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

10/7/83

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. *Chad B Sandusky*
        Acting Section Head   9/23/83
        Toxicology Branch
        Hazard Evaluation Division (TS-769)

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).

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.1. 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 █████████████ ███████████████ 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. ███████████████████████ 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

respiratory distress and abdcminal distension. At 00▮▮▮▮▮▮
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 hyperkera-
tosis, 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 characteris-
tic 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.

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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, glomerulone-phrosis, 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).

OUTLINE OF BRONOPOL DATA REVIEW, SECTION II

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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 observa-
tions 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 intraperi-
toneal 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 para-
graph 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

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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.

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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.

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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 admini-
stration 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 treat-
ment. Only the male dog showed changes in
the blood. Neutrophils and SGPT and SGOT
increased. At necropsy of the male dog the

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investigators noted intense congestion and
edema of the gastric mucosa, congested mesen-
teric 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 inha-
lation 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)

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

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 obser-
vation period.

3.   Reported Results:

The authors stated that 78 to 92% of the par-
ticles 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 e:posed 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.

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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 [redacted] (0.5% or 2% w/v) normal saline (concentration and nature of salts used was unspecified, 0.5% w/v), or [redacted] (0.5, 2, or 5% w/v). Groups of 3 female rabbits were used in each experiment.

Experiment 1:   One-tenth ml of the 0.5 or 2% solutions of bronopol in [redacted] 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 [redacted] 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

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 ███████████████ 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 ███████████████ ████ is strongly irritating to the eyes of rabbits.

5.   Core Classification: Minimum.

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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 █████████

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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

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 ███████████████████ 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 completely, 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.

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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).

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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 10 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.

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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

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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 dif-
ferences 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 dif-
ferences for males were described as statis-
tically 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.

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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 inter-
stitial 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

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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 dis-
solved 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 after-
noon 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.

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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.   Discussion

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.   Conclusion

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.

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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 con-
secutive 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
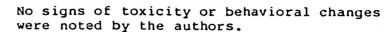weekly. Prior to the start of the experiment

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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, cardio-
vascular 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 intensi-
fication 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 excep-
tion 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, ophthalmalo-
gical, 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. <u>Rats - Teratogenicity Study</u>

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

b. <u>Materials and Methods</u>:

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

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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.

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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:

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

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

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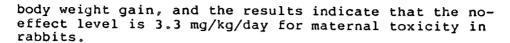group had ulceration or erosion in the stomach at necropsy.

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

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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 cita-tions 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.

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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.

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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 speci-
fied, 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 consti-
tuents.

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 measure-
ments 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.

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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:

Organ	Control group		High dose group	
	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

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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 papil-
lomas 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 dif-
ference 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 sub-
stance (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 mor-
tality, 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

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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 re-
duced 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.

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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 morta-lity, 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 particu-larly 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 samples 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 under-
lying 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 radio-
labelled 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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