2-14-85

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

004291

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chronic and oncogenic study with Bolero in rats
Accession No. 255042 through 255046
EPA Reg. No.239 2431
Tox. Chem. No. 207 DA

TO: Richard Mountfort, PM #23
Registration Division (TS-767)

FROM: Quang Q. Bui, Ph.D. 2/12/85
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Laurence D. Chitlik, DABT 2/14/85
Section Head, Section V
Toxicology Branch/HED (TS-769C)

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant:

Chevron Chemical Company
Richmond, California

Action Requested:

Review of a combined chronic and oncogenic study in rats with Technical Bolero

Recommendation:

The combined chronic and oncogenic study with Technical Bolero in rats is classified as Supplementary Data (see Conclusions and Recommendations sections for requested data and clarification).

It is requested that a laboratory audit be conducted due to some unusual findings reflected in the study data.

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STUDY REVIEW

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CHEMICAL: Thiobencarb - Bolero

TEST MATERIAL: Technical Bolero - 95.3%

STUDY/ACTION TYPE: Chronic study in rats

STUDY IDENTIFICATION:

Title: Technical Bolero: Combined Oncogenicity and Toxicity Study in Dietary Administration to the Rat.
Testing Facility: Life Science Research Ltd. Suffolk, England
Final Report No.: 83/KCI045/248
Report Date: September 7, 1984
Study Director: H.A.Cummins
Accession No.: 255042 through 255046

CONCLUSIONS:

Under the conditions of this study, a systemic NOEL apparently is demonstrated at 20 ppm (lowest dose tested). However, the oncogenic potential and cholinesterase NOEL of Bolero cannot be determined at the present time.

The registrant is requested to provide clarification concerning the identical cholinesterase activity values reported for different animals. Furthermore, the sensitivity of the test methods used to determine urinary protein concentrations should be clarified.

The oncogenic potential of Technical Bolero cannot be assessed in the absence of historical control data for pancreatic islet cell adenomas and testicular interstitial cell adenomas. Furthermore, all submitted histopathologic reports were indicated as "draft" and, hence, cannot be used for a final evaluation.

RECOMMENDATIONS:

1. This study is presently classified as Supplementary Data pending the submission of the following information:

a. The registrant is requested to provide clarification concerning:

i. The identical cholinesterase activity values noted for different animals in this study.

ii. The significant differences in cholinesterase activity values noted between males and females of the same group which appeared to be beyond the usual expected differences between sexes.

iii. The sensitivity of the test methods used to determine urinary protein concentrations.

b. The following additional data are requested:

i. Results of the test chemical analyses performed at different intervals throughout the study

ii. "Final" histopathologic reports for non-neoplastic and neoplastic findings. Only "draft" histopathologic reports were submitted and, hence, preclude a final assessment of the oncogenic potential of Bolero.

iii. Historical control data for testicular interstitial cell adenomas and pancreatic islet cell adenomas collected by this testing facility for this species and strain must be submitted. The historical control data must contain information relating to study identification, vehicle used, date, collected from a period of 3 years prior to and, if possible, 1-2 years after this study initiation.

The above mentioned tumor types were increased in the treated groups in this study. Thus, the historical data are necessary for proper assessment of this study.

2. As a result of the rat chronic and oncogenic study conducted by Life Science Research Ltd, Suffolk, England, on Technical Bolero, (Study # 83/KCI045/248), it is requested that a laboratory audit be conducted due to some unusual findings reflected in the study data.

a. Identical cholinesterase values were reported for different animals sacrificed at the same interval.

b. Significant differences in cholinesterase activities between males and females of the same group.

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MATERIAL AND METHODS

Test material: Technical Bolero, 95.3%
Light yellow liquid
Lot No. U0048
Dosage levels: 0, 20, 100, and 500 ppm
Animals: F344 rats (Charles River, Portage, Michigan)

Procedures:

A copy of the procedures used is appended.
The registrant indicated that the protocol used followed sections 163.83.1 and 163.83.2 of the 1978 EPA Proposed Guidelines. However, the following comments are noted:

1. Upon arrival at the testing facility, viral antibody screen was performed on 5 randomly selected animals of each sex. Hematological results did not reveal any abnormal findings. The batch of animals was therefore considered as suitable for testing.

2. Four groups of 100 animals/sex/group each were used in this study. Each group was divided into two phases:

a. Chronic Phase: 40 animals/sex/group
Interim sacrifice was performed on 10 animals/sex/group on weeks 28, 52, 79, and 104.
b. Oncogenic Phase: 60 animals/sex/group
All animals in this subgroup were sacrificed at week 108.

3. Histopathologic examinations were conducted on animals sacrificed after 52 weeks of the chronic phase, on all animals of the oncogenic phase, and on all unscheduled deaths. Histopathology was not conducted on animals of the chronic phase sacrificed at weeks 104. Necropsy was performed on all animals sacrificed at all intervals and on all unscheduled deaths.

4. On week 36, diets prepared for groups III (100 ppm) and IV (500 ppm) females were inadvertently fed to groups IV and III females, respectively. The registrant had been notified of this error.

5. Results of the test chemical analysis performed by the sponsor at different intervals throughout the study are not available.

6. All histopathologic sheets for non-neoplastic and neoplastic findings were indicated as "draft". Final reports approved by a pathologist should have been submitted.

RESULTS

1. Chemical Analysis

The purity of Technical Bolero used was analyzed by the sponsor at different intervals throughout the study: weeks 25, 51, 78, and 104. Respective results of 95.3, 95.7, 95.6, and 95.3% were reported. However, confirming data for these analyses were not included in the final report.

2. Diet Analysis

Dietary samples were analyzed by the testing facility at different intervals throughout the investigation. Recoveries of 93% +/- 8.9 were found by gas chromatographic methods.

3. Clinical Observations

Clinical signs suggestive of sialodacryodenitis were found in the majority of rats from all groups including the controls during weeks 11-16 and were characterized by nasal discharge, swollen necks, rales, and reddening of the eyelids. These clinical signs disappeared from most of the infected animals within 2-3 weeks.

Throughout the entire investigation, the incidences of "hair loss" and "scabs" in the 100 and 500 ppm males were higher than that of controls. The number of animals with "hair loss" for the 0, 100, and 500 ppm groups were respectively 39, 55, and 49. Respective incidences of "scabs" were 19, 38, and 39. "Sores on the hind feet" occurred more frequently in treated females than in controls. Respective incidences of 7, 17, 19, and 14 were found in the females receiving 0, 20, 100, and 500 ppm.

Palpable cutaneous or subcutaneous swellings were detected in all groups and were examined histopathologically.

4. Mortality

The mortality rates among all groups recorded throughout the investigation are tabulated as follows:

Chronic Phase (40 animals/sex/group)

	Control		20 ppm		100 ppm		500 ppm	
	M	F	M	F	M	F	M	F
Number deaths	5	4	5	3	3	6[a]	1	2
Mortality rate (%)	13	10	13	8	8	15	3	5

[a]: 2 accidental deaths

Oncogenic Phase (60 animals/sex/group)

Number deaths	27	24	23	21	14	20	15	16
Mortality rate (%)	45	40	38	35	23	33	25	27

Cummulative (100 animals/sex/group)

Number deaths	32	28	28	24	17	26	16	18
Survival rate (%)	68	72	72	76	83	74	84	82

The mortality rates of the treated groups (males and females) were not affected by Bolero administration. All groups apparently had a higher survival rate than the controls. In each group, an adequate number of animals survived until study termination for complete necropsy and histopathologic examinations.

5. Water Consumption

No differences in water consumption were noted among the treated and control groups for both males and females.

6. Food Consumption

Food consumption was measured weekly during the entire study for all animals. The following table illustrates the food consumption data over successive 13-week periods.

Food Consumption (g/rat/week)	Groups							
	Control		20 ppm		100 ppm		500 ppm	
	M	F	M	F	M	F	M	F
Weeks 1-13	111	81	108**	79**	103**	75**	98**	72**
Weeks 14-26	117	84	112**	81**	107**	77**	102**	75**
Weeks 27-39	119	85	115**	82**	109**	77**	102**	74**
Weeks 40-52	118	85	115**	82**	110**	79**	103**	75**
Weeks 53-65	122	94	120*	89**	111**	85**	105**	81**
Weeks 66-78	123	98	121	93**	113**	89**	107**	86**
Weeks 79-91	126	102	125	98**	116**	92**	110**	89**
Weeks 92-104	116	95	111**	94	106**	86**	101**	84**
Weeks 1-104(a)	119	91	116	87*	109**	83**	104**	80**
As % of control(a)			97.5	95.6	91.6	91.2	87.4	87.9

* Significantly different from controls, P<0.05
** Significantly different from controls, P<0.01
(a) calculated by this reviewer

Significant differences in food consumption were found between the treated and control groups throughout the entire study. When the mean weekly food consumption was calculated by this reviewer for the 2-year period, a dose-related decrease in food consumption was observed for both male and female treated animals. The investigators indicated that the decrease in food consumption was due to the unpalatability of the test compound and referred to study 80/KCI043/034 conducted at the same testing facility for reference.

7. Body weight

Body weight was recorded weekly during the first 6 months and bi-weekly thereafter for all animals.

The body weight gain data calculated over successive 13-week periods are presented as follows:

Body weight gain (grams)	Control M	Control F	20 ppm M	20 ppm F	100 ppm M	100 ppm F	500 ppm M	500 ppm F
Weeks 0-13	206	88	200**	82**	186**	77**	170**	70**
Weeks 13-26	64	29	59*	26**	55**	21**	48**	19**
Weeks 26-40	38	23	35*	23	31**	18**	26**	18**
Weeks 40-52	24	22	25	18**	21*	17**	18**	14**
Weeks 52-66	10	32	12	28**	4**	21**	8	17**
Weeks 66-78	8	21	9	19	6	19	2**	17**
Weeks 78-92	-7	17	-11	25**	0	16	-5	20
Weeks 92-104	-38	2	-37	5	-17**	5	-13**	6
Weeks 0-104(a)	305	234	292	226	286	194	254	181
As % of control(a)			95.7	96.6	93.8	82.9	83.3	77.4

* Significantly different from controls, P<0.05
** Significantly different from controls, P<0.01
(a) Calculated by this reviewer

Dose-related decreases in body weight gain were found during practically all 13-week periods for the 100 and 500 ppm groups and for females at 20 ppm. Significant differences from control values were observed for the males of the 20 ppm group only during the first 40 weeks of treatment. The body weight gains of the 20, 100, and 500 ppm groups for the entire study were respectively 95.7, 93.8, and 83.3% of control for males and 96.6, 82.9, and 77.4% of control for females.

The lower body weight gains observed in the treated groups were associated with a lower food intake which may have resulted from the unpalatability of the test compound.

8. Food Conversion Ratio

Weekly food conversion ratios were calculated for the first 13 weeks of treatment. This ratio was expressed as the amount of food consumed per unit of body weight gain. The overall ratios for the first 13 weeks for males were 7.0, 7.0, 7.2, and 7.4 for the control, 20, 100, and 500 ppm groups. Respective ratios of 12.0, 12.5, 12.7, and 13.5 were found for females.

These findings indicated that the food utilization of the 100 (7.2) and 500 ppm (7.4) groups was slightly less efficient than that of the control (7.0). The comparable food conversion ratio observed in the 20 ppm group suggests that the decrease in body weight gain noted at this level should be regarded as a consequence of a decrease in food consumption and not as a toxic manifestation.

9. Hematology

Hematological determinations were made before the commencement of the study, at different intervals during the study, and at study termination. Only findings of significance are discussed in this review.

	Wk 25		Wk 52		Wk 77		Wk 103		Wk 108	
	M	F	M	F	M	F	M	F	M	F
Hb (%)										
Control	16.3	16.1	16.3	16.1	15.8	15.5	15.0	14.9	14.5	14.4
20 ppm	16.2	16.2	16.1	15.9	15.7	15.6	13.6	15.0	14.8	15.3*
100 ppm	16.3	15.9	16.0	15.4*	16.0	15.6	17.4*	14.7	16.7	14.7
500 ppm	16.4	15.8	16.1	15.8*	16.4	15.5	17.1*	15.6	16.7	15.5*
RBC (mil/cmm)										
Control	9.04	8.45	9.20	8.39	8.89	8.07	7.82	7.41	7.66	7.04
20 ppm	8.98	8.41	9.06	8.36	8.77	7.95	7.04	7.41	7.59	7.5[illegible]
100 ppm	9.00	8.38	9.12	8.22*	8.98	8.19	9.30*	7.27	8.85	7.18
500 ppm	9.01	8.40	9.28	8.39	9.29*	8.29	9.16*	7.89	8.92	7.74*

* Significantly different from controls, $P < 0.05$

In the treated males, significant increases in hemoglobin and red blood cells were found at weeks 103 for the 100 and 500 ppm groups. However, the hemoglobin concentrations in treated females were significantly decreased at weeks 52 but increased at weeks 108. The red blood cell concentrations in the 500 ppm females were also significantly increased at weeks 108. Since consistent results were not obtained in males and females, the biological significance of these findings remains unclear.

No apparent compound-related effects were noted with respect to packed cell volume, platelet count, leucocyte count, leucocyte count differential, mean corpuscular hemoglobin concentration, mean corpuscular volume, and reticulocyte count.

Hematologic values presented in the final report were verified with those of the individual animal data and no discrepancies were found.

10. Clinical Chemistry

Plasma samples were collected and clinical chemistry determined at different intervals throughout the study.

No apparent compound-induced effects were noted with respect to SGPT, SGOT, uric acid, creatinine, total bilirubin, total cholesterol, electrophoretic protein fractions, sodium, potassium, calcium, chloride, and inorganic phosphorus.

Clinical values for other parameters investigated are given in the following table:

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	Wk 25		Wk 51		Wk 77		Wk 103		Wk 108	
	M	F	M	F	M	F	M	F	M	F
Alkaline Phosphatase (IU/L)										
Control	178	158	151	134	132	125	91	112	107	109
20 ppm	164*	153	143	134	142	144*	119*	103	109	120
100 ppm	170	156	142	142	145	123	124*	106	144*	119
500 ppm	156*	136*	129*	114*	145	113	137*	110	128	120
Creatinine Phosphokinase (IU/L)										
Control	230	206	298	229	140	192	127	239	234	177
20 ppm	193	274*	439*	183*	256*	223	126	187	265	206
100 ppm	259	329*	314	259	198	184	171	202	235	193
500 ppm	190	275*	276	180*	202	216	173	185	348*	254*
Lactate Dehydrogenase (IU/L)										
Control	536	456	623	487	282	517	340	581	557	485
20 ppm	470	601*	906*	396*	688*	556	359	474	605	550
100 ppm	641	730*	685	528	574*	441	460	505	567	524
500 ppm	474	581*	613	372*	549*	536	473	439	824*	694*
Urea (mg %)										
Control	51	49	46	53	58	58	89	45	96	36
20 ppm	54	55	50	52	67	64	104	47	95	39
100 ppm	63*	61*	50	63*	67*	71*	69	49	69*	45*
500 ppm	66*	57*	57*	63*	81*	69*	63	54*	64*	46*
Glucose (mg %)										
Control	145	137	134	127	147	143	125	129	105	128
20 ppm	144	138	145*	125	153	141	127	125	116	138
100 ppm	135*	125*	128	123	147	137	138*	125	116	141*
500 ppm	136*	124*	135	120	120	125	130	130	106	143*
Total Proteins (g %)										
Control	7.9	8.0	7.4	8.5	8.7	8.5	7.3	7.3	7.0	7.4
20 ppm	7.7*	7.9	7.6	8.3	9.0	8.2	7.0	7.9*	7.2	7.4
100 ppm	7.9	7.6	7.8	8.4	8.7	8.4	7.3	7.5	7.4	7.3
500 ppm	7.8	7.5*	7.4	8.2*	8.3	8.0*	6.9	7.8*	7.3	7.6
Albumin (g %)										
Control	4.1	4.2	3.5	4.3	4.2	4.2	2.1	2.7	2.1	3.0
20 ppm	3.9	4.3	3.4	4.3	3.8	4.0	2.2	2.9	2.3	3.1
100 ppm	4.1	4.1	3.8	4.4	3.5*	4.6	2.6*	2.8	2.6*	3.1
500 ppm	3.9	4.0	3.5	4.0*	3.9	4.1	2.5*	3.2*	2.7*	3.4*

* Significantly different from controls, $P < 0.05$

Significant decreases in alkaline phosphatase were observed in males of the 500 ppm group at weeks 25 and 51 and in all treated males at weeks 103. At weeks 108, all treated males had higher SAP than control but with statistical significances found only at the 100 ppm level. In the absence of consistent results, the biological significance of this finding remains unclear.

The lactate dehydrogenase enzyme in the treated males apparently was higher than control values throughout the entire investigation except at weeks 25. However, significant increases in all treated males were noted only at weeks 77.

Elevated blood urea nitrogen concentrations were noted for males at weeks 25, 51, and 77, and for females at all intervals with statistical increases found consistently at the 100 and 500 ppm dosage levels. These findings suggested that the elevated BUN observed apparently was compound-related.

Blood glucose was significantly increased in females of the two highest dosage groups at weeks 103 and 108. Other inter-group differences were recorded for creatinine phosphatase and total protein but without consistency over time and between the sexes. At weeks 108, the albumin levels of the treated animals were significantly increased for the 100 and 500 ppm males and for the 500 ppm females.

Individual values for blood chemistry were appended with the final report. No discrepancies between the final report and the raw data were found by this reviewer.

11. Cholinesterase Activity

Blood cholinesterase activity was determined at commencement of treatment, after 25, 51, 77, 103, and 108 weeks of treatment. Brain cholinesterase was recorded at study termination.

Prior to the evaluation of the cholinesterase activity, several issues should be raised and need clarification from the registrant.

a. Blood Cholinesterase

Plasma butyryl and acetyl cholinesterase and erythrocyte butyryl and acetyl cholinesterase activities were determined. The investigators indicated that the determinations were carried out by Technicon Method No. 354-75 P/A based on the method of Humiston and Wright, 1967.

This reviewer noted that certain values for cholinesterase activity were identical for different animals in different groups.

i. Plasma butyryl ChE., 25 wks, Males, Appendix 12 C, page 697

The value "238 iu/L" was repeated 16 times out of 40 determinations: 3 in the control, 5 in the 20 ppm, 6 in the 100 ppm and 2 times in the 500 ppm. A value of "259 iu/L" was also used 16 times: 4 in the control, 1 in the 20 ppm, 4 in the 100 ppm, and 7 times in the 500 ppm group. Therefore, out of 40 values reported for control and treated males at weeks 25, plasma butyryl ChE values of "238" and "259" were used 32 times (Attachment I).

ii. RBC acetyl ChE, Females, Weeks 51, Appendix 12D, p. 700

Out of 10 determinations in the control group, the number "523 iu/L" was reported 5 times.

Out of 10 determinations in the 500 ppm group, a value of "503 iu/L" was repeated 6 times and that of "523 iu/L" twice (Attachment II)

b. Brain Cholinesterase

Brain cholinesterase activity was determined at weeks 108 from samples taken from 10 males and 10 females of each group. The Technicon method was used based upon the method described by Humiston and Wright, 1967.

i. Brain butyryl ChE, Males, Weeks 108, Appendix 13, p. 711

Forty values, one each from 40 animals, were reported at this interval. The value "2068 iu/L" was used 23 times, that of "1772 iu/L" 11 times, and that of "2360 iu/l" 5 times.

It is noteworthy to indicate that all 10 determinations of the control group were reported as either "2068" or "1772 iu/L" (Attachment III).

ii. Brain butyryl ChE, Females, Weeks 108, Appendix 13, p. 712

Values of "2068" and "2360 iu/L" found repeatly in the males at this interval were also reported for females. Out of 40 determinations, the number "2068 iu/L" was used 14 times and that of "2360 iu/L" 19 times. An activity of "2656 iu/L" was also reported 7 times. All ten determinations of the 20 ppm group were reported as either "2360" or "2068 iu/L" (Attachment IV).

c. Plasma cholinesterase between males and females

At all intervals studied, the plasma butyryl ChE and acetyl ChE activities of females in all groups including the control were always 2-4 fold higher than those of the controls. Although, this reviewer recognizes that inter-sex differences in cholinesterase activity exists but is unaware of such a 2-4 fold differences. No explanation was given by the investigators.

The following table illustrates those differences:

Cholinesterase Activity (iu/L)	25 weeks M	25 weeks F	51 weeks M	51 weeks F	77 weeks M	77 weeks F	103 weeks M	103 weeks F	108 weeks M	108 weeks F
Plasma butyryl ChE										
Control	253	1475	274	3174	512	1805	838	1519	578	1207
20 ppm	242	1457	287	3270	480	1807	615	1456	692	1296
100 ppm	246	1277	288	3061	426	1746	606	1407	613	1299
500 ppm	259	1192	270	3322	450	1927	552	1501	534	1402
Plasma acetyl ChE										
Control	564	2428	743	3174	796	2499	1399	2281	1110	2034
20 ppm	550	2413	733	3270	758	2532	1199	2297	1340	2155
100 ppm	578	2134	724	3061	693	2331	1120	2227	1200	2001
500 ppm	579	1995	710	3322	758	2597	1000	2322	1049	2194

12. Urinalysis

Urinalysis on all groups was performed at study initiation and at weeks 24, 51, 77, 103, and 108 after treatment.

Slight depression in urine volume was noted in both treated males and females with significant reductions observed at weeks 103 and 108 for the two highest dose groups.

No compound-related effects on the urinary protein concentration were evident from the reported data. However, Table 11E on page 141 erroneously indicated a value of 1050 mg/dl for the control males at weeks 103. Based upon the raw data (Appendix 14E, page 726), this value should be corrected to 1000 mg/dl. The protein concentration in the urine was determined by precipitation with sulphosalicylic acid and compared wilth turbimetric standards or Multistix (Ames Company, Slough, England). From the raw data, the highest concentration of protein that could be determined from the urine was 1000/dl and this value was reported for most of the test animals at weeks 103 and 108.

No explanation was given by the investigators as to the sensitivity of the test method used. Apparently, 1000 mg/dl was the maximum level detectable by this method.

13. Organ Weights

Organs were collected from all animals sacrificed in interim and at study termination.

Significant increases in the relative brain weight were noted in males of the 100 and 500 ppm groups at weeks 52 and 104 and in females of the 100 and 500 ppm groups at weeks 52, 104, and 108. However, no changes in the absolute brain weight were associated with these dosage levels. The changes in relative brain weight were not considered as biologically significant by this reviewer since animals in these two highest dosage levels had lower terminal body weight.

Significant decreases in the absolute liver weight were noted in both males and females of the 500 ppm group at weeks 104 and 108 but without alterati in the relative liver weights. The absolute kidney weights were significantly decreased in the 100 and 500 ppm males and females at weeks 108. No changes in relative kidney weight were found.

In the males, the testes apparently were affected as indicated by increases in both relative and absolute weight observed in the treated groups. The significant increases in relative ovary weight noted ir the 100 and 500 ppm groups at weeks 108 were biologically insignificant in the absence of changes in absolute ovary weight.

Organ Weight (grams)	Brain Abs	Brain Rel	Liver Abs	Liver Rel	Kidneys Abs	Kidneys Rel	Testes or Ova Abs	Testes or Ova Rel
WEEK 52-MALES								
Control	2.0	0.46	15.9	3.7	3.3	0.76	3.2	0.75
20 ppm	2.0	0.49	14.2*	3.5	3.2	0.78	3.2	0.78
100 ppm	2.0	0.51*	14.9	3.9	3.3	0.84*	3.3	0.85*
500 ppm	1.9	0.56*	13.0	3.8	2.9*	0.85*	2.9*	0.86*
WEEK 104 MALES								
Control	2.0	0.48	16.4	4.0	3.6	0.89	5.4	1.32
20 ppm	2.0	0.51	17.5	4.4	3.9	0.99	4.5	1.11
100 ppm	2.0	0.52*	14.5*	3.8	3.5	0.90	4.2	1.10
500 ppm	2.0	0.53*	14.2*	3.8	3.2	0.86	5.0	1.33
WEEK 108 MALES								
Control	2.0	0.55	17.9	4.9	4.0	1.10	4.1	1.09
20 ppm	2.0	0.53	18.1	4.7	3.9	1.04	5.4*	1.41*
100 ppm	2.0	0.53	16.8	4.5*	3.7*	0.98*	5.3*	1.40*
500 ppm	2.0	0.57	15.6*	4.5	3.5*	1.03	4.7	1.34*
WEEK 52 FEMALES							Ovary (X 1(	
Control	1.8	0.75	9.4	3.8	2.0	0.82	62	25.2
20 ppm	1.8	0.77	8.3*	3.6	2.0	0.85	49*	21.0*
100 ppm	1.8	0.85*	8.4*	4.0	2.0	0.93*	56	26.7
500 ppm	1.8	0.85*	8.1*	3.8	2.0	0.94*	50*	24.0
WEEK 104 FEMALES								
Control	1.8	0.58	12.2	3.9	2.7	0.87	74	23.9
20 ppm	1.8	0.59	11.1	3.6	2.5	0.82	80	26.2
100 ppm	1.8	0.67*	11.7	4.2	2.5	0.90	75	27.0
500 ppm	1.8	0.67*	10.1*	3.7	2.3*	0.86	58	21.8
WEEK 108 FEMALES								
Control	1.8	0.57	13.4	4.1	2.9	0.89	64	19.4
20 ppm	1.8	0.58	13.2	4.2	2.8	0.90	67	21.2
100 ppm	1.8	0.64*	11.6*	4.0	2.6*	0.90	66	22.9*
500 ppm	1.8	0.65*	11.1*	4.0	2.5*	0.89	65	23.7*

Abs: absolute weight
Rel: relative weight - Organ to final body weight ratio
*: Significantly different from controls, $P \leq 0.05$

14. Gross Observations

There were no gross pathological observations which suggested compound-related effects from the necropsy data performed on weeks 28 or 52 of treatment (10 animals/sex/group).

After 108 weeks of treatment, the incidences of gross pathologic observations between the control and treated groups were similar except for the following findings:

° Reduced incidences of irregular or pallid cortical surfaces of the kidneys in males of the 100 and 500 ppm groups.

° Increased incidences of petechial hemorrhages in the lungs of females in the 500 ppm group.

° Decreased incidences of enlarged and hemorrhagic pituitary glands in females of the 500 ppm level.

15. Histopathology

In this review, histopathologic findings on non-neoplastic and neoplastic findings are summarized in two different tables for clarity. Note that all histopathologic reports were indicated as "Draft"

Non-neoplastic findings

After 52 weeks of treatment, no compound-related effects were noted from the reported findings.

After 108 weeks of treatment, nephrocalcinosis occurred only in the treated animals especially females but at a low frequency. Bile duct hyperplasia and focal necrosis of the liver were found at similar frequency and severity among all test groups. The incidences of perivascular lymphocytic infiltration in the lung of males in the two highest groups were higher than controls. Ovarian cysts were found at low frequency in both control and treated females.

Significant decreases in "purulent prostatitis" were noted in the treated groups but the biological significance of this finding is unclear. Increased incidences of "dilatation of gastric glands" in the stomach were found in the treated males with significant differences noted at the 100 and 500 ppm levels. Slight increase in "degeneration of the testes seminiferous tubules" was also observed in all treated males.

Non-neoplastic findings of interest collected from animals of the oncogenic phase are summarized in the next table.

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NON- NEOPLASTIC FINDINGS	CONTROL M	CONTROL F	20 PPM M	20 PPM F	100 PPM M	100 PPM F	500 PPM M	500 PPM F
Number of animals examined	60	60	60	60	60	60	60	60
Nephrocalcinosis								
Unilateral	0	0	0	4	1	2	2	5
Bilateral	0	0	0	0	0	5	0	1
Liver								
bile duct hyperplasia	50	12	53	9	54	11	56	17
focal necrosis	1	3	5	5	5	2	2	5
Lung, perivascular lympho. infiltration	15	17	15	26	29*	15	19	17
Luteal cyst								
Unilateral		3		5		7		4
Bilateral		0		0		1		0
Prostate, focal hyperplasia	11		24*		17		13	
Prostate, purulent prostatitis	30		17*		20		18*	
Stomach, pylorus								
Hyaline degeneration	24	2	21	3	4*	1	10*	1
Dilatation gastric glands	41	53	49	47	53*	55	52*	53
Testis								
Degeneration semi-tubules bilateral	41		44		52		46	
Degeneration semi-tubules unilateral	7		10		4		10	
Mineralization of tubules bilateral	6		5		5		6	
Mineralization of tubules unilateral	15		22		17		23	

* Significantly different from controls, $P < 0.05$

INCIDENCES OF RELEVANT NEOPLASMS
ANIMALS KILLED OR DYING DURING TREATMENT

Number of animals	29	26	25	21	17	21	16	17
Pheochromocytoma, benign	7	0	6	1	2	2	0	0
Pheochromocytoma, malignant	2	0	1	0	1	0	0	0
Pheochromocytoma total	9	0	7	1	3	2	0	0
Pituitary adenoma	16	16	12	7	8	9	3*	4*
Pituirary carcinoma	1	1	0	3	0	1	1	2
Pancreas islet cell neoplasms	2	0	2	0	2	0	1	0
Testes B interstitial cell adenomas (%)	18 (62)		16 (64)		14 (82)		12 (75)	

* : Significantly different from controls, $P < 0.05$

The incidences of pheochromocytoma observed in the control male animals which died or killed during the treatment period were higher than those of the treated groups.

INCIDENCES OF RELEVANT NEOPLASMS
ANIMALS SACRIFICED AT STUDY TERMINATION

	Control		20 ppm		100 ppm		500 ppm	
	M	F	M	F	M	F	M	F
Number of animals	31	34	35	39	43	39	44	43
Pheochromocytoma benign	6	3	2	4	4	0	2	2
Pheochromocytoma malignant	0	0	0	1	1	0	1	0
Pheochromocytoma total	6	3	2	5	5	0	3	2
Mammary gland adenoma	1	14	2	15	1	10	1	8
Pituitary adenoma	16	20	14	18	19	15	16	14*
Pituitary carcinoma	0	1	0	2	0	2	2	0
Pituitary neoplams total	16	21	14	20	19	17	16	14*
Pancreas islet cell neoplasms	0	3	1	0	9*	0	2	0
Testes B Interstitial cell adenoma (%)	28 (90)		35 (100)		39 (91)		42 (95)	

*: Significantly different from controls, $P < 0.05$

At study termination, significant decreases in pituitary adenomas were observed in females of the 500 ppm level. However, the incidences of pancreatic islet cell adenomas were significantly increased in males of the 100 ppm group. The findings of testes interstitial cell adenomas were similar between the control and treated groups.

The number of animals with primary neoplasms were slightly decreased in females of the two highest dosage groups. The incidences of male treated animals bearing malignant tumors were also lower than controls.

Neoplastic Findings - All animals of the oncogenic phase

The distribution of neoplastic findings in all animals of the oncogenic phase (60 animals/sex/group) is summarized in the next table.

The incidences of total primary neoplasms, total benign neoplasms, and total malignant neoplasms of the treated groups were biologically and statistically similar to those of the controls for both males and females.

The incidence of animals with pheochromocytoma (benign and malignant) was lower in treated males than in controls with significant differences found at the 500 ppm level. Similarly, treated males had lower incidence of pituitary adenoma with statistical differences found at the 500 ppm level ($P < 0.05$).

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INCIDENCE OF RELEVANT NEOPLASMS
ALL ANIMALS OF THE ONCOGENIC PHASE

	Control		20 PPM		100 PPM		500 PPM	
	M	F	M	F	M	F	M	F
Number of animals	60	60	60	60	60	60	60	60
Pheochromocytoma benign	13	3	8	5	6	0	2	2
Pheochromocytoma malignant	2	0	1	1	2	2	1	0
Pheochromocytoma total	15	3	9	6	8	2	3*	2
Mammary gland adenoma	3	23	2	17	1	15	2	9*
Pituitary adenoma	32	36	26	25	27	24	19	18*
Pituitary carcinoma	1	1	0	2	1	2	2	0
Pituitary neoplasms total	33	37	26	27	28	26	21	18*
Pancreas islet cell neoplasms	1	3	3	0	10*	0	3	1
Monocytic leukemia	12	6	9	3	12	7	11	6
Granulocytic leukemia	0	2	3	1	0	0	1	0
Testes B interstitial cell adenoma	46		51		53		54*	
Thyroid, neoplasms (para and follicular)	16	11	21	10	22	17	10	11

* : Significantly different from controls, $P < 0.05$

In females, the incidence of "mammary gland adenoma" was lower in all treated groups than in controls attaining statistical differences at the 500 ppm level ($P<0.01$).

There were two tumor types that occurred at a higher frequency in the treated males than in the controls. The incidence of "pancreatic islet cell neoplasm" increased in all treated groups. However, significant differences were attained only at the mid dose level (100 ppm) Interstitial cell testicular adenomas were found in 76.7, 85.0, 88.3, and 90.0% of the males receiving 0, 20, 100, and 500 ppm, respectively, with statistical significance ($P<0.05$) only at the highest dosage level.

DISCUSSION AND CONCLUSIONS

1. Systemic Effects

No compound-related effects were evidenced from the clinical observation, mortality, and gross necropsy data. Dose-related decreases in food consumption and body weight gain were noted in all treated groups throughout the entire study. However, the food conversion ratios of the treated groups were statistically comparable to those of the controls. These findings suggested that, at least for the 20 ppm group, the decrease in body weight should be considered as a consequence of a decrease in food intake and not as compound-related toxic manifestations. Unpalatabililty of the test material may account for the decrease in food intake.

No apparent compound-related toxic effects were observed with respects to hematologic values except for hemoglobin and red blood cell concentrations. Increases in hemoglobin and red blood cell values were noted at the 500 ppm level. The biological significance of these findings remain unclear.

Intergroup differences were noted for several clinical chemistry parameters. The significant increases in lactate dehydrogenase at week 77, in alkaline phosphatase and total proteins at week 103, and in glucose at week 108 could not be clearly related to Bolero administration in the absence of persistent and dose-response findings. However, persistent increases in blood urea nitrogen were observed in females in the 100 and 500 ppm groups and may have been compound-related.

There were no organ weight findings that could be definitely related to Bolero administration. For example, significant decreases in absolute liver and kidney weights were noted but were not associated with pathological findings. The relative liver and kidney weights of the treated animals were not consistently significant from those of the controls. Therefore, these findings were not considered by this reviewer as biologically significant. The only finding that may suggest a compound-related effect was the testis weights. Significant increases in both relative and absolute weights were found at week 108.

The incidences of "dilatation of the gastric glands" increased in the treated groups and attained statistical significances at the 100 and 500 ppm levels. Slight increases in the incidences of "degeneration of the testes seminiferous tubules" were also observed in the treated males.

No compound-related effects in urinalysis were evidenced from the submitted data. However, the registrant is requested to provide explanation concerning the sensitivity of the method used to measure urinary proteins. From the reported data, a value of 1000 mg/dl apparently was the maximum detectable level for this method. No explanations were given in the report by the investigators.

From the submitted findings, a systemic NOEL is tentatively determined at 20 ppm (lowest dose tested) with decreased food intake and body weight gain and increased blood urea nitrogen observed at the 100 ppm level.

2. Cholinesterase Activity

No conclusion could be drawn by this reviewer in terms of cholinesterase activity.

The registrant is requested to provide explanation concerning:

a. Identical values

In many occasions, certain cholinesterase activity values were repeatly reported for different animals sacrificed at the same interval.

- Plasma butyryl ChE value of "238 iu/L" was repeated 16 times out of 40 determinations performed on males at week 25.
- Plasma butyryl ChE value of "259 iu/L" was also recorded 16 times out of 40 determinations performed on males at week 25.
- Cell acetyl ChE value of "523 iu/L" was used 5 times out of 10 determinations performed on control females at week 51
- Cell acetyl ChE value of "503iu/L" was recorded 6 times out of 10 determinations performed on 500 ppm females at week 51.
- Brain butyryl ChE value of "2068 iu/L" was used 23 times out of 40 determinations performed at week 108.
- All 10 determinations of the control group at week 108 had a brain butyryl ChE value of either "2068" or "1772 iu/L".

b. Differences in ChE values between males and females

Significant differences in cholinesterase activity were found between males and females of the same group sacrificed at the same interval. At all intervals studied, the plasma butyryl ChE and acetyl ChE activities of females in all groups including the control were always 2-4 fold higher than those of the control. These differences apparently were beyond the usual expected differences between sexes and were illustrated on page 12 of this memo.

Some examples of these differences are indicated as follows:

e.g. At weeks 25, the plasma butyryl ChE activity of control males was 253 iu/L whereas that of females was 1,475 iu/L

e.g. At weeks 25, the plasma acetyl ChE activity of control males and females was respectively 564 and 2478 iu/L

(More examples are cited on pages 10-12 of this memo)

c. Sensitivity of the methods used

At weeks 108, the plasma butyryl ChE activity in all treated females was higher than controls with significant differences found at the 500 ppm level. Similarly, brain butyryl ChE activity of the 500 ppm males was also significantly higher than that of control.

No explanations were given by the investigators with respect to the increases in cholinesterase activity found in the treated animals. It is unclear as to whether Technical Bolero itself may interfere with the sensitivity of the test methods used.

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3. Neoplastic Findings

(In agreement with Louis Kasza, DVM, Ph.D., Toxicology Branch Pathologist)

No compound-related toxic effects on survival rate were noted from the submitted data. The incidences of total primary, benign, and malignant neoplasms collected from all animals of the oncogenic phase were similar between the control and treated groups. Slight increases in the percentage of animals with malignant tumors were observed in the Bolero treated animals killed or dying during the treatment period.

Two types of tumor were found at a higher incidence in the treated groups: pancreas islet cell adenomas and testes interstitial cell adenomas.

Pancreatic islet cell adenomas were found in 1 (1.6), 3 (5.0), 10 (16.6), and 3 (5.0) males (percentage) in the groups receiving 0, 20, 100, and 500 ppm, respectively. Although, statistical differences were found only for the 100 ppm group ($P<0.01$), data analysis showed a positive trend on dose ($P= 0.041$). The investigators indicated that a historical control range of 7.0 to 8.3% was found for this tumor type. However, historical control data collected at the testing facility were not submitted and therefore cannot be verified by this reviewer.

Testicular interstitial cell adenomas were observed in 76.6, 85.0, 88.3, and 90.0% of the males in the groups receiving 0, 20, 100, and 500 ppm, respectively. Statistical differences were noted at the 500 ppm ($P<0.05$, one tail test). However, data analysis did not indicate a positive trend ($P= 0.067$). The investigators also mentioned that testicular interstitial cell adenoma has a high spontaneous incidence (> 90%) in this strain. However, historical control data were not submitted.

Several tumor types occurred at a lower frequency in the treated groups than in controls. Pheochromocytoma, pituitary adenomas, and mammary gland adenomas were found less frequently in the treated animals than controls.

To fully evaluate the oncogenic potential of Bolero, the registrant is requested to provide historical control data concerning pancreatic islet cell adenomas and testicular interstitial cell adenomas for this species and strain collected at this testing facility. The historical control data must contain study identification, date, vehicle used, and should be limited to a period of 3 years prior to and 1-2 years after the conduct of this study.

CORE CLASSIFICATION

This study is presently classified as Core Supplementary Data. The requested clarification and additional data listed in the "Recommendation" section (page 2 of this memo) must be provided by the registrant. This study may potentially be upgraded depending upon the adequacy of the requested data.

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