I. DATA GAP STATUS

Chronic toxicity, rat: No data gap, possible adverse effect
Chronic toxicity, dog: Not required at this time
Oncogenicity, rat: No data gap, no adverse effect
Oncogenicity, mouse: Not required at this time
Reproduction, rat: No data gap, possible adverse effects
Developmental toxicity, rat: Not required at this time
Developmental toxicity, rabbit: No data gap, no adverse effect
Gene mutation: No data gap, no adverse effect
Chromosome effects: No data gap, possible adverse effects
DNA damage: No data gap, no adverse effect
Neurotoxicity: Not required at this time

Toxicology one-liners are attached.

All record numbers for the above study types through 267672 (Document No. 50199-0116) were examined. This includes all relevant studies indexed by DPR as of Nov. 9, 2012.

In the 1-liners below:
** indicates an acceptable study.
**Bold face** indicates a possible adverse effect.

File name: t20121219.wpd
II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

COMBINED, RAT

** 50199-0005, 016; 70691, 70692, 70693, 70699, 70700, 120866, 121138; “Bronopol Toxicity and Tumorigenicity Study in Rats by Administration in the Drinking Water for 104 Weeks” (Huntingdon Research Centre, Huntingdon, UK, Report # BTS 51/75719, 4/2/76), Bronopol (Batch # CT 92495T, 99.7% purity and CT 95274W, 100.3% purity) administered in the drinking water for 104 weeks at 0 (drinking water), 7, 32, or 142 mg/kg/day (calculated dosage) to 45 Sprague Dawley CD rats/sex/group and satellite groups of 15/sex/group. **Possible adverse effects:** squamous metaplasia with inflammatory cell infiltration and groups of atrophic acini and dilation and minimal epithelial hyperplasia of the salivary glands reported in males at 32 and 142 mg/kg/day and in females at 142 mg/kg/day. Dilatation of the sinusoids in the gastric lymph nodes in 4/12 males and 5/22 females at 142 mg/kg/day. Epithelial hyperplasia with hyperkeratosis associated with areas of ulceration in the non-glandular epithelium of the stomach at 142 mg/kg/day in both sexes. Increased incidence of progressive glomerulonephrosis in males and females at 142 mg/kg/day. No oncogenic effect reported. Decreased bodyweight gain in males at 32 mg/kg/day (10% to 16% less than controls) and in males and females at 142 mg/kg/day (13% to 44%). NOEL = 7 mg/kg/day. Oncogenic NOEL ≥ 142 mg/kg/day. Initially reviewed as unacceptable but possibly upgradeable with submission of test article purity and cage size (Green and Morgan, 12/29/88; Gee, 4/5/89); subsequently re-reviewed with additional data; acceptable; (upgraded, Leung, 7/27/93).

CHRONIC TOXICITY, RAT

See “Combined Rat,” above.

CHRONIC TOXICITY, DOG

No study on file.

ONCOGENICITY, RAT

See “Combined Rat,” above.

ONCOGENICITY, MOUSE

50199-0006 070694, 070695, “Bronopol, Potential Local and Systemic Tumorigenic Effects in Repeated Dermal Application to Mice (Final Report 0 - 80 Weeks),” (Huntingdon Research Centre, report # BTS 43/74761, 1/23/75), Bronopol, Batch # CT 89075 N and CT 92495T, no purity, no stability, applied to the shaved, intact skin of 52 CFLP mice/sex/group at 0 (90% acetone/water), 0.2% and 0.5% for 80 weeks (applied on Mondays, Wednesdays, and Fridays). # deaths/# dosed at 0, 0.2%, and 0.5% respectively: 43/104 (19 M), 44/104 (26 M), and 46/104 (25 M). Some decrease in body weight gain in males at 0.5% (6 - 8% lower total body weight...
later in study). No increase in tumor incidence reported. **No adverse effect** indicated. NOEL = 0.5%. **Unacceptable** (no test article characteristics, no dosing solution analysis, inadequate description of dosing procedure), questionable if upgradeable (only two dose levels, neither of which produced toxic signs). Record 070695 contains the pathology report from 1986 in which additional tissues were examined. (Green, Morgan 1/10/89 and Gee, 4/11/89)

001 907749, Brief summary of record numbers 070694, 070695.

**REPRODUCTION, RAT**

**50199-0088** 242143 Carney, E. W., C. L. Zabltny, R. J. Rasoulpour, K. E. Stebbins, and J. Thomas, “Bronopol: two generation drinking water reproductive toxicity study in Crl:CD (SD) rats,” The Dow Chemical Co., Midland, MI, 3/13/08. Laboratory Project Study ID 061065. Groups of 27 Crl:CD (SD) rats/sex were dosed in drinking water, pH adjusted with HCl to 4.5, with Bronopol, 99.85% purity, Lot TC0601X702, at 0, 0.01, 0.05, or 0.15%, achieving an average of 8.8, 41, or 104 mg/kg/day in treated F0 males, 10.5, 53, or 141 mg/kg/day in F1 males. F0 females during pre-mating achieved an average of 11.5, 53, or 129 mg/kg/day, and F1 females achieved 14.8, 67, or 181 mg/kg/day. Concentrations were stepped down during lactation to achieve near constant mg/kg/day exposure during that phase. Parental systemic toxicity NOEL = 0.01% in water (corresponding to 8.8 and 10.5 mg/kg/day in F0 and F1 males, and to 11.5 and 14.8 mg/kg/day in pre-mating females), based on kidney tubule degeneration with regeneration (bilateral, multifocal, very slight to slight) in mid-dose males, and on thyroid follicle dilatation (very slight) in mid-dose females. At the high dose, both sexes were affected for tubule degeneration, often with tubular dilatation and/or associated inflammation in both generations. Drinking water consumption was reduced meaningfully in both sexes of mid-dose F0 parents, particularly in the first few weeks of exposure, likely due to reduced palatability. Ulceration and/or multifocal erosion of stomach glandular mucosa of high dose females was likely also treatment-related. Parental reproductive effects NOEL = 0.05% in drinking water (56-68 mg/kg/day in females during gestation and lactation), based on high dose findings of dystocia (two F0 dams) and poor perinatal survival (two F1 dams with some dead pups at parturition, and none of these surviving past PND 2). Subsequent viability of pups was unaffected, but pup weights at weaning were slightly reduced in F0–F1 pups, likely treatment-related. Acceptable. Dystocia and poor perinatal survival were possible adverse effects. Aldous, 12/19/12.

50199-007 070701, “Bronopol: Range-Finding Reproduction Study in Rats,” (International Research and Development Corporation, report # 510-012, 1/27/86), Bronopol Pharmaceutical Grade, batch 845309 A, 99.9% purity, administered in the drinking water to 5 Charles River COBS CD rats/sex/group through 1 generation and 1 litter at 0 (tap water), 25, 50, 100 or 200 mg/kg/day (nominal). One 200 mg/kg/day male (# 84218) was sacrificed in extremis during study week 2. Water intake for males in all treated groups was reduced compared to controls. Reduced weight gain for males in all treatment groups was reduced compared to controls. NOEL parental < 25 mg/kg/day (reduced weight gain primarily in males); developmental > 200 mg/kg/day (nominal). **Supplemental.** (Green, Morgan 1/4/89)

50199-008 070702, “Bronopol: Two Generation reproduction Study in Rats,” (International Research and Development Corporation, report # 510-013, 12/23/87), Bronopol Pharmaceutical Grade, batch # 845309, 99.9% purity, administered in the drinking water for 2 generations, 2
litters/generation at 0 (tap water with pH adjusted to 4.0), 22.5, 55.2 or 147.0 mg/kg/day (mean achieved doses) with 13 male and 26 female Charles River COBS CD rats per group. Increased kidney weights in F0 females at 55.2 and 147.0 mg/kg/day. Increased incidence of progressive nephropathy in F0 females at 55.2 and 147.0 mg/kg/day. Increased severity of spontaneous nephropathy (tubular degeneration and hyaline cast formation) in F1 high dose animals of both sexes. Parental systemic NOEL = 22.5 mg/kg/day (kidney effects). Reproductive NOEL = 55.2 mg/kg/day (decreased pup weight at high dose). Unacceptable but possibly upgradeable (incomplete histopathology on F0 animals, not all F0 and F1 adults examined for histopathology of the reproductive organs although tissues apparently saved - the missing histopathology should be performed). (Green, Morgan 1/6/89 and Gee, 4/10/89).

DEVELOPMENTAL TOXICITY, RAT

006 070696, “Teratogenicity, Oral Administration (rat),” (performing laboratory not specified, no study date), bronopol, no purity, daily administration by gavage on days 1 to 20 of pregnancy at 0 (water), 10, 30 or 100 mg/kg/day (nominal) with 7 to 12 mated Boots-Wistar female rats per group. Four rats (days 9, 13, 19, and 21 respectively) at 100 mg/kg/day, 3 rats (days 2 and 5) at 30 mg/kg/day, and 3 rats (days 6, 9, and 18 respectively) at 10 mg/kg/day died from lung and gastric lesions. One rat at 30 mg/kg/day was killed after uterine hemorrhage on day 19. Reduced mean litter size at 10, 30, and 100 mg/kg/day which appears related to a smaller number of corpora lutea. Reduced maternal weight gain at 10, 30, and 100 mg/kg/day (group means 22%, 25%, and 35% less than control values respectively). No adverse effects indicated. NOEL maternal < 10 mg/kg/day, developmental NOEL > 100 mg/kg/day. Unacceptable, not upgradeable (insufficient numbers/group, dosing prior to day 6 of gestation). (Green, Morgan 12/20/88 and Gee, 4/10/89)

006 070697, “Effect of Bronopol on Pregnancy of the Rat, Dermal Administration,” (Huntingdon Research Centre, report # BTS44/73446, 7/26/73), bronopol, no purity, no stability, 6-hour daily occluded exposure on days 6 through 15 of gestation to clipped, intact skin of 20 mated female CD rats/group at 0 (2.5% Methofas), 5 or 20 mg/kg/day. No maternal or fetal toxic effects related to treatment reported other than skin reaction. No adverse effect indicated. NOEL (maternal and developmental) ≥ 20 mg/kg/day. Unacceptable, not upgradeable (lack of maternal toxicity at high dose, only 2 dose levels). (Green, Morgan 12/20/88 and Gee, 4/10/89)

001 907748  Brief summaries of record numbers 070696, 070697, and 070698.

DEVELOPMENTAL TOXICITY, RABBIT

006 070698, “Teratogenicity, Oral Administration,” (Performing laboratory not specified, no date), bronopol, no purity, daily administration by gavage on gestation days 6-18 at 0 (water), 1.0, 3.3 or 10.0 mg/kg/day with 7, 9 or 10 mated female NZW rabbits/group. No maternal or developmental effects demonstrated. No adverse effect indicated. NOEL (maternal and developmental) ≥ 10 mg/kg/day. Unacceptable, not upgradeable (Adequacy of the high dose not demonstrated, too few mated females/group). (Green, Morgan 12/21/88 and Gee, 4/11/89)
** 50199-0019 121022; “Bronopol Oral (gavage) Rabbit Developmental Toxicity (Teratogenicity) Study” (Author: Irvine, L. F. H; Toxicol Laboratories Ltd., Herefordshire, UK, Report # TX92042, 4/24/92); 833; Bronopol (batch # 888902, 99.8% purity); 0, 5, 20, 40 or 80 mg/kg/day by oral gavage on days 7 to 19 of pregnancy; 17 - 19 dams/dose; two dams were killed during the dosing period; necropsy exam of the dam from the 5 mg/kg dose group indicated hemorrhaging in the thoracic cavity and lungs suggesting dosing accident, whereas the other dam at 80 mg/kg exhibited gastric ulceration; loss of mean body weight with reduced food consumption at the onset of dosing at 80 mg/kg/day between days 7 and 9, which continued to day 11; however, by day 15 most of the animals had regained the lost weight with compensatory increase in food consumption; **no adverse effects;** decreased mean fetal weight and increased incidence of fetuses with retarded skeletal ossification at 80 mg/kg/day; maternal NOEL = 40 mg/kg/day (gastric ulceration with loss of body weight and reduced food consumption), developmental NOEL = 40 mg/kg/day (based on reduced fetal weight and retarded skeletal ossification); acceptable; (Leung, 7/15/93).

50199-0079; 234461-SUP; “Bronopol: Oral gavage developmental toxicity probe study in New Zealand white rabbits,” (Carney, E., Tornesi, B., Stebbins, K. and Card, T.; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674; 12/23/05); Groups of 7 time-mated female New Zealand white rabbits were administered by gavage with 0, 10, 40, 80 or 120 mg/kg/day bronopol on gestation days 7 to 27. Cage-side examination of the animals were conducted twice daily, clinical observations were conducted on all animals at least once daily. Body weights were recorded on GD (Gestation Day) 0, daily during the dosing period and on GD 28. Daily feed consumption was recorded and statistically analyzed for all animals from GD 4-28. On GD 28, all surviving adult females were submitted for a complete necropsy and a detailed examination of the reproductive tract was performed. The number and position of implantations, viable fetuses, and resorptions were recorded. The animals in the highest dose group were euthanized on GD 9 due to loss of body weights, reductions in mean feed consumption, and clinical signs. Rabbits given 80 mg/kg/day bronopol were euthanized on GD 16 due to progressive decline in feed consumption and body weight loss. One rabbit from the 40 mg/kg/day bronopol group was euthanized on GD 19 due to no feed consumption for 3 consecutive days and clinical signs. Sporadic signs of decreased/soft feces and reduction of mean feed consumption was observed in rabbits from the 40 mg/kg/day group. There were no treatment related effects on reproductive parameters. Maternal NOEL: 10 mg/kg/day [based on reduced body weight, food consumption and clinical signs], Embryo/Fetal NOEL : 40 mg/kg/day. Study supplemental. Pan and Leung, 1/25/12.

**GENE MUTATION**

50199-009 070703, “Mutagenicity Testing by Means of In Vitro Microbial Tests, the Host-Mediated Assay, and the Dominant Lethal Assay in Mice,” (performing laboratory not specified, no date), bronopol, no purity, single daily oral administration on six succeeding days at 0 (0.9% saline), 12.5, 25 or 50 mg/kg/day with 5 female OLAC mice/group. Intraperitoneal injection of Salmonella typhimurium TA1530 concomitant with last dose. Sampled after 3 hours. Three plates per sample. **No increase in revertants. Unacceptable**, not upgradeable (adequacy of high dose not established). (Green, Morgan 1/11/89 and Gee, 4/11/89)
50199-009 070703, “Mutagenicity Testing by Means of In Vitro Microbial Tests, the Host-Mediated Assay, and the Dominant Lethal Assay in Mice,” (performing laboratory not specified, no date), bronopol, no purity, tested at 1% in the diffusion bead assay and at 40 μg/plate in the semi-quantitative plate incorporation assay, duplicate plates. Positive controls included N-methyl-N’-nitro-N-nitrosoguanidine (in DMSO), 9-aminoacridine hydrochloride, and 2,7-diaminofluorene (in DMSO). No increase in revertants reported. Unacceptable, not upgradeable (insufficient number of plates, inadequate strains). (Green, Morgan 1/13/89 and Gee, 4/11/89)

50199-009 070704, “Bronopol-Boots: In Vitro Bacterial Mutagenicity Testing,” (The Boots Company PLC, Research Department, report # TX 86004, 1/23/86), bronopol pharmaceutical grade, 99.7% purity, reverse mutation assay with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 with and without activation (Aroclor 1254 induced S9 male rat liver fraction), 2 trials in triplicate, at 0 (distilled water), 3.9, 7.8, 15.6, 31.2, 62.5 or 125.0 μg/plate. No increase in reversion rate reported. Unacceptable with minor variances - no individual plate counts - upgradeable. (Green, Morgan 12/21/88 and Gee, 4/11/89)

**009 070705, “Bronopol-Boots: In Vitro Mammalian Cell Mutation Assay,” (The Boots Co. PLC, Research Department, report # TX 86043, 8/21/86), Bronopol Pharmaceutical Grade, 99.7%, forward mutation assay with Chinese hamster V79 cells with and without (Aroclor 1254 induced S9 male rat liver fraction) activation, multiple trials at 0 (distilled water), 0.5, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0, 8.0 or 16.0 mg/ml. No conclusive evidence for forward mutation. Acceptable. (Green, Morgan 12/22/88 and Gee, 4/11/89)

001 907750, Brief summary of record numbers 070703, 070704, and 070705.

CHROMOSOME EFFECTS

50199-009 070703, “Mutagenicity Testing by Means of In-Vitro Microbial Tests, the Host-Mediated Assay, and the Dominant Lethal Assay in Mice,” (conducting laboratory not specified, no date), bronopol, no purity, no stability, administered orally once daily for six consecutive days at 0 (water), 20 or 100 mg/kg/day or as a single intraperitoneal dose at 10 mg/kg with 10 or 20 OLAC male mice/group. Mated 1 male to 3 females for 4 consecutive weeks. Adverse effect: reduced numbers of live implants/female in weeks 2 and 3 post-treatment in groups where males received 100 mg/kg and in week 4 where males were treated intraperitoneally with a single dose at 10 mg/kg. Intraperitoneal NOEL < 10 mg/kg, oral NOEL = 20 mg/kg. Unacceptable, not upgradeable (inadequate period of testing, test article characteristics, date and performing laboratory). (Green, Morgan 1/11/89 and Gee, 4/11/89)

** 50199-009 070707, 070708, 070709, “Bronopol-Boots: In Vitro Human Lymphocyte Clastogenicity Testing,” (The Boots Co. PLC, Research Department, report # TX 86049, 8/21/86), Bronopol, 99.7% purity, batch # 845454C, in vitro chromosomal damage assay in human lymphocytes with and without activation (Aroclor 1254 induced S9 male rat liver fraction) at 0 (distilled water), 10, 20, 30, or 40 mg/ml with 1 or 2 trials; scored 100 cells per concentration and mitotic index reported. Positive controls - Cyclophosphamide with activation, Mitomycin C without. Adverse effect: increased percentage of cells containing chromosomal aberrations, both including and excluding gaps, reported in both trials at 30 mg/ml without
activation. Record 070708 reported the decomposition of bronopol in culture medium in 2 and 24 hours; Record 070708 reported the formation of formaldehyde over a 24 hour period. **Acceptable.** (Green, Morgan 12/23/88 and Gee, 4/14/89)

**NOTE:** Records 121084 and 121085 are supplemental to 070707.

**Note:** Formaldehyde is reportedly released (peak concentration of 4.2 mg/ml reached after 2 hours incubation at 37 °C) as bronopol decomposes in culture under the conditions of the in vitro human lymphocyte clastogenicity assay and is thought to cause the chromosomal damage seen. See record # 070710.

50199-009 070711, “Bronopol-Boots: Genotoxicity in Short-Term Tests,” (The Boots Company PLC, Research Department, report # TX 86054, 8/21/86), bronopol, summarizes results of mutagenicity studies (Ames test, V79 cell mutation assay, host-mediated (mouse) bacterial assay, dominant-lethal assay in male mice, micronucleus assay in male and female mice, and in vitro human lymphocyte chromosome assay). **Weak in vitro clastogenic activity** in cultured human lymphocytes reported - probably record 070707 in 009. (Green, Morgan 1/13/89)

001 907751, Brief summary of record number 070703.

**DNA DAMAGE**

**50199-009 070706, “Bronopol-Boots: Micronucleus Assay in Mice,”** (The Boots Co. PLC, Research Department, report # TX 86001, 1/21/86), Bronopol, 99.7% purity, batch 845454C, administered by gavage at 0 (distilled water), 80 or 160 mg/kg with 12 or 24 CD-1 mice/sex/group. Four or 8 mice/sex/group were sacrificed at 24, 48, and 72 hours and femoral bone marrow smears taken. Four (4) mice/sex died within 48 hours of dosing at 160 mg/kg, one female at 80 mg/kg died within 72 hours. **No increase in micronuclei** reported. The proportion of polychromatic cells in the smears decreased at 72 hours in males at both doses and in females at 80 mg/kg. **Acceptable.** (Green, Morgan 12/22/88 and Gee, 4/14/89)

** 50199-0059 185160; “In Vivo/In Vitro Unscheduled DNA Synthesis in Rat Primary Hepatocyte Culture at Two Timepoints with a Dose Rangefinding Assay with Bronopol,” (M. A. Cifone; Covance Laboratories, Inc., Vienna, VA; Study No. 22387-0-494OECD; 10/11/01); Male Fischer 344 rats were dosed by oral gavage with 0 (vehicle control-water), 100 or 200 mg/kg of Bronopol (lot no. SKF-PD015-00001, purity: 98.5%) and euthanized at 2 to 4 hours or 14 to 16 hours after dosing. Ten animals/group were dosed with 0 or 100 mg/kg of the test material. Fourteen animals were treated with 200 mg/kg. Four animals each in the positive control group were treated with 10 or 15 mg/kg of N-dimethylnitrosamine (DMN) and euthanized at 2 to 4 hours or 14 to 16 hours, respectively, after dosing. Upon recovery of the hepatocytes, a primary culture was established and the cells were exposed to ³H-thymidine (10 µCi/ml) for 4 hours, followed by further incubation overnight with unlabeled thymidine. There were 3 cultures/animal in one trial used for the unscheduled DNA synthesis analysis. Three of the animals in the 200 mg/kg group died. There were 3 cultures/animal in one trial. There was no treatment-related increase in unscheduled DNA synthesis. **No adverse effect indicated.** The positive control was functional. **Study acceptable.** (Moore, 10/10/02). Record 185159 at the beginning of this volume is a re-statement of the abstract.
50199 - 052; 182806; “Bronopol UDS Dose Range Finding Study” (M. A. Cifone, Covance Laboratories, Inc., Vienna, VA, Study ID # 011069, 7/2001). Three Fischer 344 rats/sex/dose were treated orally via gavage at 100, 200, 300, 500, and 600 mg/kg body weight with Bronopol (Lot SKF-PD015-00001, purity not provided). Animals were observed within 0.5 hours and 2 - 4 hours of dosing daily for three days for toxic signs and mortality. All animals dosed at 600 and 500 mg/kg were found dead by 2 days after dosing, as were two males and three females dosed at 300 mg/kg, and 1/3 females at 200 and at 100 mg/kg. Ataxia [Possible adverse effect] was reported in both sexes (females at all dose level and males treated at doses ≥ 500 mg/kg). Other clinical signs included paleness, rough haircoat, squinted eyes, irregular respiration and lacrimation were observed. Supplemental (Leung, 10/1/2001). (Pilot study for Record No. 185160, above).

NEUROTOXICITY

Not required at this time.

METABOLISM

50199-0076, 234458, “Bronopol: Oral Absorption, Distribution and Elimination in Crl:CD(SD) – Sprague Dawley derived Rats ,” Metabolism study; 851; Rat; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674, 1/23/07; Saghir, S. A. and Clark, A. J., study ID: 061134, a.i.: Bronopol, purity: 99.85%, radiolabeled purity: 96.6% Lot # for non-radiolabeled: TC0601X702, Reference # for radiolabeled: 35096-64E. Groups of 4 male rats/dosing group were administered a single oral dose of 1 or 30 mg/kg 14C- Bronopol or multiple oral doses of 1 mg/kg non-radiolabeled Bronopol followed with 14C-radiolabeled Bronopol. Radioactivity in urine, feces expired volatiles, expired CO2 were measured for 5 days after dosing. The mean percentage of administered doses recovered were 92.47% and 90.59% respectively for 1 and 30 mg/kg groups. The total radioactivity excreted in urine was 69.05% and 69.90% of the administered dose for 1 and 30 mg/kg groups, respectively. Excretion via feces was lower: 17.21% and 13.47% of the administered doses in the 1 and 30 mg/kg groups respectively. The excretion via volatiles and CO2 was low: less than 2% in the 1 and 30 mg/kg groups. At termination, the liver and kidney in groups sacrificed 5 days after dosing had the highest concentrations of radioactivity. Study supplemental due to lack of identification of major metabolite(s). Pan and Leung (1/23/12).

50199-009 070712, “The Metabolism of Bronopol (2-Bromo-2-Nitropropane-1,3-Diol) After Oral Administration to Rats and Dogs, and Application to the Skins of Rats and Rabbits,” (Huntingdon Research Centre, report # BTS31/74149, 5/15/74), [14C]-Bronopol, labeled in the 2-carbon atom position of the molecule; specific activity 21 μCi/mg; radiochemical purity estimated by TLC at 99%. A single record contains several species and routes. All reviews originally by Green and Morgan (reviews completed 1/20/89 to 4/19/89). Some edits and organization of data by species and route by Aldous (Nov. 9, 2012).

Rat gavage:
Six rats received a single dose by gavage at 1 mg/kg (numbers/sex not specified). 80.9% of radioactivity was excreted in urine and 6.3% in expired air at 24 hours. In 48 hours, 5.2% was excreted in feces. During 5 days, rats excreted 83.3% of radioactivity in urine, 5.8% in feces (via
the bile) and 8.4% in expired air. Peak blood levels of radioactivity were reported within 2 hours after dosing. Five metabolites were found in urine with “A” being the major one. “A” was the only one extractable from plasma, no $^{14}$C bronopol was detected in blood. Unacceptable, not upgradeable (insufficient numbers of animals and insufficient tissues for % recovery measurements. No dose level justification). (Green, Morgan 1/20/89). Notes by Aldous: Metabolite “A” was determined by investigators to most likely be 2-nitropropane-1,3-diol. Other metabolites were not characterized. This study included two bile-cannulated rats, which passed about 7% of administered dose in bile, suggesting that fecal excretion represented absorbed bronopol. Expired air contained 6.3% of administered dose.

**Oral, dog, (gel capsule):**

Beagle dogs (numbers/sex not included) received gelatin capsules at 1 mg/kg. Up to 63.9% of radioactivity was excreted in urine in 12 hours after dosing. After 5 days, 81.1% had been excreted in the urine and 3.1% in feces. No adverse effect indicated. Unacceptable, not upgradeable (one dose level without justification).

**Rat dermal:**

Six rats received a single occluded dose in acetone at 4.8 mg/ml to clipped, intact skin (volume of 0.05 ml/rat applied to 1 cm²). > 77% remained on or in the skin at the site of application after 6 to 96 hours. Five metabolites were found in the urine with “A” being the major one. No adverse effect indicated. Unacceptable, not upgradeable (6 animals, inadequate area exposed, limited tissue distribution, influence of vehicle on results).

**Rabbit dermal:**

$[^{14}C]$-Bronopol, labeled in the 2-carbon atom position of the molecule; specific activity 21 μCi/mg; radiochemical purity estimated by TLC at 99%, single occluded treatment administered in acetone at 4.8 mg/ml to clipped (3) or cream depilated (1), intact skin of 4 rabbits (2/sex) (volume of 0.2 - 0.4 ml/rabbit applied to 10 cm²). 37% to 61% of dose remained at treatment site 6, 12, 24 and 48 hours post-treatment. 0.27% to 6.6% of radioactivity was found in the urine of rabbits with clipped skin after from 6 to 24 hours and 25% in urine of the female with dipilated skin at 48 hour sampling. No adverse effect indicated. Unacceptable, not upgradeable (4 animals only). (Green, Morgan 1/24/89, with edits by Gee 4/19/89).
mg/kg/day with 3 Beagle dogs/sex/group. Vomiting reported approximately 30 min after dosing at 20 mg/kg/day through week 6. Thereafter dosing routine was changed so that the dogs were fed 2 hours prior to dosing. Increased group mean liver and spleen weights (as % of bodyweight) at 20 mg/kg/day. No abnormal histopathology was observed. **No adverse effects** indicated. NOEL = 8 mg/kg/day (increased liver and spleen weight). **Unacceptable**, not upgradeable (insufficient number of animals). (Green, Morgan 12/16/88). NOTE: report 50199-0018 121018 and 121020 are duplicates of these records.

50199-0004 070686, “12-Week Feeding Trial (Rat),” (performing laboratory not specified, no date), bronopol, no purity, fed in the diet for 12 weeks at 0, 100 or 1000 ppm with 8 or 12 Boots-Wistar rats/sex/group. No treatment related effects reported. **No adverse effect** indicated. Reported NOEL = 1000 ppm. **Unacceptable**, not upgradeable (no ophthalmology, no diet analysis, 2 dose levels, incomplete histopathology, insufficient numbers). (Green, Morgan 1/9/89).

50199-004, -016; 70687, 120866; “Bronopol Oral toxicity to Rats Repeated Administration for 13 Weeks,” (author: Hunter, B., et. al., Huntington Research Center, Huntingdon, UK, Report # BTS34/73268, 8/29/73); 821; bronopol (batch # CT 89075N, purity 99 - 100%); daily (7 days/week) administration by gavage at 0 (distilled water), 20, 80, and 160 mg/kg/day at a volume of 5 ml/kg with 20/sex/group. At 160 mg/kg/day respiratory distress, abdominal distension, reduced food consumption, and reduced body weight gain were widely noted with all animals either dead or sacrificed in extremis on or by day 9. At 80 mg/kg/day: 7 males and 9 females died on test, respiratory distress and abdominal distension that subsided by week 8 in survivors was reported in a majority of animals, reduced body weight gain and food consumption during first week of treatment was also reported. At 20 mg/kg/day: 1 male reported with respiratory distress during week 1 and with reduced body weight gain through week 2. Thereafter, weight gain was similar in this group and respiratory distress was no longer apparent. **Possible adverse effect**: renal changes (dilated tubules containing eosinophilic material) seen in 2 males at 20 mg/kg/day and in 2 males at 80 mg/kg/day; NOEL < 20 mg/kg/day (abnormal histopathological changes in the kidney); originally reviewed as unacceptable but possibly upgradeable with submission of test article characteristics (Green and Morgan, 12/15/88); subsequently reviewed with additional data and was found to be acceptable; (Leung, 7/16/93).

50199-0075, 232406, “Bronopol: 28-Day drinking water toxicity study in Beagle dogs,” subchronic oral toxicity study; 821; Beagle dogs; MPI Research, Inc., 54934 North Main Street, Mattawan, Michigan 49071-9399, 12/15/06; Stewart, C., study #: 133-070, k-081547-042, a.i.: Bronopol. Groups of 2/sex Beagle dogs were administered 0, 0.005, 0.025 and 0.05% Bronopol in drinking water for at least 28 consecutive days. [Mean uptake levels were: 4.47, 20.73 and 40.59 mg/kg/day for males and 4.27, 15.40 and 32.65 mg/kg/day for females]. All animals were observed for mortality, morbidity, injury and availability of food and water twice daily, for clinical signs including neurobehavioral findings, body weights and food consumption weekly. Water consumption was measured and recorded daily from day -3 to 29. Ophthalmoscopic examinations were conducted, and blood samples for clinical pathology evaluations were collected pretest and prior to scheduled necropsies. Urine samples were collected from all animals at scheduled necropsies. At scheduled necropsies, all animals were examined macroscopically and microscopically for abnormalities and lesions, weights of selective organs were measured. Multifocal, subacute to chronic inflammation of the nasal mucosa was observed in all Bronopol treated males and ½ per group of all treated females. Local irritation and inflammation of the nasal mucosa in all treated animals is likely caused by intermittent reflux of
drinking water into the nasopharynx and caudal nasal passages. Multifocal hypertrophy of mucus cells in the stomach was observed in some dogs treated with 0.025 or 0.05% Bronopol. This was interpreted to be a treatment-related adaptive response to gastric irritation from direct contact with the test article. NOEL for systemic toxicity was 0.05% Bronopol in drinking water due to lack of evidence for systemic toxicity. NOEL for local effects was not established. Study supplemental. Pan and Leung, 1/30/12.

VARIOUS SHORT-TERM STUDIES (other than routine acutes on end-use products)

50199-0116  267672  This study was submitted as an “adverse effects report,” which is a designation not usually given to an acute oral study. A report of this study was previously submitted to DPR, and was reviewed by Kellner. Study identification from Kellner one-liner reads: 50199-0067 204670;  Acute oral toxicity; 811; Rat; The Dow Chemical Company, Toxicology & Environmental Research and Consulting, Midland, MI; Study # 011128; 9/25/01.

50199-0087  242071  Wiescinski, C. M., and L. K. Sosinski, “Bioban™ QK-PRO: local lymph node assay in CBA/J mice,” The Dow Chemical Co., Midland, MI, 8/13/08. Laboratory Project Study ID 081077. There were 6 female mice/dose, at 0, 0.4%, 2%, or 10% Bioban™ QK-PRO formulation in propylene glycol. The high dose was justified by severe erythema at 25% test article in preliminary testing. Positive control was α-hexylcinnamaldehyde (HCA), diluted to 30% in propylene glycol. Investigators applied 25 µl/ear to the dorsal surface of each ear on days 1, 2, and 3. Mice were evaluated for body weight and for erythema (neither was affected at Bioban™ QK-PRO doses tested). To determine proliferative response by stimulation index (SI), mice were injected iv (tail vein) with 20 μCi ³H-thymidine 5 hrs before sacrifice. Auricular lymph nodes were excised at sacrifice and gently homogenized. Cells were suspended in trichloroacetic acid, and prepared for scintillation counting. An SI ≥ 3 was considered to indicate sensitization. Body weight gains with test article were not affected by treatment. Positive control mice lost over 10% of body weight by day 6. SI’s for controls through progressive test article concentrations were 1 (control reference), 0.7, 0.6, and 5.4, respectively, hence highest dose indicated a sensitizer. The SI for positive control was 9.3. No test article levels caused erythema. Positive control elicited erythema scores of 2 on treatment days 2 and 3, and score of 1 in all mice on day 6. Study is acceptable. Aldous, Nov. 2, 2012. [Note: various sensitization studies have shown bronopol to be at least a weak sensitizer, including one human epidemiological study (Record 184250). This is reflected in some or all use label statements].

50199-0057  184250  Frosch, P. J. et al., “Contact Allergy to Bronopol,” Contact Dermatitis 1990: 22: 24-26. Patch tests, principally conducted in London, found 0.21% of human subjects to have “clinically relevant” allergic reactions. About one-third of affected persons also reacted to formaldehyde, which is a breakdown product of bronopol. This 3-page report was justifiably not considered to be “reviewable” when read by Kellner in 2002. Aldous, 11/9/12.

50199-0004  070690. “Effect of Repeated Administration of Bronopol to the Skin of Rabbits for Three Weeks,” (Huntingdon Research Centre, 12/18/73), bronopol, no purity, no stability, 6 hour daily exposure for 3 weeks without occlusion to clipped, abraded skin of 5 NZW rabbits/sex/group at 0 (2.5% methyl cellulose), 0.2% or 0.5%. No deaths. Slight to well-defined erythema reported days 1 through 21 in all groups. Moderate to severe erythema reported days 4
through 21 at 0.5%. Well-defined edema reported at 0 and 0.2%, moderate edema reported at 0.5%. **Adverse effect**: histopathology revealed dermal scarring at 0.2% and 0.5%. NOEL < 0.2%. **Supplemental**. (Green, Morgan 12/19/88).

50199-0001 907741 This first volume for the a.i. included a very brief summary of toxicity studies available as of 1979. No data are in such detail as to be reviewable. Aldous, 11/9/12.

**STUDIES ON METABOLITES, ENVIRONMENTAL BREAKDOWN PRODUCTS, OR PRODUCT CONTAMINANTS**

**50199-009 070710**, “Formaldehyde: In Vitro Human Lymphocyte Clastogenicity Testing,” Everest, R. P., and C. V. Williams (Study relates to formaldehyde as breakdown product of bronopol). The Boots Co. PLC, Research Department, report # TX 86050, 8/21/86. Formaldehyde solution (Fisons A.R.), batch # 93, 38% w/v, in vitro chromosomal damage assay in human lymphocytes without activation at 0 (distilled water), 0.5, 1.0, 2.0, 4.0, 6.0 or 8.0 mg/ml, no replicates, scored 100 cells per concentration and recorded mitotic index. **Adverse effect**: increase in chromosomal damage reported at 6 and 8 mg/ml. **Supplemental** to record # 070707. (Green, Morgan 12/23/88 and Gee, 4/14/89). Note by Aldous on Nov. 2, 2012. This study evaluated formaldehyde at 0, 4, 6, and 8 µg/ml for gaps, chromosome deletions or exchanges, and for chromatid deletions or exchanges. There was no response at 4 µg/ml, a modest response at 6 µg/ml (mostly gaps), and a marked response at 8 µg/ml (additional gaps, but many chromatid deletions and exchanges). This strong (non-linear) dose-response was quite similar to that of bronopol in Record No. 070707.

**DUPLICATE RECORDS** (often due to studies being submitted by different companies)

50199-009 070703 is the same report as Record 50199-024 121079
50199-009 070704 is the same report as Record 50199-024 121080
50199-009 070705 is the same report as Record 50199-024 121081
50199-009 070706 is the same report as Record 50199-024 121082
50199-009 070707 is the same report as Record 50199-024 121083
50199-009 070711 is the same report as Record 50199-024 121087
50199-0018 121016 is the same report as Record 50199-004 70686
50199-0018 121017 is the same report as Record 50199-004 70687
50199-0020 121024 is the same report as Record 50199-007 70701
50199-0022 121027 is the same report as Record 50199-004 070690
50199-0024 121086 is the same report as Record 50199-009 070710
50199-0024 121088 is the same report as Record 50199-009 070712
50199-0024 121089 is the same report as Record 50199-009 070713