REACH

Bronopol

EC number: 200-143-0 | CAS number: 52-51-7

Toxicological information

Repeated dose toxicity: oral

001 Key | Experimental result

Administrative data

Endpoint:	chronic toxicity: oral
Type of information:	experimental study
Adequacy of study:	key study
Reliability:	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies:	other: see 'Remark'
Remarks:	The test substance bronopol was determined in terms of batch numbers (batch No: CT 92495T used for the test period ranging from week 1 to week 51, and batch No: CT 95274W used for the test period ranging from week 52 to week 104), but no further details were provided. The study was conducted prior to the implementation of guideline and GLP was compulsory at this time. The stability of the test substance in aqueous solution was monitored at a later time point . However, all data taken together are of scientific acceptability.

Data source

Reference

Reference 1

Reference Type:	study report
Title:	Unnamed
Year:	1976

Reference 2

Reference Type:	study report
Title:	Unnamed
Year:	1998

Reference 3

Reference Type:	other: Report
Title:	Unnamed
Year:	1985

Reference 4

Reference Type:	study report
Title:	Unnamed
Year:	1985

Reference 5

Reference Type:	study report
Title:	Unnamed
	1993

Year:	

Reference 6

Reference Type:	study report
Title:	Unnamed
Year:	1986
Report date:	1986

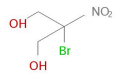
Materials and methods

Principles of method if other than guideline:	The study was conducted prior to the implementation of guideline and GLP was compulsory at this time.
GLP compliance:	not specified

Test material

Test material information

Constituent 1



Reference substance name:	Bronopol
EC Number:	200-143-0
EC Name:	Bronopol
Cas Number:	52-51-7
Molecular formula:	C3H6BrNO4
IUPAC Name:	2-bromo-2-nitropropane-1,3-diol
Test material form:	not specified
Details on test material:	No data on purity.

Test animals

Species:	rat
Strain:	Sprague-Dawley
Sex:	male/female
Details on test animals or test system and environmental conditions:	A total of 480, 28 +/- 1 day old CD rats, a caesarian-derived strain of Sprague-Dawley origin, was obtained from Charles River Laboratories, St. Aubin-les-Elbeuf, France, on 14 May 1973. Following an initial quarantine and acclimatisation period of four days to accustom the rats to the environmental conditions existing in our laboratories, each animal was weighed and allocated to one of several arbitrary weight ranges, each containing rats with bodyweights differing from one another by no more than +/- 2.5 g. Equal numbers of animals from within each weight range were randomly allocated to each of the four treatment groups, and all animals then identified by earmark. In this way we ensured that each group contained a similar population of rats arid initial. mean bodyweights were also approximately equalised. Treatment conmmenced after a predosing period of one week during which time the water intake, food consumption and bodyweights were measured. The rats were housed five to a cage (unless the number was reduced by mortality) in suspended cages with wiremesh floors. Animal room temperature and relative humidity were controlled at 21 +/- 20C and 50 +/- 5% respectively, and lighting was controlled to give 12 hours light (8 a.m. to 8 p.m. B.S.T.) and 12 hours dark per 24 hours. The cages constituting each group were dispersed in batteries so that possible environmental influences arising from their spatial distribution were equilibrated, as far as possible, for all treatments. All rats had free access to standard laboratory rat food, Spratt's Laboratory Diet 1.

Administration / exposure

Route of administration:	oral: drinking water
Vehicle:	water

Duration of treatment / exposure:	104 wk
Frequency of treatment:	7days/week

Doses / concentrations

Doses / concentrations 1

Dose / conc.:	10 mg/kg bw/day (nominal)
Remarks:	re-evaluated dose: 7 mg/kg bw/day

Doses / concentrations 2

Dose / conc.:	40 mg/kg bw/day (nominal)
Remarks:	re-evaluated dose: 32 mg/kg bw/day

Doses / concentrations 3

Dose / conc.:	160 mg/kg bw/day (nominal)
Remarks:	re-evaluated dose: 142 mg/kg bw/day

No. of animals per sex per dose:	45 male and 45 female rats per dose in main groups. 15 male and 15 female rats per dose in satellite groups.
Control animals:	yes
Details on study design:	The test consisted of a main test series and a satellite test series. Each test series consisted of 4 test groups. Each test group of the main test series comprised 45 animals/sex/group whereas each test group of the satellite series comprised 15 animals/sex/group. Main test series: The animals of the main test series were used for evaluation of the carcinogenic potential of the test substance. Satellite test series: The animals of the satellite series were used for the evaluation of haematological and clinical-chemical parameters, and for urinalysis. These animals were not considered for carcinogenicity evaluation.

Examinations

Observations and examinations performed and frequency:	The body weight of each rat was recorded at test initiation and weekly thereafter. The quantity of food consumed was recorded for each cage of rats (each cage contained 5 animals) and the mean weekly intake was calculated. Food efficiency was assessed by calculation of the mean food conversion ratios (FCR values) during the period of fastest growth (week 1 to 24) as weights of food consumed per unit gains in body weight. Water consumption was measured daily and the mean weekly intake was calculated. The animals were regularly observed for clinical symptoms of toxicity, changes in behaviour, and mortalities. Ophthalmoscopic examinations were conducted on the animals of the control and the high dose groups (i.e. 0 and 160 mg/kg bw/day) of the main test series only. Examination time points were: prior test initiation, week 0 (pre-dosing period), week 26, week 52, week 78 and week 103. Haematology: Test groups 1 and 4 (i.e. control and 160 mg/kg bw/day), 10 animals /sex /group, blood samples removal from the orbital sinus of fasted animals, Time points: Week 26, 52, 78 and 102. Parameters: Packed cell volume, haemoglobin, red cell count, mean corpuscular haemoglobin concentration, mean cell volume, total white cell count, differential count (neutrophils, lymphocytes, eosinophils, basophils, monocytes), platelet count, and thrombotest. Clinical chemistry: Time points: Week 26, 52, 78 and 102. Parameters: Plasma urea, plasma glucose, total serum proteins, serum protein electrophoresis and albumin/globulin (AG) ratio, serum alkaline phosphatase (SAP), serum glutamic-pyruvic transaminase (SGPT), sodium and potassium. Urinanalyses: Number of animals: Satellite test series Test groups 1 and 4 (i.e. control and 160 mg/kg bw/day), and additionally test groups 2 and 3 (i.e. 10 and 40 mg/kg bw/day), 10 animals /sex /group, Urine samples were collected overnight from fasted animals. Time points: Test groups 1 and 4: Week 26, 52, 77 and 103. Test groups 2 and 3: Week 77 and 103 Parameters: Test groups 1 and 4: Specific gravity, pH, protein, reducing substances, glucose, ketones, bile pigments, urobilin, haemoglobin.

	Test groups 2 and 3: Haemoglobin, microscopy of spun deposits (examination was repeated for verification in case of animals showing haemoglobinuria).
Sacrifice and pathology:	At test ending, all surviving animals of both test series (main and satellite) were sacrificed for the purpose of necropsy and were subjected to organ weighing and to gross pathological and histopathological examination. Animals that died or were sacrificed in extremis during the test period also were subjected to gross pathological and histopathological examination for determination of the cause of death. Gross pathology: All superficial tissues including urogenital orifices, tails, auricles, auditory meatus, and painted skin areas were checked for abnormalities such as swelling, distortion or other evidence of tumor formation. The nares, mouth, tongue, pharynx and auditory region were examined. The cranial roof was removed for examination of the brain, the pituitary gland and the cranial nerves. The subcutaneous tissues including regional lymph nodes, mammary glands and salivary glands were examined. The abdominal and thoracic contents were examined. All gross abnormalities were recorded. Organ weights: All surviving animals of both test series, at terminal sacrifice Absolute and relative weights were determined for liver, kidneys, adrenals, testes, uterus, ovaries, spleen, lungs, brain, heart, seminal vesicles, prostate, pituitary and thyroid. Histopathology: All surviving animals of both test series, at terminal sacrifice. Samples of following organs/tissues were taken, fixed in buffered 10% formalin and processed for histological examination: All abnormal tissues, brain, pituitary, thymus, salivary glands, stomach, caecum, ileum, liver, kidneys, spleen, heart, lungs, gonads, uterus, adrenals, urinary bladder, lymph nodes, pancreas, bone marrow, eye (fixed in Davidson´s fixative), blood smears. For microscopical examination, the tissue samples were embedded in paraffin; the embedded samples were then sectioned, and the sections were Haematoxylin/Eosin stained. Liver and kidney samples were frozen and frozen sections were stained for fat with Oil Red O and for glycogen (liver) or basement membranes (kidney) with periodic acid Schiff reagent. Additional sections from tissues in which colonies of microorganisms were seen were prepared and stained with Gram stains. Selected samples for microscopical examination: All tissue listed above and collected from 10 animals/sex from the control group and from 12 males and 15 females of the high dose group (main test series) were subjected to microscopical examination. Samples of following organs/tissues were taken, fixed in buffered 10% formalin and were not processed for histological examination in the first instance: Oesophagus, spinal cord, tongue, jejunum, trachea, aorta, prostate, seminal vesicles, female mammary gland, sciatic nerve, bone, skeletal muscle, skin, second eye (fixed in Davidson´s fixative). Neoplastic findings: All rats of the main test series were examined for neoplastic changes. Gross abnormalities and lesions suggestive of neoplasia were recorded. Following series of organs was considered for tumorigenicity screening: Adrenals, thyroids, ovaries, liver, spleen, lymph nodes and pituitary gland. Blood smears also were examined for abnormalities indicative of neoplastic change, and when such abnormalities were seen, an examination of bone marrow was added.

Results and discussion

Results of examinations

Clinical signs:	effects observed, treatment-related
Description (incidence and severity):	Clinical symptoms of toxicity: During the last year of treatment, a reduction in grooming activity was noted in the groups treated with 160 mg/kg bw/day of test substance. No further treatment-related effects were reported. From week 9 to 10 of treatment all animals suffered from a viral disease, which occasionally affects rats of the used strain. The viral disease was identified as sialodacryoadenitis and persisted for 2 to 3 weeks; during this time, the rats showed reduced appetite and decrease in body weight gain and body weight. Thereafter, the rats recovered and by week 12, they appeared normal. This disease did not affect the test conduct.
Mortality:	mortality observed, treatment-related
Description (incidence):	An increase in mortality was observed in the 160 mg/kg bw groups when compared to controls; mortality affected more male animals than females. In the high dose group of the main test series the difference to control was statistically significant.

	For the remaining groups, recorded mortalities were within control range (see Table 4).
Body weight and weight changes:	effects observed, treatment-related
Description (incidence and severity):	For the males of the high dose group, body weight gain was decreased in comparison to control starting from week 3 of treatment: The differences against control values were of statistical significance throughout the whole test period. From week 78 till test ending, a significant loss in mean body weight could be evidenced, which however also was related to the high mortality observed towards test ending. For the males of the 40 mg/kg bw group, body weight gain was found to be significantly below control for the period ranging from week 26 to week 78 of treatment. Body weight gain of the males treated with the lowest test dose of 10 mg/kg bw/day was similar to control and therefore inconspicuous. For the females of the high dose group, no differences in body weight gain were seen during the first weeks of treatment. Thereafter and throughout the remaining time of treatment, body weight gain almost was significantly lowered compared to control, and from week 78 until test ending, a significant loss in mean body weight could be evidenced. Body weight gains for the females of the low and mid dose groups almost were similar to control and inconspicuous.
Food consumption and compound intake (if feeding study):	effects observed, treatment-related
Description (incidence and severity):	For the males of the high dose group, food consumption was significantly reduced in comparison to control starting from week 14 of treatment till test ending. A slight reduction in food consumption also was reported for the males of the 40 mg/kg bw group for the period between week 53 and 78 of treatment. For the females of the high dose group, food consumption was similar to control during the first 78 weeks of treatment; thereafter, a slight reduction in food consumption was observed.
Water consumption and compound intake (if drinking water study):	effects observed, non-treatment-related
Description (incidence and severity):	Prior initiation of treatment, water consumption was similar for all groups of animals. Starting from week 1 of dosage and throughout the whole test period, all treated animals showed reduced water consumption compared to control, which furthermore was dose-related. The effect was more pronounced for treated males than for treated females. The reduction in water consumption rather was due to a palatability problem that to the treatment as such. Test substance intake: The group mean bronopol intakes throughout the whole treatment period of 104 weeks were calculated on the basis of water consumption and body weight and were as follows: Group Expected dosage Achieved dosage (mg/kg bw/day) Males Females 2 10 mg/kg bw/day 10.5 mg/kg bw/day 10.4 mg/kg bw/day 3 40 mg/kg bw/day 40.2 mg/kg bw/day 40.7 mg/kg bw/day 4 160 mg/kg bw/day 152.2 mg/kg bw/day 158.4 mg/kg bw/day
Ophthalmological findings:	no effects observed
Description (incidence and severity):	Examination of the eyes revealed no treatment-related effects.
Haematological findings:	no effects observed
Description (incidence and severity):	No treatment-related and/or biologically relevant differences between treated and control animals could be evidenced.
Clinical biochemistry findings:	no effects observed
Description (incidence and severity):	No treatment-related and/or biologically relevant differences between treated and control animals could be evidenced.
Urinalysis findings:	effects observed, treatment-related
Description (incidence and severity):	In the 160 mg/kg bw group a decrease in urine volume was seen during weeks 26 and 52, which was related to the decreased water consumption. During week 77, urine volumes of the high dose animals were similar to those of control animals; however, towards test ending (i.e. week 103), the urine volume produced by the high dose animals again was lower than control.
Histopathological findings: non-neoplastic:	effects observed, treatment-related
Description (incidence and severity):	Kidney: Histopathology of the kidney revealed no treatment-related changes, which would explain the increase in relative kidney weight reported. Moreover, progressive glomerulonephrosis of varying degree was reported for animals of both treated and untreated groups; however, the incidence of this lesion in rats treated with 160 mg/kg bw group was statistically significantly increased compared to control. With regard to histopathology, glomerulonephrosis was characterized by glomerular abnormalities, atrophic or

	cystically dilated nephrons, which often were lined by basophilic epithelium, and interstitial fibrosis with foci of lymphocytic infiltration. Glomerulonephrosis is not untypical for the rat strain used. The treatment with bronopol at a test dose of 160 mg/kg bw/day probably exacerbed the occurrence of this spontaneous lesion as a consequence of the reduced renal output observed at the high dosing level. Salivary glands: Squamous metaplasia was seen in the ducts of the salivary glands, and was often associated with minimal mixed or chronic inflammatory cell infiltration, and with groups of atrophic acini. The affected glands often were dilated and displayed minimal epithelial hyperplasia. The incidence and gravity of these effects were increased in the males of the 40 and the 160 mg/kg bw groups and in the females of the 160 mg/kg bw group when compared to control. Squamous metaplasia in the salivary glands is regarded as a spontaneous changes occurring in the rat strain used; the treatment with bronopol probably exacerbed the occurrence of this spontaneous lesion. Lymph nodes: A treatment-related dilatation of the sinusoids in the gastric lymph nodes was reported for 4/12 males and 5/22 females of the 160 mg/kg bw group. The finding generally was associated with hyperplasia and ulceration of the non-glandular epithelium of the stomach. No such changes were seen in the control and the low and mid doses groups.
Histopathological findings: neoplastic:	effects observed, treatment-related
Description (incidence and severity):	Treatment-related macroscopical findings were reported for the high dose animals and affected the stomach (table 5). Lesions in the stomach included thickening of the non-glandular region, raised area with central depressions, warty excrecences and ulceration; these lesions were seen in 9 males and 10 females treated with 160 mg/kg bw of bronopol. Incidental/spontaneous findings were reported, which occurred in both treated and untreated animals, and/or showed no dose-response relationship. Especially in the stomach of high dose rats that died during the experiment, histopathological examination revealed squamous cell papillomata associated with epithelial hyperplasia and ulceration. These findings were rather related to the irritant potential of bronopol than indicative of a tumorigenic potential for this substance. Re-evaluation of the findings: The more recent re-evaluation of the histopathological findings confirmed the findings of the older evaluations, and treatment-related papillomas were reported for the forestomachs of the rats treated with bronopol. The fact to the papillomas were mainly seen in the high dose group and were associated to ulcerations indicates that the tumours rather were a consequence of the irritant potential of bronopol than of a carcinogenic potential of the test substance as such. Carcenogenicity: negative
Details on results:	Body weigth gain: For the males of the high dose group, body weight gain was decreased in comparison to control starting from week 3 of treatment: The differences against control values were of statistical significance throughout the whole test period. From week 78 til test ending, a significant loss in mean body weight could be evidenced, which however also was related to the high mortality observed towards test ending. For the males of the 40 mg/kg bw group, body weight gain was found to be significantly below control for the period ranging from week 26 to week 78 of treatment. Body weight gain of the males treated with the lowest test dose of 10 mg/kg bw/day was similar to control and therefore inconspicuous. For the females of the high dose group, no differences in body weight gain were seen during the first weeks of treatment. Thereafter and throughout the remaining time of treatment, body weight gain almost was significantly lowered compared to control, and from week 78 until test ending, a significant loss in mean body weight could be evidenced. Body weight gains for the females of the low and mid dose groups almost were similar to control and inconspicuous. Food consumption: For the males of the high dose group, food consumption was significantly reduced in comparison to control starting from week 14 of treatment til test ending. A slight reduction in food consumption also was reported for the males of the 40 mg/kg bw group for the period between week 53 and 78 of treatment. For the females of the high dose group, food consumption was similar to control during the first 78 weeks of treatment; thereafter, a slight reduction in food consumption was observed. Water consumption: Prior initiation of treatment, water consumption was similar for all groups of animals. Starting from week 1 of dosage and throughout the whole test period, all treated animals showed reduced water consumption compared to control, which furthermore was dose-related. The effect was more pronounced for treated males than for treated females. The reduction in water consumption rather was due to a palatability problem that to the treatment as such. Test substance intake: The group mean bronopol intakes throughout the whole treatment period of 104 weeks were calculated on the basis of water consumption and body weight and were as follows: Group Expected dosage Achieved dosage (mg/kg bw/day) Males Females

2 10 mg/kg bw/day 10.5 mg/kg bw/day 10.4 mg/kg bw/day
3 40 mg/kg bw/day 40.2 mg/kg bw/day 40.7 mg/kg bw/day
4 160 mg/kg bw/day 152.2 mg/kg bw/day 158.4 mg/kg bw/day

Result (carcinogenicity): negative

Clinical symptoms of toxicity: During the last year of treatment, a reduction in grooming activity was noted in the groups treated with 160 mg/kg bw/day of test substance. No further treatment-related effects were reported.
From week 9 to 10 of treatment all animals suffered from a viral disease, which occasionally affects rats of the used strain. The viral disease was identified as sialodacryoadenitis and persisted for 2 to 3 weeks; during this time, the rats showed reduced appetite and decrease in body weight gain and body weight. Thereafter, the rats recovered and by week 12, they appeared normal. This disease did not affect the test conduct.
Mortality (see table 4): An increase in mortality was observed in the 160 mg/kg bw groups when compared to controls; mortality affected more male animals than females. In the high dose group of the main test series the difference to control was statistically significant. For the remaining groups, recorded mortalities were within control range (see Table 4).

Ophthalmoscopic examination: Examination of the eyes revealed no treatment-related effects.
Haematology: No treatment-related and/or biologically relevant differences between treated and control animals could be evidenced.
Clinical Chemistry: No treatment-related and/or biologically relevant differences between treated and control animals could be evidenced.
Urinanalysis: In the 160 mg/kg bw group a decrease in urine volume was seen during weeks 26 and 52, which was related to the decreased water consumption. During week 77, urine volumes of the high dose animals were similar to those of control animals; however, towards test ending (i.e. week 103), the urine volume produced by the high dose animals again was lower than control.
Neoplastic findings:
Treatment-related macroscopical findings were reported for the high dose animals and affected the stomach (table 5). Lesions in the stomach included thickening of the non-glandular region, raised area with central depressions, warty excrecences and ulceration; these lesions were seen in 9 males and 10 females treated with 160 mg/kg bw of bronopol.
Incidental/spontaneous findings were reported, which occurred in both treated and untreated animals, and/or showed no dose-response relationship. Especially in the stomach of high dose rats that died during the experiment, histopathological examination revealed squamous cell papillomata associated with epithelial hyperplasia and ulceration. These findings were rather related to the irritant potential of bronopol than indicative of a tumorigenic potential for this substance. Re-evaluation of the findings: The more recent re-evaluation of the histopathological findings confirmed the findings of the older evaluations, and treatment-related papillomas were reported for the forestomachs of the rats treated with bronopol. The fact to the papillomas were mainly seen in the high dose group and were associated to ulcerations indicates that the tumours rather were a consequence of the irritant potential of bronopol than of a carcinogenic potential of the test substance as such.

Histopathology:
Kidney: Histopathology of the kidney revealed no treatment-related changes, which would explain the increase in relative kidney weight reported above. Moreover, progressive glomerulonephrosis of varying degree was reported for animals of both treated and untreated groups; however, the incidence of this lesion in rats treated with 160 mg/kg bw group was statistically significantly increased compared to control. With regard to histopathology, glomerulonephrosis was characterized by glomerular abnormalities, atrophic or cystically dilated nephrons, which often were lined by basophilic epithelium, and interstitial fibrosis with foci of lymphocytic infiltration. Glomerulonephrosis is not untypical for the rat strain used. The treatment with bronopol at a test dose of 160 mg/kg bw/day probably exacerbed the occurrence of this spontaneous lesion as a consequence of the reduced renal output observed at the high dosing level.
Salivary glands: Squamous metaplasia was seen in the ducts of the salivary glands, and was often associated with minimal mixed or chronic inflammatory cell infiltration, and with groups of atrophic acini. The affected glands often were dilated and displayed minimal epithelial hyperplasia. The incidence and gravity of these effects were increased in the males of the 40 and the 160 mg/kg bw groups and in the females of the 160 mg/kg bw group when compared to control. Squamous metaplasia in the salivary glands is regarded as a spontaneous changes occurring in the rat strain used; the treatment with bronopol probably exacerbed the occurrence of this spontaneous lesion.
Lymph nodes: A treatment-related dilatation of the sinusoids in the gastric lymph nodes was reported for 4/12 males and 5/22 females of the 160 mg/kg bw group. The finding generally was

associated with hyperplasia and ulceration of the non-glandular epithelium of the stomach. No such changes were seen in the control and the low and mid doses groups.

Stomach: With regard to histopathology, ulceration in the non-glandular epithelium of the stomach often was accompanied by epithelial hyperplasia of varying degree and hyperkeratosis. Occasional squamous cell papillomata also were seen. The increased incidence and severity of the hyperplasia and areas of ulceration seen in the high dose group compared to control indicated that the findings were treatment-related.

In none of the remaining examined organs and tissues, treatment-related changes were seen. In fact, changes such as foci of myocarditis in the heart, small areas of interstitial pseumonitis in the lung, cytoplasmic vacuolisation in hepatocytes of the liver, changes in endocrine glands (e.g. vacuolisation and distention of cells) and changes in the reproductive systems (e.g. testicular atrophy in males or follicular cysts in ovaries) were reported, which occurred in both, treated and untreated animals and therefore were of no toxicological relevance.

Effect levels

1

	Key result
Dose descriptor:	NOAEL
Effect level:	7 mg/kg bw/day (nominal)
Based on:	test mat.
Sex:	male/female
Basis for effect level:	other: Systemic toxicology

2

Dose descriptor:	LOAEL
Effect level:	32 mg/kg bw/day (nominal)
Based on:	test mat.
Sex:	male/female
Basis for effect level:	other: Systemic toxicology

Target system / organ toxicity

Critical effects observed:	not specified

Any other information on results incl. tables

Table 1: Group mean body weight gains (g), data summary (extract from tables presented in the study report).

Period (week)	Males				Females			
	G1	G2	G3	G4	G1	G2	G3	G4
0 – 3	166	168	171	166	83	86	84	83
3 – 6	105	102	98	90	53	57	52	55
0 – 6	271	270	269	256**	136	137	136	138
6 – 13	100	98	95	73***	45	47	47	40**
0 - 13	371	368	364	329***	181	184	183	178
13 - 26	103	108	98	52***	45	47	57*	38
26 – 52	110	107	88**	18***	60	58	59	28***
52 – 78	98	74	47***	36***	69	80	83	41**
78 – 104	40	7	23	-104***	35	39	0	-7***

G1, control group, G2, 10 mg/kg bw/day group, G3, 40 mg/kg bw/day group,

G4, 160 mg/kg bw/day group; *, p<0.05; **, p<0.01;***, p<0.001

Table 2: Group mean food consumption (g/rat/week), data summary (extract from tables presented in the study report):

Period (week)	Males				Females			
	G1	G2	G3	G4	G1	G2	G3	G4
1 – 13 (T)	2221	2208	2193	2169	1649	1678	1671	1657
1 – 13 (%)	-	99	99	98	-	102	101	100
14 – 26 (T)	2186	2164	2133	2000	1649	1648	1636	1589
14 – 26 (%)	-	99	98	91***	-	100	99	96
27 – 52 (T)	4233	4260	4171	3954***	3239	3185	3136	3116
27 – 52 (%)	-	101	99	93	-	98	97	96
53 – 78 (T)	4820	4687	4477*	4065***	3702	3670	3637	3640
53 – 78 (%)	-	97	93	84	-	99	98	98
79 – 104 (T)	4797	4659	4649	4021	3936	3909	3737	3659
79 – 104 (%)	-	97	97	84***	-	99	95	93

G1, control group, G2, 10 mg/kg bw/day group, G3, 40 mg/kg bw/day group, G4, 160 mg/kg bw/day group;

T, Total (g); %, percentage of control; *, p<0.05; **, p<0.01;***, p<0.001

Table 3: Group mean water consumption (ml/rat/week) and achieved dose of bronopol, data summary (extract from tables presented in the study report).

Period (week)	Males				Females			
	G1	G2	G3	G4	G1	G2	G3	G4
1 – 13 (T)	3769	2838	2435	1840	2574	2273	2014	1589
1 – 13 (%)	-	75	65	49	-	88	78	62
14 – 26 (T)	3493	2595***	2196***	1657***	2454	2181***	1925***	1560***
14 – 26 (%)	-	74	63	47	-	89	78	64
27 – 52 (T)	5829	5010***	4338***	3264***	5225	4687***	4250***	3457***
27 – 52 (%)	-	86	74	56	-	90	81	66
53 – 78 (T)	5997	5418	4692***	4076***	5661	5270	4836**	4279***
53 – 78 (%)	-	90	78	68	-	93	85	76
79 – 104 (T)	6747	6397	6306	3652***	6695	6268	5601*	4021***
79 – 104 (%)	-	95	93	54	-	94	84	60

G1, control group, G2, 10 mg/kg bw/day group, G3, 40 mg/kg bw/day group, G4, 160 mg/kg bw/day group;

T, Total (g); %, percentage of control; *, p<0.05; **, p<0.01;***, p<0.001

Group	Expected dosage	Achieved dosage (mg/kg bw/day)	
		Males	Females
2	10 mg/kg bw/day	10.5 mg/kg bw/day	10.4 mg/kg bw/day
3	40 mg/kg bw/day	40.2 mg/kg bw/day	40.7 mg/kg bw/day
4	160 mg/kg bw/day	152.2 mg/kg bw/day	158.4 mg/kg bw/day

Table 4: Mortality in the main and satellite test series after treatment with bronopol.

Dose level	Main test series		Satellite test series	
	Males	Females	Males	Females
0 mg/kg bw/day	21/45	19/45	8/15	9/15
10 mg/kg bw/day	20/45	19/45	5/15	10/15
40 mg/ kg bw/day	20/45	22/45	7/15	8/15
160 mg/kg bw/day	36/45***	28/45*	12/15	10/15

*, p<0.05; ***, p<0.001

Table 5: The incidences and severity of histopathological findings in the stomach.

Lesion	Male							
	G1		G2		G3		G4	
	D	S	D	S	D	S	D	S
Squamous cell papilloma							2 (1)	
Papillomatous/marked epithelial hyperplasia							5 (3)	3
Moderate epithelial hyperplasia	1				1		3 (1)	4 (1)
Minimal focal epithelial hyperplasia								
Ulceration	1						5 (3)	5

No of rats affected/No of rats in which salivary glands were examined	1/4	0/24	1/1	0/25	1/1	0/21	8 (4)/ 8 (4)	7 (1)/ 8 (3)

Lesion	Females							
	G1		G2		G3		G4	
	D	S	D	S	D	S	D	S
Squamous cell papilloma							(1)	
Papillomatous/marked epithelial hyperplasia	2						0	(2)
Moderate epithelial hyperplasia	1				1		2	3 (2)
Minimal focal epithelial hyperplasia								5
Ulceration	1						(2)	6 (4)
No of rats affected/No of rats examined	2/2	0/26	1/1	0/26	0/0	0/23	(3)/(3)	8 (4)/ 17 (5)

G1, control group, G2, 10 mg/kg bw/day group, G3, 40 mg/kg bw/day group,

G4, 160 mg/kg bw/day group; (), satellite group animals

Applicant's summary and conclusion