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

12/3/84

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

To: John H. Lee
Product Manager #31
Registration Division (TS 767C)

Thru: Christine F. Chaisson, Ph. D C.F. Chaisson 11/29/84
Section Head, Review Section 4
Toxicology Branch
Hazard Evaluation Division (TS 769)

From: Roger Gardner
Toxicologist Roger Gardner 11/29/84
Toxicology Branch
Hazard Evaluation Division (TS 769)

Subject: Review of acute toxicity studies on Bronacide 10A and additional information on previcusly submitted studies. EPA Reg. Nos. 47374-E and 33753-R. Tox. Chem. No. 116A

Action Requested

1. Review acute oral, dermal, and inhalation studies, primary skin and eye irritation studies, and a skin sensitization study.

2. Review additional information on previously submitted rat and mouse oncogenicity, acute, subchronic, and teratology studies.

Recommendations and Conclusions

1. The acute oral and dermal toxicity studies and the primary eye and skin irritation studies with Bronacide 10A indicate that the formulation should be placed into Toxicity Category III with respect to oral and dermal toxicity as well as eye and skin irritation (See Section II. A. and Appendix I, pages I-1 to I-4 and I-7 to I-13).

2. Actual concentration measurements, particle size distribution, and other test atmosphere conditions during

exposure were not reported in the acute inhalation toxicity study (See review in Appendix I, pages I-5 to I-6). The operating characteristics and detailed description of the aerosol generating aparatus were also not reported. Until this information is made available, the report is considered to be incomplete, and a data gap exists for acute inhalation toxicity of Bronacide 10A.

3. The toxicity data on Bronopol generally indicate that the chemical's primary effect is the irritation that results from direct exposure (gastrointestinal, dermal, etc.). A rat study demonstrated that dermal penetration of Bronopol is 4 to 10% (recovered in the urine over a 5-day period following treatment) at a concentration of 4 mg/ml. A second dermal experiment (tissue distribution) demonstrated that 11% of the applied dose was excreted in the urine during the 24 hours following treatment, and the majority of the dose was found in the skin of the test site.

4. The concentration of Bronopol to which the general population could be exposed ranges from 0.01 to 0.03% (in household laundry products, fabric softeners, and cleaners) which is from 16.7 to 50 times less than the highest dose used in the chronic dermal study. That solution (0.5% applied to the shaved skin of mice three times a week for 80 consecutive weeks) did not induce tumors, and no signs of dermal irritation were observed. The 0.01 and 0.03% concentrations are also 167 to 500 times less than one which induced no dermal irritation in a primary skin irritation study (5% [redacted]

5. Some of the proposed uses for Bronopol involve textiles (See Section I. A., below) , and the Agency has proposed a policy for assessing data submitted in support of such uses (See 40 CFR Part 162 (Vol. 47, No. 240, December 14, 1982 as well as the discussion in Section III. A., below). On the basis of that proposed policy, exposure studies (leachability studies with treated textiles) are needed before a complete evaluation can be completed. Therefore, the Toxicology Branch cannot comment on data gaps for those uses until the Exposure Assessment Branch has evaluated information submitted on exposure.

6. Data requirements for the remaining proposed uses (See Section I. A. for a list of the industrial uses) are identified according to §158.135 published on October 24, 1984 (FR Vol. 49, No. 207, pages 42892-93). Consideration of points 1. through 4. above indicate that the acute inhalation toxicity study on Bronacide 10A represents the

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only data gap. However, this conclusion is subject to change pending comment from the Exposure Assessment Branch on the likelihood of significant inhalation and dermal exposures from the proposed uses

I. Background

A. Proposed uses and formulations

Bronopol (2-bromo-2-nitropropane-1,3-diol) is a biocide which is proposed for use as an industrial preservative to prevent bacterial degradation of fiber lubricants, spin finishes, and sizes in textiles, an "in-can" preservative for paints, coatings, adhesives, and water-based printing inks, and an inhibitor of bacterial growth in contaminated pigment slurries. All of these uses employ concentrations of the active ingredient ranging from 0.01 to 0.03%. Bronopol is also to be used as a preservative in detergents, fabric softeners, household cleaners, and laundry products.

According to the Confidential Statement of Formulation, Bronacide 10A, which is proposed for the above uses contains 13% active ingredient, and the inert ingredients include [redacted] Toxicity data on the formulation are summarized in Section II., Data Evaluation Records in Appendix I., and Toxicology Branch "One-liners" in Appendix II.

B. Summary of previously submitted toxicity data (Appendix II)

Toxicological studies reviewed previously (Memorandum dated October 7, 1983. From: R. Gardner, To: J. H. Lee, Registration Division, Subject: Review of toxicology data on Bronopol. EPA Reg. No. 47374-I. Tox. Chem. No. 116A) indicated the following toxicity profile.

The Toxicity Categories indicated for the technical grade material are summarized as follows:

Type of Study	Toxicity Category
Acute oral toxicity	II
Acute inhalation toxicity	I
Acute dermal toxicity	III
Primary eye irritation	I
Primary skin irritation	I

As noted in the previous review (cited above), the acute dermal LD_{50} value was based on use of 2 rats per dose group, and the results were considered along with those from other

studies with diluted test substance. The undiluted technical grade material is likely to be severely irritating to the skin or eyes because the effects observed in the other studies increased with the concentration of the active ingredient used.

No skin sensitization was observed in guinea pigs.

No-observed-effect levels (NOEL) (mg/kg/day) and lowest effect levels (LEL) with respect to body weight decreases or mortality are summarized as follows:

Study	NOEL	LEL
13-week rat gavage study*	20	80
13-week dog study**	4	8
2-year rat study***	10	40

*See Section III. B., below
**Liver and spleen weight increases were observed
***Test substance was administered in the drinking water (see Section III B., below)

Bronopol did not induce tumors in the long-term rat study mentioned above.

A series of specific teratology studies (following protocols that differed from those recommended by the Agency's Guidelines) indicated that the NOEL for maternal toxicity in rats (mortality) was below 10 mg/kg/day. A second study in which the treated group of rats was given the 10 mg/kg/day dose during gestation and allowed to nurture the offspring had no mortality, but two of the dams were sacrificed on day 19 of lactation because of poor condition. When doses of 0, 20, or 40 mg/kg/day were given to pregnant rats from day 15 to day 21 of gestation, no mortality was observed. These results considered together suggest that the NOEL for maternal effects is below 10 mg/kg/day in rats.

In another study pregnant rats were given 0, 20, or 40 mg/kg/day by dermal application on days 6 through 15 of gestation, and the only effect in treated rats was dermal irritation.

Reproduction studies were submitted, but they have not been reviewed for the following reasons:

1. One of the two studies used only one dose and produced one generation of offspring.

2. The other study only used two doses and one generation of offspring from approximately half the animals needed in a standard study.

Several metabolism experiments indicate that rats and dogs rapidly absorb oral doses of 1 or 2 mg bronopol per kg body weight. The chemical is excreted primarily in the urine (approximately 80-85% in 24 to 120 hours after dosing). The feces and expired air are also routes of excretion.

A rat study demonstrated that dermal penetration of Bronopol is 4 to 10% (recovered in the urine over a 5-day period following treatment) at a concentration of 4 mg/ml. A second dermal experiment (tissue distribution) demonstrated that 11% of the applied dose was excreted in the urine during the 24 hours following treatment, and the majority of the dose was found in the skin of the test site.

Bronopol does not accumulate in any particular organ, and the tissues with the highest residue concentrations are involved in excretion (liver, kidneys, and lungs).

II. New Data Summary (Appendix I)

A. Bronacide 10A

Acute studies were submitted in support of registration of a 13% formulation. The acute LD_{50} values are summarized as follows (see Data Evaluation Records in Appendix I, pages I-1 to I-13):

Route	Species	Sex	LD_{50} (g/kg)	Toxicity Category
Oral	Rat	Males	1.138	III
		Females	1.138	III
Inhalation	Rat	Both	>56.8 (mg/l)	III
Dermal	Rabbit	Both	>2	III

Survivors in the acute oral study exhibited ataxia, nasal discharges and rough coat. The signs were noted during the 72 hours following treatment, and most deaths occurred within 72 hours also. Gross necropsy of animals that died revealed severe gastrointestinal irritation, and microscopic examination of tissues from these animals revealed congestion in the gastrointestinal tract and liver. Some of those animals occasionally had congestion in the kidneys. Surviving animals did not exhibit any treatment related effects at necropsy. In the inhalation study, test animals exhibited lethargy during the exposure period, and they appeared normal at 2

hours after the end of the exposure. None of the test animals died according to the report.

There were no deaths in the acute dermal toxicity study. Histological observations of treated skin indicated inflammatory infiltration in 2 of the 5 male and all of the female rabbits in the treated group. These animals also exhibited a thickening of the epidermis and stratum corneum.

Bronacide 10A should be classified into Toxicity Category III for eye irritation (mean irritancy score = 0.43), and primary dermal irritation (mean primary irritation score = 3.31).

Bronacide 10A should be classified as a skin sensitizer based on results from a study in guinea pigs.

B. Technical grade Bronopol

The results of a long-term mouse study indicate that Bronopol is not oncogenic under the test conditions (dermal application of up to 0.5% solutions of bronopol (See Appendix I, pages I-20 to I-25.).

The mutagenicity studies (Ames tests, host mediated assay, and dominant lethal assay) are not acceptable for reasons discussed in Section III., and Appendix I (pages I-14 to I-19).

The additional individual animal data submitted for the rabbit teratology study indicate that only 10, 9, 7, and 9 rabbits were used in the control, low, mid, and high dose groups, respectively. The reported decrease in body weight gain is also of questionable toxicological significance (see Data Evaluation Record in Appendix I, page I-19).

III. Discussion

A. Regulatory considerations

Identification of data gaps is done on the basis of a proposed policy which was published in 40 CFR Part 162 (Vol. 47, No. 240, December 14, 1982) because some of the proposed uses (Section I. A.) involve textiles. The Agency states in the Notice that the variety of use patterns precludes a consistent set of data requirements to support the assessment of potential risks associated with biocides in textiles. The Agency further states that the data requirements will be linked to the potential for exposure associated with the uses.

Specific requirements are described as follows:

> Acute toxicity data will be required to determine the appropriate precautionary labeling for textile biocides. Special concern over exposure of workers, including those who handle and install treated fabric, may arise on a case-by-case basis...
>
> ...The risk to the general population from treated fabrics range from nil to a real and perceptible risk, commensurate with the toxicity characteristics and the potential for exposure...

There are three categories for classification of textile uses upon which data requirements are based. They include: (1) items with infrequent or no direct human contact, (2) items with frequent but indirect human contact, and (3) fabric and other items which come into direct contact with the body. The proposed uses of Bronopol in household laundry detergents and fabric softeners fall into the third category which involves the most extensive data requirements. The data requirements for Bronopol will be assessed on that basis.

In the above cited notice, the Agency recommends that a two tier approach to data development be followed by the Registrant. The first tier involves determining the leachability of the chemical from various types of treated fabric. The need for the second tier of studies requested by the Agency depends on the results of the first tier studies. If the chemical leaches from treated fabrics, the following recommendations are made:

> ...a determination of skin penetration using a radiolabeled compound should be made...the Agency would prefer to review the protocols prior to the start of the study in order to provide input on such parameters as levels of exposure, target organs, metabloism, and excretion.
>
> In cases where the second tier of studies demonstrates a significant dermal exposure with no dermal penetration, the Agency will require sufficient data to assess the hazard of topical skin contact. Data may include subchronic dermal exposure studies and dermal sensitization studies. The chemical nature of the biocide, length and amount of exposure, and the results of subchronic studies will determine the need for the long-term dermal studies, or in liue of the long-term studies, at least short-term mutagenicity assays.

> In cases where the second tier studies show both significant dermal exposure and dermal penetration, long-term/oncogenicity and reproductive effect studies may be required. The need for such studies will be evaluated in light of additional information developed or already at hand such as subchronic studies, genotoxicity studies, and metabolism studies.

As indicated in Sections I. B., II. A., and II. B., as well as the Appendices, much of the data requirements mentioned in the Federal Register Notice has been submitted. In addition to the acute studies, there are dermal penetration, long-term and subchronic dermal toxicity studies, skin sensitization studies, mutagenicity assays, teratology studies (including dermal studies in rats), and metabolism studies. All of these studies are discussed below as they relate to the proposed policies described in the Federal Register Notice.

B. Hazard assessment

Previous discussions of the toxicity data on Bronopol indicate that the chemical's primary effect is the irritation that results from direct exposure. In acute oral, subchronic oral, teratology, and chronic (drinking water) studies the primary effect in treated animals was gastrointestinal irritation which frequently was associated with death. Surviving animals given doses at or near LEL's exhibited body weight decreases. Dermal studies indicated that Bronopol caused skin irritation which increased in its intensity as the concentration applied increased.

Although no rationale was given for the routes of administration chosen in various studies (see Section I. B., page 4, above), the primary effect of the test substance and the type of exposures likely to be associated with the proposed uses were apparently considered, particularly with respect to the subchronic, chronic, teratology, and metabolism studies. The predominant route of administration chosen for many of the studies on Bronopol is dermal.

The concentration of Bronopol to which the general population could be exposed ranges from 0.01 to 0.03% (in household laundry products, fabric softeners, and cleaners) which is from 16.7 to 50 times less than the highest dose used in the chronic dermal study. That solution (0.5% applied to the shaved skin of mice three times a week for 80 consecutive weeks) did not induce tumors, and no signs of dermal irritation were observed. The 0.01 and 0.03% concentrations are

also 167 to 500 times less than the test concentration which induced no dermal irritation in a primary skin irritation study (5% [redacted] These comparisons consider direct exposure from the products containing bronopol rather than exposures that could result from treated fabrics.

C. Data gaps

For reasons mentioned in Appendix I (page I-5) the acute inhalation study on Bronacide 10A is considered incomplete. The Toxicology Branch reserves comment on the need for additional data until exposure data associated with the proposed textile and industrial uses become available and have been reviewed by the Exposure Assessment Branch.

APPENDIX I

Data Evaluation Records for
Submitted Studies

Bibliography

Pharmichem Testing Services, Inc. June 15, 1984. Acute oral toxicity test. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.....I-1

Pharmichem Testing Services, Inc. June 15, 1984. Acute dermal toxicity test. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750..I-3

Product Safety Labs. Undated. Acute inhalation toxicity study. Unpublished report no. T-3754. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.....I-5

Pharmichem Testing Services, Inc. April 10, 1984. Eye irritation study. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.....I-7

Pharmichem Testing Services, Inc. April 10, 1984. Skin irritation study. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.....I-10

Pharmichem Testing Services, Inc. Undated. Dermal Sensitization study. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.....I-12

Everest, R. P. September 12, 1974. Mutagenicity testing by means of in vitro microbial test, the host mediated assay, and the dominant lethal assay in mice. Unpublished report. Submitted by Inolex Chemical Company. EPA Acc. No. 247199. Resubmitted under EPA Acc. No. 252632.....I-14

Inolex Chemical. Undated. Rabbit: Oral administration. Part 3 (4). Toxicology. EPA Acc. No. 247199.....I-19

Hunter, B., C. Graham, and D. E. Prentice. January 23, 1975. Bronopol: Potential local and systemic tumorigenic effects in repeated dermal application to mice. Unpublished report prepared by Huntingdon Research Centre. Submitted by Inolex Chem. EPA Acc. No. 247197.....I-20

The Registrant submitted individual animal data from several studies cited below (see Acc. No. 252631 submitted with the current action). Since those data confirm the conclusions reached in the previous review dated October 7, 1983 (From: R. Gardner, To: J. H. Lee, Registration Division, Subject: Review of toxicology data on Bronopol. EPA Reg. No. 47374-I. Tox. Chem. No. 116A), they will not be discussed further. Data which permit review of studies which were previously regarded as incomplete are discussed in DATA EVALUATION RECORDS which are included in this appendix (see citations below).

Studies reviewed previously

Inolex Chemical. November, 1981. Acute toxicity: Mouse and rat (Dog). Part 3 (1). Toxicology. EPA Acc. No. 247193.

Inolex Chemical. November, 1981. Primary eye irritation: Rabbit. Part 3 (1). Toxicology. EPA Acc. No. 247193.

Inolex Chemical. November, 1981. Primary dermal irritation: Rabbit. Part 3 (1). Toxicology. EPA Acc. No. 247193.

Inolex Chemical. November, 1981. Dermal sensitization. Part 3 (1). Toxicology. EPA Acc. No. 247193.

Palmer, A. K., and A. M. Neuff. July 31, 1973. Effect of bronopol on peri- and postnatal development of the rat. Part 3 (4). Toxicology. Unpublished report submitted by Inolex Chemical. EPA Acc. No. 247199.

Hunter, B., P. Batham, R. Heywood, A. E. Street, D. E. Prentice, and J. M. Offer. January 23, 1975. Bronopol Toxicity and tumorigenicity study in rats by administration in the drinking water. Unpublished report prepared by Huntingdon Research Centre. Submitted by Inolex Chem. EPA Acc. No. 252633.

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DATA EVALUATION RECORD

Citation: Pharmichem Testing Services, Inc. June 15, 1984. Acute oral toxicity-test. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.

Materials and Methods

Test substance: Bronacide 10A (lot no. 9463A, 2-bromo-2-nitro-1,3-propanediol at an unspecified purity) was used.

Test species: Male and female Sprague Dawley rats were used.

Experimental procedure: Four groups containing 5 male and 5 female rats each were given single doses of 0.5267, 0.7901, 1.185, or 1.778 undiluted test substance per kg body weight by oral gavage.

Test animals were weighed on the day of treatment, one week after treatment, and at sacrifice two weeks after dosing. Observations for the occurrence of toxic signs and mortality were made daily throughout the 14-day observation period. At the end of the observation period surviving animals were sacrificed and subjected to gross necropsy. Animals dying during the observation period were also subjected to necropsy as soon after their deaths as possible. Tissues processed and examined microscopically included kidney, intestine, stomach, liver, lung, spleen, testis, cecum, and mesenteric nodes.

The only other description of the protocol or methods for calculation of the LD_{50} was a citation of 21 CFR 191.1, page 12 dated 1973; Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Association of Food & Drug Officials of the U. S., dated 1965; and Statistical Tables for Dose Evaluation, EBY, Report #5711.

Reported Results

The authors noted that there were no deaths or signs of toxicity observed in females given the 0.5267 and 0.7901 g/kg doses and males given the 0.5267 g/kg dose. All animals given the 1.778 g/kg dose died within 72 hours after treatment.

According to the report, survivors exhibited ataxia, nasal discharges and rough coat. The signs were noted during the 72 hours following treatment, and most deaths occurred within

72 hours also. Gross necropsy of animals that died revealed severe gastrointestinal irritation, and microscopic examination of tissues from these animals revealed congestion in the gastrointestinal tract and liver. Some of those animals occasionally had congestion in the kidneys. Surviving animals did not exhibit any treatment related effects at necropsy.

Discussion and Conclusions

There were adequate data presented in the report to indicate that the acute oral LD_{50} is 1.138 g/kg ($\pm$ 0.146) for male rats and 1.138 g/kg ($\pm$ 0.013) for female rats. These results place the formulation into Toxicity Category III.

Core classification: Minimum

DATA EVALUATION RECORD

Citation: Pharmichem Testing Services, Inc. June 15, 1984. Acute dermal toxicity test. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.

Materials and Methods

Test substance: Bronacide 10A (lot no. 9463A, 2-bromo-2-nitro-1,3-propanediol at an unspecified purity) was used.

Test species: Male and female New Zealand White rabbits were used. They weighed from 2.5 to 3.5 kg.

Experimental procedure: The trunks of test rabbits were shaved and the exposed skin was abraded. A group of 5 male and 5 female rabbits received a dermal application of 2 g undiluted test substance per kg body weight spread over the prepared skin (an area equivalent to 10% of the body surface). The test substance was applied on a gauze pad, and the test sites were occluded with plastic wrapping to maintain contact between the test substance and skin. After 24 hours the occulusive dressings were removed and the treated skin was washed to remove excess test substance.

A group of 2 male and 2 female rabbits were treated in the same manner except that physiological saline was substituted for the test substance.

Test animals were weighed on the day of treatment and at weekly intervals thereafter. All test rabbits were observed for mortality and the occurrence of toxic signs frequently on the day of dosing and twice daily for the subsequent 14-day observation period. At the end of that time the surviving rabbits were weighed and sacrificed. Treated skin sites as well as the thoracic and abdominal cavities were examined grossly, and lesions were noted.

Skin samples from test sites on treated and control rabbits were taken and examined microscopically.

Reported Results

No mortalities or signs of toxicity were observed according to the report. The only histological observations noted by the authors were inflammatory infiltration in 2 of the 5 male

and all of the female rabbits in the treated group. These animals also exhibited a thickening of the epidermis and stratum corneum.

Discussion and Conclusions

The reported results supported the conclusion that the application of 2 g Bronacide 10A to the abraded skin of rabbits had no toxic effects. The acute dermal LD_{50} is greater than 2 g/kg, and the results place the formulation into Toxicity Category III for acute dermal toxicity.

Core classification: Minimum

DATA EVALUATION RECORD

Citation: Product Safety Labs. Undated. Acute inhalation toxicity study. Unpublished report no. T-3754. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.

Materials and Methods

Test substance: Bronacide 10A (lot no. 9463A, 2-bromo-2-nitro-1,3-propanediol at an unspecified purity) was used.

Test species: Male and female Wistar derived rats were used.

Experimental procedure: One group containing 5 rats of each sex was exposed to a test atmosphere with 56.8 mg test substance per liter of air per hour (nominal concentration). The exposure period was reported to last for 3.5 hours.

The aerosol was generated by a nebulizer containing the liquid test substance. The test atmosphere was generated into a 90 liter chamber in which the rats were placed during exposure. Conditions such as the temperature, humidity, and actual concentration of the test substance during the exposure period were not reported.

The animals were observed frequently during exposure and daily for the subsequent 14-day observation period. Mortality and the occurrence of toxic signs were noted, and body weights were botained on the day of dosing and on day 14 of the observation period. Surviving animals were sacrificed at the end of the observation period, and necropsies were performed.

Reported Results

The investigators reported that the test animals exhibited lethargy during the exposure period, and the animals were described as normal in appearance 2 hours after the end of the exposure. None of the test animals died according to the report.

Discussion and Conclusions

The authors stated that there was a problem with a pressure valve in the test chamber aparatus during the last half hour of the exposure period. The concentration of test substance increased to 233 mg/l during that time, and that concentration was not included in the calculation of the LC_{50}. The LC_{50}

was reported to be >56.8 mg/l/hr (for a 3.5 hour exposure).

Core classification: Supplementary. Actual concentration measurements, particle size distribution, and other test atmosphere conditions during exposure were not reported. The operating characteristics and detailed description of the aerosol generating aparatus were also not reported. The study might be upgraded if this information is provided.

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DATA EVALUATION RECORD

Citation: Pharmichem Testing Services, Inc. April 10, 1984. Eye irritation study. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.

Materials and Methods

Test substance: Bronacide 10A (lot no. 9463A, 2-bromo-2-nitro-1,3-propanediol at an unspecified purity) was used.

Test species: Young adult New Zealand White rabbits were used.

Experimental procedure: Nine rabbits, previously found to exhibit no signs of eye defects or irritation, were used in the experiment. One-tenth ml of undiluted test substance was instilled into one eye of each rabbit, and the eyelids were gently held together for one second. Thirty seconds after instillation the treated eyes of three rabbits were washed for one minute with lukewarm water. The treated eyes of the six remaining rabbits were left unwashed.

All eyes of test animals were examined 24, 48, and 72 hours after treatment. Examinations were also conducted 4 and 7 days after instillation of the test substance. Corneas were examined for the presence and extent of opacities. The condition of the iris was also noted, and the conjunctivae were evaluated for chemosis, redness, necrosis, and discharge. These observations were scored according to the following scales:

Corneal opacity

Degree of density

1 - scattered or diffuse area, details of iris visible
2 - easily discernible transluscent areas, details of iris slightly obscured
3 - opalescent areas, no details of iris visible, size of pupil barely discernible
4 - opaque, iris invisible

Area of cornea involved

1 - one-quarter (or less but not zero)
2 - greater than one-quarter to less than one-half

3 - greater than one-half to less than three-quarters
4 - greater than three-quarters

score = score for degree x score for extent x 5
maximum = 80

Iris

1 - folds above normal, congestion, swelling, circumcorneal injection (any one or a combination of these), iris still reacting to light (sluggish reaction is positive)
2 - no reaction to light, hemorrhage, gross destruction (any one or all of these)

score = score for iris x 5
maximum score = 10

Conjunctivae

Redness

1 - vessels definitely injected above normal
2 - more diffuse, deeper crimson red, individual vessels not discernible
3 - diffuse beefy red

Chemosis

1 - any swelling above normal (including nictitation membrane
2 - obvious swelling with parital eversion of the lids
3 - swelling of lids about half closed
4 - swelling of lids about half to completely closed

Discharge

1 - any amount different from normal (does not include small amount in inner canthus of normal animals)
2 - discharge with moistening of the lids and hairs just adjacent to the lids
3 - discharge with moistening of the lids and considerable area around the eye

Score = sum of values for redness, chemosis, and discharge multiplied by 2. Maximum = 20

Scores were determined for each reading (see above), and the two group scores were averaged for each reading. The average scores for each reading time were added to provide a final

total which was then divided by the product of 110 (maximum possible score for a reading) and the number of reading periods. The result was used to determine the irritancy of the test substance when it was compared with the following scale:

Range of scores	Classification
0 - 0.005	non-irritating
0.006 - 0.17	slightly irritating
0.18 - 0.34	mildly irritating
0.35 - 0.67	moderately irritating
0.68 - 1.0	severely irritating

Reported Results

The average eye irritation scores reported for test rabbits are as follows:

Time after treatment	Group	
	Unwashed	Washed
24 h	55.67	55.0
48 h	50.0	55.67
72 h	49.0	48.67
4 days	48.17	48.67
7 days	26.33	32.33
10 days	16.67	20.67
13 days	12.17	15.67

The calculated irritancy score was reported to be 0.43 which clacsifies the test substance as a moderate irritant.

Corneal opacities persisted for 7 to 10 days after treatment, and the iris and conjunctivae showed signs of mild irritation which persisted for 4 to 7 days.

Discussion and Conclusions

The report included adequate information to support the conclusions drawn by the investigators. The results suggested that Bronacide 10A should be classified into Toxicity Category III for eye irritation.

Core classification: Minimum

DATA EVALUATION RECORD

Citation: Pharmichem Testing Services, Inc. April 10, 1984. Skin irritation study. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.

Materials and Methods

Test substance: Bronacide 10A (lot no. 9463A, 2-bromo-2-nitro-1,3-propanediol at an unspecified purity) was used.

Test species: Male and female New Zealand White rabbits were used. They weighed from 2.5 to 3.5 kg.

Experimental procedure: On the day before treatment the hair was clipped from the backs of rabbits to expose the skin for application of the test substance. Only rabbits without skin defects were selected for the study, and 3 rabbits of each sex were used. Two sites on each animal were abraded deep enough to penetrate the stratum corneum without causing bleeding and two sites were left intact.

One-half ml undiluted test substance was applied to 1 x 1 inch gauze pads which were then placed over each of the four test sites on each animal. The trunks of each rabbit were then wrapped with plastic. The plastic and gauze pads were removed 24 hours after they were applied, and the skin was then scored for signs of irritation. Test sites were also evaluated 72 hours after treatment.

Erythema and eschar formation as well as edema were scored on a 5-point scale (0-4) with a maximum possible score of 8 for any site. Scoring was done according to the following classifications:

Erythema and eschar		Edema	
No erythema	0	No edema	0
Slight erythema	1	Very slight edema	1
Well-defined erythema	2	Slight edema	2
Moderate to severe erythema	3	Moderate edema	3
Severe erythema to slight eschar formation	4	Severe edema	4

Mean irritation grades for intact and abraded skin sites were calculated by adding the scores for the two sites (of each type) observed at 24 and 72 hours after treatment. Irrita-

tion grades were calculated separately for intact and abraded skin. According to the report the test substance was then rated as follows:

Score range	Rating
0 - 0.04	Nonirritating
0.05 - 2.0	Mildly irritating
2.1 - 3.5	Moderately irritating
3.6 - 8.0	Severely irritating

Reported Results

The reported mean irritation scores for intact and abraded skin sites were 1.58 and 2.08 at 24 hours after treatment, respectively. The respective values at 72 hours following application of the test substance were reported to be 3.66 and 5.91. The calculated irritation grade was reported to be 3.31. By the criteria described above, the test substance is classified as a moderate skin irritant.

Nine of the 12 abraded sites were reported to have slight erythema and no edema at 24 hours, and there were no reactions reported at any of the abraded sites examined 72 hours after treatment.

The report stated that two of the test animals died (one on day 2 and the other on day 3 of the study), but no explanation of these deaths was provided.

Discussion and Conclusion

The report included adequate information to support the conclusions drawn by the investigators. The results suggest that Bronacide 10A should be classified into Toxicity Category III for primary dermal irritation.

Core classification: Minimum

DATA EVALUATION RECORD

Citation: Pharmichem Testing Services, Inc. Undated. Dermal Sensitization study. Unpublished report no. 92994. Submitted by Inolex Chemical Company. EPA Acc. No. 253750.

Materials and Methods

Test substance: Bronacide 10A (lot no. 9463A, 2-bromo-2-nitro-1,3-propanediol at an unspecified purity) was used. The positive control substance was 1-chloro-2,4-dinitrochlorobenzene.

Test species: Male albino guinea pigs were used.

Experimental procedure: The hair was clipped from the backs of the test animals. One guinea pig was used to determine a non-irritating or slightly irritating concentration of the test substance to be used in the main study.

A group of 10 animals were given 10 intradermal injections each of a 0.1% solution of the test substance in physiological saline, and a second group of 2 animals was given a series of intradermal injections of the positive control substance. The concentrations used in the study were based on the preliminary study mentioned above and described in the report. The animals were treated every second day for 3 consecutive weeks, and a challange dose was administered two weeks after the end of the initial 3-week dosing period.

Injection sites were examined and scored for reactions (erythema and eschar formation = S, and edema = E) 24 and 48 hours after each administration. Reactions were scored according to the following scales:

Erythema and eschar		Edema	
No erythema	0	No edema	0
Slight erythema	1	Very slight edema	1
Well-defined erythema	2	Slight edema	2
Moderate to severe erythema	3	Moderate edema	3
Severe erythema to slight eschar formation	4	Severe edema	4

The authors stated that an average irritation score was obtained by adding the scores for each treatment during an observation period and dividing by the number of observations for that period. They further noted that a sensitizing

reaction was indicated by an increase in positive reactions after the challange dose was given (on day 35) above that observed after the first injection was given (on day 0).

A sensitization score (SS) was calculated as follows:

$$SS = \frac{(S_c)^2 (E_c)}{(S_p)^2 (E_p)}$$

where:

S_c = erythema and eschar score after challange dose given on day 35

S_p = erythema and eschar score after doses administered during the first 3 weeks of the study

E_c = edema score after challange dose

E_p = edema score after prechallange dose

If the index is 3 or more the test substance is considered to be a sensitizer.

Reported Results

The authors reported that the positive control substance (DCNB) cuased sensitizing reactions with average indices of 7.02 and 18.68 at 24 and 48 hours after the challange dose was given, respectively. The two irritation indices reported for the test substance were 3.81 and 0 at 24 and 48 hours after challange, respectively. The 24-hour score was the result of 3 of the 10 animals which had sensitization indices of 20, 4.76, and 13.33.

Discussion and Conclusions

The report included adequate information to support the conclusions drawn by the authors. The results suggest that Bronacide 10A should be classified as a skin sensitizer.

Core classification: Minimum

DATA EVALUATION RECORD

Citation: Everest, R. P. September 12, 1974. Mutagenicity testing by means of in vitro microbial test, the host mediated assay, and the dominant lethal assay in mice. Unpublished report. Submitted by Inolex Chemical Company. EPA Acc. No. 247199. Resubmitted under EPA Acc. No. 252632.

Materials and Methods

Test substances: Technical grade bronopol was used. Reference mutagens used in this study included 9-aminoacridine, 5-bromouracil, proflavin sulfate, B-naphthylene, MNNG, benz(a)pyrene, 2-aminofluorene, 2,7-diaminofluorene, cyclophosphamide, hydrazine, captan, B-propiolactone, EMS, MMS, METEPA, trimethyl phosphate, quinacrine, and dimethyl sulfoxice.

Test species: Salmonella typhimurium strains TA1535, TA1536, TA1537, and TA1538 were used. Samples of each bacterial strain were grown overnight at 37°C in nutrient broth, and subsequently centrifuged and resuspended in 0.9% saline. One-half ml of each culture was added to 10 ml molten saline agar (45° C) along with a sterile solution of 0.5 ml histidine and biotin (1 mM/ml). This molten agar mixture was layered over Vogel-Bonner minimal agar in 2 ml aliquots.

In assays with metabolic activation, a 0.4 ml aliquot of the test strain in saline (see previous paragraph) was mixet with 0.4 ml of the test substance or reference mutagen solution. This mixture was added to 1.5 ml of the S9 mix (see below), and that mixrure was added to 6 ml molten agar. Two ml aliquots of the agar mixture were layered onto Vogel-Bonner plates as described above.

Esherichia coli strains WP2, UvrA, CM561, and CM611 were used along with the S. typhimurium strains mentioned above in spot tests. The E. coli are grown in nutrient broth overnight and resuspended in phophate buffer (0.67 M, pH 7.0) as described above for S. typhimurium. A 0.5 ml aliquot of inoculum is mixed with 9 ml molton salt agar (0.6% agar, 0.6% NaCl) which is layered onto broth supplemented Davis-Mingoli minimal agar plates. The necessary tryptophan is provided in the nutrient broth supplement.

Preparation of Rat Liver S9 mix: Male Boots Wistar arts were given water containing 0.1% phenobarbitone ad libidum. After one week the animals were sacrificed. The livers were

removed, homogenized 0.15 M KCl, and centrifuged at 9000 x g. The supernatant was decanted and retained. One part of the liver 9000 xg supernatant was mixed with nine parts of a cofactor solution to make the S9 mix. The cofactor solution was made up of 70 ml phosphate buffer (100mM, pH 7.4) containing 31 mg TPN, 18 mg Glucose-6-phosphate, 16 mg $MgCl_0$ and 25 mg KCl. Seven ml of the cofactor solution was added to 3 ml of the

Experimental procedure---spot tests: The test substance as a 0.1% solution in water in fishspine beads was placed on each agar plate for diffusion into the medium. These plates were incubated for 3 days at 37° C after inoculation with tester strains. The known mutagens were evaluated as 1% solutions in water or DMSO.

Experimental procedure---semi-quantitative assay: For these assays 0.5 ml of the test solution was added to the molten agar layered onto the Vogel-Bonner plates (see above). The Bronopol solution tested contained 40 ug/plate. All test solutions were evaluated for sterility, and a vehicle control and negative control were assayed. Each test solution was evaluated on 3 plates.

Experimental procedure---host mediated assay: Test species: The tester strain of S. typhimurium used in this assay was TA1530. Female OLAC mice were used as the host.

Groups of 5 female mice were given 6 daily doses of 12.5, 25, or 50 mg test substance per kg body weight in water by oral intubation. On the seventh day the animals received the same dose along with an intraperitoneal injection of the test strain of bacteria. A fourth group received a doses of 330 mg dimethyl nitrosamine by intramuscular injection on the day before i. p. injection of the bacteria. A second dose of the positive control substance was administered on the day of the bacterial injection.

Three hours after injection of the bacteria, the organisms were recovered by intraperitoneal aspiration, and triplicate plates were made for determination of the number of organisms recovered as well as the number of revertants. The plating procedures were the same as those used for the non-activated assays described above.

Experimental procedure---dominant lethal assay: Groups of 10 male mice were given single daily oral doses of 20 or 100 mg test substance per kg body weight. Doses were administered for 6 consecutive days prior to mating. Female mice received

no test substance. Each male was housed with 3 females for one week, and the females were maintained for 14 days beyond the midpoint of the mating period. At that time they were sacrificed and examined. The three females were replaced each week for the four consecutive weeks following dosing.

Gross necropsies were performed on the females to determine the pregnancy rate, number of live implants, and the number of dead implants.

Reported Results

Spot test: According to the report, a 1% aqueous solution of bronopol was not mutagenic in the E. coli or S. typhimurium strains tested. The positive control substance (MNNG) was mutagenic under the test conditions.

Semi-quantitative assay: The average number of revertants observed on the replicate plates for each compound were reported as follows:

Compound	G46	TA1535	TA1536	TA1537	TA1538
		With S-9 mix			
Bronopol	-	10.5	0	6.5	25.18
MNNG	-	>5000	-	-	-
9-aminoacridine HCl	-	-	0	>1000	40.35
2-aminoflourene	-	-	0	8.1	>200
Water	-	4.9	0	9.8	12.20
DMSO	-	5.7	0	8.3	6.8
		Without S-9 mix			
Bronopol	0	0	0	0	0
MNNG	>5000	>5000	-	-	-
9-aminoacridine HCl	-	-	0	>1000	20.22
2-aminoflourene	-	-	0	10.4	29.30
water	4.5	11.12	0	12.14	32.40
DMSO	3.7	12.18	0	8.4	6.5

Host mediated assay: The reported mutation frequencies are as follows:

Dose (mg/kg)	Frequency
0	0.9×10^{-8}
12.5	0.5×10^{-9}
25	0.7×10^{-8}
50	0.7×10^{-8}
330*	21.5×10^{-8}

*Positive control (dimethyl nitrosamine)

Dominant lethal assay: Cummulative mortality in treated males was reported as follows:

Mating week	Intraperitoneal 10	Intraperitoneal METEPA*	Oral doses (mg/kg) 0	Oral doses (mg/kg) 20	Oral doses (mg/kg) 100
1	0	0	0	0	1
2	0	0	0	0	4
3	1	0	0	0	4
4	1	0	0	0	4

*Positive control (25 mg/kg, i.p.)

The pregnancy rate (%) was reported as follows:

Mating week	Intraperitoneal 10	Intraperitoneal METEPA*	Oral doses (mg/kg) 0	Oral doses (mg/kg) 20	Oral doses (mg/kg) 100
1	33	83	70	67	44
2	45	67	57	73	39
3	48	70	55	80	50
4	44	56	56	76	56

The mean number of live implants per dam were reported as follows:

Mating week	Intraperitoneal 10	Intraperitoneal METEPA*	Oral doses (mg/kg) 0	Oral doses (mg/kg) 20	Oral doses (mg/kg) 100
1	9.2	7.4	9.4	9.8	8.5
2	8.1	4.2	9.6	8.4	6.7
3	9.9	6.9	9.4	9.3	6.0
4	8.7	8.7	10.4	10.6	9.6

The mean number of dead implants was reported as follows:

Mating week	Intraperitoneal 10	METEPA*	Oral doses (mg/kg) 0	20	100
1	0.7	3.5	0.6	1.0	0.5
2	1.2	3.9	0.5	0.9	0.4
3	0.3	3.1	1.0	0.5	0.2
4	0.2	1.2	0.7	0.7	0.5

The mean total implantations per dam were reported as follows:

Mating week	Intraperitoneal 10	METEPA*	Oral doses (mg/kg) 0	20	100
1	9.9	10.9	10.0	10.8	9.0
2	9.3	8.1	10.1	9.3	7.1
3	10.2	10.0	10.4	9.8	6.2
4	8.9	9.9	10.0	11.2	10.1

Discussion and Conclusions

The bacterial assays indicated that, under test conditions, the test substance did not cause mutations. However, the studies are not acceptable since replicate plate counts used to determine reported means were not included. In addition, only one dose was used for the semiquantitative assay.

The dominant lethal assay is also unacceptable because the test mice were not mated over a period long enough to fully evaluate spermatogenesis. The authors noted a decrease in the "implantation rate" during the second and third mating weeks for the 100 mg/kg dosed group. A decreased implantation rate was also observed in the females mated with males given the 10 mg/kg dose i. p. during the fourth mating week. These decreases were atributed by the investigators to the decrease in pregnancy rate which they associated with the occurrence of overt toxicity (indicated by mortality) in test animals in that group (see Reported Results section above).

DATA EVALUATION RECORD
(Supplementary Review)

Citation: Inolex Chemical. Undated. Rabbit: Oral administration. Part 3 (4). Toxicology. EPA Acc. No. 247199.

NOTE: The following is a discussion of additional individual animal data submitted in response to questions raised by a previous Toxicology Branch review (Memorandum dated October 7, 1983. From: R. Gardner, To: J. H. Lee, Registration Division, Subject: Review of toxicology data on Bronopol. EPA Reg. No. 47374-I. Tox. Chem. No. 116A). Materials and methods, most of the reported results, and discussion of those data are not repeated herein unless they are relevant to the additional data recently submitted.

The previous Toxicology Branch review stated that a no-effect level of 3.3 mg/kg/day was established for maternal toxicity. The highest dose (10 mg/kg/day) caused a significant decrease in body weight gain during the dosing period (0.13 kg for the control group and 0.04 kg for the high dose group), but the individual maternal body weight data submitted did not show significant differences between the control and treated groups. For example, at day 17 of gestation (last day of dosing), the group mean maternal body weights, as determined from the reported individual weights, were 3.930, 4.097, 4.100, and 4.077 kg for the control, low, mid, and high dose groups, respectively. The body weight gain effect is minor when compared with body weights themselves, and such a comparison implies that the significance of the decrease in body weight gain is not necessarily toxicologically significant.

The individual animal data indicated that there were 10, 9, 7, and 9 does in the control, low, mid, and high dose groups, respectively. These numbers are somewhat less than the number of individuals recommended by the Agency for teratology studies in rabbits, and the study is somewhat less sensitive. Based on this consideration, the additional data submitted do not provide the means of upgrading the study from the supplementary classification originally assigned to it.

DATA EVALUATION RECORD
(Supplementary Review)

Citation: Hunter, B., C. Graham, and D. E. Prentice. January 23, 1975. Bronopol: Potential local and systemic tumorigenic effects in repeated dermal application to mice. Unpublished report prepared by Huntingdon Research Centre. Submitted by Inolex Chem. EPA Acc. No. 247197.

Note: The materials and methods are discussed in a previous memorandum dated October 7, 1983 (From: R. Gardner, To: J. H. Lee, Registration Division, Subject: Review of toxicology data on Bronopol. EPA Reg. No. 47374-I. Tox. Chem. No. 116A). That review summarized the mouse study as follows:

> Bronopol was dissolved in a solution of acetone and water (9:1) at concentrations of 0, 0.2, or 0.5%. The solutions were painted on clipped skin of mice 3 times each week for 80 consecutive weeks.

The summary further stated:

> The stated purpose of the study was to evaluate the potential for bronopol to cause skin tumors in mice, and results were summarized rather than being reported in detail so that conclusions could not be independently evaluated.

The review also noted (page 61) that no appendices containing the individual animal data on body weight and food consumption or time to diagnosis of tumors (other than skin tumors) were not included with the report.

The results that are discussed herein were in appendices which were missing from the original report, and they are evaluated below.

Reported Results

The individual animal data for body weight and food consumption was provided. These results were consistent with reported group means as indicated by a spot check of the statistical analysis of the control and high dose group males. There is no apparent reason to change the previous evaluation of these data. Those results were described in the previous review as follows:

> A reduction in group mean body weight gain was noted during weeks 26 to 52. However, mean body weights for

each group of males or females were comparable at weeks 26, 52, and 80, and no differences were noted between groups with respect to food consumption or efficiency of food utilization.

The lungs, liver, and ovary of the animals contained the most frequently observed non-neoplastic lesions according to the report. The incidence of some of those lesions (number with lesion/ number examined) is summarized as follows:

Lesion	Dose group	Incidence	
		Males	Females
lung			
Lymphoid aggregations (perivascular or peribronchiolar)	Control	14/50	9/51
	Low	11/50	12/50
	High	14/50	11/49
Alveoli with alveolar macrophages	Control	9/50	6/51
	Low	6/50	7/50
	High	11/50	5/49
liver			
Hepatocytes vacuolated or distended	Control	26/50	28/51
	Low	21/50	26/50
	High	13/50	22/49
Hepatocyte degeneration	Control	8/50	14/51
	Low	10/50	6/50
	High	9/50	5/49
Inflammatory cell infiltration	Control	26/50	30/51
	Low	19/50	21/50
	High	23/50	11/49
ovary			
cysts	Control	-	8/50
	Low	-	9/50
	High	-	10/50

A variety of other lesions were also reported, but they occurred in 5 or fewer animals from each group and did not show a dose-related incidence in the treatment groups.

The most common neoplastic lesions according to the report occurred in the lungs of both sexes. These tumors were graded

on a five point scale using the following criteria:

Grade 1 - non-invasive tumor
Grade 2 - locally invasive tumor, sometimes extending into airway
Grade 3 - tumor multifocal in lobe of origin
Grade 4 - tumor extending beyond lobe of origin into mediastinum, or into other lobes, but not outside thorax
Grade 5 - distant metastases

The incidence of all grades of lung tumors in each group is summarized as follows:

Dose group	Incidence	
	Males	Females
Control	13/50	10/51
Low	13/50	9/50
High	13/50	11/49

The incidence of tumor-bearing animals in the control, low, and high dose groups were 24, 21, and 23 of 50 males, respectively. The respective incidence of tumor-bearing female mice was reported to be 25/51, 18/50, and 22/49 in the control, low, and high dose groups.

The number of animals with grossly or microscopically observed lesions is summarized as follows:

Gross observations

	Males			Females		
	Control	Low	High	Control	Low	High
Subcutaneous						
abscesses	9	2	8	1	1	1
masses	5	3	3	3	1	3
edema	0	1	1	2	0	0
Cutaneous						
ulceration	1	4	3	1	1	3
scab formation	0	0	2	0	0	2
hair loss	0	1	0	0	1	0
suspected papilomas	1	0	1	1	1	3
edema	0	0	0	0	1	0
No. examined	50	50	50	51	50	48

Microscopic observations

	Males			Females		
	Control	Low	High	Control	Low	High
Subcutaneous tissue						
abscesses	5	1	6	0	1	2
edema	0	1	0	-	-	-
hemorrhage	-	-	-	0	1	2
inflammatory cell infiltration	0	1	0	-	-	-
Cutaneous tissue						
ulceration	0	2	3	0	1	2
inflammatory cell infiltration	0	3	1	1	1	2
abscess	0	0	1	-	-	-
folliculitis	0	1	1	-	-	-
hyperplasia/hyperkeratosis/parakeratosis	2	3	3	1	1	3
keratitis	0	0	2	-	-	-
Subcutaneous tissue						
anaplastic sarcoma	1	0	0	-	-	-
fibrosarcoma	0	0	1	2	0	0
Cutaneous tissue						
squamous cell papilloma						
treated skin	1	0	1	0	0	3
untreated skin	-	-	-	1	1	0
No. examined	50	50	50	51	50	48

Discussion and Conclusions

Most of the liver and lung lesions were found after the 50th week of the study as indicated in the individual animal data sheets submitted. The ovarian cysts were diagnosed during the last 20 weeks of the test. These results and the absence of a dose-related effect on the incidence of the lesions supported the authors' conclusion that the observations were not manifestations of test substance toxicity. As tabulated above the incidences of skin lesions indicated almost no effects resulting from the dermal application of the test substance during the experiment.

The incidence of tumor bearing animals or those with lung tumors (number with tumor/number examined) with respect to time of diagnosis is summarized as follows:

Dose group	Weeks on test 0-20	21-40	41-60	61-80	Termination
			Tumor-bearing animals		
Males					
Control	0/0	0/2	1/5	5/9	18/32
Low	0/0	1/4	0/2	7/14	13/26
High	0/2	1/2	2/11	5/8	15/27
Females					
Control	0/0	0/0	7/8	10/15	9/28
Low	0/2	1/2	1/6	2/7	14/34
High	0/0	0/3	4/6	4/9	14/31
			Lung tumors		
Males					
Control	0/0	0/2	0/5	4/10	9/32
Low	0/0	0/4	0/2	5/14	8/26
High	0/2	0/2	0/11	3/8	9/27
Females					
Control	0/0	0/0	4/8	2/15	4/27
Low	0/2	0/2	0/6	1/7	7/34
High	0/0	0/3	2/6	0/9	8/31

With the exception of the incidence of lung tumors in females examined at termination of the study, there is no apparent dose-related increase in tumors. Contingency table analysis of the three groups examined at termination indicated no statistically significant differences (2 X 3 analysis, Chi square (2) = 0.540, P = 0.6886; analysis of the control and high dose groups, Chi square = 0.498, P = 0.4874). The total incidence of lung tumors in female rats during the study (corrected for early mortality) was 10/50, 8/47, and 10/46 in the control, low, and high dose groups, respectively. These results indicate that the test substance is not oncogenic under the test conditions (dermal application of up to 0.5% solutions of bronopol).

There were discrepancies in tumor counts from the individual animal data sheets and those summarized in the original report.

The group totals for tumor bearing animals and animals with lung tumors as indicated by the data discussed on page 5 herein are as follows:

Data from individual animal sheets

Dose group	Tumor bearing		Lung tumors	
	Males	Females	Males	Females
Control	24/48	26/51	13/49	10/50
Low	21/46	18/51	13/46	8/47
High	23/50	22/49	12/50	10/46

Data from reported summary tables

Dose group	Tumor bearing		Lung tumors	
	Males	Females	Males	Females
Control	24/50**	25/51*	13/50**	10/51**
Low	21/50**	18/50**	13/50**	9/50***
High	23/50	22/49	12/50*	11/49***

*Number with tumor is different
**Number of animals examined is different
***Number with tumor and and number examined are both different.

The discrepencies may arise from counting errors or differences in the criteria for selection of the animals for examination (losses due to autolysis for example). However, neither of the data sets indicate that bronopol is oncogenic in mice under the test conditions.

The results indicate that the test substance is not oncogenic when applied to the skin of mice at concentrations of 0.2 and 0.5% in water:acetone for 80 weeks.

Core classification: Minimum

APPENDIX II

Toxicology Branch
"One-liners"

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; Inolex Chem. Corp; undated	Technical	247199	10 mg/kg/day caused 37.5% mortality Since this was the lowest dose tested, no NOEL was determined. Insuffient numbers of litters were available for evaluation of fetal effect. Levels tested = 0, 10, 30 or 100 mg/kg/day		Supplementary 003859
Teratology - rat; Inolex Chem. Corp; 7/26/73	Technical	247199	NOEL > 40 mg/kg/day (highest dose tested) applied dermally on days 6-15 of gestation. Levels tested- 20, & 40 mg/kg/day.		Minimum 003859
Teratology - rabbit; Inolex Chem. Corp; undated	Technical	247199	Teratogenic NOEL > 10 mg/kg/day(HDT) Maternal NOEL = 3.3 mg/kg/day Maternal LEL = 10 mg/kg/day (decreased body weight) Feto Toxic NOEL = > 10 mg/kg/day (HDT) Levels tested = 0, 1, 3.3 and 10 mg/kg/day		Supplementary 003859
13-Week oral - rat; Research Centre; report no. BT534/73268; 8/29/73	Technical	247195	NOEL = 20 mg/kg/day LEL = 80 mg/kg//day (7/20 males & 9/20 females died). Levels testtted = 0, 20, 80 or 160 mg/kg		Minimum 003859
13-Week oral - dog; Huntingdon Res. Centre; 7/27/73	Technical	247194	NOEL = 8 mg/kg/day LEL = 20 mg/kg/day (effects noted were emesis and increased liver and spleen wts. Levels tested by gavage = 0, 4, 8, and 20 mg/kg/day		Minimum 003859