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

MAR 30 1993

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Bronopol (2-Bromo-2-nitropropane-1,3-diol): Review of Rabbit Developmental Toxicity Study (83-3b)

Barcode No.: D188042	Submission No.: S435175
Case No.: 816637	PC Code No.: 216400
CAS No.: 52-51-7	Tox. Chem. No.: 116 A
EPA ID No.: 216400-033753	**Rereg. Case No.: 2770**

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (H7509C) Krystyna K. Locke 2/23/93

TO: Barbara Briscoe / Ron Kendall, PM 51
Accelerated Registration Branch
Reregistration Division (H7505C)

THRU: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C) Roger Gardner KB 3/25/93 3-23-93

Action Requested

Toxicology Branch I/HED has been asked to review the following study: " Bronopol: Oral (Gavage) Rabbit Developmental Toxicity (Teratogenicity) Study ". **Author:** Lorraine F. H. Irvine; **Study Number:** BON/3/91; **Testing Facility:** Toxicol Laboratories Limited, Bromyard Road, Ledbury, Herefordshire, England; **Report Issued:** March 13, 1992; **Study Completed On:** August 17, 1991. **MRID No.:** 42648201

According to PM 51, this study was submitted in response to a DCI, for fulfillment of Guideline 83-3.

Response from Toxicology Branch I/HED

This study had already been reviewed and the review was sent to the Product Manager 31 (John H. Lee, Disinfectant Branch, Registration Division) who submitted this study to Toxicology Branch I/HED. The date of the review is January 8, 1993 and the MRID number is 42319601. The study was classified as Core-Guideline. The study was submitted with Barcode No. D178575 and Submission No. S418272.

DP BARCODE: D188042 REREG CASE # 277

CASE: 816637 DATA PACKAGE RECORD DATE: 02/11/9
SUBMISSION: S435175 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 604 PHASE 4 RESPONSE SUBMIS
CHEMICALS: 216400 Bromo-2-nitropropane-1,3-diol 100.00

ID#: 216400-033753
COMPANY: 033753 BOOTS CO PLC
PRODUCT MANAGER: 51 BARBARA BRISCOE 703-308-8177 ROOM: CS1 3H3
PM TEAM REVIEWER: RON KENDALL 703-308-8068 ROOM: CS1 4L3
RECEIVED DATE: 09/01/92 DUE OUT DATE: 12/30/92

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 188042 EXPEDITE: N DATE SENT: 02/11/93 DATE RET.: / /
CHEMICAL: 216400 Bromo-2-nitropropane-1,3-diol
DP TYPE: 999 Miscellaneous Data Package
ADMIN DUE DATE: 03/04/93 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : HED	2/12/93	/ /
BRAN: TB-1	/ /	/ /
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

Please review MRID 426480[illegible]1 for fulfilment of GDLN 83-3.
This is a submission response to a DCI.

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

JOHN W. KENNEDY CONSULTANTS, INC.

9101 CHERRY LN., SUITE 113, LAUREL, MD 20708-1133
TELEPHONE: (301) 490-1600, FAX: (301) 490-5793

426482-00

August 28, 1992

Emily Mitchell
Chemical Review Manager
U.S. Environmental Protection Agency
Accelerated Review Branch
Registration Division (H-7505C)
401 M Street, S.W.
Washington, DC 20460

Subject: Transmittal Document: Response to Data Call-In; Phase 4 Response

Names and Addresses of Submitters:

John W. Kennedy Consultants, Inc.
9101 Cherry Lane, Suite 113
Laurel, Maryland 20708

and

The Boots Company, PLC
Nottingham, England
NG2 3AA
United Kingdom

John W. Kennedy Consultants, Inc. is authorized by the Boots Company to act on its behalf in all matters related to pesticide registrations.

Regulatory action in support of which this package is submitted:

Phase 4 response to DCI for EPA #283267-1, Bronopol Technical; 2-bromo-2-nitropropane-1, 3-diol.

Transmittal Date: August 28, 1992

List of Submitted Studies:

Volume 1 - Administrative documents

Volume 2 - Bronopol: Rabbit Development Toxicity (Teratogenicity) 83-3

42648201

Company Name: John W. Kennedy Consultants, Inc.

Company Official/Contact: David L. Ritter
301-262-9278
410-643-6981

Signature: David L. Ritter

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Primary Review by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 12/9/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

Secondary Review by: Roger Gardner, Section Head Roger Gardner 12/10/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

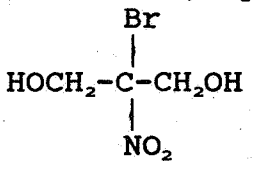
DATA EVALUATION RECORD

STUDY TYPE: Teratology - Developmental Toxicity
Guideline § 83-3
Species: Rabbit

EPA IDENTIFICATION NUMBERS:

MRID No.: 42319601
Barcode No.: D178557
Data Submission No.: S418272
PC Code: 216400
Tox. Chem. Code: 116A

TEST MATERIAL: Bronopol; Pharmaceutical grade; CAS chemical name: 2-Bromo-2-nitro-1,3-propanediol
Structure:



SYNONYMS: Bronopol-Boots

STUDY NUMBER: BON/3/91

SPONSOR: The Boots Company PLC; the Priory, Thurgarton, Nottingham, NG14 7GX, England

TESTING FACILITY: Toxicol Laboratories Limited, Bromyard Road, Ledbury, Herefordshire, HR8 1LH, England

TITLE OF REPORT: Bronopol: Oral (Gavage) Rabbit Developmental Toxicity (Teratogenicity) Study

AUTHOR: Lorraine F. H. Irvine, Study Director

REPORT ISSUED: March 13, 1992
Study completed on: August 17, 1991

CONCLUSIONS:

Groups of 18, 19 or 20 mated female New Zealand White rabbits received pharmaceutical grade Bronopol (purity: 99.8%) by gavage during gestation days 7 through 19 and were sacrificed on gestation day 28. Aqueous solutions of Bronopol, prepared just

before use, were administered daily at the nominal dose levels of 0 (vehicle control), 5, 20, 40 or 80 mg/kg (Groups 1 through 5, respectively) and the dose volume of 2 mL/kg. Separate solutions were prepared for each dose level and individual body weights were obtained daily during the treatment period. The analytical concentrations of Bronopol in dosing solutions were very close to nominal concentrations. The dose levels used were based on a preliminary (range-finding) study. Day 0 of pregnancy was the day of mating. Maternal and developmental toxicities were observed only in the 80 mg/kg/day groups. The NOEL and LOEL were as follows:

Maternal NOEL: 40 mg/kg/day
Developmental NOEL: 40 mg/kg/day

Maternal LOEL: 80 mg/kg/day (Body weight loss during the first 4 days of treatment; decreased body weight gain during the remainder of the treatment period; decreased food consumption during the dosing period; and reduction in the quantity and size of fecal pellets ----- each finding in comparison with the concurrent control group).

Developmental LOEL: 80 mg/kg/day (Decreased fetal body weights in both sexes; slight increase in runted fetuses; increase in fetuses with major external/visceral and skeletal abnormalities*; increase in fetuses with minor skeletal abnormalities; and increased incidence of fetuses with unossified forelimb and hindlimb epiphyses (skeletal variants) ----- each, but one (*), in comparison with the concurrent controls.

*Compared with historical controls, as there were no major external/visceral and skeletal abnormalities in the concurrent control group.

CORE CLASSIFICATION: GUIDELINE

This study satisfies the guideline requirements (§ 83-3) for a developmental toxicity study in rabbits.

MATERIALS AND METHODS

(1) Test Animals:

Species: Rabbit
Strain: New Zealand White
Source: Interfauna U.K. Limited, Huntingdon, Cambridgeshire, England

Age: Approximately 4 months at study initiation
Weight: 3-4 kg on gestation day 0.

(2) Test Compound:

Purity: 99.8% by hplc and IR spectrum
Batch No.: 888902
Description: White crystalline material, readily soluble in water
Supplier: The sponsor
Impurities: [redacted]
Date of receipt at testing facility: July 8, 1991
Storage: At room temperature (18 ± 3° C) in the dark.

(3) Vehicle:

Tap water, purified by reverse osmosis in an Elgastat water purification unit, was used as a vehicle.

(4) Dosing Solutions:

Bronopol was dissolved for dosing in purified water. Fresh solutions were prepared daily and separate solutions were prepared for each dose level. After the weighed amount of Bronopol was dissolved in the appropriate amount of water, the pH of each solution was adjusted to approximately pH 4 using hydrochloric acid. One 10 mL sample from each formulation prepared on June 3, 1991 (first day of dosing) and on June 17, 1991 (one day before the end of dosing period) was analyzed for the concentration of Bronopol (a detailed analytical procedure was provided). The analytical concentrations of Bronopol were very close to the nominal (intended) concentrations: 97.8, 97.8, 99.8 and 100.1% for Groups 2, 3, 4 and 5, respectively, for the June 3 samples; and 96.1, 94.6, 98.5 and 98.5%, respectively, for the June 17 samples. The amount of Bronopol administered was based on body weights which were determined daily for each rabbit during the treatment period. According to the sponsor, aqueous (acidic) solutions of Bronopol are stable for at least 7 days.

(5) Mating Procedures:

Mating was conducted at the supplier's (Interfauna, U.K. Limited) premises. Each female had been observed to copulate with one stud buck and then given an intravenous injection of 25 IU chorionic gonadotrophin (Chorulon-Intervet Laboratories, Cambridgeshire) to stimulate ovulation. The day of mating was termed day 0 of pregnancy. Mated females were delivered to Toxicol (testing facility) by day 2 of pregnancy. The supplier provided documentation of the parentage of each female, the identification of each stud male used for mating and the body weight of each female at mating. Before acceptance of females to groups, the mating and parentage records were checked to ensure

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that sibling females were distributed across groups and also that no female within the group was mated with the same stud male. There were 94 mated females and 54 stud males in this study. Fifteen males were mated once (male:female ratio 1:1), 38 males were mated twice (male:female ratio 1:2) and one male was mated 3 times (male:female ratio 1:3). The distribution of these ratios within groups was as follows:

Group	1	2	3	4	5	Total
Male:Female Ratio	Number of females mated*					
1:1	5	3	1	4	2	15
1:2	13	14	17	14	18	76
1:3	0	1	1	1	0	3

*Tabulated by the reviewer, using data in APPENDIX 13, pages 148-152 of this submission (MRID No. 42319601)

(6) Study Design:

Groups of 18, 19 or 20 mated female New Zealand White rabbits were assigned to study groups on gestation day 3 by a randomization procedure based on body weight. The rabbits were dosed once daily during the gestation days 7 through 19. Aqueous solutions of Bronopol were administered by gavage at dose levels shown below.

Test Group		Dose Level (mg/kg/day)	Number Assigned
1	Vehicle control	0	18
2	Low dose	5	18
3	Low-mid dose	20	19
4	High-mid dose	40	19
5	High dose	80	20

80 mg/kg/day was selected as a level which would elicit maternal effects. However, in the event that the effects may have been too severe, 40 mg/kg/day was selected as the next highest level known to be tolerated by the pregnant rabbit, according to the study director.

The dose levels were selected by the sponsor after examination of data from a preliminary range-finding study in mated rabbits (Report No. BON/2/91). This study was not

submitted to the Agency for review.

As reported in this submission, there was no apparent maternal or embryotoxicity at doses between 1 and 40 mg/kg/day in the range-finding study. At 160 mg/kg/day, there was severe maternal toxicity with gastric ulceration and consequent reduction in food consumption and body weight. At 80 mg/kg/day, there were indications of similar, but less marked, effects but this dose level was not fully evaluated. Effects on embryonic development at 80 and 160 mg/kg/day were not assessed.

In both studies (range-finding and actual study), the rabbits were housed individually in grid-bottomed metal cages suspended over paper-lined trays. The rabbits were given a pelleted diet, SQC Rabbit Standard (Special Diet Services, Witham, Essex, U.K.) and tap water (via an automatic watering system) without restrictions. Each batch of diet was delivered with an accompanying certificate of analysis detailing nutritional composition and levels of specified contaminants (heavy metals, aflatoxins and insecticides). These data were included in the current submission (MRID No.: 42319601).

(7) Observations:

After allocation to groups, the females were observed daily for changes in clinical condition. Body weights were recorded by the supplier on gestation day 0 and at the testing facility on gestation days 3, 7 through 19, 22, 25 and 28. Food consumption was recorded every two days from days 3 to 27 and over one day from day 27 to 28 of pregnancy. For days 25 to 27 and for days 27 to 28, the food consumption was combined (days 25 to 28 for reporting). The animals were sacrificed by the intravenous injection of sodium pentobarbitone on gestation day 28 and all were necropsied. The thoracic and abdominal cavities were opened and the major organs examined. Organs and tissues showing macroscopic abnormalities were removed and fixed in buffered formal saline. The following data were also collected for pregnant females on gestation day 28:

(a). Weight of gravid uterus

(b). Number of corpora lutea

(c). Number and distribution of implantation sites. The implantations were classified as early resorptions, late resorptions, dead fetuses or live fetuses. The implantation sites were numbered separately for the right and left horns. Numbering was sequential, starting at the ovarian end and through to the cervix.

The live fetuses were removed and examined for external abnormalities. They were then killed by an intravenous

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injection of sodium pentobarbitone solution. Each fetus was weighed and then briefly fixed in alcohol. Later the same day, the fetuses were skinned, dissected, the viscera were examined and the sex was determined by inspection of the internal genitalia. The fetuses were eviscerated and placed back in alcohol. After at least 12 hours in alcohol fixation, a razor blade cut was made through the head along the frontal parietal suture and the brain examined. The fetuses were subsequently cleared in potassium hydroxide, stained with alizarin red S and examined for skeletal variants and abnormalities. Structural congenital abnormalities that impair or potentially impair the survival or development of the fetus were classified as major abnormalities. Other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification from that expected of a day 28 gestation fetus were classified as variants. Also classified as variants were commonly observed deviations in the number of thoracic vertebrae and ribs, lumbar vertebrae and caudal vertebral centrae and neural arches. All skeletal specimens were stored in aqueous glycerol (to prevent fungal growth). No procedures were referenced in the above description of the preparation of fetuses for the examination of visceral and skeletal abnormalities.

(8) Historical Control Data:

A document entitled BACKGROUND DATA, NEW ZEALAND WHITE RABBIT (INTERFAUNA), January 1987 to November 1990, was included in the submission (MRID No. 42319601; APPENDIX 19, pages 168 through 184). This document contains very detailed pregnancy and fetal abnormalities data for 1045 animals (319 controls and 726 inactive treatments). These data, obtained from a number of studies (not specified how many) which were conducted by Toxicol Laboratories Limited during 1987-1990, were reported under the following headings: (1) Summary of Pregnancy Data; (2) Fetal Examination/Summary of Group Mean Data; (3) External and Visceral Abnormalities; and (4) Skeletal Abnormalities. The term "inactive treatments" was not defined.

(9) Statistical Analysis:

Maternal group mean body weights, body weight gain, uterus weights, food consumption, number of corpora lutea, number of live fetuses, total number of implantation sites, percent of preimplantation and postimplantation losses, fetal sex ratio, fetal body weights, and the percentage of fetuses with variations (variants) and abnormalities were evaluated statistically as is detailed in Attachment I of this review. The tests used (analysis of variance, Kruskal-Wallis, Student's and Dunn's) were appropriate.

REPORTED RESULTS

Maternal Observations

(1) Mortality:

There were no mortalities, but two females (one from Group 2 and another from Group 5) had to be sacrificed before the termination of the study.

The Group 2 (5 mg/kg/day) female was killed on gestation day 17 due to poor clinical condition arising from a dosing intubation error. About 30 minutes after dosing, this animal appeared lethargic, had irregular breathing and dilated pale pupils, and extensive hemorrhaging in the thoracic cavity and lungs was observed at necropsy. The animal was pregnant: 5 live implants and 2 resorptions were found in the left uterine horn, whereas the right uterine horn contained 4 live implants and 1 resorption.

The Group 5 (80 mg/kg/day) female was killed on gestation day 16 due to persistent low food consumption and low body weight gain. An extensive ulceration of the gastric mucosa was observed at necropsy. Since the deterioration of the clinical condition of this female began before the start of dosing, Bronopol was not apparently the primary cause. However, Bronopol treatment could have potentiated the condition, according to the study director. The animal was pregnant: 5 and 2 live implants were found in the left and right uterine horn, respectively.

Data from these two females have been excluded from statistical analyses.

(2) Clinical Observations:

Most females (95%) in Group 5 showed a reduction in the quantity and size of fecal pellets during most of the dosing period. The study director considered this to be a consequence of the observed treatment-related decrease in food consumption. However, a reduction in the size and quantity of fecal pellets was also noted for 50, 67, 58 and 74% of females in Groups 1, 2, 3 and 4, respectively. This reduction occurred throughout the study for a total of 1-9 days/animal and was regarded by the study director as an expected incidence for pregnant New Zealand White rabbits.

Other abnormalities, such alopecia on paws, limbs, abdomen, flanks and neck, and scabs on forepaws and ears, occurred at low incidence (1-4/group) and in a dose-unrelated manner.

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(3) Body Weights:

Compared with the controls (Group 1), Bronopol had no effect on body weight gains of females in Groups 2, 3 and 4. At the onset of dosing (gestation day 7), there was a significant ($p < 0.001$) mean body weight loss in Group 5. This weight loss continued through gestation day 11. By gestation day 15, most animals regained the lost weight and, thereafter, their weight gain was similar to or slightly greater than that of the controls. The weight gain data are summarized below.

Group Mean Maternal Body Weight Gains (Kg)#					
Group	1	2	3	4	5
Bronopol (mg/kg/day)	0	5	20	40	80
Body weights on g.d. 0	3.82	3.78	3.83	3.84	3.80
Body weight gains during:					
Predosing (g.d. 0 to 7)	0.10	0.11	0.01*	0.13	0.14
Dosing (g.d. 7 through 9)	0.05	0.06	0.06	0.03	-0.06***
Dosing (g.d. 7 through 19)	0.23	0.28	0.27	0.27	0.05*
Postdosing (g.d. 19 to 28)	0.20	0.23	0.22	0.23	0.27
Entire study (g.d. 0 to 28)##	0.02	0.09	-0.02	0.11	0.04

#This table is based on TABLES 1 and 2, pages 27 and 28, of the submission (MRID No.: 42319601). Standard deviations, additional body weight gains and body weights are in Attachment II of this review.

##Body weight gain minus the gravid uterus weight.

g.d.: Gestation day

*Significantly different from control, $p < 0.05$, Student's t test

***Significantly different from control, $p < 0.001$, Student's t test

(4) Food Consumption:

Compared with the control group, Bronopol had no effect on food consumption of females in the treated Groups 2, 3 and 4. At the onset of dosing, the mean food consumption in Group 5 was decreased. This decrease was statistically significant ($p < 0.001$) during the first 4 days of dosing (g.d. 7 to 11) and statistically insignificant during the remaining 9 days of dosing (g.d. 11 through 19). After the end of the dosing period, there

was an increase in food consumption in this group. During g.d. 23 to 28, the mean food consumption in Group 5 was significantly ($p < 0.05$) greater than in the control group. The food consumption data (including standard deviations) for all five test groups are in Attachment III of this review, whereas data for Groups 1 and 5 are summarized below.

Group Mean Maternal Food Consumption (g/rabbit/day) ± S.D.#		
Group	1	5
Bronopol (mg/kg/day)	0	80
Gestation days:		
3 to 7	167 ± 36	174 ± 50##
7 to 11	181 ± 31	113 ± 78***
11 to 15	147 ± 46	119 ± 63
15 to 19	150 ± 29	145 ± 59
19 to 23	167 ± 39	185 ± 26
23 to 28	136 ± 30	162 ± 29*

#This table is based on TABLE 3, page 29, and APPENDIX 4, page 65, of the submission (MRID No.: 42319601).

##This value is a mean for 17 rabbits and the remaining values are means for 19 rabbits. Two spare mated females were allocated to this group on g.d. 7, as contingency against the possible replacement of two females, already allocated to the study, but showing poor food consumption and body weight gain prior to dosing.

g.d.: Gestation day

*Significantly different from control, $p < 0.05$, Student's t test

***Significantly different from control, $p < 0.001$, Student's t test

(5) **Necropsy Findings**:

The only abnormality observed at the termination of the study was a necrotic kidney in one control female. Gastric ulceration, observed in one Group 5 female that was sacrificed on g.d. 16, was not observed in any froup at the terminal sacrifice.

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(6) Pregnancy and Implantation Data:

The pragnancy rate was 100% for each group. All females were pregnant, including the two which had to be sacrificed before the termination of the study, and there were no abortions. The mean number of corpora lutea and implantations were similar in all groups. The pregnancy and implantation data are summarized below.

Group	1	2	3	4	5
Bronopol (mg/kg/day)	0	5	20	40	80
No. Pregnant rabbits/ No. mated*	18/18	18/18	19/19	19/19	20/20
No. with litters on day 28	18	17**	19	19	19**
Mean No. of corpora lutea SD(±)	11.6 2.4	11.2 1.8	12.0 1.9	12.2 1.9	12.8 2.2
Mean No. of implantations SD(±)	10.2 2.7	10.2 1.5	9.7 3.4	11.0 2.3	10.8 1.9

*This table is based on TABLE 4, page 30 of the submission (MRID No. 42319601).

**One animal from each of these groups was sacrificed before the termination of the study.

Developmental Observations

The developmental toxicity endpoints examined in this study included cesarean section observations (number of live and dead fetuses, percent of pre- and postimplantation losses, number of early and later resoptions, sex ratio, and weight of male and female fetuses) and external/visceral and skeletal abnormalities. Fetal length was not examined.

(1) Cesarean Section Observations:

With the exception of tetal weights in Group 5 (80 mg/kg/day), Bronopol had no effect on any of these endpoints examined, for the following reasons: (a) The mean numbers of the live fetuses/doe was slightly higher in the treated groups than in the control group; (b) There were no dead detuses in the treated groups; (c) The mean preimplantation losses (%) were within expected (historical control) ranges in all five groups; (d) The mean postimplantation losses (%) and the number of early resorptions were lower in the treated groups than in the control group and (e) The number of late resorptions in Groups 2, 3 and 5 were lower than in the control group. The mean fetal weight was

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significantly ($p < 0.05$) lower in Group 5 than in the control group. Both sexes were affected although, when analyzed separately by sex, statistical significance ($p < 0.05$) was only achieved for female fetuses. The lower fetal weight was attributed partly to the slightly greater litter size, compared with the concurrent control group, but mainly to the retardation of embryonic growth, as a probable consequence of the effects of Bronopol on maternal food consumption and body weight. The above data are summarized below.

Cesarean Section Observations#					
Group	1	2	3	4	5
Bronopol (mg/kg/day)	0	5	20	40	80
Mean no. of live fetuses/doe SD(±)	8.8 2.3	9.7 1.7	9.4 3.3	9.6 2.0	10.2 2.0
Total no. of dead fetuses/group	1	0	0	0	0
Mean preimplantation loss (%)					
Current study	11.7	8.7	19.0	9.8	14.7
Historial control data##	18.7 (Range of groups means: 5.5-38.0%)				
Mean postimplantation loss (%)	13.2	4.8**	3.6**	12.1	6.1*
No. of early resorptions/group	11	3	3	10	7
No. of does with 1 resorption	4	3	3	5	1
No. of does with 2 resorptions	2	0	0	1	3
No. of does with 3 resorptions	1	0	0	1	0
No. of does with >3 resorptions	0	0	0	0	0
No. of late resorptions/group	13	5	4	17	6
No. of does with 1 resorption	1	3	4	7	3
No. of does with 2 resorptions	0	1	0	3	0
No. of does with 3 resorptions	4	0	0	0	1
No. of does with >3 resorptions	0	0	0	1	0
Sex ratio/group					
Males (%)	45	57	43	52	45
Females (%)	55	43**	57	48	55

Cesarean Section Observation# -- continued.					
Group	1	2	3	4	5
Bronopol (mg/kg/day)	0	5	20	40	80
Group mean fetal weights (g)					
Males SD(±)	36.0 6.0	35.3 4.1	36.7† 5.0	34.6 3.8	32.9† 5.3
Females SD(±)	35.6 5.6	35.6 4.8	36.4 5.0	34.5 4.5	31.7* 5.1
Both sexes SD(±)	35.8 5.6	35.5 4.4	36.8 5.0	34.6 3.9	32.1* 5.0

#This table is based on TABLE 4 (page 30), TABLE 5 (page 31) and APPENDIX 6 (pages 69-73) of the submission (MRID No.: 42319601).

##This information is on page 169 of the submission (BACKGROUND DATA; APPENDIX 19).

†Male fetuses in these groups were obtained from 18 rather than 19 litters because one doe in each group had only female fetuses in the uterus (pages 76 and 78 of the submission).

*($p < 0.05$) and **($p < 0.01$), significantly different from control; Dunn's multiple comparison test and Kruskal-Wallis test.

(2) Fetal Abnormalities:

Fetal abnormalities included external/visceral and skeletal major and minor abnormalities, and skeletal variants. The major and minor abnormalities are summarized below.

External/Visceral and Skeletal Major and Minor Abnormalities#					
Group	1	2	3	4	5
Bronopol (mg/kg/day)	0	5	20	40	80
Total No. of litters examined	18	17	19	19	19
Total No. of fetuses examined	159	165	178	182	193
Abnormality	Number of fetuses with major abnormalities				
External/visceral	0	3	4	1	6
Skeletal	0	2	4	1	7

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External/Visceral and Skeletal Major and Minor Abnormalities'-Continued					
Group	1	2	3	4	5
Bronopol (mg/kg/day)	0	5	20	40	80
Abnormality	Number of fetuses with minor abnormalities only				
External/visceral	38	36	23	29	30
Skeletal	18	22	10	27	60
	Mean % of fetuses with major abnormalities				
External/visceral	0	1.7	2.5	0.9	3.3
Skeletal	0	1.0	2.5	0.9	4.2
	Mean % of fetuses with minor abnormalities only				
External/Visceral	24.7	20.9	16.9	16.3	17.5
Skeletal	10.2	13.0	4.5	13.5	29.5**
No. of litters with major abnormalities	0	4	6	2	6
Fetuses with one or both types of major abnormalities:					
Number	0	4	7	2	12
Mean percent	0	2.1	4.1	1.8	6.9

'This table is based on TABLE 6A, page 32, of the submission (MRID No.: 42319601).

**($p < 0.01$), significantly different from control; Dunn's multiple comparison test and Kruskal-Wallis test.

Major Abnormalities

According to the author of this submission, the incidence of fetuses (mean %: 6.9) with major external/visceral and skeletal abnormalities in the 80 mg/kg/day group was slightly higher than expected, referring, presumably, to the historical control data (there were no fetuses with major abnormalities in the concurrent control group). According to the historical control data (Attachment IV in this review), the mean percent of the external/visceral and skeletal abnormalities in the New Zealand White rabbit was 1.8 (range: 0.0-6.6) and 1.5 (range: 0.0-6.4), respectively. However, the difference from the controls in the

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80 mg/kg/day group, in the current study, was not statistically significant (Kruskal-Wallis test). Although the incidences of major abnormalities in the 5 mg/kg/day group (mean %: 2.1) and the 20 mg/kg/day group (mean %: 4.1) were higher than the incidence in the 40 mg/kg/day group (mean %: 1.8), they were within the historical control range and were considered by the author to be coincidental and unrelated to Bronopol treatment. The following major abnormalities were observed:

Group		Major Abnormality[#]
1		None
2	(a)	Severe craniofacial abnormality, with central proboscis, anophthalmia and hydrocephaly. Associate scull defects ----- in one fetus.
	(b)	Pulmonary valvular atresia ----- in two fetuses.
	(c)	Asymmetry of thoracic vertebrae and fused ribs (slight scoliosis) ----- in one fetus.
		Each of the above (4) fetuses affected belonged to a different litter.
3	(a)	Asymmetry of thoracic vertebrae and free floating rib (slight scoliosis) ----- in one fetus.
	(b)	Hydrocephaly, frontal bones short ----- in one fetus.
	(c)	Aortic arch enlarged, ductus arteriosus constricted ----- in one fetus.
	(d)	Pulmonary valvular atresia ----- in two fetuses.
	(e)	Asymmetry of pelvic girdle ----- in one fetus.
	(f)	Major fusion of sternebrae ----- in one fetus.
		Five of the above (7) fetuses affected each belonged to a different litter and two fetuses belonged to the same litter. Of those belonging to the same litter, one had (c) and another had (d).
4	(a)	Sacral neural arches - one or more absent of, misplaced ----- in one fetus.
	(b)	Major blood vessel abnormalities ----- in one fetus.
		Each of the above fetuses affected belonged to a different litter.

[#]This summary is based on TABLE 6B, page 33, of the submission (MRID No.: 42319601).

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5	(a)	Gastroschisis
	(b)	Major fusion of sternebrae
	(c)	Major blood vessel abnormalities
	(d)	Tail agenesis, hydrocephaly, asymmetry of lumbar vertebrae (scoliosis)
	(e)	Asymmetry of thoracic vertebrae
	(f)	Umbilical hernia
	(g)	Sternebrae duplicated
	(h)	Aortic arch enlarged
	(i)	Umbilical hernia
	(j)	Asymmetry of thoracic vertebrae, one or more fused or absent ribs (scoliosis)
	(k)	Asymmetry of thoracic vertebrae (slight scoliosis)
	(l)	Asymmetry of lumbar vertebrae (slight scoliosis)
		Each of the above (12) abnormalities, observed in Group 5, occurred in a different fetus. Three of the fetuses affected each belonged to a different litter and the remaining 2, 3 and 4 fetuses affected belonged to three separate litters.

Minor Abnormalities

The most frequent minor external and visceral abnormalities included alterations in the position of blood vessels, additional minor blood vessels arising from aortic arch, stomachs distended with gas and runted fetuses (TABLE 7, pages 35 and 36 of the submission; MRID No.: 42319601). The incidence of the first two abnormalities was highest in the control group (mean %: 9.6-11.2 and 3.1, respectively) and lowest in the 80 mg/kg/day group (mean %: 2.6-5.7 and 0.7, respectively), and was, therefore, treatment-unrelated. Although more fetuses had distended stomachs in the 80 mg/kg/day group (mean %: 5.4) than in the control group (mean %: 1.9), this finding was not significant by the Kruskal-Wallis test. The mean percent incidence of runted fetuses (< 20 g body weight) in the 0, 5, 20, 40 and 80 mg/kg/day groups was 0.4, 1.1, 0.4, 0.9, and 2.2, respectively. Since the retardation of the fetal weight was treatment-related in the 80 mg/kg/day group, the slightly higher incidence of runted fetuses in this group was also regarded by the author of this submission (study director) as treatment-related.

Compared with the control group, there was a significantly (**p < 0.01) higher incidence of fetuses with minor skeletal abnormalities in the 80 mg/kg/day group (mean %: 10.2 vs 29.5**) when these abnormalities are combined (page 13 of this review). This was due to a general retardation of skeletal ossification resulting in higher incidence of fetuses with unossified or partially ossified pubic bones, skull, vertebrae, sternum and

limbs (Attachment V in this review).

The incidences (mean percent) of fetuses with minor skeletal abnormalities in the remaining Bronopol-treated groups were similar to, or lower than, those in the control group.

Skeletal Variants

Compared with the concurrent control group, there was an increased incidence of fetuses with unossified forelimb and hindlimb epiphyses in the 80 mg/kg/day group. There were the only treatment-related , although statistically insignificant, skeletal variants observed in this study. The incidence of these findings in the control and 80 mg/kg/day groups was as follows:

Finding	Mean % of fetuses	
	Control group	80 mg/kg/day group
Forelimbs		
Epiphyses: One or more not ossified	38.4	46.0
Proximal or distal epiphyses of humerus only not ossified	25.2	34.4
Hindlimbs		
Epiphyses: One or more not ossified	58.2	74.4

The incidence of unossified forelimb and hindlimb epiphyses in the 5, 20 and 40 mg/kg/day groups was similar to or lower than that in the control group (Attachment V in this review).

Compared with the concurrent control group, there was a significantly higher incidence of fetuses with 6 instead of the usual 7 lumbar vertebrae in the 20 mg/kg/day group (mean %: 8.9 vs 22.3**) and in the 80 mg/kg/day group (mean %: 8.9 vs 22.6*). However, since a dose-related trend was lacking (Attachment V in this review), no biological significance was attached to this finding. (*$p < 0.05$ and **$p < 0.01$; significantly different from control; Dunn's multiple comparison test).

Predominant, but treatment-unrelated, skeletal variants included (a) increased or decreased numbers of thoracic, lumbar and caudal vertebrae; (b) unilateral or bilateral vestigial 13th rib; (c) unilateral or bilateral extra 13th rib; (d) unossified

5th and 6th sternebrae; and (e) retarded ossification of 5th and 6th sternebrae (Attachment V in this review).

DISCUSSION

This study was conducted according to EPA 1982 Guideline 83-3 and meets the December 24, 1989 ACCEPTANCE CRITERIA for 83-3 Teratology (Developmental) Studies (attached). A protocol for this study was submitted to EPA in 1991 (No MRID number; Submission No. S398602) and Toxicology Branch I/HED classified it as Acceptable on November 25, 1991. This protocol, with slight amendments, is also included in the current submission.

In general, this study is well planned and well reported. Maternal and fetal data have been reported as group means and as individual data, and each parameter examined is adequately discussed. Toxicology Branch I/HED agrees with the author's (study director's) interpretation of the experimental data.

Although the range-finding study (No. BON/2/91) has not been submitted to EPA for review, the results obtained are summarized in the current submission (No. BON/3/91; MRID No. 42319601). Four, rather than three, dose levels have been selected for the current study because, in the event that the highest dose is too toxic for the does, there will still be three dose levels left, as required by "regulatory authorities."

In the current study, the preparation of fetuses for examination is described in detail. However, none of the generally used procedures (such as Wilson's, Staples' or Dawson's procedure) is referenced.

Although comments are made in the submission about the effects of Bronopol on embryotoxicity, embryolethality or embryonic growth retardation, two NOELs are reported: Maternal NOEL and a Developmental NOEL.

The following signed and dated statements have been included in the submission:

(1) Statement of Data Confidentiality Claims.
(2) Good Laboratory Practice (GLP) Compliance Statement.
(3) Flagging Statement (There are no 6(a)(2) data in this study).
(4) Quality Assurance Authentication. This study has been inspected five times by the Quality Assurance Unit of Toxicol Laboratories Limited during 5/29-6/24/91. The draft report and the final report were audited on 11/22/91 and 3/12/92, respectively.

Although, overall, this study is reported in a clear and detailed manner, the following ambiguities and omissions should

be noted:

(1) The numbering of pages in CONTENTS is off by 2 pages for the entire report. To illustrate: SUMMARY is on page 7 in the report. However, according to the table of contents, it should be on page 5.

(2) Food consumption was reported as g/rabbit/day. A more meaningful way to report these data, as is commonly done, would have been as g/kg of body weight/day.

(3) Fetal length was not determined.

The above omissions and ambiguities are not significant enough to downgrade this study by classifying it as Core-Supplementary or Core-Minimum, instead of Core-Guideline. Also, new data for points (1) and (2) need not be submitted for this study. However, food consumption data, reported as g/animal/day in future studies, will not be accepted.

Attachment I

RIN 0220-95

BRONOPOL 3/30/93 REVIEW

Page_____ is not included in this copy.

Pages 23 through 39 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.

MRID No.: 42319601
Study No.: BON/3/91
Study Date: 3/13/92

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Subdivision F
Guideline Ref. No. 83-3
December 24, 1989

83-3 Teratology Studies (Rabbit)

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 20 pregnant animals/dose group for mice, rats or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).
3. ✓ At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.* ✓ At the low dose, no developmental toxicity is reported.
5. ✓ Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.* ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual food consumption.
10. ✓ Necropsy on all animals
11. ✓ Individual uterine examination including number of fetal deaths, early and late resorptions and numbers of viable fetuses per sex.
12. ✓ All ovaries examined to determine number of corpora lutea.
13. ✓ Individual litter weights and/or individual fetal weights per sex/litter.
14. ✓ Individual fetus external examination.
15. ✓ Individual fetus skeletal examination for 1/3 to 1/2 of each litter for rodents and all for all rabbits.
16. ✓ Individual fetus soft tissue examination.

Criteria marked with a * are supplemental and may not be required for every study.