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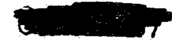
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460



10/7/83

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of toxicology data on bronopol.
EPA Registration No. 47374-R. Tox. Chem. No. 116A
and 47374-1.

TO: John H. Lee
Product Manager 31
Registration Division (TS-767)

THRU: Chad B. Sandusky, Ph.D.
Acting Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769)

Chad B Sandusky 9/23/83

Action Requested:

Review of toxicological data in support of a manufacturing use registration of bronopol (2-bromo-2-nitropropane-1, 3-diol).

Recommendations and Conclusions

1. Acute Toxicity studies (See Section II. A, B, C, D and E below) indicate that technical grade bronopol should be classified into Toxicity Category I for eye and skin irritation and acute dermal toxicity, Toxicity Category II for acute oral toxicity, and Category III for acute inhalation toxicity.

2. It should be noted that the acute dermal LD_{50} study used 2 male rats per dose group, and is considered to be supplementary data. However, those limited results suggest that bronopol may belong in Toxicity Category I for Acute Dermal Toxicity. This conclusion is supported by the results of other toxicity studies which indicate that the chemical is severely irritating to the skin (Sections II. D, F.3, G.3., and K.2).

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3. A no-effect level of 20 mg/kg/day was established in a 13-week study with rats. The lowest effect level was 80 mg/kg/day. That dose caused significant body weight loss and mortality. (Section II. F.l. below.).

4. A no-effect level of 3.3 mg/kg/day was established for maternal toxicity in a rabbit teratogenicity study. The lowest effect level was 10 mg/kg/day and pregnant rabbits and rats given that dose showed significant body weight decreases (Section II. G.4. below). No effects were noted on the fetuses from treated animals.

5. Reports on microbial and dominant lethal mutagenicity assays (Section II. J. below) are incomplete. More detailed descriptions of the methods used in these studies are needed.

6. There are sufficient toxicity data to support the requested manufacturing use registration. This recommendation is made in view of points 1 through 5 above.

7. No final statement can be made with regard to reproduction toxicity, chronic toxicity, oncogenic toxicity, teratogenicity, and mutagenicity testing since no registrations for specific uses were requested. However, the following points should be noted.

7a. The microbial and mammalian assays discussed above are appropriate for evaluating the potential of bronopol to induce gene mutations in bacteria and the potential to cause structural chromosome damage in mammals. However, additional testing is needed to satisfy the purposes of the Agency's proposed data requirements for mutagenicity (Federal Register, November 29, 1982, Vol. 47, No. 227, Sections 158.105 and 158.135, pages 53206 and 53210; Section IV, page 53193-4; and Section V E, pages 53195-6). These additional tests should include forward or reverse mutation assays with mammalian cells *in vitro* to establish the relevance of microbial results to mammals and tests to assess the potential of bronopol to cause chromosomal damage and other genotoxic effects. The choice of tests, including those already described above, should be justified with respect to the objectives of sensitive screening and establishment of relevance to mammals. The justification of the battery of tests selected should also consider the nature of the test substance. When any genetic activity of the chemical is found, an assessment of heritable or other related health risks should be made.

7b. Many of the studies of reproduction toxicity, teratogenicity, subchronic, oncogenic and chronic effects followed unique protocols. These studies are discussed in Sections II

F., G., H. and K. below. Final conclusions regarding their acceptability depend upon the requested uses those studies are to support.

As stated above (See 5.), complete reports on the microbial and dominant lethal assays are needed along with a justification for selecting those and any additional tests conducted. The justification should demonstrate that the proposed purposes and objectives for the battery of mutagenicity tests have been satisfied.

I. Data Summary

A. Acute Toxicity

Bronopol was found to cause primary irritation associated with the route of administration. Animals given oral doses were found at necropsy to have adhesions of stomach to liver, ulceration of the stomach and duodenum, and thickening of the intestinal walls. Acute oral LD_{50} values (mg/kg) were reported as follows:

Species	Males	Females
Rat	307	342
Mouse	374	327

These results place bronopol in Toxicity Category II.

An acute dermal toxicity study was conducted in male rats using 2 animals per dose. The results indicated that a dermal LD_{50} is likely to be between 64 (no deaths reported) and 160 mg/kg (both animals died). In view of the apparently preliminary nature of this study, the results suggest that bronopol should be classified in Toxicity Category I for acute dermal toxicity. Those rats that died had subcutaneous edema and hemorrhaging, while the survivors showed scabbing of the treated skin. The severity of the dermal reaction of male rats treated with a dermal dose of 64 mg/kg and reactions observed in dermal studies discussed below indicate that additional acute dermal toxicity studies on the technical grade material would provide no additional useful information about bronopol. Based on these considerations the chemical is classified into Toxicity Category I for acute dermal toxicity.

Rats in an acute inhalation toxicity study showed signs of eye irritation, dyspnea as well as nasal and ocular discharges containing traces of blood (in animals exposed to the 5 mg/L concentration) during the 4 hour exposure

period. Although some of these signs persisted for 3 days after exposure, no deaths were noted. The LC_{50} is greater than 5 mg/L (highest concentration tested) which suggests that bronopol should be classified into Toxicity Category III for acute inhalation toxicity.

Solutions of 0.5, 2, or 5% bronopol in [redacted] or aqueous saline were instilled in the eyes of test rabbits. Slight swellig and redness of the conjunctivae was noted to persist for up to 5 hours after instillation of the 0.5 and 2% solutions. The 5% solution caused redness of the conjunctiva which persisted for up to 7 days after treatment. Therefore, the 5% solution of bronopol is placed in Toxicity Category II for eye irritation. However, in view of the severe irritation noted in other toxicity studies the technical grade material should be classified in Toxicity Category I.

Concentrations of up to 4% bronopol in 2.5% aqueous methyl cellulose had no effect on the skin of rats. A 2% solution of bronopol in acetone caused mild erythema in a rabbit, and the effect persisted for 72 hours. [redacted] solutions containing 1.25, 2.5 or 5% bronopol did not irritate the skin of rabbits. Bronopol in methyl cellulose solution caused slight to moderate edema and erythema in rabbit skin. These effects persisted for 24 hours in one rabbit treated with 0.5% bronopol. The response of treated skin in human volunteers was slight (erythema) after application of 1% solution of bronopol in soft paraffin. Slight erythema was also noted after application of an aqueous solution of 0.25% bronopol (buffered to pH 5.5).

This severity of response in laboratory animals and human volunteers increases with the increase in concentration. These results and those reported in other dermal studies discussed herein suggest that technical grade bronopol should be classified a Category I dermal irritant despite the lack of results from conventional tests.

No skin sensitization was observed in guinea pigs challenged with 0.5 ml of a 0.5% bronopol solution.

B. Subchronic Toxicity

The only dose-related pathology in rats given bronopol by gavage for 13 weeks was noted in those animals which died during the study. Animals given 80 or 160 mg/kg/day were reported to have signs of

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respiratory distress and abdominal distension. At necropsy high dose animals had hemorrhagic foci of the mucosal lining in the glandular stomach. Histological observation of gastrointestinal tissues revealed superficial ulceration with underlying inflammation, epithelial hyperplasia and hyperkeratosis, and congestion of vessels in the mucosa. By the end of the study all animals given the 160 mg/kg/day dosage were dead. Mortality in the 80 mg/kg/day dose was 35 or 45% for males and females, respectively. Only one female from the 20 mg/kg/day group died, and no histopathology was noted in that animal. On the basis of concommitant mortality and pathological observations a no-observed-effect level (NOEL) is established at 20 mg/kg/day and the lowest-effect level (LEL) is 80 mg/kg/day in the study.

A similar study in beagle dogs showed that bronopol is emetic at doses of 8 or 20 mg/kg. These doses were also reported to increase the spleen and liver weights of treated dogs. The NOEL for those effects after 13 weeks was 4 mg/kg/day and the LEL is 8 mg/kg/day.

A 21-day dermal toxicity study was conducted in rabbits with bronopol (0, 0.02, or 0.5%) in 2.5% aqueous methyl cellulose. The treated skin was described as hardened and brown with sloughing during the third week of the experiment. These reactions occurred only at the high dose. It should be noted that the skin of test animals was abraded.

C. Teratogenicity and Reproduction

A series of studies following protocols characteristic of specific rather than more generalized testing procedures were submitted. These studies showed that 30 to 50% mortality occurred in groups of pregnant rats given doses from 10 to 100 mg/kg/day by gavage during gestation. The animals that died were found at necropsy to have lung congestion and gastrointestinal effects including erosion of the glandular stomach. A subsequent study in which a group of 10 pregnant rats was given 10 mg/kg/day doses during gestation and allowed to deliver and nurture their young showed no mortality. However, two of the dams were sacrificed because of their poor condition on day 19 of lactation.

In another study pregnant rats were given 0, 20, or 40 mg/kg/day from day 15 through day 21 of gestation. No compound related effects were noted on the dams or their offspring.

A teratogenicity study was conducted in which doses of 0, 20, or 40 mg/kg were applied to intact shaved skin of pregnant rats. Doses were applied on days 6 through 15. Signs of dermal irritation were noted on the 8th day of gestation in treated rats. These signs included edema and erythema, and by the 11th day of gestation scabbing was reported in the rats treated with bronopol. No other maternally toxic, fetotoxic, or teratogenic effects were observed.

A briefly reported teratogenicity study in rabbits showed that a maternal toxicity NOEL of 3.3 mg/kg/day administered by gavage (days 8 through 16 of gestation) is likely. Because the study was reported in summary form, the NOEL can only be considered as provisional. The LEL is apparently 10 mg/kg/day based on decreased maternal body weight gain.

Reproduction studies were submitted but not reviewed for the following reasons:

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

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

The results of these studies should be considered along with those from the other toxicity studies (see Section I below).

D. Mutagenicity.

Reports of host-medicated and dominant lethal assays were not reviewed because test procedures were not described beyond citation of other references and reports which were not included in the submitted data.

E. Chronic Toxicity and Oncogenicity.

Rats were given bronopol in their drinking water for two years at concentrations to approximate daily

doses of 0, 10, 40, or 160 mg/kg. The highest dose decreased survival significantly (to 16% in male rats and to 38% in female rats after 2 years). Decreased food and water consumption were also noted at that dose. Other pathological effects observed included ulceration and thickening of the glandular stomach wall, glomerulonephrosis, hemoglobinuria, and decreased body weight and urinary volume (related to decreases in food and water consumption). At the mid dose, male rats also exhibited signs of chronic inflammation and squamous metaplasia in the salivary glands (also in both sexes given the highest dose). Those males also showed decreases in body weight during the study. Based on these results an LEL can be established for chronic toxicity at 40 mg/kg/day, and a NOEL was reported to be 10 mg/kg/day. No dose related oncogenic effects were observed.

Bronopol was dissolved in a solution of acetone and water (9:1) at concentrations of 0, 0.02, or 0.5%. The solutions were painted on clipped skin of test mice 3 times each week for 80 consecutive weeks. No compound related effects were noted on the incidence of skin tumors or other pathological findings. 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 study is therefore considered to be supplementary information.

F. Metabolism

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

Dermal absorption in rats and rabbits is low (10 to 20% in acetone and 4% in water/acetone (9:1) and the pattern of excretion is the same as for animals treated orally.

Bronopol does not accumulate in any particular organ, and tissues with the highest concentrations are in organs involved in excretion of the chemical (kidney, liver, and lungs).

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I. Data Review

A. Acute oral and dermal toxicity

1. Citation:

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

2. Materials and Methods:

Test substance: Bronopol (2-bromo-2-nitropropane-1,3-diol), technical (described by the authors as pharmaceutical grade).

Test species: Male and female C.S.1 mice weighing between 17 and 28 g and Boots-Wistar rats of both sexes weighing between 70 and 95 g were used. Male and female beagle dogs were also used.

Test procedures: a. Acute oral toxicity - Mice (i): Two groups of 6 and one group of 2 male mice were given single doses of 250, 500 or 1,000 mg/kg by gastric intubation. The test substance was dissolved in 10% acacia. The animals were observed at an unspecified frequency for 7 days following treatment, and mortality and toxic signs were noted.

b. Acute intraperitoneal toxicity - mice: Groups of 2 male mice received single intraperitioneal injections of 1, 2, 5, 100, or 200 mg bronopol in 10% acacia, while groups of 4 male mice were given i.p. injections of 10, 25 or 50 mg/kg in the same vehicle. These animals were observed for 7 days with respect to mortality and signs of toxicity.

c. Acute oral toxicity - mice (ii): The test substance was dissolved in distilled water and given by oral intubation to groups which contained 10 male and 10 female mice. Doses were 250, 300, 360, 430 or 520 mg/kg. The animals were observed for the 2 hours following dosing and necropsied when they died or when they were sacrificed at 7 days after treatment. Although the frequency of observations during the period following dosing were

not specified, the authors noted signs of toxicity and mortality. Necropsies were confined to gross examination of internal organs.

d. Acute intraperitoneal toxicity - rats: Single doses were administered by intraperitoneal injection and dosages were 10, 13, 18, 24, 32, 42, or 56 mg/kg. All other aspects of this experiment are the same as those discussed in paragraph c., above.

e. Acute oral toxicity - rats: Groups of 4 male rats were given single oral doses of 200, 400, or 800 mg bronopol (in 10% acacia) per kg body weight by gastric intubation. The animals were observed for mortality and toxic signs during the 7 days following dosing. They were also necropsied after death or sacrifice.

f. Acute subcutaneous injection - rats: Groups of 2 male rats were given a single subcutaneous injection of 25 or 50 mg/kg, while groups of 4 male rats received single injections of 100, 200, or 400 mg/kg. These animals were observed in the same manner as those described in paragraph e., above.

g. Acute oral toxicity - rats: An experiment similar to that of Experiment c. above was conducted with rats. Single doses of 200, 280, 390, 550, or 770 mg/kg were administered by gavage.

h. Acute intraperitoneal toxicity - rats: This experiment followed the same procedure as that of Experiment d. described above. Groups of male and female rats received single i.p. injections of 20, 28, 40, 56 or 78 mg/kg. All other aspects of this experiment were similar to procedures described in paragraph d., above.

i. Acute dermal toxicity - rats: The authors stated that the procedure of Noakes and Sanderson (1969) was followed. (Noakes, D. N., and Sanderson, D.M. 1969. Brit. J. Indust. Med. 26:59). They further stated that bronopol was dissolved in acetone and applied to the

backs of groups of 2 male rats. Doses were 64, 160, 400, or 1000 mg/kg. The treated animals were observed for 4 hours after dosing and then observed at an unspecified frequency during the 3 weeks following treatment. No further information regarding the protocol was reported.

j. Acute oral toxicity - dogs: Bronopol was given by oral intubation in single doses of 0, 40, 100, or 250 mg/kg. The test chemical was administered in aqueous solution, and dogs had been fasted overnight. Food was offered to them 3 hours after dosing. Blood samples were collected 24 hours before as well as 1 and 6 days after dosing. Hematological and biochemical observations were made in these samples. Urine was collected and assayed 24 hours before and 7 days after treatment. Erythrocyte, platelet and differential white cell counts, hemoglobin and hematocrit were determined along with bilirubin, sugar, urea nitrogen, GPT, GOT, alkalin phosphatase, sodium, and potassium. Urine was examined for color, pH, bilirubin, protein, reducing substances, sugar, and presence of blood. Respiration, pulse rate, rectal temperature, and the appearance of gingivae, ears, eyes, and nose were observed at dosing and 45 min, 90 min, 3, 6 and 24 hr. after dosing. In addition, blood samples were collected at these times for determination of blood sugar. The authors stated that the dogs were observed for 7 days and sacrificed on the 8th day following dosing. Prior to sacrifice animals were weighed. At necropsy the adrenals, brain, gonads, heart, kidney, liver, lungs, pituitary, secondary sex organs, major organs and tissues were examined microscopically, but no list of specific tissues was presented.

3. Reported Results:

a. Acute oral toxicity - mice (i): The authors stated that deaths occurred within 24 hours of oral administration. None of the 6 mice given the 250 mg/kg dose died, but all animals given 500 or 1000 mg/kg died. The authors stated that the LD_{50} was approximately 350 mg/kg without explaining how the value was determined.

b. Acute intraperitoneal toxicity - mice: Deaths were reported to occur within 4 days after i.p. injections and time to death was described as being longer at lower doses. None of the animals given 1, 5, or 10 mg/kg died, while all of these receiving 50, 100, or 200 mg/kg died. Three of the 4 given 25 mg/kg died. No further information on signs of toxicity or methods used to estimate the 20 mg/kg LD_{50} value were given.

c. Acute oral toxicity - mice (ii): The only signs of toxicity noted by the authors in the male mice given 250 mg/kg doses was persistent vocalization in 2 animals. The other groups also contained 1 or 2 animals exhibiting this type of behavior. One female given the 250 mg/kg dose died 5 days after treatment. Most of the other deaths occurred between 2 and 19 hours after dosing. Signs of toxicity included inactivity (in animals given doses of 360 mg/kg or more) and ataxia (in female mice given 430 mg/kg or higher doses). At necropsy the authors noted ulceration of the stomach and duodenum, thickening of the intestinal wall, adhesions of the stomach to the liver, and lesions on the surface of the liver.

The authors determined by probit analysis that the oral LD_{50} for male mice is 374 mg/kg, and for female mice it was found to be 327 mg/kg (no confidence limits were reported).

d. Acute intraperitoneal toxicity - mice: No deaths were reported in groups of rats given 10, 13, or 18 mg/kg injections. The lowest dose at which the authors noted signs of toxicity was 13 mg/kg. These signs were slow respiration and inactivity (in one male mouse), ataxia (in mice given 18 mg/kg or more) and sedation (in mice receiving doses of 32 mg/kg or more). Deaths in mice given 56 mg/kg occurred between 2 and 19 hours after treatment, while most of the deaths in groups given lower doses were reported at varying times throughout the 7 day observation period. Most of the animals were found at necropsy to have gross adhesions of all the abdominal organs or adhesions involving the liver and diaphragm. The authors noted that some males had ascites and small white spots on the surface of the liver.

The LD_{50} values for male and female mice were determined by probit analysis. The respective values reported were 32.8 and 34.7 mg/kg.

e. Acute oral toxicity - rats: The animals appeared less active and subdued after dosing according to the authors. Deaths occurred from a few hours to 2 days after dosing and the authors observed that these rats had large volumes of fluid and mucus in the stomach, desquamation and congestion in the secretory stomach, and enteritis. No deaths were reported in the 200 mg/kg group; 2 or 4 died in the 400 mg/kg group; and three of those given 800 mg/kg died. The authors estimated that the oral LD_{50} was 200 mg/kg.

f. Acute subcutaneous injection - rats: Rats given s.c. injections of bronopol were described as appearing to be in pain. The animals also appeared subdued and less active according to the authors. Deaths were noted within 2 days following dosing, and those animals exhibited subcutaneous hematoma and edema. Lung congestion, bleeding in the secretory stomach, and edema in both organs were also noted. In animals surviving to day 7 after dosing, the only gross lesions observed were at the site of injection. Subcutaneous adhesions and fibrosis were reported to be at the injection sites in these animals.

g. Acute oral toxicity - rats: Most of the animals appeared sedated after treatment. The authors also noted animals with nasal exudates, gasping, wheezing, and cyanosis. Four animals convulsed and died immediately after dosing (one from each of 4 dose groups including the 280, and 530 mg/kg groups of males and the 280 and 770 mg/kg groups of females). These animals also had white foam around their mouths according to the authors. Most deaths occurred within 19 hours after dosing with a few deaths reported during the 3 days that followed dosing (the latter deaths were in the two or three lower dose groups). The authors stated that no gross lesions were found at necropsy. The LD_{50} for male rats was determined by probit analysis to be 307 mg/kg, while that for female rats was reported to be 342 mg/kg.

h. Acute intraperitoneal toxicity - rats: The authors stated that treated animals became sedated or lethargic, atoxic, and breathed slowly soon after dosing. Most deaths occurred within 19 hours of dosing. At necropsy the authors reported adhesions of organs in the upper abdomen with ascites. The intraperitoneal LD_{50} values for male and female rats were reported to be 22 and 30.2 mg/kg, respectively.

i. Acute dermal toxicity - rats: The authors stated that no signs of toxicity were observed during the first 4 hours after dosing. Treated areas of skin were stained yellow on all rats. Subcutaneous edema and hemorrhaging occurred in rats treated with the 400 and 1000 mg/kg applications, and those animals were found dead on the morning of the day after dosing. The animals in the 160 mg/kg dose group had similar skin reactions according to the authors, and the rats also were cold, had labored breathing and were prostrate. One of these rats died 2 days after dosing and the other died 6 days after treatment. The authors noted lung congestion in dead rats which were necropsied. Slight scabbing of skin treated with the 64 mg/kg dose was reported to occur during the first week after dosing. The authors emphasized the difficulty in accurately determining a dermal LD_{50} when only 2 animals were used for each dose level, but they concluded that the dermal LD_{50} for male rats is between 64 and 160 mg/kg.

j. Acute oral toxicity - dogs: The authors reported that the dogs exhibited signs of severe gastric irritation. The male dog given 250 mg/kg had emesis within 3 min. after treatment that continued overnight. Feces were tarry in appearance according to the report. The dog lost 1 kg of weight overnight, and the loss was attributed to dehydration. Within 3 min. after administration of the 250 mg/kg dose the female dog vomited a large volume of fluid which the authors found to contain the test chemical. The male dog was sacrificed 7 days after treatment. Only the male dog showed changes in the blood. Neutrophils and SGPT and SGOT increased. At necropsy of the male dog the

investigators noted intense congestion and edema of the gastric mucosa, congested mesenteric lymph nodes, and ulcerative lesions of the gingivae. In addition to confirming these findings, microscopic examinations revealed atrophy of the prostate according to the authors. The organs of the female dog had normal weight and microscopic appearance 7 days after dosing.

Both dogs given the 100 mg/kg dose were reported to have emesis during the first 45 min. after treatment. Respiration rate increased shortly after treatment according to the authors, but the increase was described as transient. The only other effect that the investigators described as possibly compound related was slight atrophy of the prostate. Similar results were noted in the dogs given 40 mg/kg. No prostate changes were reported.

4. Discussion and Conclusions

The series of studies adequately demonstrates that bronopol causes irritation of a type which is dependent upon the route of exposure. The irritation was noted in the gastrointestinal tracts of rats, mice, and dogs receiving oral doses of the test chemical, and the LD_{50} values for oral toxicity place bronopol in Toxicity Category II. The oral LD_{50} values (mg/kg) are summarized as follows:

Species	Males	Females
Rat	307	342
Mouse	374	327

Intraperitoneal LD_{50} values (mg/kg) were as follows:

Species	Males	Females
Rat	22.0	30.2
Mouse	22.8	34.7

5. Core Classification:

Minimum for the acute oral studies when considered together. Supplementary for the dermal study which used only 2 male rats per dose group. The dermal study can be used with other data

to classify the chemical in Toxicity Category I for acute dermal toxicity.

B. Acute Inhalation Toxicity.

1. Citation:

Binns, R., Z.S. Berczy, P. H. Hague, and E. J. F. Spicer. February 3, 1971. Acute inhalation toxicity of bronopol to the rat. Huntingdon Research Centre. Report No. 3878/71/36. Submitted by Inolex Chemical. EPA Acc. No. 247193.

2. Materials and Methods:

Test substance: Technical grade bronopol. Batch No. 78077, 77.8.70. Purity unspecified.

Test species: CFY strain albino rat.

General procedures: A Wright dust generator was used to produce 3 dust concentrations for the study. Dust concentrations were obtained by varying the rates of air flow and powder feed into the system. Particles were collected on glass slides for determination of size distributions. Dusts were generated for 6 hours through a central inlet of a Perspex chamber. Nominal dust concentrations were determined as follows:

weight of packed powder = G (g/mm reservoir height)
rate of powder feed = M (minutes/mm packed powder)
airflow rate = L (liters/minute)

$$\text{nominal concentration, } C = \frac{G \times 1000}{M \times L} \text{ mg/liter air (mg/m}^3\text{)}$$

The authors stated that the airflow and powder delivery rates were adjusted to provide nominal concentrations of 5, 0.5, or 0.05 mg/liter of air.

Experimental procedure: Four groups containing 4 male and 4 female rats were placed into the test chamber for the 6 hour exposure period. Each of these groups was exposed to anticipated nominal concentrations of 0, 0.05 or 0.5 mg/L of air. One group of 6 male rats and two groups of 4 male and 4 female rats were exposed to nominal

concentrations of 5 or 0 mg/kg, respectively. The animals were observed at intervals (length of time unspecified) during the exposure period, and they were observed for 10 days (5 mg/L group) or 14 days (0.5 mg/L group) following the exposure. Animals exposed to the lowest concentration were sacrificed immediately after their exposure. At the end of the observation periods the animals from the 0.5 and 5 mg/L groups were sacrificed and examined grossly. Tissues of the respiratory tract and organs with gross lesions were prepared for microscopic examination.

Animals were weighed on the day of exposure and on days 5, 7, 9, 12, and 14 of the observation period.

3. Reported Results:

The authors stated that 78 to 92% of the particles examined from samples were respirable (1 to 15 microns) and 50 to 81% were small enough for alveolar deposition (1 to 5 microns). The lower percentages were detmerined for the highest nominal dust concentration and the highest respirable proportions were reported for the lowest dust concentration. The percentage of respirable particles at the intermediate dust concentration was 85% and the percentage which was small enough to be deposited in alveolae was 66%.

Rats exposed to 5 mg dust per liter of air exhibited signs of eye irritation, dyspnea, profuse mucous production and lethargy during exposure according to the investigators. After 30 minutes of exposure the rats began sneezing, and after 2 hours the nasal and ocular discharges were described as increasingly tainted with blood as the exposure period progressed. Blinking of the eyes was noted throughout the exposure period. These signs and the occurrence of labored movement persisted in these animals for 3 days after the exposure. The fur around the snout, mouth, and ventral areas was stained yellow. The rats also lost weight during the first 3 days after exposure (5 to 6% of their body weight on the day of exposure). Three of the 6 male rats in this group were reported to have subpleural brown foci (1-2 mm in diameter)

on their lungs. No histopathological observations were reported for this group.

The rats exposed to 0.5 mg/L were reported to exhibit similar signs of toxicity to those reported for the 5 mg/L exposure group. However, the signs were described as slight, and they disappeared soon after the exposure period. Body weights were comparable with those in control groups during the observation period, and subpleural punctate foci were observed macroscopically in rats from the treated group (1 of 4 males and 4 of 4 females) and one of two control groups (1 of 4 males and 1 of 4 females). Microscopic examinations of the lungs were reported to show minimal chronic pneumonitis in all treated animals. Two of the treated males were found to have small predominantly fibroblastic inflammatory foci. These effects were associated by the authors with exposure to air containing bronopol dust.

No remarkable differences between control and treated animals exposed to 0.5 mg/L were noted.

4. Discussion and Conclusion

This study did not establish an LC_{50} value for acute inhalation toxicity in rats. However, it characterizes the irritation effects of bronopol in the respiratory tract of rats and provides sufficient data to classify bronopol in Toxicity Category III (LC_{50}>5 mg/L) for acute inhalation toxicity.

5. Core Classification: Minimum.

C. Eye Irritation:

1. Citation:

Inolex Chemical Corporation. Undated. Primary eye irritation: Rabbit. Part 3(1) Toxicology. EPA Acc. No. 247193.

2. Materials and Methods:

Test substance: Technical grade bronopol (no further description provided).

Test species: New Zealand White rabbits.

Test procedures: Bronopol was dissolved in (0.5% or 2% w/v) normal saline (concentration and nature of salts used was unspecified, 0.5% w/v), or (0.5, 2, or 5% w/v). Groups of 3 female rabbits were used in each experiment.

Experiment 1: One-tent. ml of the 0.5 or 2% solutions of bronopol in was instilled in one eye of each of the rabbits. The other eye was treated with the same volume of the vehicle. The 2% solution was instilled in treated eyes once, while the 0.5% solution was instilled once daily for 4 consecutive days.

Experiment 2: The 0.5% saline solution was instilled on 4 consecutive days into one eye in three rabbits, and normal saline was instilled in the other eye of each rabbit.

Experiment 3: Groups of three rabbits had one drop of a 0.5, 2, or 5% solution of bronopol in instilled into one eye. The other eye was not treated. Eyes were irrigated with lukewarm water 24 hours after treatment. The authors stated that the eyes were assessed according to FDA methods described in the Federal Register (Vol. 37. No. 83 Section 191.12, 1972). The examinations were conducted 1, 24, 48, and 72 hours after treatment and on days 7, 14, and 21 after treatment.

No further explanation of methods used for Experiments 1, 2 and 3 were presented.

3. Reported Results:

Experiment 1: The investigators noted inflammation and edema of the conjunctiva in eyes with the 2% solution. These effects subsided within 5 hours of treatment. The 0.5% solution was reported to cause only slight inflammation with lacrimation at the time of

INERT INGREDIENT INFORMATION IS NOT INCLUDED

instillation. These effects were similar to those observed in eyes treated with the vehicle.

Experiment 2: One rabbit was reported to have slight inflammation and moderate edema of the conjunctiva in the treated eye 2 to 4 hours after the first treatment with the test solution. The irritation was gone 24 hours later. No reaction was observed in that animal after the three subsequent treatments. The remaining rabbits were unaffected by the test solution, and control eyes also remained unchanged.

Experiment 3: The authors stated that the rabbits' eyes were scored according to an FDA scale and they reported that the 5% solution was a strong irritant in one treated animal. One of the other two rabbits in the same group responded marginally, and bronopol was an irritant in the third rabbit. The observed effects were confined to the conjunctiva which exhibited slight redness and swelling with moderate discharge. These effects were noted at the first observation (one hour after dosing), and they subsided in all but one of the rabbits by the seventh day after treatment. Slight redness was noted in one rabbit on the seventh day of observation.

The other two solutions (0.5 and 2%) had only slightly irritating effects on the eyes of rabbits during the first hour after instillation as did the [redacted] vehicle. However, the effects were not observed 24 hours after treatment.

4. Discussion and Conclusions:

The methods used were briefly described and included a citation of FDA guidelines for these tests. The authors provided adequate data supporting their conclusion that a 5% solution of bronopol in [redacted] [redacted] is strongly irritating to the eyes of rabbits.

5. Core Classification: Minimum.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

D. Dermal irritation

1. Citation:

Inolex Chemical Corp. Undated. Primary dermal irritation Part 3(1). EPA Acc. No. 247193.

2. Materials and Methods:

Test substance: Technical grade bronopol. No further description was given.

Test species: Female rats of an unspecified strain; female New Zealand white rabbits.

Experiment 1: Three groups of 5 female rats had the hair shaved from their backs. The test substance was applied to the skin in 0.5 ml volumes of 0.5, 2, or 4% bronopol (w/v) in 2.5% aqueous methylcellulose. Application sites were occluded with polyethylene sheets and "sleek adhesive plaster" for 6 hours. A fourth group of 5 female rats was shaved, treated with the methylcellulose solution alone, and had application sites occluded in the same manner. After the occluding dressings were removed, the shaved skin of test rats was washed, blotted dry, and examined for signs of dermal irritation.

Experiment 2: Three groups of 6 female rabbits had the hair on their backs clipped. The rabbits were restrained and the test substance was then applied in acetone at a concentration of 0.5, 1, or 2% (w/v) to one of two sites. The other site was treated with acetone alone.

For application, 1 ml of each solution was placed on a 1 x 1 inch gauze pad which was put on the back of a rabbit. The animals were wrapped with polythene sheeting and "Sleek adhesive plaster." These dressings were removed 6 hours after application of test and control solutions. The skin was washed, blotted dry, and scored for reactions according to the Draize method at 6, 24, and 72 hours after the test substance was applied to the skin.

Experiment 3: Three groups of 6 rabbits were treated as in Experiment 2 above with bronopol solution (1.25, 2.5, or 5% w/v) in [redacted]

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The application sites were occluded, cleaned, and scored by the same methods described above for Experiment 2.

Experiment 4: Four sites (one-inch square) on the clipped backs of 6 female rabbits were treated with 2.5% aqueous methylcellulose containing 0, 0.5, 2, or 5% (w/v) bronopol. A 0.5 ml amount of each solution was applied directly to intact skin. The application sites were occluded in the same manner as in Experiments 2 and 3 described above, and the dressings were removed after 6 hours. Treated sites were washed, blotted dry and scored 6 hours after the application of test and control solutions.

Experiment 5: Bronopol was prepared in soft parraffin at concentrations of 0, 0.5, 1, or 2% or in aqueous buffer (pH 5.5) at concentrations of 0, 0.05, 0.1, or 0.25%. Each test solution was placed on a 1cm "lint" square backed by surgical tape.

The squares were then applied to the forearms of human volunteers and kept in place for 24 hours. At the end of that time the tape was removed and treated skin was examined for signs of inflammation. Reactions were scored as follows:

Normal	0
Slight erythema	1
Moderate erythema	2
Marked erythema	3
Marked erythema and papula	4
Marked erythema, vessicles, and induration	5

3. Reported Results:

Experiment 1: No skin reactions were reported on rats treated with 0.5, 2, or 4% bronopol in methylcellulose after 6 hours or on the day following the dermal applications.

Experiment 2: The authors reported that three of the rabbits treated with the 2% bronopol solution in acetone had mild erythema at the 6 hour observation. They noted that the effect had almost completely subsided by the 72-hour observation. A fourth rabbit from the group

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was reported to have mild erythema at 24 hours which persisted to the 72 hour observation. One of the rabbits treated with the 10% concentration was noted to have mild erythema at 24 hr. No other erythema was reported in the other rabbits. No edema was reported.

Experiment 3: The investigators reported that bronopol in [redacted] at concentrations of 1.25, 2.5, or 5% did not induce skin reactions in the rabbits tested.

Experiment 4: Severe edema was noted at skin sites treated with 5% bronopol in 2.5% methylcellulose at 6 hours (when occlusions were removed). The edema was described as slight at 24 hours, but 5 of the 6 sites had slight to moderate erythema. The responses at sites treated with the 2% bronopol solutions were described as more variable. Four of these sites had moderate to severe edema at 6 hours which were described as reduced at 24 hours. Five sites also showed slight to moderate erythema at that time. Only one of six skin sites treasted with 0.5% bronopol showed slight erythema and slight edema at 24 hours according to the authors.

Experiment 5: Applications of 1% bronopol in soft paraffin caused slight erythema on the skin of 2 volunteers, and the 5% preparation caused moderate erythema in 4 of the 10 subjects. Slight erythema was noted in one of 10 volunteers treated dermally with 0.25% bronopol in an aqueous buffer.

4. Discussion and Conclusions:

Results from this and other studies (see section I.A.1., above) suggest that bronopol is severely irritating to the skin of test animals.

5. Core Classification:

Minimum when this study is considered along with the other acute studies.

E. Dermal Sensitization

1. Citation:

Inolex Chemical. Undated. Dermal sensitization. Part 3(1). Toxicology. EPA Acc. No. 247193.

2. Materials and Methods:

Test substances: Technical grade bronopol (no further description given).

Experimental procedures: Experiment 1: Results of a preliminary study were cited by the authors without a complete description. The authors stated that intradermal injections of 0.2% aqueous bronopol in guinea pigs caused edema and necrosis at the injection site. On the basis of those results the authors stated that 0.05% solutions were used in the sensitization studies with guinea pigs.

In the main study, two groups of 10 guinea pigs were used (sex unspecified). The first group was given a series of 10 injections of the 0.05% solution and the second group received injections of distilled water. Injections were administered at 2 to 3 day intervals. The first injection consisted of 0.05 ml, and the remaining injections were 0.1 ml. Two weeks after the last of these was administered the challenge injection of 0.05 ml of the 0.05% bronopol solution was given. The animals were examined for skin reactions at 24 hours after the first sensitizing dose was administered and at 24 and 48 hours after the challenge dose. Three guinea pigs from each of the two groups were sacrificed 24 hours after the challenge dose and section of skin were preserved for microscopic examinations.

Experiment 2: The authors described a preliminary experiment in which groups of six guinea pigs were tested. Each group had the hair of the flanks clipped and 0.1 or 1% solutions (vehicle unspecified) of bronopol were applied to 1 square cm areas on the flanks of each animal. A second group of animals was tested with 1 or 5% solutions. Application sites were then examined for signs of primary dermal irritation. The authors noted irritation

at sites treated with the 5% solution and trace reactions at sites where the 1% solution was applied.

The methods for the main study were not described complecely, but the authors stated that the ear-flank method (Stevens, M.A. 1967. Brit. J. Ind. Med. 24:189.) was followed. Dinitrochlorobenzene (DNCB) was used as a positive control. Three groups containing six female guinea pigs were used. Bronopol or DNCB as 1% solutions in acetone or acetone alone were applied to the outer surface of the animals' ears. A 0.1 ml aliquot of the solutions was applied daily for 3 consecutive days. One week after the first ear application the backs and flanks of the animals were shaved, and challenge doses were applied in 0.2 ml amounts to four sites on each animal. Challenge doses consisted of 1% bronopol, 0.25% DNCB, or acetone (only one site treasted). The sites were examined for erythema at 24 and 48 hours after aplication of the challenge doses.

3. Reported Results:

Experiment 1: The authors stated that 4 of the 6 guinea pigs injected with the 1% bronopol solution had edema surrounded by erythema. The lesion was described as 5 to 20 mm in diameter. The remaining test and control animals had erythema in an area of 5 mm in diameter according to the authors. These observations were made 24 hours after the first sensitization injection.

At the first observation which followed the challenge dose, the authors noted similar skin reactions in animals from the test and control groups. These reactions consisted of foci of necrosis surrounded by erythema. The authors stated that the lesions varied from 5 to 20 mm in diameter. They further reported that at the subsequent observation (48 hours after challenge) the lesions showed signs of healing, but the erythema was more pronounced. Microscopic changes reported by the authors included edema of the dermis with necrosis in the epidermis in all bronopol-treated animals. Three of the six control animals were reported to have necrosis of the epidermis with a slight cellular reaction which was not described further.

Experiment 2: The authors noted that 24 hours after the challenge dose, 12 of 20 DCNB treated skin sites were bright pink and 8 were pink. At 48 hours after challenge, all 20 sites were described as bright pink. Twenty of 24 sites treated with bronopol showed no reaction 24 hours after challenge according to the authors. Four others were scored as slightly pink. At 48 hours after challenge with bronopol no reactions were reported for 20 treated sites and 4 showed barely perceptible reactions.

Results of irritation controls (6 sites, one per test animal) showed that the 1% bronopol solution caused no reaction 24 or 48 hours after treatment. The DCNB solution caused no reaction at 2 of the 6 sites. One site was slightly pink, and 1 was bright pink 24 hours after treatment according to the authors. By 48 hours following treatment 3 sites were bright pink, 2 had a barely perceptible reaction, and 1 showed no reaction.

4. Discussion and Conclusions:

There are sufficient data presented to support the authors' conclusion that bronopol is not a skin sensitizer.

5. Core Classification: Minimum.

F. Subchronic Toxicity

1. 13-Week Oral Rat Study

a. Citation:

Hunter, B., P. Batham, R. Heywood, A. E. Street, A. J. Newman. August 29, 1973. Oral Toxicity to rats: Repeated administration for 13 weeks. Huntingdon Research Centre unpublished report No. BT534/73268. Submitted by Inolex Chemical Corporation. EPA. Acc. No. 247195.

b. Materials and Methods

Test material: Bronopol (technical grade, batch No. 29075N, purity unspecified).

Test species: SPF rats of the CD strain.

Test procedures: Four groups of 20 male and 20 female rats were given doses of 0, 20, 80, or 160 mg bronopol per kg body weight. Dosages were administered by oral gavage in distilled water seven days each week for 13 weeks.

Animals were apparently observed frequently for occurrence of behavioral or other signs of toxicity. The authors stated that animals showing severe signs of toxicity were isolated, and those found in extremis were sacrificed to prevent cannibalism.

Animals that died after the first dose (4 males and 5 females) were replaced. The authors stated that replacement rats were taken from groups of "spare" animals treated with the same dose as the individual rats they replaced.

Mean food consumptions for each cage of 5 animals were calculated weekly during treatmen'. Individual body weights were obtained at the beginning of the study and at weekly intervals through the end of the study.

Food was withdrawn overnight from those rats selected for urinalysis, hematology and blood chemistry evaluations. Overnight urine samples were collected from 1C male and 10 female rats from the control and mid-dose groups at weeks 6 and 12 of the study. The pH, specific gravity, protein, reducing substances, glucose, ketones, bile pigments, and urobilin were measured. The sediments from centrifuged urine samples were examined microscopically.

Blood was taken from the orbital sinus of each of 10 male and 10 female rats from each group during the 6th and 12th weeks of the study. The authors stated that packed cell volume, hemoglobin, total and differential white cell counts were measured. A thrombotest and platelet count were obtained at week 12, according to the report. Blood chemistry analyses included blood urea, glucose, serum protein, serum alkaline phosphatase (SAP), serum glutamic, pyruvic transaminase

(SGPT), and electrolytes. Blood samples were from the control and mid-dose groups.

At terminal sacrifice organ weights were obtained for adrenals, brain, heart, kidneys, liver, gonads, pituitary, spleen, thymus, thyroid, and uterus. The authors stated that organ to body weight ratios were used for intergroup comparisons. Tissues from the organs which were weighed as well as from the aorta, cecum, colon, duodenum, eye, femur, ileum, jejunum, lungs, lymph nodes, mammary glands, esophagus, pancreas, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, stomach, tongue, trachea, urinary bladder and gross lesions were preserved for microscopic examination in some animals (See following paragraphs).

The authors stated that the microscopic examination of animals found dead during the study included abnormal tissues so that a cause of death could be determined. These animals as well as 120 of each sex from the 160 mg/kg/day group that were sacrificed in extremis, 10 rats of each sex from the control and all survivors from the 80 mg/kg/day group did not have microscopic examinations on the aorta, colon, jejunum, mammary glands, esophagus, prostate, salivary glands, sciatic nerve, seminal vescles, skeletal muscle, skin, tongue, and trachea. The authors further stated that the kidneys of 10 male and 10 female rats from the control group and all animals surviving in the 20 mg/kg/day group to the end of the study were examined.

c. Reported Results:

Gasping and wheezing were noted by the authors in 9 males and 5 females from the high dose group. These signs were observed 16 hours after the first dose was administered. The authors reported that 12 males and 7 females exhibited these signs, which were more pronounced immediately after the second daily dose. After 9 days the authors stated that 19 males and 14 females in the high dose group showed these signs of respiratory distress.

The high-dose group also contained animals with abdonomal distention 3 days into the study (approximately half the animals were affected).

The authors also noted respiratory distress in animals given the 80 mg/kg/day dose. They described the distress as less pronounced, and it was first observed 18 hours after the first treatment. Survivors in this group were reported to show gradual improvement so that by week 8 no signs of respiratory distress were noted.

The 6 males and 6 females from the mid-dose group that exhibited abdominal distension were reported to have died during the study (see discussion below).

Only one male in the low-dose group was noted to have a reaction to bronopol administration. Mild respiratory distress was reported and decreased body weight gain was observed for the first two weeks. Subsequently, body weight returned to normal levels and respiratory distress was not seen according to the authors.

The authors stated that during the 24 hours following the first dose, 4 male and 5 female rats in the high-dose died and were replaced. After 9 days of treatment, 22 males (including all replacements) and 14 females (including 3 replacements) died.

In the mid-dose group, most deaths were reported to occur during the first 3 weeks of the study, and a few were noted during the 6th through 7th weeks. One male was sacrificed in extremis during the 12th week. A total of 7 males and 9 females in the mid-dose group died or were sacrificed during the study.

At necropsy the authors reported the majority of rats that died showed gaseous or fluid distention of the gastrointestinal tract. A few animals in the high dose group were found to have hemorrhagic foci in the mucosa of the glandular stomach. Raised white areas on the glandular stomach mucosa were also observed in a few other animals.

The authors stated that despite respiratory signs of toxicity, the only gross lung pathology noted was distension in 4 male and 2 female rats in the high-dose group.

Histologically, the gastrointestinal lesions observed in the 160 mg/kg group were described as superficial ulceration with underlying inflammation, epithelial hyperplasia and hyperkeratosis, and congested vessels in the mucosa.

In many of these animals the authors noted regressive changes in the thymus marked by almost complete absence of lymphatic tissue.

Only one death (a female rat) was noted in the 20 mg/kg/day group. It occurred during the 10th week of the study and the authors stated that no cause of death could be determined.

Total mortality at the end of the 13-week study was reported as follows:

Dose group	Males	Females
Control	0/20	0/20
Low	0/20	1/20
Mid	7/20	9/20
High	24/24	25/25

Mean food consumption for male rats in the 80 mg/kg group was 48% less than that of control animals during the first week of the study. Thereafter, the group means were comparable for control, mid- and low-dose group males. A similar response was reported for females in the 80 mg/kg/day group. The mean food consumption in that group was 43% less than that for the control group during the first week, and thereafter food consumption was comparable to that for controls.

Group mean body weight for the 80 mg/kg/day group was decreased from control mean weights by 23% in male rats and 15% in female rats after the first week. The difference between the appropriate control groups and the mid-dose

group males and females gradually decreased during the study. For males the mean weight at termination was 9% less than that for control males, while mean terminal body weight for females in the mid-dose group was comparable to that for control females.

The authors noted that the body weight decreases found in the first week were related to the food consumption. Food consumption to body weight ratios for the control, 20, and 80 mg/kg groups were comparable.

No group differences with respect to urinalysis were reported.

Hematological values which were reported to be statistically, significantly different included red blood cell count in 80 mg/kg group females (7.3×10^6 cells/cmm compared with 7.5×10^6 cells/cmm in the control group, $p < 0.01$) at 6 weeks, and neutrophil count in the 80 mg/kg group males (2.2×10^3 cells/cmm compared with a control mean count of 1.3×10^3 cells/cmm).

There were no statistically, significantly different hematological measurements found at 12 weeks. The authors stated that the differences found were within normal ranges for rats. Decreases in blood glucose levels were reported for males given 80 mg/kg/day at 6 weeks (91 mg% in treated males compared with 113 mg% in controls). Glucose levels in the two groups were comparable at 12 weeks. These values in 80 m/gkg group females were comparable at both 6 and 12 weeks. The differences for males were described as statistically significant ($P < 0.001$) but within normal ranges for the strain of rat tested.

At 12 weeks urea, total protein, and globulin concentrations were slightly increased above controls in male rats given the 80 mg/kg dose. These differences were not statistically significant.

No differences between the female rats given 80 mg/kg/day and the untreated animals were noted for blood chemistry measurements. No blood chemistry or hematology data were reported for the 20 mg/kg group.

No dose-related pathology was noted in rats surviving to the end of the study.

d. Discussion

The macroscopic and microscopic pathology, which is dose-related, was noted in those rats that died before the end of the treatment period. Those changes indicated that bronopol is a severe gastrointestinal irritant. On the basis of mortality, a no-effect level for the test substance is 20 mg/kg/day. However, the authors noted unusual pathological observations in the kidneys of males given the 20 or 80 mg/kg dosages. Two males (one from the 20 mg/kg group and one from the 80 mg/kg group) had distended tubules which contained eosinophilic material. The surrounding interstitial tissues were infiltrated with mononuclear cells. One other male rat in each of the 20 and 80 mg/kg/day groups had dilated tubules containing the eosinophilic material in the corticomedullary junction. These results are, as the authors concluded, equivocal since there is no dose-related increase, and their occurrence involved so few animals.

e. Conclusion

Based on mortality, a no-effect level of 20 mg/kg/day is established in this study. The lowest-effect level was 80 mg/kg/day.

f. Core Classification

Minimum.

2. 13-Week Oral Dog Study

a. Citation:

Rivett, K. F., H. Chesterman, K. Skerrett, A. E. Street, and A. J. Newman. July 27, 1973. Oral Toxicity Study in the beagle dog. Unpublished

report prepared by Huntingdon Research Centre. Submitted by Inolex Chemical. EPA Acc. No. 247194.

b. Materials and Methods

Test substance: Technical grade bronopol (batch number CT 87376M).

Test species: Male and female beagle dogs (17 to 23 weeks of age) were used.

Experimental procedure: Bronopol was dissolved in water to make a 0.5% solution (w/v), and appropriate amounts of the solution were administered once each day, 7 days each week for 13 consecutive weeks. Each group of 3 male and 3 female dogs received a daily dose of 4, 8, or 20 mg bronopol per kg body weight, and one group received the vehicle without test substance. The volume of solution given to the high dose group (4 ml/kg/day) was the same as that of the vehicle administered to the control group.

The authors stated that clinical signs were noted daily. Food was offered to each dog in the morning and in the afternoon each day, and the amount consumed was recorded. Body weights were obtained weekly throughout the study, and water consumption was monitored at intervals (unspecified) during the study.

Ophthalmic examinations were performed before the study was started and during the sixth and twelfth weeks. During these same times dogs were fasted for 16 hours before blood samples were drawn. Hematological examinations of the samples included hemoglobin, red cell count, white cell counts (total and differential), erythrocyte sedimentation rate, packed cell volume, clotting time, and platelet count. Blood chemistry analyses included plasma urea and glucose, serum protein analysis, serum alkaline phaosphatase (SAP), serum glutamic-pyruvic transaminase (SGPT), serum bilirubin, and electrolytes.

For urine collection, water was withdrawn from animals at midday and collection of urine was conducted from 5 pm to 9 am (overnight). Specific gravity, pH, protein content, as well as the presence of reducing substances, ketones, glucose, bile pigments and, urobilinogen were evaluated. Urine specimens were centrifuged and the sediment was examined microscopically for epithelial cells, red blod cells, white blood cells, microorganisms, casts, and other abnormal constituents.

At the end of the 13 week test period animals were sacrificed. At necropsy a gross external and internal examination was conducted. The brain, pituitary, heart, lungs, liver, spleen, pancreas, thymus, prostate or uterus, kidneys, thyroid, adrenals, and gonads were removed and weighed. Samples of tissues from the weighed organs and from the aorta, trachea, esophagus, lymph nodes, jejunum, ileum, duodenum, gall bladder, urinary bladder, salivary gland, tongue, stomach, colon, skin, mammary gland, skeletal muscle, bone marrow, peripheral nerve, eye and optic nerve were prepared for histological examiantion.

c. Reported Results

The authors stated that no animals died during the experiment. Vomiting was noted predominantly in the high dose group. It was reported to occur half an hour after dosing and was observed mostly duirng the first 6 weeks of the study. The authors stated that during the first 6 weeks of the study the dogs were fed each morning an hour after dosing. They stated that this routine was changed because of the vomiting. The dogs were fed first and dosed 2 hours after feeding and the investigators noted that vomiting was rarely observed during the time the new routine was followed.

No compound related effects on food consumption, water consumption, body weight or ophthalmological observations were reported. Hematological and biochemical observations also showed no compound related effects. All urinary observations,

with the exception of the presence of blood in the urine of two mid dose and one high dose dogs at 6 weeks, were also uneffected by treatment.

No gross abnormalities were noted by the authors to appear consistent with dose.

The authors noted that there was a tendency toward increased liver weight in the high dose group and the liver-to-body weight ratio for one male dog was described as approaching the upper limit of the normal range. The same effect was noted with respect to spleen weight, and the authors noted that one male from the high dose group had a particularly high spleen weight. When that dog's spleen weight was eliminated from consideration, there were no statistically significant differences between the groups for spleen weights.

Although isolated histological findings were reported none could be related to administration of the test substance.

d. <u>Discussion</u>

Because of the observation of vomiting and the changes made in feeding schedule relative to the timing of dosage administration in the high dose group, it is unlikely that these animals absorbed toxic levels of bronopol. The lack of toxic effects late in the study and the response of treated dogs early in the study suggests that further testing in dogs would not provide any additional information about the toxicity of the test chemical.

e. <u>Conclusion</u>

The results of the study indicate that a no-observed-effect level with respect to emesis and slight liver and spleen weight increases is 8 mg/kg/day. The lowest effect level is 20 mg/kg/day.

f. Core Classification

Minimum.

3. 3-Week Rabbit Dermal Study

a. Citation:

Davies, R.D., J. C. Holliday, A.E. Street, R. Heywood, A.J. Newman, Dec. 18, 1973. Effect of Repeated Administration of Bronopol to the Skin of Rabbits for Three Weeks. Huntingdon Research Centre. Submitted by Inolex Chem. Acc No. 247194.

b. Materials and Methods

Test substance: Technical grade bronopol. No further description was provided.

Test species: A strain of New Zealand White rabbits was used. On the day prior to the beginning of the experiment hair was shaved from a 10 cm^2 area in the dorsal lumbar region of each animal.

The animals had that region clipped again as needed during the experiment. In addition to clipping, the exposed skin was abraded on the first day of the study and weekly thereafter.

Experimental procedure: Bronopol was dissolved in 2.5% methyl cellulose at concentrations of 0, 0.2, or 0.5% (w/v). A 1 ml aliquot of each test solution was applied once each day to the clipped areas of skin of 5 male and 5 female rabbits. Applications were made 7 days a week for 3 consecutive weeks. The rabbits were restrained in "Elizabethan" collars, and the treated areas were left unoccluded. After each daily 6 hours exposure period the treated skin was washed with warm soap solution and rinsed. The skin was then blotted dry.

Observations: The animals were observed for signs of toxicity and behavioral changes, and the treated skin was scored each day for the appearance of edema and escher. The rabbits were weighed and food consumption was measured weekly. Prior to the start of the experiment

and at termination blood samples were taken from the lateral ear vein for hematological evaluation. Blood chemistry was evaluated at termination of the study. Ophthalmalogical examinations were also conducted before the study began and at termination.

At the end of the three week dosing period test animals were sacrificed, and they were grossly examined externally and internally. Organ weights were obtained for the thyroid, heart, liver, kidneys adrenals, and gonads. Tissue samples preserved for observation were from the organs of the endocrine system, gastrointestinal tract, lymphatic system, cardiovascular system, urogenital tract, and central nervous system. Tissues preserved but not examined included the aorta, esophagus, sciatic nerve, and skeletal muscle. All macroscopic lesions were preserved and examined.

c. Reported Results

All 5 males and 4 of the 5 females in the control group had slight to well-defined erythema according to the authors. The fifth female exhibited slight erythema. The authors noted that all rabbits in the control group exhibited edema and they stated that intensification of slight reactions occurred in separate rabbits at various times during the study. These intense reactions were described as ameliorated by the end of the study.

Skin reactions in the low dose group were similar to the control groups with the exception of more intense edema (well defined in 3 males and four female rabbits). The high concentration was more irritating in that it caused well-defined erythema which progressed to moderate erythema in all but one female. All rabbits in this group also exhibited well defined edema which progressed to moderate edema in 3 males. The authors noted that the skin reactions in the high dose group were associated with hardened brown skin. This condition persisted throughout the study, and the authors reported that there was sloughing of these areas during the third week.

No signs of toxicity or behavioral changes were noted by the authors.

Food consumption was decreased in two low dose group and one high dose group rabbits and body weights were reduced in individual animals from all three groups. The body weight decrease was noted in a female from the low dose grup which consumed less food than the other animals. The weight losses did not exhibit dose related trends and the authors noted that two males and one female from the control group, and two females from each treated group lost from 180 to 430 g during the study.

No significant hematological, ophthalmalogical, or blood chemistry changes were found. There were no compound related changes with respect to organ weights, gross observations (other than treated skin), and histopathology (other than skin).

d. Discussion

Since all animals had their skin abraded, this study is supplementary information. The results should be considered with those from a second study in which the test substance is applied to intact skin.

e. Conclusion

The 0.2% solution intensified the erythema and edema noted in control group rabbits, and the 0.5% solution caused thickening, hardening, and sloughing of treated skin in addition to erythema and edema. No other signs of toxicity or mortality were noted.

f. Core Classification:

Supplementary. The study could be upgraded if data for an experiment with intact skin are submitted.

G. Teratogenicity

1. Rats - Teratogenicity Study

a. Citation: Inolex Chemical. Undated. Teratogenicity: Rat, Oral administration. Bronopol. Part 3(4) Toxicology. EPA Acc. No. 247199.

b. Materials and Methods:

Experiment 1: Groups of 7 or 8 females were mated with males of proven fertility. Day 1 of gestation was the day sperm were found in vaginal smears from the mated females. Each group of mated females was given 0, 10, 30 or 100 mg bronopol per kg body weight on days 1 through 20 of gestation. The test substance was administered in water by oral intubation.

Maternal body weights were measured on days 1, 4, 7, 10, 13, 16, 19, and 21 of gestation. The dams were sacrificed on day 21 of gestation and the ovaries and uteri were examined. The numbers of corpora lutea, implantation sites, resorptions and fetal weights were determined. All fetuses were grossly examined externally and internally for abnormalities. They were then prepared for skeletal examinations, and skeletal variations were noted. Samples of liver, kidney, lung, and brain tissue were prepared for histological examination in five litters from dams treated with 100 mg/kg/day and from 6 control group litters. Maternal animals were grossly necropsied and tissues with lesions were preserved for histological examination. No other procedural details were described.

Experiment 2: A group of 10 mated female rats was given daily doses of 10 mg bronopol per kg body weight from day 16 of gestation until parturition. Doses were administered in water by oral intubation, and treatment was discontinued at parturition. The dams and their offspring were sacrificed when the offspring were weaned (21 days of age). At birth, litters were culled to 9 pups. Any pups appearing underweight or neglected were culled at birth or during lactation. All of the offspring were necropsied and

examined for external and internal abnormalities. Those surviving to weaning were prepared for skeletal examinations. The dams were also necropsied and organs with gross lesions were preserved for microscopic examination.

A control group of 5 mated female rats was treated with the water vehicle and evaluated according to the same procedures used for treated dams and their offspring. Maternal body weights were measured on days 1, 4, 7, 10, 13, 16, 19 and 21 of gestation.

c. Reported Results:

Experiment 1: The authors reported that four dams in the 100 mg/kg/day group, 3 in the 30 mg/kg/day group, and 3 from the 10 mg/kg/day group died. They further stated that the cause of death was lesions in the lung and gastrointestinal tract. A fourth rat from the 30 mg/kg dose group was sacrificed on day 19 of gestation because of what the authors described as a uterine hemorrhage. The animal had two live fetuses. Mean maternal weight gain during gestation for survivors was 79.7, 61.9, 59.4, and 51.5g for the control, 10, 30, and 100 mg/kg/day groups, respectively. Respective mean body weights for the 10, 30, or 100 mg/kg dose groups were 6.1, 7.9, and 10.2% less than that for the control group.

At necropsy of animals which died during the study, the authors noted congestion in the lungs and erosion of the secretory stomach. Fluid and blood were also found in the thorax. The authors also reported lung damage in rats sacrificed on day 21 of gestation. One from each of the 100 and 30 mg/kg groups and 3 from the 10 mg/kg group were reported to show these effects. One of the three from the 10 mg/kg group also had a gastric ulcer according to the investigators.

None of the control rats died or were found at necropsy to have gross pathology.

Experiment 2: The mean-maternal body weight gain during gestation was 74.8g for the untreated control group and 62.6g for the 10 mg/kg treated group. The mean body weight for the treated group was 6.4% less than that for untreated rats on day 21 of gestation. No mortalities were reported in treated animals and delivery of offspring was described as normal.

The authors reported that two control rats were weak at parturition, and those dams were sacrificed along with their offspring. On the 19th day of lactation one rat had what was described as respiratory embarrassment and the animal was sacrificed with its offspring at that time. At necropsy of the remaining animals the investigators noted that three animals from both the control and treated groups showed signs of pneumonia.

Mean litter sizes at birth were 8.1 and 7.6 for the control and treated groups, respectively. Respective mean pup weights at birth were 33.6 and 34.1g. The viability index (the number of pups weaned given as a percentage of the number born alive less those which were culled at birth) was 80.8% for the control group and 74.3% for the treated group.

d. Discussion and Conclusions:

Experiment 1: Although the authors discussed fetal and litter data the results reported for maternal animals showed that all doses caused maternal toxicity and mortality. The mortality was from 37.5% in the low-dose group to 50% in the highest-dosed group. There were 3 to 4 litters left for examination, and therefore there were too few litters left to support any conclusion about the teratogenic potential of bronopol in the rat.

The gastrointestinal and pulmonary effects as well as the mortality observed at dosages of 30 or 100 mg/kg are consistent with results from subchronic studies with rats (see Section II.F. above). However, these effects were also noted at a level of 10 mg/kg which is below the 20 mg/kg no-effect level in the subchronic study.

Experiment 2: The protocol is not designed to evaluate embryonic and fetal development. However, it demonstrated maternal effects in dams exposed to 10 mg/kg/day during the last 5 days of gestation.

e. Core Classification: Supplementary. The data are sufficient to show that doses of 10 mg/kg/day are toxic to pregnant rats.

2. Rats - Peri- and Post-natal Effects.

a. Citation: Palmer, A. K., and A. M. Neuff. July 31, 1973. Effect of bronopol on peri- and post-natal development of the rat. Part 3(4). Toxicology. EPA Acc. No. 247199.

b. Materials and Methods:

Test substance: Technical grade bronopol (batch number CT 89075N).

Test species: CD strain Specific Pathogen Free rats.

Experimental procedures - preliminary experiment: Four groups of 5 pregnant rats were given daily doses of 0, 10, 20, or 40 mg/kg. Dosages were admininstered in distilled water by gastric intubation. Dosages were given from day 15 of gestation through the end of gestation. Day 1 of gestation was determined by the presence of sperm in vaginal smears. Test animals were observed daily for signs of toxicity, and they were weighed on days 1, 7, 14, and 20 of gestation and at birth and days 7 and 12 post partum. The duration of gestation was noted, and the young were counted, weighed and examined at birth. The authors stated that litters were examined for abnormal or dead pups each day during lactation with a minimum of nest disturbance. Pups were individually weighed on the 4th and 12th days of lactation. On day 12 of lactation, pups were sacrificed and examined for external and internal abnormalities, and their sex was determined by gonadal examination. Pups which died before sacrifice were also examined in a similar manner. Abnormal pups were preserved for serial sectioning or skeletal examination in accordance with the type of abnormality they exhibited.

The authors stated that cumulative group mean pup losses were derived from individual litter percentages as follows:

$$\frac{\text{(No. viable young at birth - No. young at stated time)}}{\text{No. viable young at birth}} \times 100$$

The authors also stated that mean values were calculated from individual observation as follows:

Mean A: All surviving animals bearing young at birth, including those subsequently losing the entire litter.

Mean B: All animals rearing some young to weaning.

Main study: The only procedural differences from the preliminary study were dose levels and group sizes. Three groups of 20 pregnant rats were given doses of 0, 20, or 40 mg/kg/day. In addition, dams were weighed on day 21 of post partum. Pups were also maintained for 21 days after birth instead of 12.

c. Reported Results:

Preliminary study: No mortality or signs of toxicity were noted by the authors, and body weight changes were comparable for all groups. No effect on the duration of gestation was noted. However, one rat in the high-dose group exhibited dystocia. Necropsy of that rat revealed one dead pup in the vagina and 15 dead fetuses in the uterus. The authors noted that the uterus was filled with blood.

No signs of toxicity or mortalities were reported in any of the four test groups after parturition. Maternal body weights, litter sizes, pup weights, and incidence of abnormalities in pups were not significantly different in treated and untreated groups of rats.

Main study: The authors reported that rats from all test groups showed signs of respiratory infection, but no other compound-related effects were observed. The infection was described as mild and occurred during the second week of gestation.

Two deaths (one in the 20 and another in the 40 mg/kg group) were noted, but the authors could not relate them to treatment. They stated that the two rats had lower body weights

than other animals No effects on body weight changes, pregnancy rate, or length of gestation were noted.

Total litter loss was reported for one rat in the control group and one in the 40 mg/kg group. Two others were reported with total litter loss in the 20 mg/kg group.

Mean litter sizes for all groups were comparable although the authors noted statistically significant pup mortality during lactation. The significance was attributed by the authors to a low mortality rate in control group pups when compared with background data for the laboratory (not provided in the report).

A slight decrease in mean litter and mean pup weights was noted. However, the authors stated that the differences were not statistically significant.

One pup had hydrocephalus in the control group. No other abnormalities were observed in the pups.

d. Discussion and Conclusions:

There were no compound related effects noted in adult or young rats. In view of other studies conducted in rats with comparable doses (Sections II E and II F. 1., above), these findings are not unusual. The dosage period was for the last 5 days of gestation. In the other studies dosages were administered for 10 days to 13 weeks. Studies reviewed above suggest that bronopol is acutely toxic, and overt effects on adult animals are generally noted after more than 5 consecutive daily doses.

e. Core Classification: Not Applicable.

3. Rat, Dermal Exposure Teratogenicity Study:

a. Citation: Palmer, A. K., and P. James. July 26, 1973. Effect of bronopol on pregnancy of the rat: Dermal administration. Part 3(4). Toxicology. EPA Acc. No. 247199.

b. Materials and Methods:

Test substance: Technical grade bronopol (Batch No. CT89075N).

Test species: CD strain Specific Pathogen Free rats.

Experimental procedure: Three groups of 20 pregnant rats were prepared for dermal application of the test substance on day 3 of gestation (day 1 of gestation was the day sperm were found in vaginal smears). The investigators clipped the hair from the dorsal and dorso/lateral surfaces of each animal.

This procedure was repeated when necessary during dosing.

Solutions of bronopol were prepared in 2.5% Methotas. On days 6 through 15, appropriate solutions of the test substance were applied to the intact clipped skin of rats in doses of 0, 20 or 40 mg/kg/day. Each solution was spread over a 5 x 5 cm area of skin which was occluded with a cloth bandage held in place by Elastoplast adhesive bandage waterproofed with "sleek." After a 6-hour exposure the occlusive dressings were removed and the treated area cleaned with mildly soapy water, rinsed, and blotted dry. Control animals were sham treated with the vehicle.

Animals were observed daily for signs of toxicity and body weights were obtained on days 1, 3, 6, 10, 14, 17, and 20 of gestation. On day 20 the animals were sacrificed. At necropsy the ovaries and uteri were examined. Corpora lutea, viable young, and resorption sites were counted and litter weights were recorded. All of the fetuses were examined externally, and one-third of them were preserved for soft tissue examination. The remainder were prepared and examined for skeletal abnormalities.

Mean fetal weights were calculated from the litter weights. Preimplantation losses were calculated as follows:

$$\frac{(\text{No. corpora lutea} - \text{No. implantations})}{\text{No. corpora lutea}} \times 100$$

Post-implantation losses were calculated by the following formula:

$$\frac{(\text{No. implantations} - \text{No. viable fetuses})}{\text{No. implantations}} \times 100$$

According to the authors, group mean values were derived in two ways. The first (Mean A) included all survivors showing signs of nidation (including total resorptions). The second mean (Mean B) included only animals with viable fetuses at terminal sacrifice. The authors stated that statistical analyses were done on litter results using non-parametric procedures (Wilcoxon test) with a two tailed criterion.

c. Reported Results:

All of the treated animals had skin reactions at the site where the test substance was applied. These signs included erythema and edema which appeared on the 8th day of gestation. By day 11 the authors noted thickening of the skin and scab formation, and signs of healing were seen between day 15 and 20 of gestation. No other signs of toxicity were noted. Mean body weight changes during gestation were reported to be comparable for treated and control groups for dams having viable young at termination of the study. Pregnancy rates for all groups were also comparable.

Group mean litter sizes were reported to be 12.3, 11.0, and 11.5 for the control, low- and high-dose groups, respectively. Mean fetal weights for the control, 20, and 40 mg/kg groups were 3.64, 3.66, and 3.78g, respectively. Fetal losses were reported to be 3.4, 7.5, and 7.5% for control, low- and high-dose dams, respectively.

The authors reported that only one fetus had a major malformation which was described as severe thoracic hemorrhage which caused displacement of thoracic viscera. The incidence of minor soft tissue and skeletal anomalies was not affected by treatment.

d. Discussion and Conclusions:

There were adequate data presented to indicate that dermal applications of bronopol to pregnant rats had no teratogenic effects. A no-effect level for teratogenicity or fetotoxicity is 40 mg/kg/day (highest dose tested). However, it should be noted

that the doses tested were severely irritating to the skin.

e. Core Classification: Minimum.

4. Rabbit Teratology Study:

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

b. Materials and Methods:

Test substance: Technical grade bronopol.

Test species: Female New Zealand white rabbits.

Experimental procedure: Groups of mated female rabbits were given 0, 1, 3.3, or 10 mg/kg/day by oral intubation on days 8 through 16 of gestation. Group sizes were not specified. Day 1 of gestation was the day of mating. The test substance was administered in water.

Does were weighed on days 0, 8 through 17, 23, and 30 of gestation. The animals were sacrificed on day 30 and the contents of their uteri were examined. Corpora lutea, live and dead fetuses, and resorptions were counted, and each fetus was weighed. The fetuses were then examined for external and visceral abnormalities, and they were cleared and stained for skeletal examination. Samples of fetal liver, kidney, lungs, gonads and brain tissues were taken and prepared for histological observation. These samples were from the control and high-dose groups. Tissue samples from all does were taken from the adrenals, lungs, liver, kidneys and any tissues which were found to have gross lesions. No other details of the test procedure were given.

c. Reported Results:

The authors stated, "All the rabbits on bronopol survived treatment without any serious adverse effects." A depressed body weight gain for day 8-17 of gestation (during dosing) was reported for the 10 mg/kg group. The mean weight gain during that period was 0.04 kg for the high dose group compared with 0.13 kg for untreated does. The authors noted that 2 animals in the control and 3.3 mg/kg group, 3 given 10 mg/kg and 1 in the 1 mg/kg

group had ulceration or erosion in the stomach at necropsy.

The group mean numbers of live fetuses per litter were 7.2, 8.0, 8.6, and 10.3 for the 0, 1, 3.3, and 10 mg/kg/day groups, respectively. The respective percentage of fetal mortalities for these groups were reported to be 10.0, 21.7, 20.0, and 4.1. Group mean implantation indices (number of implantations per number of corpora lutea multiplied by 100) for the control 1, 3.3, and 10 mg/kg/day dosage groups were 78, 91, 97, and 92%, respectively. The average fetal weights for each group ranged from 47.6 in the highest dose group to 50.7g in the low dose group (the average fetal weight in untreated rabbits was 48.2g).

The numbers of litters used in deriving these values were 10, 9, 7, and 9 for the control, low-, mid-, and high-dose groups, respectively.

Two fetuses from a control group litter were reported to have major malformations. One was reported to have shortened and malformed nasal bones and small dome shaped frontals and the other had no right kidney and ureter, a large right ovary, diaphragmatic hernia with protrusion of the intestine into the thorax, and small lungs. The third fetus was from a doe given 1 mg/kg/day doses. It was without a left kidney and the left ovary was enlarged. These malformations were classified by the authors as major. Minor malformations of the gall bladder were noted in one fetus from each of the low- and mid-dose groups (double gall bladder) and the high-dose group had 2 fetuses with this type of malformation (one without a gall bladder and 1 with a double gall bladder). Minor skeletal effects were reported in several fetuses. Two from the high-dose group and one from the control group had sterna without a V according to the report, and slight fusion of the sterna was noted in 4 fetuses from the 10 mg/kg/day group. Rib swelling was reported in 4 control group fetuses and 1 fetus from the high dose group. No skeletal examinations were made of fetuses from the mid- or low-dose groups.

d. Discussion and Conclusions:

The description of the protocol and results was brief. However, there were sufficient data to indicate that no dose-related effects were observed in the fetuses of treated rabbits. The highest dose caused decreased

body weight gain, and the results indicate that the no-effect level is 3.3 mg/kg/day for maternal toxicity in rabbits.

e. Core Classification: Supplementary. The study could be upgraded if more detailed information on the protocol and results is reported.

H. Reproduction

Discussion and Conclusions

The studies reported under Accession No. 247199 do not follow a complete protocol. In the first study only one dose and one mating were used. In the second study two doses and one mating were used, and pregnancies of approximately half of the animals were interrupted on day 13 of gestation. There were approximately half the minimum number of animals usually evaluated in standard reproduction studies. . Based on these considerations, two studies are not valid evaluation of the potential of bronopol for reproductive toxicity (See Sections 3.6.2.1.1 and 3.6.3.1.1 of the submission).

J. Mutagencity

1. Citation: Inolex Chemical. Undated. Mutagencity testing by means of in vitro. microbial test. The host mediated assay, and the dominant lethal assay in mice. Part 3(4). Toxicology. EPA Acc. No. 247199.

2. Materials and Methods:

Test substance: Technical grade bronopol.

Test species: *Salmonella typhimurium* strains G46, TA1535, TA1536, TA1537, and TA1538. *Escherichia coli* strains WP2, UVRA, CN561, and CN611. Strain OL/C mice (males and females).

Experimental procedures - *In vitro* microbial assays: Methods were not described in detail beyond citations of the literature including Bridges, B.A., 1972. J. Lab. Pract., 21:411-424; Ames, B.N., 1973, in Chemical Mutagens, Vol. 1. ed. Hollaender, Plenum Press; and Ames, B.N., Less, F.D., Durston, W.E., and Yamasaki, E., 1975., Proc. Nat. Acad. Sci. 70:2281-2285.

The authors stated that a detailed description of the methods is in an internal report which was not submitted.

Host-mediated assay - The procedures were described by referring to Legator (1973) (reference not cited in bibliography) and an internal report which was not included in the documents submitted.

Dominant lethal assay - The methods used were described in a separate report according to the authors.

3. Discussion and Conclusions:

These studies are not reviewed here because the methods were not described along with the results. No final conclusions can be made without complete reports (see Section III.C below).

C. Chronic Toxicity and Oncogenicity

1. Rats - Oral (drinking water)

a. Citation: Hunter, B., P. Batham, R. Heywood, A.E. Street, D.E. Prentice J.M. Offer. April 2, 1976. Bronopol toxicity and tumorigenicity study in rats by administration in the drinking water for 104 weeks. Unpublished report prepared by Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. Submitted by Inolex Chemical. EPA Acc. No. 247197.

b. Materials and Methods:

Test substance: Technical grade Bronopol (batch number CT 92495T used during weeks 1 to 51 and batch number 95274W used during weeks 52 to 104) was used; no further description was given.

Test species: Sprague-Dawley CD strain rats were used.

Experimental procedure: Rats were assigned to four main groups which contained 45 of each sex or 4 satellite groups containing 15 of each sex. Assignments were made to ensure that each group contained similar populations on the basis of body weights. After acclimation, the water and food consumption as well as body weights were determined during the week prior to the beginning of dosing.

The test substance was disolved in tap water at three concentrations which would result in daily doses of 0, 10, 40, or 160 mg/kg. Concentrations were adusted weekly to accommodate for changes in water consumption and body weight. New solutions were prepared weekly and aliquots were placed in plastic bottles for each cage of 5 rats.

Although frequencies of observation were not specified, the authors stated that all animals were observed for appearance of toxic signs, behavioral changes, or mortality. Food consumption and body weight were measured weekly throughout the 104 week treatment period. Water consumption was measured daily.

Urinalysis, hematology, and blood chemistry were examined in fasted animals from the statellite groups. Urine was collected from 10 rats of each sex from each of the four satellite groups during weeks 26, 52, 77, and 103. Urine was tested for pH, specific gravity, protein, reducing agents, glucose, ketones, bile pigments, urobilin, and hemaglobin. Sediment from certifugation was microscopically examined for epithelial cells, leukocytes, erythrocytes, micro-organisms, casts, sperm, and abnormal constituents.

Blood samples were withdrawn from the orbital sinus of 10 male and 10 female rats in satellite groups at weeks 26, 52, 78, and 102. Hematological measurements from animals in the control and high dose groups included packed cell volume, hemoglobin, red cell count total and differential white cell counts, platelet count and clotting time. Blood chemistry tests on samples from the same groups of rats included urea, glucose, total serum protein, serum protein electrophoresis, serum alkaline phosphatase, serum pyruvic-glutamic transaminase, and electrolytes.

Ophthalmic examination of all rats in the control and high dose groups was conducted during the week before start of treatment and weeks 26, 52, 78, and 103.

All surviving rats were sacrificed at 104 weeks. Gross external and internal examinations were conducted. During these examinations the following organs were removed and weighed: brain, pituitary, thyroid, spleen, lungs, heart liver, kidneys, seminal vesicles, prostate, adrenals, testes, ovaries, and

uterus. Tissues from these organs and others were preserved for microscopic examination. The other preserved tissues included pancreas, bone marrow, thymus, lymph nodes, stomach, ileum, cecum, urinary bladder, eye, salivary glands, and any macroscopically abnormal tissues. Tissues which were preserved but not examined according to the authors included trachea, esophagus, aorta, second eye, spinal cord, tongue, jejunum, mid-colon, sciatic nerve, mammary gland, prostate, seminal vesicles, skin and bone. The tissues of all animals in the main groups that were routinely examined microscopically included adrenals, ovaries, spleen, liver, lymph nodes, and pituitary gland, as well as all macroscopically observed lesions. Blood smears were also routinely examined, and if abnormalities were seen, the bone marrow sections were examined. Tissues examined from animals in the control (10/sex) and high dose groups (12 males and 15 females) included kidney, gonads, uterus, adrenals, brain, pancreas, bone marrow, thymus, lymph nodes, stomach, ileum, cecum, urinary bladder, eye, heart, liver, lungs, spleen, pituitary, salivary glands, and tissues with grossly observed lesions.

c. Reported Results

The authors noted the occurrence of a viral infection (sialodacryoadenitis) in the rats. Decreased body weight gain during weeks 9 and 10 of the study was attributed to the infection, and the authors stated that rats with the disease had recovered by the 12th week.

During the last year of the study the rats given the highest dose were reported to have reduced grooming activity with brown stained areas of fur. No other signs of toxicity were noted.

Mortality for the main groups at the end of the study was 80% in the high dose males compared with 47% in the control male rats. The mortality for female rats by the end of the study was 62% in the high dose group compared with 42% in the control group. Mortality in the low and mid dose groups was comparable to that in the control groups for both sexes.

According to the auhtors the only treatment related lesions in rats dying after the 82nd week involved the stomach. These lesions were described as thickening of the mucosa, ulceration, raised areas, and excrescences. There were 10 of 16 males and 3 of 14 females from the high dose group which were reported to have these lesions. No control rats exhibited similar effects.

Decreased food consumption was reported in male rats given the highest dose. The amount consumed by the high dose group males was 84% of that consumed by control male rats during the first year of the study. Female rats in the high dose group consumed less food than control females after the 78th week of the study. The food consumption was 84% of the control value during weeks 79 through 104 for the high dose females. The authors also noted a marginal decrease in food consumption for the mid-dose males during weeks 53 through 78. The group mean total consumption was 84% of control values for that period. All other groups consumed comparable amounts of food during the study.

By the 10th week group mean body weight for the high dose group males was 12.5% less than that of control males. At the end of the first year of treatment the group mean body weight for high dose males was 25% less than that for the control group. At the end of the study the high dose group mean body weight was 45% less than that of controls for males rats and 21% less for female rats. Reported weight differences between controls and the remaining treatment groups were less than 10% throughout the study.

By the 10th weeks group mean body weight for the high dose group males was 12.5% less than that of control males. At the end of the first year of treatment the group mean body weight for high dose males was 25% less than that for the control group. At the end of the study the high dose group mean body weight was 45% less than that of controls for male rats and 21% less for female rats. Reported weight differences between controls and the remaining treatment groups were less than 10% throughout the study.

The authors reported that addition of bronopol to the drinking water resulted in a dose-related decrease in water consumption. The concentrations of bronopol in the drinking water were adjusted on a weekly basis to maintain expected dose levels and the authors noted that retrospective calculations resulted in the following mg/kg/day dosages:

Group	Males	Females
Low	10.5	10.4
Mid	40.2	40.7
High	152.2	158.4

The volume of excreted urine in high dose group rats was reported to be less than controls as the result of decreased water consumption. Hemoglobinuria was reported in 1 of 10 control males and 5 of 10 in high dose male rats examined at week 52. Three females of the 10 high dose group rats examined also exhibited hemoglobinuria at week 52. No control females of the 10 examined were reported with hemoglobinuria. At week 103 the incidence of hemoglobinuria was reported as follow:

Group	Males	Females
Control	5/10	1/10
Low	3/10	0/10
Mid	3/10	0/10
High	10/10	2/10

Statistically significant differences between the control and high dose groups were noted. There were statistically significant decreases noted for hemoglobin (g %) and erythrocyte count at week 52, but there were no statistically significant differences noted for these two parameters in the high dose rats at week 78. During week 102 the packed cell volume hemoglobin, and erythrocyte count were statistically significantly increased in male rats of the high dose group, while the females exhibited decreased packed cell volume and red cell count (see below for further discussion).

The authors stated that all hematological parameters were within normal ranges for rats of the same strain and age as those tested.

Blood chemistry results were reported to be within normal limits.

No ophthalmological abnormalities were observed which the authors could relate to treatment.

The authors stated that mean kidney-to-body-weight ratios were higher than expected in the high dose group for rats of the same age and body weight. The relative weights of most of the organs weighed in the high dose group were also statistically significantly higher than the respective ratios for control group rats. This finding was attributed by the authors to the large difference in group mean body weight for the control and high dose groups (see discussion above). Absolute organ weights reported to be statistically significantly less than control values are summarized as follows:

	Control group		High dose group	
Organ	Males	Females	Males	Females
Heart	2.1	1.4	1.5	1.2
Lungs	2.5	NDS	2.2	NSD
Liver	24.2	NDS	15.9	NSD
Prostate	1.0	---	0.7	---
Thyroid	35	43	26	36
Testes	5.4	---	4.3	---
Kidney	NSD	3.9		3.6
Body	860	528	474	423

NSD = No significant difference.

The authors stated that the incidence of progressive glomeralonephrosis in the kidneys of male and female rats from the high dose group was compound related. The relationship was characterized as an exacerbation of a spontaneous lesion by the test substance. The incidence of this lesion is summarized from the main and satellite groups as follows:

Dose Group	Males	Females
Control	14/49	4/52
Low	10/47	4/53
Mid	14/48	4/59
High	26/54	20/52

The authors noted squamous metaplasia in the ducts of salivary glands. Groups of atrophic acinie were also reported, and signs of chronic inflammation were observed. The incidence of affected salivary glands in these animals examined was summarized as follows:

Dose Group	Males	Females
Control	3/24	2/26
Low	5/24	1/26
Mid	12/25	2/23
High	11/12	12/20

The authors reported that gastric lymph nodes exhibited sinusoid dilatation (4 of 12 males and 5 of 22 female rats from the high dose group). These changes occurred in animals found to have epithelial hyperplasia and ulceration of the non-glandular stomach. These changes did not occur in rats from the control, low, and mid-dose groups.

Lesions found in the stomach were described as hyperplasia and hyperkeratosis of the epithelium in the non-glandular stomach. These changes were found associated in some cases with ulceration according to the authors. Of the 23 high dose group males examined, 12 had these types of lesions, while 15 of 48 females in the high dose group were affected. The incidence of these stomach lesions in controls was 1 of 28 and 2 of 28 for the male and female rats, respectively. The low and mid dose groups were comparable to controls with respect to the incidence of these stomach lesions.

No other non-neoplastic lesions were found to be toxicologically significant according to the authors.

The number of tumor bearing animals in each main group was reported to be comparable. The incidence of animals with tumors among those examined in the main groups is summarized as follows:

Dose Group	Males	Females
Control	28/43	35/44
Low	30/43	42/45
Mid	22/42	39/42
High	11/41	30/38

The most frequently observed tumors in control and treated rats included pituitary adenomas in both sexes and mammary fibroadenomas. Other tumor types were noted in 5 or fewer animals from each group. With the exception of squamous cell papillomas in the non-glandular stomach, none of these tumor types had an apparent relationship to dose. The squamous cell papillomas in the stomach were reported along with epithelial hyperplasia or ulceration, and they were associated by the authors with the chronic irritation caused by the test substance in the gastrointestinal tract.

Although no dose-response relationships were reported for incidences of any tumor, the incidence of mammary fibro-adenomas in the high-dose group females was significantly increased above controls (36/45 in the treatment group vs. 25/44 in control rats, $X^2 = 5.544$, p=0.019). When the incidence of mammary fibroadenomas in the main and satellite groups are combined for each treatment, there is marginal statistical significance of the difference between the control and low dose groups (29/52 in controls vs. 38/53 in the low dose group, $X^2 = 2.833$, p=0.089).

d. Discussion

The authors attributed the apparently decreased tumor incidence in the high dose groups to the early deaths of the tested rats. This effect is more apparent in males since they appeared to be more susceptible to the toxicity of the test substance (See Reported Results section above). In these rats the first tumor diagnosed in the control group was noted at week 50. In correcting the incidence of tumor bearing male rats for early mortality, one rat with lymphocytic lymphosarcoma is censored. Although the rat is from the low dose group and died during the 25th week, the tumor was not found in any other treated or untreated rat in the study. In addition, only 7 other animals in all groups of males died before that time. One of those 7 deaths occurred in the high dose group at week 22 with no reported neoplastic lesions. Therefore, the low-dose group male that died with a tumor is considered incidental, and the animal is censored with all others dying before the 50th week of the study. (Note: This approach is an attempt

to assure that a dose-related increase in tumor incidence is not observed by an isolated and unusual occurrence that could lead to including animals that would ordinarily be censored.)

The adjusted incidence of tumor bearing male rats is as follows:

Dose Group	Tumor bearing/No. examined
Control	33/45
Low	31/44
Mid	25/48
High	13/44

These results suggest that factors in addition to mortality may be contributing to the decreased tumor incidence in treated males. The most frequently observed tumor was described as pituitary adenoma which is a common tumor in older rats. There are data reported in the literature (Roe, 1981) that suggest a reduction in the incidence of common tumors in rats given nutritionally restricted diets for two years. As noted above, the food consumption of male rats in the mid and high dose groups was reduced. A statistical analysis of food consumption and tumor incidence in the male rat of this study is beyond the scope of this discussion, and the study itself was not designed to evaluate such a relationship. However, there is evidence that reduced food consumption may be a factor along with early deaths, which could have affected the results of this study with respect to the high-dose group male rats.

The results described above (see Reported Results) show that tumor incidence is generally higher for females, and, as is the case in the male rats, the most frequently occurring tumors are common in older rats (i.e., pituitary adenomas and mammary fibroadenomas). A review of individual animal pathology reports indicates that each tumor bearing animal has a pituitary adenoma, or a mammary fibroadenoma, or both. Further discussion will be limited to the reported results with respect to mammary fibroadenomas, since they suggest an increase which may be related to treatment.

In view of the possible effects of mortality on tumor incidence, the data on mammary fibroadenoma incidence is adjusted for early mortality. The first diagnoses were made in weeks 78, 68, 73, and 64 for the control, low, mid and high dose groups, respectively. A better perspective of the time-to-tumor results for the main and satellite groups is indicated by the following summary:

Weeks on study	No. with mammary fibroadenoma/No. examined			
	Control	Low	Mid	High
1-52	0/0	0/1	0/1	0/5
53-78	5/8	2/4	2/3	1/6
79-105	11/19	15/23	16/22	18/24
Termination	15/26	21/26	17/23	17/22
Total	31/58	38/54	35/49	36/57

After these results are adjusted for early mortality, the total incidences become 31/57, 38/53, 35/48, and 36/49 for the control, low, mid, and high dose groups repectively. A trend analysis shows no statistically significant dose related trend with respect to the incidence of mammary fibroadenomas in treated female rats. The incidence of other tumors in male and female rats was low (5 or less in each group) and was unrelated to compound administration.

e. Conclusion

The irritation of the gastrointestinal tract, mortality, and decreased food consumption particularly in male rats given 40 or 160 mg/kg/day in their drinking water, indicates that bronopol has toxic effects at daily doses as low as 40 mg/kg/day. The no-observed effect level is 10 mg/kg in rats.

The study also indicates that, under the test conditions described above, bronopol is unlikely to be oncogenic in the rat.

f. Core Classification: Minimum.

2. Mouse - Dermal

a. Citation: Hunter, B., C. Graham, 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. Acc. No. 247197.

b. Materials and Methods

Test substance: Two batches of technical grade bronopol were used. Batch No. CT89075N was used for the first 13 weeks of the study and Batch No. CT 92495T was used until the end of the study. Test species: (FLP strain Swiss mice of both sexes were used.

Experimental procedure: Mice were randomly distributed in three groups each containing 52 males and 52 females. All test mice had the hair of their backs closely clipped before the start and at weekly intervals during the 80 week treatment period.

The test substance was dissolved in 90% acetone and water at concentrations of 0, 0.2 or 0.5%. Each solution was applied to the prepared skin of the mice from each group. Applications were made 3 times each week (Monday, Wednesday, and Friday) for 80 consecutive weeks.

There is no description of how treated skin areas were occluded, and animals were housed four to a cage. All mice were examined daily for appearance of toxic signs and observed for changes in behavior. Descriptions of skin lesions and palpable masses were recorded. Individual animals were weighed weekly during the first 12 weeks and bi-weekly during the remainder of the study. Food consumption for each cage of four mice was recorded weekly. The results were used to calculate efficiency of food utilization. Moribund animals were sacrificed. These animals and mice found dead were prepared for subsequent postmortem observations.

The animals that died during the study and those sacrificed at the end of the 80 week test period were subjected to gross necropsy. Tissues which were processed for microscopy, according to the report, included the adrenals, liver, lymph nodes, ovaries, pituitary, spleen, thyroid, and skin from treated sites. Other organs preserved but not processed for microscropic examination included the

aorta, brain, bone, mid colon, eyes, gall bladder, heart, jejunum, kidneys, liver, lungs, mammary gland, esophagus, pancreas, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, untreated skin, testes, thymus, tongue, trachea, urinary bladder, and uterus. Bone marrow smears were prepared, and grossly abnormal tissues were prepared for microscropic examination.

c. Reported Results

The only sign of toxicity noted by the authors during the study was hair loss around the clipped area of mice treated with the 0.5% solution.

A reduction in group mean body weight gain was noted during weeks 26 to 52 in males treated with the 0.5% bronopol solution. 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.

Survival of males was 33/52, 26/52, and 27/52 for the control, low and high dose groups, respectively.

Respective survival of female mice was 28/52, 34/52, and 31/52 for the control, low, and high dose groups.

No significant differences in group mean body weights at the end of the study, and food consumption was not affected by the test substance. The authors also stated that food use efficiency was unaffected by treatment.

No compound related effects were noted by the authors with respect to gross or histological observations. The lesions and neoplasms which were reported were attributed to the age of the test animals.

d. Discussion and Conclusions:

The authors stated that the purpose of the study was to determine the likelihood that bronopol causes skin tumors after chronic dermal exposure. As indicated in the above description of methods, the animals were not examined as mice usually are in a general oncogenicity study.

The results were not reported in detail (only group means for body weights and food consumption or summary tables of the incidence of gross and microscopic lesions in each group were reported). Appendices containing individual animal data were omitted for unspecified reasons, and time to diagnosis data were included only for skin tumors.

These considerations preclude a complete and independent review of the study, and the stated intent to evaluate the potential of bronopol to cause skin tumors limits the study's usefulness in a full evaluation of the oncogenic potential of the test substance.

e. Core Classification: For reasons described in the Discussion above, the study is considered supplementary.

L. Metabolism

1. Citation: Chassecud, L.F. D.H. Moore, J.D. Lewis, and D. Bucke. May 5, 1974. The metabolism of Bronopol (2-bromo-2-nitropropane-1,3-diol) after oral administration to rats and dogs and application to the skin of rats and rabbits. Unpublished report No. BTS31/74 149 prepared by Huntingdon Research Centre. Submitted by Inolex Chemical. EPA Acc. No. 247191.

2. Materials and Methods:

Test substance: 2-bromo-2-nitropropane-1,3-diol. ^{14}C label at the second carbon. Specific activity 21 uC/mg. Radiochemical purity of 99% as detemined by thin layer chromatography (tlc). Unlabelled bronopol was also used.

Test species: CFY rats (males and females) beagle dogs (males and females), rabbits (males and females).

Analytical procedures: Plasma samples were extracted with ethyl acetate (twice with 3 volumes). Freeze dried urine residues were extracted with methanol. Extracts were concentrated and chromatographed on thin layer plates.

Urine extracts evaporated to dryness were dissolved in acetate buffer solution (pH 5.0) and mixed with a glucuronidase and sulfatase mixture. These

mixtures were chromatographed and compared with chromatograms of untreated extracts.

Autoradiography was conducted on the tlc plates. Radioactive zones were eluted in methanol and water. These eluates were then concentrated and analyzed by mass and infrared spectroscopy. The tlc plates and their radioautographs were compared with tlc plates developed with unspecified standard chemicals. The silica was removed from radioactive zones of tlc plates and eluted with methanol. The eluates were then counted by liquid scintillation.

Fecal samples, tissues, organs, and carcasses were mascerated in methanol and centrifuged. Urine, plasma, solvent extracts, CO_2 traps and cage washings were also assayed for radioactivity by liquid scintillation. Residues from extraction procedures were oxidized and the radiolabelled CO_2 produced was absorbed in liquid scintillation cocktails. These samples were then counted. Radioactivity absorbed by occlusive dressings used in dermal studies were removed with toluene, and the washings were assayed by liquid scintillation also. External standard quench correction curves were used in these procedures.

Experimental Procedures: Experiment 1. Six rats (sex unspecified) were given a single oral dose of 1 mg radiolabelled bronopol per kg body weight in aqueous buffered solutions (pH 5.0). Urine was collected over 24 hour intervals for 5 days following treatment. Expired air was monitored for the 5 day observation period and $^{14}CO_2$ was trapped for radioassay. Feces were also collected at unspecified intervals.

Doses were administered by gavage.

Three male rats were given the 1 mg/kg dose. Blood sampler were drawn from the tail at unspecified intervals. The whole blood was collected in heparinized tubes and certrifuged, and aliquots of the plasma were counted for radioactivity.

One male and one female rat were anesthetized with halothane and oxygen and their bile ducts were cannulated. After recovery these rats were given the 1 mg/kg dose by stomach tube and placed in restraining cages. Bile urine, and feces were collected for 48 hours.

Six male rats were given the 1 mg/kg dose, and at 15 min, 1, 4, 8, 24, or 72 hours one of the animals was sacrificed. The carcass was frozen and sectioned sagitally. The sections were freeze dried and autoradiographed.

Experiment 2: Single doses of 2 mg radiolabelled bronopol combined with 6 to 8 mg unlabelled bronopol were administered to beagle dogs in gelatin capsules. Urine and feces from one male and one female were collected daily for 5 days following dosing. Blood was drawn from the cephalic vein of these dogs at daily intervals after treatment. The blood samples were centrifuged and aliquots were analyzed for radioactivity or extracted with ethyl acetate and chromatographed. One male dog was sacrificed at 1-1/2 or 6 hours after dosing, and the organs were removed and frozen for subsequent analysis.

Experiment 3: Groups of 6 rats (sex unspecified) had their backs clipped of hair. Radiolabeled bronopol was applied to a 1 cm^2 area of the shaved skin in a solution with water, acetone or water/acetone (9:1 v/v) (4 mg bronopol/ml solvent). The bronopol dose applied was stated to be 1 mg/kg. Treated skin was then covered with polythene sheets and "Sleek" adhesive dressing. The rats were housed in metabolism cages, and urine and feces were collected. Expired air from 2 rats in each solvent group was monitored for $^{14}CO_2$. The authors noted that animals found gnawing the occlusive dressings were not evaluated since ingestion of the test material was likely. Based on the amount of urinary radioactivity recovered from the group receiving applications of bronopol in acetone, the authors stated that they used the organic solvent in subsequent dermal studies.

The hair was removed from the backs of 6 rats and 4 rabbits for the main study. Hair was clipped or removed by a depilatory agent (one rabbit). Animals received applications of ^{14}C-bronopol and were handled in the same manner as the rats in the preliminary study described above. The dose for rats was 1.2 mg/kg, while that for rabbits was 0.72 mg/kg. One rat was sacrificed at 6, 12, 24, 48, 72, or 96 hours after application, and a rabbit was sacrificed 6, 12, 24, or 48 hours after the dermal application of the radiolabelled bronopol.

3. Reported Results:

Experiment 1: The authors stated that radioactivity was absorbed rapidly. They noted that most of the recovered radioactivity was in the urine (80.9% in the urine 24 hours after administration). Fecal recovery was reported to be 5.2% in the feces (48 hours after treatment) and 6.3% in the expired air. Five days after oral administration, the authors reported that 83.3, 5.8, and 8.4% of the radiolabel was recovered in the urine, feces and expired air respectively.

The cannulated rats were reported to have up to 7% of the administered radioactivity in the bile, and urinary excretion accounted for up to 80.5% of the activity 48 hours after dosing. The investigators concluded from these results that the activity recovered in feces resulted from that encountered in the bile, and the results indicated to the investigators that almost all of the administered dose is absorbed by the treated rats.

Blood levels were reported to peak 2 hours after dosing in two rats and 30 min. after dosing in a third. The authors noted that the initial halflife for the decline of blood concentrations was 3 to 5 hours. The authors stated the peak blood concentrations were 0.2 to 0.3 % of the administered dose per ml of blood, and by 24 hours after dosing these levels were 0.02 to 0.03%/ml.

The authors stated that whole-body autoradiography of treated rats indicated rapid absorption of the test substance. The kidney and urine were the only places where high radioactivity, relative to that in the stomach, was noted. The brain and spinal cord were reported to show no radioactivity until 4 hours after administration of labelled bronopol. Elevated radioactivity in the kidney was not noted by the investigators in animals sacrificed 7, 8, 24, or 72 hours after dosing. The tissue distribution suggested by the autoradiographs in this experiment was described as even, and there was no evidence that radioactivity persisted in any organs. No radioactivity was observed in the bile capillaries.

Experiment 2: The authors noted that dogs given radiolabelled bronopol excreted the label rapidly. Twelve hours after dosing, 63.9% of the administered

radioactivity was recovered in the urine. The authors further stated that after 5 days 81.1% of the administered activity was accounted for in the urine and 3.1% in the feces. Expired air was not monitored.

Blood concentrations peaked between 30 min. and 2 hours after dosing. According to the investigators the peak concentrations had declined by 10 fold in 24 hours, and they estimated the halflife to be 4 hours. Based on the assumption that blood volume is equivalent to 5% of the body weight, the authors concluded that peak blood concentrations represented 5.9 to 9.0% of the administered dose.

Tissue levels in treated dogs expressed as mg/g of tissue indicated that the kidneys contained the highest amount (0.0168 mg/g) while the plasma contained 0.0099 mg/g. The concentrations reported for the remaining tissues analyzed ranged from approximately 0.003 to 0.008 mg/g. The radioactivity measured in muscle accounted for 27.06% of the administered dose (0.00771 mg/g), and plasma accounted for 4.46% of the dose. The investigators noted that 22.04% of the dose was excreted in the urine 1.5 hours after treatment.

Six hours after treatment the concentration of test substance in the kidneys was 0.0091 mg/g. The plasma concentration was 0.0056 mg/g. The tissue concentrations were generally decreased to approximately half their values 1.5 hours after treatment.

Experiment 3: The amount of radioactivity excreted after 5 days in the urine of rats given dermal applications of bronopol showed that dermal absorption is less than gastrointestinal absorption. When the test chemical was applied in acetone, up to 12% of the applied radioactivity was excreted. When water/acetone (9:1 by volume) or water was used as the solvent only 4% was recovered in the urine.

The authors reported that during a distribution study of rats receiving a dermal application of bronopol, up to 11% of the applied activity was excreted in the urine during the first 24 hours after treatment. Less than 3% of the dose was accounted for in the carcass, and most of the administered radiolabel was found at or near the

site of application. No tissue concentrations were measured.

Tissue distribution of radioactivity in rabbits after dermal application was similar to that reported in rats. The kidneys had the highest concentration and the authors noted that most of the organ to plasma concentration ratios were less than 1. The exceptions included the liver, kidney, and lungs which the authors associated with excretion of bronopol and its metabolites. Plasma concentration peaked approximately 12 hours after application (0.0031 mg/g).

Autoradiography of treated skin from rabbits showed that the epidermis had the greatest radioactivity. The authors noted superficial penetration of the dermis by radiolabelled bronopol around the hair follicles. The remainder of the dermis and underlying tissues contained no radiolabel. These results prompted the authors to investigate the ability of bronopol to bind to rat skin and fur. The treated skin (in vivo) and fur (in vitro) were washed with water, extracted with methanol, and burned in oxygen to produce $^{14}CO_2$ which was counted to determine the amount that bound to the skin or fur. The skin contained up to 20% of the applied bronopol after a 24 hour exposure but the fur retained only 2%.

Results from thin layer chromatography, infrared and mass spectroscopy indicated that the major metabolite of bronopol is 2-nitropropane-1,3-diol. This metabolite was found in plasma and urine. The authors stated that efforts to identify 4 other urinary metabolites failed. The nitropropanediol metabolite constituted 40 to 50% of the radioactivity recovered, and no bronopol was detected. Similar results were reported for both rats and dogs.

The authors further stated that addition of radiolabelled bronopol to plasma or saline (pH 7.4) in vitro showed that the test substance converts to 2-nitropropane-1,3-diol in plasma. It is relatively stable in saline solution.

4. Discussion and Conclusions

The experiments described above indicate that rats and dogs rapidly absorb oral doses of 1 to 2 mg bronopol per kg body weight. The chemical is also

rapidly excreted primarily in the urine (approximately 80-85%). The feces and expired air are also routes of excretion.

Dermal absorption in rats and rabbits is low (10 to 20%) and the pattern of excretion is the same as for animals treated orally.

Bronopol does not accumulate in any particular organ, and tissues with the highest concentrations are in organs involved in excretion of the chemical (kidney, liver, and lungs).

5. Core Classification: Not applicable

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