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068196

DATA EVALUATION REPORT

STUDY TYPE: 83-4. Reproductive and Fertility Effects - Rat

TOX. CHEM. NO.: 116A
MRID NO.: 406609-01

TEST MATERIAL: Bronopol, pharmaceutical grade, white crystals, purity (a.i.): 99.9%, batch no.: 845309, readily soluble in water, and stable in aqueous solutions at pH 4.0 and lower.

SYNONYMS: Bronopol-Boots

STUDY NUMBER(S): 510-013

SPONSOR: The Boots Company, PLC, Nottingham, England

TESTING FACILITY: International Research and Development Corporation (IRDC), Mattawan, Michigan

TITLE OF REPORT: Bronopol: Two Generation Reproduction Study in Rats

AUTHOR(S): Margery J. Wirth

REPORT ISSUED: December 23, 1987

CONCLUSIONS:

Systemic NOEL: 25 mg/kg*
LEL: 70 mg/kg; increase in the absolute and relative weights of kidney of F_0 females; decrease in the absolute and relative weights of liver of F_1 males and females; and an increased incidence of progressive nephropathy in F_0 males and females.

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*Compared with the control values, an increased (6%; $p < 0.05$) absolute weight of kidneys in the F_0 females, a decreased (10%) absolute weight of livers in the F_1 males, a decreased (10%; $p < 0.05$) relative weight of liver (liver/body weight ratio) in the F_1 females, and decreased (7.5-16.1%) water consumption in the F_0 and F_1 males and females were observed at this level. However, changes in organ weights were small and these organs were histologically similar to those of the control rats. Since Bronopol was administered in drinking water, decreased water consumption reflected palatability rather than toxicity.

Reproductive NOEL: 70 mg/kg
LEL: 200 mg/kg; decreased female fertility index (number of pregnant females/number of females mated) during the F_{1a} mating.

Developmental NOEL: 25 mg/kg
LEL: 70 mg/kg; slight increase in the number of stillborn F_{1b} pups and slight decrease in body weights of the live F_{1b} pups, both on lactation day 0.

The anticipated doses of Bronopol were 25, 70 and 200 mg/kg of body weight/day, but the actual (achieved) median doses for the F_0 and F_1 males and females were 22.55, 55.2 and 147.0 mg/kg/day, respectively. The lower than intended doses resulted from the reduced consumption of drinking water, which contained Bronopol, by all treated groups.

Classification: Core-Guideline

EXPERIMENTAL PROCEDURES

This study was started on 9/13/85 and terminated with the sacrifice of the last F_1 rat on 12/18/87. It involved parental groups F_0 and F_1 and two litters per group, F_{1a} and F_{1b}, and F_{2a} and F_{2b}, respectively. The F_{1b} rats were used as the F_1 parents. Bronopol was administered in drinking (tap) water during the premating, mating, gestation and lactation periods. The pH of water was adjusted to 4.0 with hydrochloric acid in order to ensure the stability of Bronopol. The target concentrations of Bronopol used were 0.025, 0.07 and 0.2%, corresponding to 25, 70 and 200 mg/kg/day, respectively (anticipated dose levels). The control group received tap water adjusted to pH 4.0. Dose concentrations were based on the results of a range-finding study, conducted at IRDC (No. TX 860009; see separate review). Dosing solutions were prepared twice weekly (Monday and Thursday) and were dispensed in glass water bottles. Remaining solutions were stored in amber bottles at room temperature (< 25° C). Samples of dosing solutions (drinking water) were analyzed for concentration of Bronopol on study weeks 1, 4, 8 and 13. The rats (Charles River COBS® CD® strain) were:

1. Obtained from Charles River Laboratories, Inc. Portage, Michigan.

2. Acclimated for 15 days.

3. Six weeks old and weighed 159-194 g (males) and 121-147 g (females) at the initiation of the study.

4. Assigned to groups randomly, on a weight bases, by the computer-generated system and the groups were evaluated for homogeneity using Bartlett's test.

5. Fed basal laboratory diet of Purina Certified Rodent Chow® #5002.

6. Housed individually, except during mating, in suspended wire-mesh cages, at temperature of 23.5 ± 0.72° C, humidity of 41.1 ± 12.2%, and 12-hour light/dark cycle.

7. Identified by cage and group, and individually by Monel® metal ear tag bearing the animal number.

F_0 rats, 13 males and 26 females/group, were treated with Bronopol for at least 80 days before they were mated (1 male: 2 females) to produce the F_{1a} litter. A maximum of 15 days was allowed for the mating period. Following weaning of the F_{1a} pups, the females "rested" for 10 days and then were remated to produce the F_{1b} litters. The F_{1b} pups selected to become F_1 parents, 13 males and 26 females/group, received Bronopol at weaning and continuing for 87 days prior to mating, and then until sacrifice. A maximum of 17 days was allowed for the F_{2a} mating period. Following weaning of the F_{2a} pups, the females "rested" for 10 days and then were remated to produce the F_{2b} litters.

On the 25th day following separation from the male, any F_0 or F_1 female that did not deliver was sacrificed. The necropsied uteri from females that appeared nonpregnant were opened and placed in 10% ammonium sulfide solution for detection of implantations.

After weaning of the F_{1b} pups, 10 F_0 females, randomly selected from each group, were necropsied and selected tissues were retained in fixative. The remaining F_0 rats were externally examined, sacrificed and discarded. The F_1 rats remained on the study between 33 and 47 days following weaning of their F_{2b} pups. During that time, 10 F_1 females, randomly selected from each group, were necropsied and examined histopathologically. The remaining F_1 rats were externally examined, sacrificed and discarded.

The following parameters were examined for the F_0 and F_1 rats:

1. Reproductive: copulatory and fertility indices, copulatory interval and gestation length.

2. Appearance, Behavior and Mortality (animals were observed twice daily).

3. Body weights. Males were weighed weekly, from the initiation of treatment until sacrifice. Females were weighed weekly, from the initiation of treatment until evidence of copulation and during the "rest" periods between matings; on gestation days 0, 6, 15 and 20; and on lactation days 0, 7, 14 and 21. Females with no evidence of copulation were weighed on a weekly basis until sacrifice.

4. Food consumption. Was recorded weekly (except during mating) for all animals, from the initiation of treatment, until sacrifice. In addition, food consumption for mated females was calculated for gestation day intervals 0-6, 6-15, 15-20 and 0-20; and lactation day intervals 0-7, 7-14, 14-21 and 0-21.

5. Water and Bronopol consumption. Were calculated for the same time intervals as food consumption.

6. Necropsy. Ten randomly selected F_0 and F_1 parents/sex/group were examined. Rats dying prior to scheduled sacrifice were also examined.

7. Organ weights. The following organs were weighed from the 10 selected F_0 and F_1 parents/sex/group: adrenals, heart, kidneys, liver, ovaries, testes and thyroid/parathyroid complex.

8. Histopathology. The following organs/tissues were preserved (in phosphate-buffered neutral formalin) from the 10 selected F_0 and F_1 parents/sex/group:

Adrenals*	Pancreas
Aorta	Pituitary
Brain	Peripheral nerve
Colon*	Prostate*
Duodenum	Salivary gland
Epididymides*	Seminal vesicles
Esophagus*	Skeletal muscle
Eyes	Skin
Heart*	Spleen*
Ileum*	Stomach*
Jejunum*	Testes*
Kidneys*	Thymus
Liver*	Thyroid/parathyroid complex*
Lung*	Trachea*
Lymph node (mesenteric)	Urinary bladder*
Mammary gland	Uterus and cervix*
Ovaries*	All gross lesions*

Only kidneys were examined for all of the F_0 groups, but tissues marked with an asterisk (*) were examined for all of the F_1 groups.

The litters (F_{1a}, F_{1b}, F_{2a} and F_{2b}) were examined after delivery to determine litter size, number of viable and stillborn pups, and any gross abnormalities. Pups were weighed on lactation days 0, 4, 7, 14 and 21. The sex of pups was recorded on lactation day 4 and, on that day, the litters were culled to 10 pups of equal sex distribution, when possible, in order to provide a homogenous group size for evaluation of nursing, survival and growth. The extra pups were examined externally, sacrificed and discarded. The retained pups were caged with their mothers for three weeks, at which time all but the F_{1b} pups were examined externally for abnormalities, sacrificed and discarded. Necropsy and histopathology were performed on 5 weaned F_{1b} and F_{2b} pups of each sex from each group. The intact pups found dead were also necropsied. Tissues marked with an asterisk (above) were also examined histopathologically for the F_{1b} and F_{2b} pups. Organs weighed for the F_0 and F_1 adults (above) were also weighed for the F_{1b} and F_{2b} pups.

Statistical analyses were performed, as detailed in Attachment I, on parental body and organ weights, male and female fertility indices, pup survival indices, litter size and pup body weights.

RESULTS

Bronopol: Stability and Concentration in Dosing Solutions (Drinking Water)

The test material (pharmaceutical grade Bronopol), analyzed for active ingredient at the beginning and termination of the study, was stable for the duration of the study. The compound was stored in the dark at room temperature (< 25° C).

The drinking water, analyzed periodically for the concentration of Bronopol, contained 95-107% of the theoretical (target) concentrations.

F_0 and F_1 Generations (Parental Data)

Appearance, Behavior and Mortality

Nothing remarkable was observed in the surviving animals.

A total of 11 animals died or were sacrificed in extremis during the study, 8 in the F_0 generation and 3 in the F_1 generation. The following F_0 rats did not survive the study: 1 female from each the control and low-dose groups, and 1 male and 5 females from the high-dose group. The F_1 nonsurvivors included 1 female from each the low-, mid- and high-dose groups. Four of the F_0 rats (1 control and 3 high-dose) died during study weeks 1-11 (premating period) and the remaining 4 during study weeks 18 (lactation day 21 of the F_{1a} mating), 25 (gestation day 22 of the

F_{1b} mating) and 26 (lactation day 3 of the F_{1b} mating). One F_1 female died on gestation day 14 of the F_{2a} mating and the remaining 2 at the beginning of the F_{2b} mating.

The following findings were noted in the F_0 animals prior to death or at necropsy:

1. Control female: fractured nasal bone.
2. Low-dose female: intrauterine bleeding.
3. High-dose male: emaciation.
4. High-dose females (number affected):
   - Nothing remarkable (1).
   - Cool to touch (1).
   - Emaciation, dehydration, labored breathing, and very much enlarged adrenal gland (1).
   - Emaciation, labored breathing, absence of righting reflex, mild hydronephrosis and clear fluid in uterine horns (1).
   - Lesions in heart, liver, stomach mucosa, and mucosa of ileum and cecum (1).

The following findings were noted in the F_1 animals at necropsy:

- Nothing remarkable (1 high-dose).
- Congested lungs (1 low-dose).
- Discoloration of liver, stomach lesions, and enlargement of kidneys, lymph nodes, spleen and thymus (1 mid-dose).

Histopathology was not performed on the nonsurvivors.

Because there were no consistent trends with respect to clinical signs, time of death and necropsy findings, the testing laboratory regarded these deaths as incidental to Bronopol.

Body Weights

Relative to the control values, consistently lower mean body weights were observed for the following high-dose animals:

- F_0 males and especially the F_1 males, where decreases in mean body weights ranged from 11 to 22% and were statistically significant ($p < 0.05$ or 0.01).
- F_1 females during both premating periods, F_{2a} and F_{2b} (7-24%; $p < 0.05$ or 0.01).
- F_0 females during the F_{1a} lactation period (8-11%), and F_{1b} gestation and lactation periods (6-14%).
- F_1 females during the F_{2a} and F_{2b} gestation and lactation periods (5-16%).

Decreases in body weights were regarded by the testing laboratory as treatment-related.

Food Consumption

Food consumption was reported as g/animal/day and as g/kg of body weight/day. Relative to the control values and calculated as g/kg/day, decreased food consumption was observed for the following high-dose rats:

- F_0 males; during weeks 1 (18%), 8(7%) and 18-20 (5-9%).
- F_0 females, during study week 1 (13%) and lactation periods F_{1a} (6-13%) and F_{1b} (8%; first week).
- F_1 females, during lactation period F_{2a} (7-16%).

All of these decreases in food consumption were considered by the testing laboratory was treatment-related; they were not statistically analyzed.

Water Consumption

Water consumption, reported as g/animal/day and g/kg of body weight/day, was reduced in all treated groups, in a dose-related manner, throughout most of the study. The reduced water intake was considered by the testing laboratory as treatment-related at all dose levels. However, no reference was made to the palatability of the Bronopol-containing drinking water. Data on water consumption, reported as g/kg/day, are summarized below.

Animals	Time	Bronopol (mg/kg/day)		
		25	70	200
		Water consumption as percentage of control value[a]		
F_0 males	Study weeks 1-31[b]	91.5	84.2	76.3
F_0 females	Study weeks 1-12	91.3	76.7	69.2
	F_{1a} mating			
	g.d. 0-20	92.5	79.4	71.0
	l.d. 0-21	98.3	87.6	78.6
	F_{1b} mating			
	g.d. 0-20	92.4	82.2	77.6
	l.d. 0-21	99.7	91.0	83.3

Animals	Time	Bronopol (mg/kg/day)		
		25	70	200
		Water consumption as percentage of control value[a]		
F_1 males	Study weeks 1-38[c]	91.1	88.9	83.7
F_1 females	Study weeks 1-14	87.3	83.9	76.1
	F_{2a} mating			
	g.d. 0-20	83.9	72.3	64.8
	l.d. 0-21	100.3	85.3	83.4
	F_{2b} mating			
	g.d. 0-20	100.1	88.4	79.0
	l.d. 0-21	99.6	92.8	94.1

g.d. = gestation days; l.d. = lactation days

[a]This table, prepared by the toxicologist who evaluated this study, is based on TABLES 11-14, pages 59-66 of the submission.

[b]Excluding study weeks 13 and 23 (mating periods).

[c]Excluding study weeks 15 and 26 (mating period). The term "study weeks" was used by the toxicologist who evaluated this study in order to facilitate evaluation. The testing laboratory used the term "Age (weeks)" when reporting findings for the F_1 males and females.

The water consumption values during each lactation period were very high. The testing laboratory commented that these values were unreliable because pups might have contributed to the consumption values.

Bronopol Consumption

The anticipated doses of Bronopol were 25, 70 and 200 mg/kg of body weight/day, but the actual (achieved) median doses for the F_0 and F_1 males and females were 22.55, 55.2 and 147.0 mg/kg/day, respectively. The lower than intended doses resulted from the reduced consumption of drinking water, which contained Bronopol, by all treated groups. The mean achieved doses of Bronopol received by the F_0 and F_1 rats during various study periods, as reported by the testing laboratory, are in Attachment II.

Necropsy Findings at Terminal Sacrifice

Granular kidneys, regarded by the testing laboratory as treatment-related, were observed in the following high-dose animals: 2 F_0 males, 4 F_0 females, 1 F_1 male and 2 F_1 females. Other, treatment-unrelated findings included hydronephrosis (1 F_0 low-dose male and 1 mid-dose female; 1 F_1 control female and 1 mid-dose male and female) and calculi (1 F_1 control female and 1 mid-dose male and female).

Organ Weights

Dose-related increases in absolute kidney weights were observed in the treated F_0 females, although the difference between the control and low-dose groups was small. Dose-related increases in relative kidney weights (kidney/body weight ratio) were noted only in the mid- and high-dose F_0 females. Other organ weight changes were observed mostly in the high-dose group, as follows:

Animals and absolute organ weights affected	Bronopol (mg/kg/day)		
	25	70	200
	Organ weight as percentage of control value[a]		
F_0 Females			
Adrenal (relative)			122.0*
Kidneys	106.1*	114.5**	128.2**
Kidneys (relative)		108.9*	136.1**
F_1 Males			
Heart			85.5*
Kidneys (relative)			114.1*
Liver	90.0	88.9	78.7**
Thyroid/Parathyroid			125.8*
F_1 Females			
Liver (relative)	89.6*	88.6*	91.7

[a]This table is based on TABLES 22-24, pages 87-98 of the submission.

* $p < 0.05$ ** $p < 0.01$

Histopathology Findings at Terminal Sacrifice

Treatment-related increase in the incidence of progressive nephropathy, a spontaneous kidney disease in rats, was observed in the mid-dose and high-dose F_0 males. In the F_0 females, the incidence increased in a dose-related manner and the nephropathy was more prominent than that seen in the F_0 males. Tubular dilatation, a constituent lesion of progressive nephropathy, was observed in the high-dose F_0 males and females.

Nephropathy (tubular degeneration and hyaline cast formation) was less pronounced in the F_1 rats, compared with that observed in the F_0 rats, but all of the F_1 male and female groups were affected. According to the testing laboratory, although there was no difference in the incidence of the lesion, the high-dose F_1 animals appeared to be rather more markedly affected. The difference in the high-dose group was considered to be a treatment-related exacerbation of a common spontaneous condition. Other findings observed in the F_1 rats (perivascular lymphoid infiltration and vascular mineralization in the lungs, and thyroid cysts) occurred with similar frequency in the control and treated groups, and were therefore Bronopol-unrelated. The incidence of nephropathy is summarized below.

Group/Observation[a]		Bronopol (mg/kg/day)			
		0	25	70	200
Number of rats examined[b]		10	10	10	10
		Number of rats affected			
F_0 Rats					
Progressive nephropathy	- M[c]	2	0	4	6
	- F	0	1	3	9
Tubular dilatation	- M	0	0	0	2
	- F	0	0	0	5
F_1 Rats					
Progressive nephropathy	- M	0	0	0	1
	- F	0	0	0	1
Tubular degeneration	- M	3	3	3	1
	- F	0	0	0	2
Hyaline casts	- M	0	2	2	2
	- F	1	1	3	0

[a]This table is based on TABLES 26 (page 103) and 28 (pages 111 and 115) of the submission.

[b]Ten rats/sex/group were examined.

[c]M - male; F - female

Reproductive Parameters: F_0 and F_1 Parents

In the low-dose and mid-dose groups, Bronopol had no effect on copulatory index (number of females mated/number of females paired), male fertility index (number of fertile males/number of males paired), female fertility index (number of pregnant females/number of females mated), copulatory interval and gestation length, the reproductive parameters examined during each of the four matings. Nothing remarkable was also observed in the reproductive parameters during the F_{1b} and F_{2a} matings, in the high-dose group. During the F_{1a} mating, the female fertility index for the high-dose group was slightly lower than that calculated for the control group (75% vs. 87.5%, respectively). During the F_{2b} mating, two high-dose females that did not deliver were found pregnant at necropsy; one female had one dead fetus in the uterus and another had one early resorption.

Litter (Developmental) Parameters: F_{1a}, F_{1b}, F_{2a} and F_{2b} Pups

In the low-dose group, Bronopol had no effect on any of the following developmental parameters examined: numbers of viable and nonviable pups at birth (lactation day 0), survival during the lactation period, body weights, size of the pups at birth and during the lactation period, and organ weights. Histopathology of the F_{1b} and F_{2b} pups at the termination of the study (F_{1a} and F_{2a} pups were not examined), and necropsy of the pups found dead also revealed nothing remarkable. According to the testing laboratory, the mean body weight of the F_{1b} pups from the low-dose group was slightly lower than control on lactation day 21. However, examination of these data (TABLE 15, page 72 of the submission) indicated that, on lactation day 21, the group mean body weights of the F_{1b} male and female control pups were 53.5 g and 50.5 g, respectively; the corresponding values for the low-dose pups were 52.4 g (97.9% of control) and 50.4 g (99.8% of control), respectively, and statistically insignificant.

In the mid-dose group, slight but treatment-related findings were observed only in the F_{1b} pups. On lactation day 0, there were more stillborn F_{1b} pups in the mid-dose group than in the control group (6.2% vs. 1.2%, respectively). This increase was attributed primarily to one dam with 100% of pup mortality on lactation day 0. Also, the mean body weights of the F_{1b} pups from the mid-dose group were slightly lower than those of the control pups on lactation day 21 (males, by 6.7% and females, by 6.5%). Neither an increase in the number of the stillborn pups nor a lower body weight was statistically significant.

In the high-dose group, treatment related findings included an increase in the percentage of stillborn pups and decreases in body weight gain and size of the pups. On lactation day 0, there were more stillborn F_{1b} pups in the high-dose group than in the control group (14.0% vs. 1.2%, respectively). This increase was

attributed primarily to two dams with high pup mortality on lactation day 0 (one, 100% and another, 93.8%). Mean body weights of the F_{1a}, F_{1b}, F_{2a}, and F_{2b} pups were significantly lower ($p < 0.05$ or, mostly, $p < 0.01$) than those of the control throughout the entire lactation period. Relative to the control values, body weight decreases for the F_{1a} and F_{2a} pups ranged from 6.4% to 13.3% during lactation days 0-14, and from 16.1% to 19.7% (males and females) on lactation day 21. Relative to the control values, body weight decreases for the F_{1b} and F_{2b} pups ranged from 13.4% to 22.0% during lactation days 0-14, and from 20.0% to 20.9% (males and females) on lactation day 21. Twenty-eight F_{1b} high-dose pups (3 nonsurvivors and 25 survivors) and 17 F_{2b} pups (1 control, 2 low-dose and 14 high-dose) were smaller in size than the remaining pups in these groups. The pups were smaller in size (other details not provided) during most of the lactation period. Histopathology of the F_{1b} and F_{2b} weanlings revealed nothing remarkable, but the following changes in organ weights were observed in the F_{2b} pups (percent of control values): liver (66.7, males and 64.7, females; $p < 0.05$), kidneys (69.2, females; $p < 0.05$) and thyroid/parathyroid (154.2, males; $p < 0.01$).

COMMENTS

This study, reported in great detail, meets the November 7, 1989 Acceptance Criteria and is classified as Core-Guideline. Although the submitted report contains a few errors and ambiguities which should be corrected by the registrant, these are not serious enough to affect acceptance of this study as a valid 2-generation rat reproduction study. The study was inspected (Quality Assurance Inspections) 56 times during its course (9/12/85-12/21/87). A range-finding study (separate report) and reproductive historical control data (Appendix N, pages 1272-1275) were also submitted. The selected route of administration was oral, in the drinking water, since this was considered the most likely route of human exposure. Two litters were produced and evaluated in each generation to more fully define any interlitter variability which might have occurred.

The historical control data (not individual studies but a summary table) were submitted for the Charles River COBS® CD® rats, a strain used in the current study, for studies terminated January, 1984 to January, 1986. The following parameters (average values and ranges) were reported: male and female fertility indices, female copulatory indices and intervals, gestation length, number of implantation sites at weaning/dam, postimplantation loss/dam, litter size at birth (viable and stillborn), pup sex distribution at weaning, pup survival indices and body weights at birth and throughout lactation, and pup body weight (separate for males and females) on lactation day 21. It was not reported how many studies were included in the compilation of the historical control data.

The following errors in Table 15, entitled Summary of Gestation and Lactation Data - F_0 Parents (F_{1b} Mating), require corrections:

Page 70:

- Female fertility index data were typed under heading Male Fertility Index.
- Male fertility index data were typed under heading Copulatory Index (Days).
- Copulatory interval data were typed under heading Female Fertility Index.

Page 71:

- Data on numbers of live pups at lactation day 0 were typed under the heading Number of Dead Pups at Lactation Day 0, and vice versa.

Page 73:

- Heading for this table, Summary of Gestation and Lactation Data - F_0 Parents (F_{1b} Mating), should read -- F_1 Parents (F_{2a} Mating). Also, this should be TABLE 16 and not TABLE 15.

The following pup mortality data, reported in the RESULTS section, PARTURITION AND LITTER OBSERVATIONS, pages 27-31, could not be verified in the submission: pups found dead and pups missing (presumed completely cannibalized) during the lactation period.

Pups Found Dead During Lactation

Bronopol (mg/kg/day)	Litters			
	F_{1a}	F_{1b}	F_{2a}	F_{2b}
Lactation Days	0-7	0-21	0-11	0-21
	Number of pups found dead			
0	5	6	10	4
25	6	10	7	7
70	3	23	4	4
200	9	40	3	4
Total dead	23	79	24	19

Pups Missing (Presumed Completely Cannibalized) During Lactation

Bronopol (mg/kg/day)	Litters			
	F_{1a}	F_{1b}	F_{2a}	F_{2b}
Lactation Days	0-21	0-14	0-9	0-21
	Number of pups missing			
0	2	3	4	0
25	4	3	2	5
70	5	4	4	1
200	8	11	3	3
Total missing	19	21	13	9

The above values cannot be obtained from the lactation data summarized in TABLES 15 AND 16 (pages 68, 71, 74 and 77), which show few deaths, or from other litter data in the submission. Since after culling of litters on lactation day 4, the extra pups were examined externally and discarded, the above deaths are difficult to explain. Considering also that different time intervals are involved in reporting pups found dead, it is difficult to correlate this mortality with treatment.