection of Doses:

In a pilot study rabbits were administered 0, 100, 200, 400, or 600 mg/kg by gavage. Pregnancy rates were 7/8, 5/8, 8/8, 8/8, and 8/8, respectively. All females survived until the scheduled sacrifice except for one female in the 400 mg/kg group. Two does in this group had totally resorbed litters. Food consumption and bodyweight change were reduced in a treatment related manner during the treatment period. Significant decreases were only found in the 400 and 600 mg/kg groups. No significant changes were noted for clinical signs, postmortem examinations, mean absolute maternal liver weights, mean numbers of resorptions or non-viable fetuses/doe, mean fetal bodyweights, or external alterations as compared to the control groups. Based upon these findings 0, 30, 100, 300, and 500 mg/kg were selected for the doses to be used in the main study.

Dosing:

All doses were administered as a volume of 2 ml/kg of bodyweight/day. The volume administered was based on the bodyweight of the animal on the morning of each dosing day. The vehicle was prepared at least every two weeks. Suspensions of the test material (in the vehicle) were prepared on the morning of each dosing day. Initially a thin paste was formed. This was finally brought to the correct volume and mixed on a Polytron® homogenizer until the suspension appeared uniform. Prior to dosing or sampling for analyses, mixtures were manually shaken. Concentrations of the test material were adjusted for purity.

Analyses of Test Article

At the beginning, during, and at the end of the treatment period, two samples of 10 ml were taken of each test suspension. One sample was stored at $\leq$-16°C just after mixing and the other sample was kept at room temperature for five hrs. before being stored at $\leq$-16°C. Concentration and stability analyses were performed by personnel of the Molecular and Genetic Toxicology Section of Haskell Laboratory.

Husbandry

Animals were quarantined for 22 days. Water from the Wilmington Suburban Water Corporation (WSWC) was available ad libitum. Each animal was provided approximately 150 g of Purina Certified Rabbit Chow® 5322 daily. The humidity in the animals' room was 40-60%; the temperature was 64-74°F. The animals were maintained on a 12 hour light/dark cycle.

Observations

Bodyweights were recorded within one day of arrival, each week during quarantine, and on the morning of days 0, 7-20, 24, and 29 of gestation.

Food consumption was monitored during quarantine and feed was measured daily, days 0-29 of gestation. Food consumption was measured daily during gestation by subtracting the weight of food remaining in the feeder and spillage, from the weight of the feed in the full feeder.

Clinical signs were recorded within one day of arrival, each week during quarantine, on the mornings of days 0-29 of gestation, and on the afternoons of days 7-19 of gestation. Does were sacrificed on day 29 of gestation by injection of T-61® (distributed by American Hoechst Corporation, Somerville, N.J.). Examinations at sacrifice consisted of: a gross exam of the internal organs, absolute and relative liver weight (maternal weight at sacrifice on day 29 of gestation minus the weight of products of conception), the intact and empty uterine weights, number of corpora lutea, and the number and relative position of implantations in utero. The uterus of females that did not exhibit obvious imlantation sites were stained with ammonium sulfide.

Within 24 hours females that were found dead were autopsied. Their internal organs were examined for pathological changes and attempts were made to determine the cause of death. The pregnancy status of the animal was determined by presence or absence of implantations. Implantations were counted; attempts were made to determine whether fetuses were "viable" or "resorbed" at the time of the mother's death (they died before or after the mother).

At the time of the scheduled cesarean, the location of each live and dead fetus in utero was recorded. Fetuses were weighed individually and examined for external alterations. Viable fetuses were euthanized by an i.p. injection of sodium pentobarbital, then examined for visceral alterations. Fetuses were sexed on the basis of the exam of their internal organs. A transverse section between the parietal and frontal bones was

008345

made through the unfixed fetal head to examine the brain. On the day of cesareans the eyelids were removed, and the eyes were examined visually to detect alterations. Upon completion of the visceral exam, the fetuses were fixed in 70% ethanol, macerated in an aqueous potassium hydroxide solution, and stained with Alizarin red S.

In the instance of abortions and early deliveries, the condition of the fetus was recorded. If possible, all live and dead fetuses were examined for congenital external and visceral malformations.

Historical control data were not provided to allow comparison with concurrent controls.

Statistical Analysis

The litter was considered as the experimental unit for statistical analysis. The section on statistical analyses is appended.

Compliance

A signed Statement of No Confidentiality Claim was provided which was dated April 5, 1988.

A signed Statement of Compliance with EPA GLP's was provided which was dated December 4, 1987.

A signed Quality Assurance Documentation Statement was provided which was signed by the quality assurance auditor.

A Statement for Flagging of Studies for Potential Adverse Effects was provided which was dated December 30, 1988. The statement indicates that the study neither meets nor exceeds any of the applicable criteria.

Results

Analyses of Dosing Suspensions

The theoretical concentrations of the dosing suspensions were 0, 15, 50, 150, and 250 mg/ml (animals were dosed in a volume of 2 ml/kg). Only one sample of the vehicle was analyzed; no detectable levels of INN-976 were found. In samples held for 5 hours at room temperature, the mean concentrations were (3 samples/level) 14.5 ± 0.6, 48.7 ± 0.6, 146.7 ± 4.2, and 244.3 ± 15.6 mg/ml. The mean concentrations found in the fresh frozen samples were reported to be comparable to those obtained from 5 hour samples.

008345

Maternal Toxicity

Mortality

There were no treatment related deaths during this study. One high dose female (21754) died on gestation day 25. During necropsy, a large trichobezoar was found that filled the stomach and blocked the intestinal tract; multiple hemorrhages of the stomach mucosa were found. In addition, multiple dark red areas were found in the lungs. This animal ate almost no food after the start of dosing and lost weight during and after the treatment period prior to death. No feces or urine were found in this animal's cageboard 4 days prior to death; blood was found in the cageboard 2 days prior to death.

A second high dose female (21741) was found dead on gestation day 29. Postmortem examination did not reveal a cause of death. The animal had one early resorption and 7 fetuses which appeared to be viable at the time of her death. This female ate more than most of the other females in this group for the first 6 days of treatment. Thereafter, she ate almost no feed. This female had severe weight changes for gestation days 13-16 and 16-20; she continued to loose weight after the treatment period. Due to the fact that she survived over 9 days after receiving the last dose, the death was not judged to be related to the test article. Furthermore, in the pilot study all 8 of the animals treated with 600 mg/kg survived until sacrifice on day 21.

Clinical Observations

There were no significant differences in the incidence of clinical observations noted when comparing the treated and control groups.

008345

Bodyweight

The investigators supplied the following data:

Table I: Bodyweight Gains (grams)[a]

Days of Gestation	Dosage (mg/kg/day) 0	30	100	300	500
N=	16	17	17	14	16
0-7	147.3	112.3	133.0	123.7	143.6
7-10[α]	-21.7	-19.1	-13.6	-116.8*	-255.4*
10-13[α]	61.2	54.9	38.9	0.7*	-11.9*
13-16[ß]	52.7	76.8	74.7	13.4	16.1
16-20	-40.9	-28.4	32.2	-37.7	-10.4
7-20[α]	51.4	84.2	132.1	-140.5*	-261.6*
20-24	83.5	94.0	55.2	99.6	147.0
24-29[ß]	44.3	45.7	13.1	100.2	126.8
20-29[ß]	127.8	139.7	68.4	199.8	273.8*
(7-20) -GUW	-353.45	-299.59	-287.77	-500.95	-618.05
ABW -Day 0	-78.29	-45.69	-86.42	-221.44	-200.69

[a] Some of these values were calculated by this reviewer

GUW = Gravid Uterus Weight (weight of products of conception)

ABW = Adjusted Bodyweight (weight day 29 of gestation excluding the products of conception).

* Significantly different from control ($p \leq 0.05$)

α Significant trend in groups ($p \leq 0.05$) by orthogonal polynomial of dose ranks. When the high dose group is excluded from this test, a trend continues to be indicated for the remaining groups.

ß Significant trend in groups ($p \leq 0.05$) by orthogonal polynomial of dose ranks. When the high dose group is excluded from this test, no trend is indicated.

Data extracted from (Haskell Lab Report No. 527-87, Table 1 p. 37)

008345

The data in the above table does not include females that were not pregnant, aborted, delivered early, had total resorptions or died prior to scheduled sacrifice. Significant downward trends for bodyweight were found days 7-10, 10-13, 13-16 and 7-20. Significant upward trends were found on gestation days 24-29 and 20-29. With exclusion of the 500 mg/kg group, the trends continued for gestation days 7-10, 10-13, and 7-20. The 300 and 500 mg/kg groups had significant loss of bodyweight as compared to the control and other test groups. The most marked loss of weight occurred during the first three days after the initiation of treatment. The 300 and 500 mg/kg groups had a significant reduction in bodyweight gain days 10-13 as compared to the controls. Upon cessation of treatment, both the 300 and 500 mg/kg groups had weight gains that were greater than control. Days 20-29 the 500 mg/kg group had a significantly greater weight gain than the control.

Significant decreases in bodyweight were found at all measured intervals after the beginning of treatment (days 8-20, 24 and 29 of gestation). The 500 mg/kg group mean bodyweights were significantly lower than the controls for all of these periods except days 8 and 29 of gestation. The 300 mg/kg group had lower mean bodyweights than the controls beginning day 8 of gestation; this was only significant on day 14. No significant differences were found for the bodyweight of the 30 or 100 mg/kg groups as compared to the control.

Food Consumption

The investigators supplied the following data:

Table II: Mean Daily Food Consumption (g)[a]

Days of Gestation	Dosage (mg/kg/day) 0	30	100	300	500
N=	16	17	17	14	16
0-7	150.1	149.5	149.9	150.7	150.6
7-10[α]	149.0	150.3	142.9	93.5*	42.6*
10-13[α]	144.1	147.2	131.4	69.7*	35.0*
13-16[α]	141.7	144.7	129.7	72.5*	54.4*
16-20[α]	127.5	137.0	141.1	78.7*	74.5*
7-20[α]	139.6	144.2	136.7	78.6*	53.4*
20-24	133.0	140.6	139.3	138.4	145.3
24-29[β]	100.1	111.7	96.7	130.8	147.8*
20-29[β]	114.7	124.6	115.6	134.2	146.7*

* Significantly different from control ($p \leq 0.05$)

α Significant trend in groups ($p \leq 0.05$) by orthogonal polynomial of dose ranks. When the high dose group is excluded from this test, a trend continues to be indicated for the remaining groups.

β Significant trend in groups ($p \leq 0.05$) by orthogonal polynomial of dose ranks. When the high dose group is excluded from this test, no trend is indicated.

Data extracted from (Haskell Lab Report No. 527-87, Table 3 p. 40)

Significant downward trends were found for all intervals during treatment. This was apparently due to significant reductions in food consumption in the 300 and 500 mg/kg groups as compared to the controls. During the posttreatment period (days 24-29 and 20-29), there was a significant upward trend. This apparently was due to increased consumption in the 500 mg/kg group. Significant differences were not found for the 30 and 100 mg/kg groups when compared to the controls.

008345

Gross Pathological Observations

No pathological findings were observed at necropsy that were attributable to treatment. All test groups showed a slight increase in the absolute mean liver weight; these differences were not significant. The increases in the absolute and relative liver weights were treatment related. A significant upward trend was found for the relative liver weight. A significant downward trend was found for the adjusted mean bodyweights. The data in Table III does not include females that were not pregnant, aborted, delivered early, had total resorptions, or died prior to sacrifice.

Table III Mean Maternal Liver Weights (g)

Dose: (mg/kg)	Adjusted Day 29 Bodyweight	Absolute Liver Weight	Relative Liver Weight
0	3865.0	114.4	3.0
30	3980.0	123.9	3.1
100	3869.6	124.9	3.2
300	3793.5	126.4	3.3
500	3737.4	126.4	3.4

Adjusted Bodyweight = bodyweight day 29 of gestation excluding the products of conception).
Relative Liver Weight = grams of liver/100 g of adjusted bodyweight

Significant trend in groups ($p \leq 0.05$) by orthogonal polynomial of dose ranks. When the high dose group is excluded from this test, a trend continues to be indicated for the remaining groups.

Significant trend in groups ($p \leq 0.05$) by orthogonal polynomial of dose ranks. When the high dose group is excluded from this test, no trend is indicated.

Data extracted from (Haskell Lab Report No. 527-87, Table 5 p. 43)

Cesarean Section Observations

Table IV: Cesarean Section Observations[a]

Dose (mg/kg):	0	30	100	300	500
#Animals Assigned	20	20	20	20	20
#Animals Mated/ Inseminated	20	20	20	20	20
Pregnancy Rate (%)	100	85	100	85	95
Maternal Wastage					
#Died	0	0	0	0	2
#Non pregnant	0	3	0	3	1
#Aborted	2	0	0	0	1
#Premature Delivery	1	0	2	1	0
Total Corpora Lutea	166	178	172	152	158
Corpora Lutea/Doe[d]	10.4 ±0.7	10.5 ±0.6	10.1 ±0.8	10.9 ±0.5	9.9 ±0.5
Total Implantations	137	134	147	108	126
Implantations/Doe[d]	8.6 ±0.5	7.9 ±0.8	8.6 ±0.6	7.7 ±1.0	7.9 ±3.1
Total Live Fetuses	129	125	142	92	110
Live Fetuses/Doe[d]	8.1 ±0.6	7.4 ±0.8	8.4 ±0.7	6.6 ±0.8	6.9 ±0.7
Live ♂	3.3	3.3	3.8	3.6	3.9
Live ♀	4.8	3.9	4.6	3.0*	3.0*
Total Resorptions	8	9	5	16	16
Early	5	5	2	5	10
Late	3	4	3	11	6
Resorptions/Doe	0.5	0.5	0.3	1.1	1.0
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Doe	0	0	0	0	0
Mean Fetal Weight[c] (gm)	42.19	44.26	41.86	45.04	42.81
Preimplantation Loss(%)	15.9	22.9	14.7[b]	26.7[b]	20.8
Postimplantation Loss(%)	6.5	5.7	3.8	11.4	10.8
early	4.4	3.4	1.2	3.5	7.2
late	2.1	2.2	2.6	7.9*	3.6
Total No. of Stunted Fetuses	4	1	7	1	0

008345

[a] Data extracted from (Haskell Lab Report No. 527-87, Table 6 p. 44 and Appendix I pp. 161-169). Some of the values in this table were calculated by this reviewer from the raw data.

[b] One female in this group had a greater number of nidations than corpora lutea. For the purpose of calculating preimplantation loss, 0 was used instead of a negative number.

[c] The mean fetal weights per group do not include the stunted fetuses.

[d] numbers represent mean ± SEM

* Significantly different from control ($p \leq 0.05$).

One doe in the 30 mg/kg group had uterine abnormalities which prevented pregnancy. No fetal sex was entered for 3 fetuses in 3 litters; therefore, these fetuses were not included in either male or female calculations, but were included in calculations of total means. The animal in the high dose group that "aborted" actually delivered early on day 29. However, since only resorptions were found, the animal was considered to have aborted. Both of the does that died were pregnant. The animals that delivered early were considered to have aborted; all data from these animals were excluded from calculations.

There was an upward trend for the total mean number and percentage of resorptions per doe. When the high dose group is excluded, no trend was indicated. Significance was only detected for the 300 mg/kg group for the mean number and percentage of late resorptions per doe. A downward trend was found for the mean number of female fetuses per doe. When the high dose group is excluded, no trend was indicated. The mean number of female fetuses per doe was significantly decreased in the 300 and 500 mg/kg groups.

2. Developmental Toxicity

Table V: External Examinations

Dose (mg/kg):	0	30	100	300	500
Observations*					
#pups(litters) examined	129(16)	125(17)	142(17)	92(14)	110(16)
#pups(litters) affected	0(0)	0(0)	1(1)	0(0)	1(1)
Domed Head	0	0	1(1)	0	0
Cleft Palate	0	0	0	0	1(1)

008345

(+) some observation may be grouped together
(a) fetal [litter] incidence

Data extracted from (Haskell Lab Report No. 527-87, Tables 7 and 8 pp. 47- 56).

Table VI: Visceral Examinations

Dose (mg/kg):	0	30	100	300	500
Malformations+					
#pups(litters) examined	129(16)	125(17)	142(17)	92(14)	110(16)
#pups(litters) affected	0	0	3(3)	0	1(1)
Great Heart Vessels- Malformation	0	0	2(2)	0	1(1)
Heart- R. vent. absent	0	0	1(1)	0	0
Septal Defect	0	0	0	0	1(1)
External Hydrocephaly	0	0	1(1)	0	0
Variations					
#pups(litters) examined	128(16)	121(17)	136(17)	92(14)	108(16)
#pups(litters) affected	92(16)	94(17)	86(17)	70(12)	59(15)
Bladder- Hemorrhage	28(12)	8(6)	12(7)	14(6)	10(7)
Gallbladder - Small	1(1)	3(2)	0	6(2)	1(1)
Great Heart Vessels- L. Carotid off Innominate	63(16)	71(14)	65(16)	43(11)	52(14)
Kidney- Renal Calculi	0	0	1(1)	0	0
Subcapsular Hemorrhage	0	0	0	1(1)	0
Supernumary Vessel[a]	46(15)	36(14)	31(12)	27(11)	21(10)

[a] Small vessel(s) originating from either the innominate, aorta, left or right carotid, or the left or right subclavian.

The fetus observed with a dome shaped head during the external exam was found to have external hydrocephaly.

Table VII: Skeletal Examinations

Dose (mg/kg):	0	30	100	300	500
Malformations*					
#pups(litters) examined	129(16)	125(17)	142(17)	92(14)	110(16)
#pups(litters) affected	1(1)	3(3)	4(4)	0(0)	2(2)
Rib-					
Branched	1(1)	1(1)	0	0	0
Fused	0	1(1)	0	0	1(1)
Skull-					
Malformation	0	0	1(1)	0	0
Sternebra-					
Fused	0	1(1)	3(3)	0	1(1)
Lumbar Vertebra-					
Hemi	0	1(1)	0	0	0
Thoracic Vertebra-					
Fused	0	0	0	0	1(1)
Hemi	0	1(1)	0	0	1(1)
Misaligned Centra or Arches	0	1(1)	0	0	1(1)
Misshapen Vertebral Arch	0	1(1)	0	0	0
Variations					
#pups(litters) examined	128(16)	122(17)	136(17)	92(14)	108(16)
#pups(litters) affected	79(16)	77(16)	80(15)	62(12)	93(16)
mean % affected	59.9	66.4	62.8	65.7	87.3*
Hyoid - bent	0	6(3)	0	0	1(1)
Rib-					
Beaded	0	0	0	1(1)	0
Calloused	0	1(1)	0	0	0
Thickened	10(8)	4(3)	9(5)	9(7)	7(5)
Rudimentary Lumbar 1	26(13)	22(11)	24(13)	18(8)	11(8)
Extra Lumbar 1	55(14)	58(13)	58(14)	46(11)	83(16)
Sternebra-					
Extra	0	0	1(1)	0	0
Cervical					
Vert.[a]	1(1)	0	0	0	0

a = extra ossification site above centrum # 1
* Significantly different from control.

D. Discussion/Conclusions

a. Maternal Toxicity:
Maternal toxicity was observed at 300 and 500 mg/kg, as evidenced by decreased bodyweight gain and food consumption.

b. Developmental Toxicity:

i. Deaths/Resorptions:
There was a statistically significant increase in the percentage of late postimplantation loss at the 300 mg/kg group. There was a slight increase in early postimplantation loss at the 500 mg/kg group that was not significant. The number of live females in the 300 and 500 mg/kg groups was significantly reduced as compared to the control. This may be due to the slightly increased number of females in the control group.

ii. Altered Growth:
There were no significant or treatment related alterations in fetal growth.

iii. Developmental Anomalies:
There was a statistically significant increase in the mean percentage of skeletal variations at the 500 mg/kg level as compared to the control (87.3 vs 59.9).

iv. Malformations:
There were no treatment related malformations detected in this study.

D. Core Classification: Core Minimum Data.

Maternal NOEL = 100 mg/kg
Maternal LOEL = 300 mg/kg
Developmental Toxicity NOEL = 100 mg/kg
Developmental Toxicity LOEL = 300 mg/kg

Bromacil RIN: 1607-93

Page_____ is not included in this copy.

Pages 42 through 51 are not included.

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 quality control procedures.

____ Identity of the source of product ingredients.

____ 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.

II. MATERIALS AND METHODS (CONTINUED)

Parameters	Test for Linear Trend	Pair-Wise Test Between Control and Test Substance Groups
Incidence of pregnancy Clinical signs Deaths Dams with total resorptions	Cochran-Armitage[10]	Fisher's Exact[11]
Maternal body weight Maternal body weight change	Orthogonal polynomial of dose ranks[10,a]	Dunnett's[12,a] when one-way ANOVA is significant
Maternal feed consumption Maternal liver weights (absolute & relative)		
Nidations Live fetuses Dead fetuses Resorptions Corpora lutea Fetal weights Incidence of fetal alterations	Jonckheere's[13,b]	Mann-Whitney U[14,15,b]

---

[a] When Bartlett's test (for homogeneity) was significant ($p<0.005$), analyses were conducted on the ranks of the original values.

[b] When more than 75% ties occurred in reproductive and fetal parameters, the Cochran-Armitage test was used to detect trends and the Fisher's Exact test was used to detect significant differences between the control and test substance groups.

II. MATERIALS AND METHODS (CONTINUED)

The use of the words "significant" or "significantly" indicates a statistically significant difference between the control and test substance groups unless otherwise noted.

M. Control Of Bias

In addition to random assignment to treatment groups (see II,G), all adult animals were coded prior to scheduled sacrifice and remained coded during the collection of postmortem and fetal data.

N. Archiving

Raw data and the final report are stored in the Information Section, Haskell Laboratory, or at the Du Pont Records Management Center, Wilmington, Delaware. All skeletal, head and selected visceral specimens are stored in the Pathology Division Archives, Haskell Laboratory.

III. RESULTS AND CONCLUSIONS

A. Analyses of INN-976 Suspensions

The nominal concentrations of the suspensions for analysis were 0, 15, 50, 150 and 250 mg/ml, representing dose levels of 0, 30, 100, 300 and 500 mg INN-976/kg of body weight, respectively. Only one sample of the vehicle (used for the control group) was analyzed, and it had no detectable INN-976. The mean concentrations (±S.D.) of

Tox Chem No. 111 File Last Updated _____ Current Date 4/4/91

STUDY/LAB/STUDY #/DATE	MATERIAL	MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. # RR
83-3(b) Developmental Toxicity Study Species: Rabbit Haskell Lab for EI du Pont HLR 527-87; 12/87	Bromacil 95.1% purity	409848-01	Bromacil was administered to presumed pregnant New Zealand White Rabbits by gavage at 0, 30, 100, 300, and 500 mg/kg. Maternal toxicity was observed at 300 and 500 mg/kg as evidenced by decreased bodyweight gain and food consumption. Developmental toxicity was evidenced by an increased incidence of resorptions at these levels. Maternal NOEL = 100 mg/kg Maternal LOEL = 300 mg/kg Developmental NOEL = 100 mg/kg Maternal LOEL = 300 mg/kg		Minimum T