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DATA EVALUATION REPORT

STUDY TYPE: Chronic/Oncogenicity - Mouse (83-1, 83-2)

TOX. CHEM. NO.: 628C

ACCESSION NUMBER/MRID NO.: 407625-01

TEST MATERIAL: Paclobutrazol

SYNONYMS: Clipper 2SC (formulation)

LABORATORY PROJECT ID NUMBER(S): CTL/C/1759 A, B, C AND E

REPORT NUMBER: 5014-72/274

SPONSOR: Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, England

TESTING FACILITY: Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, England

TITLE OF REPORT: Paclobutrazol: 104 Week Oral (Dietary Administration) Combined Toxicity and Carcinogenicity Study in the Mouse With a 52 Week Interim Kill

AUTHOR(S): D. C. Shaw

REPORT ISSUED: September, 1986

CONCLUSION:

This was a combined chronic feeding/oncogenicity study in Crl:CD-1(ICR)BR albino mice. Fifty-one animals/sex/group were fed 0, 25, 125 or 750 ppm technical Paclobutrazol (92.4% pure) in the diet for 2 years (0, 3.75, 18.75 or 112.5 mg/kg/day). Two control groups of 51 animals/sex were utilized. Nine additional animals/sex in the first control and high dose groups were kept as microbiological sentinals.

At 112.5 mg/kg/day, there was an increase in the severity of steatosis of the liver (males). There were also minor increases in relative and absolute liver weights (27% greater than controls for both absolute and relative liver weights in males and 18 and 19% greater than controls for absolute and relative liver weights, respectively for females). **The LOEL is 750 ppm (112.5 mg/kg/day) based on increases in severity of steatosis of the liver in males. The NOEL for chronic effects is 125 ppm (18.85 mg/kg/day).**

There appeared to be a borderline increase in Leydig cell tumors in high dose males (2/53, 0/50, 2/51, 1/52 and 5/52 for the control 1, control 2, low, mid- and high dose groups, respectively). A pairwise comparison was statistically significant at the highest dose level ($p<0.05$). In addition, a positive trend was observed with increasing dose ($p<0.05$). Although the authors stated that this was not considered to be biologically significant because the control values were low, it is considered to be a possible positive response. However, the study is considered to be inadequate for classification of the chemical for carcinogenicity for the following reason: although the chronic effects are considered to be sufficient to establish a NOEL and an LEL for chronic toxicity, they are not considered to be severe enough to indicate that the animals were tested at a sufficiently high dose level for an adequate carcinogenicity study.

This study is classified as Core Minimum as a chronic feeding study and Core Supplementary as an oncogenicity study (see discussion). It does not satisfy the regulatory requirement for an oncogenicity study. Due to the low dose levels tested and to the possible positive response for Leydig cell tumors, a repeat study will be needed if the use pattern for the chemical requires it.

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl)pentan-3-ol

Description: Off-white powder
Batch #(s), Other #(s): Batch # P29; test substance control reference #'s Y00001/001/032, 033 and 037; HLE Dispensary # HD 160/83-72
Purity: 92.4%
Source: ICI PLC
Vehicle (if applicable): None
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Crl:CD-1(ICR)BR albino mice
Age: 28 days at arrival, 40 days at start of study
Weight(s): not given
Source(s): Charles River (UK) Ltd., Margate, England

3. Procedure:

a. Dietary Preparation: A weighed amount of test article was mixed with a small amount of the powdered diet. The mixture was then diluted with powdered diet to obtain the desired dietary concentration for each group.

Frequency of preparation: Weekly for the first 7 weeks and then at fortnightly intervals.

Storage conditions: Not given; however, unless the powdered diet had to be refrigerated, it is assumed that it was stored at room temperature because the bulk test article was stored at room temperature in the dark, since it was known to be stable under these conditions.

Stability Analyses: Stability analyses were performed for another study conducted at the same laboratory (project number of study given, but type of study not given).

Homogeneity Analyses: Homogeneity analyses were performed for another study conducted at the same laboratory (project number of study given, but type of study not given).

Concentration Analyses: The concentration of the test article was determined in duplicate for each dietary level (including controls) for weeks 1, 3, 7, 9 and every 2 weeks thereafter until week 39, and then in week 42 and for every 4 weeks thereafter until week 102. The report stated that "if duplicate results were not in close agreement, the formulation was remixed and reanalysed, but a new batch was not prepared and if the concentrations of test article [were] found to be outside the limits of +/- 10% of nominal, a new batch was prepared and analysed".

b. Basis For Selection of Dose Levels: Selected on the basis of a preliminary 28 day dietary study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm w/w	Main Study 104 weeks male	Main Study 104 weeks female	Interim Sac. 52 weeks male	Interim Sac. 52 weeks female
Contr. 1	0	51+9*	51+9*	12	12
Contr. 2	0	51	51	12	12
3	25	51	51	12	12
4	125	51	51	12	12
5	750	51+9*	51+9*	12	12

*Microbiological Sentinals

d. Clinical Observations and Mortality: All animals were examined twice daily for mortality and morbidity. Moribund animals were killed and necropsied. Detailed clinical examinations were conducted weekly.

e. Body Weight Determinations: Individual body weights were recorded on the first day of treatment, at weekly intervals for the first 12 weeks and then every 2 weeks thereafter.

f. Food and/or Water Consumption: Food consumption was measured in the same pattern as body weights. Water consumption was measured from the bottles from each cage at weekly intervals until week 12. The authors stated that "since no differences between the groups were apparent no further measurements were made and the animals had access to water ad libitum from an automatic watering system".

g. Ophthalmological Examinations (if applicable): Not done.

h. Microbiological Sentinels: 18 male and 18 female sentinels were included in the study design (9 of each sex in the control and high dose groups). They were not part of the study and the survivors were killed at the end of the study. The authors stated that there had been no need to use them during the study.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Blood samples were collected during weeks 52 and 104 from the orbital sinus. Ten males and 10 females from each group were used.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc. (MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*		Reticulocytes
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

Collection times for blood (including # of animals): Blood samples were collected during weeks 52 and 104 from the orbital sinus. Ten males and 10 females from each group were used. These were different animals than the ones in which blood was withdrawn for the hematological measurements.

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
	Calcium*	x	Albumin*
	Chloride*		Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
	Phosphorus*	x	Cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	Enzymes:		Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides
	Creatine phosphokinase*	x	A/G Ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
	Serum aspartate aminotransferase (also SGOT)*		
	Gamma-glutamyl transpeptidase (GGTP)		

3) Urinalysis: Not done

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: not specifically stated, however, since the preparation of tissues for microscopic examination was to be conducted on all animals, including those which died prior to termination of the study, it is assumed that the gross necropsy was conducted on all animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: all animals.

k. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: all animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: all animals.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
Digestive system		**Cardiovasc./Hemat.**		**Neurologic**	
	Tongue		Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	xx	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Aorta	x	Pituitary*
x	Duodenum*	x	Lymph nodes*	x	Eyes (optic nerve.)*
x	Jejunum*	xx	Spleen*	**Glandular**	
x	Ileum*	xx	Thymus* (interim)	xx	Adrenals*
x	Cecum*	**Urogenital**			Lacrimal gland
x	Colon*	xx	Kidneys*	x	Mammary gland*
x	Rectum*	x	Urinary bladder	x	Parathyroids*
xx	Liver*	xx	Testes*	x	Thyroids*
x	Gall bladder*	x	Epididymides	**Other**	
x	Pancreas*	x	Prostate	x	Bone* (femur)
Respiratory		x	Seminal vesicle	x	Skeletal muscle*
x	Trachea*	xx	Ovaries	x	Skin
xx	Lung*	x	Uterus*	x	All gross lesions and masses
x	Larynx	x	Cervix	x	Hardarian glands
				x	Skull[1]

[1] Preserved but not processed further in the first instance.

1. Statistical Analyses: Group mean values and standard deviations were calculated where possible. Biological outliers were excluded from analysis. The authors stated that "survival probability functions were estimated by the Kaplan-Meier technique [and] survival curves were compared by the log-rank procedure. One-directional tests for trend across all groups and one-directional pairwise tests of control groups against treated groups were performed." One-way analysis of variance, pairwise comparisons using the t-test, analysis of covariance, the Kruskal-Wallis test, pairwise comparisons using the Wilcoxon rank-sum test, the Armitage test for a trend in proportions and the Chi-squared test with Yates' correction were all used for statistical analyses.

B. RESULTS:

1. Dietary Preparation: The authors stated that with some exceptions (almost all in the low dose group), the concentrations of all the diets were within 10% of nominal. Those concentrations that were greater than 10% outside of the nominal concentrations were all lower than the nominal and ranged as low as 78%). Some new batches were prepared and analysed and some were remixed and reanalysed. The results from the previously conducted homogeneity and stability tests were not reported.

2. Clinical Observations and Mortality: There were no dose-related effects on morbidity, mortality or clinical signs of toxicity. The percent surviving by the end of 104 weeks were as follows: 38, 44, 36, 47 and 53% for males and 55, 46, 56, 56 and 52% for females for the control 1, control 2, 25 ppm, 125 ppm and 750 ppm dose groups, respectively. The following tables summarize the mortality for males and females.

Group Survival for Males (# Alive)

Week	0 ppm	0 ppm	25 ppm	125 ppm	750 ppm
Start	63 (100)	63 (100)	63 (100)	63 (100)	63 (100)
4	63 (100)	63 (100)	63 (100)	63 (100)	63 (100)
12	62 (98)	63 (100)	63 (100)	63 (100)	63 (100)
28	61 (97)	63 (100)	63 (100)	61 (98)	61 (97)
44	60 (95)	61 (97)	60 (95)	59 (95)	58 (92)
52	59 (94)	60 (95)	60 (95)	59 (95)	57 (90)
68	45 (86)	46 (91)	45 (89)	43 (87)	46 (90)
76	39 (75)	42 (83)	44 (87)	40 (91)	46 (90)
84	36 (69)	38 (75)	38 (75)	36 (73)	42 (83)
92	30 (58)	32 (64)	29 (58)	32 (65)	38 (75)
104	20 (38)	22 (44)	18 (36)	23 (47)	27 (53)

() = % actuarially adjusted.

Group Survival for Females (# Alive)

Week	0 ppm	0 ppm	25 ppm	125 ppm	750 ppm
Start	63 (100)	63 (100)	63 (100)	63 (100)	63 (100)
4	63 (100)	63 (100)	63 (100)	63 (100)	63 (100)
12	63 (100)	63 (100)	63 (100)	63 (100)	63 (100)
28	63 (100)	63 (100)	61 (97)	63 (100)	62 (98)
44	62 (98)	63 (100)	60 (95)	62 (98)	60 (95)
52	62 (98)	61 (98)	58 (92)	62 (98)	60 (95)
68	50 (96)	46 (92)	48 (92)	47 (93)	47 (91)
76	50 (96)	44 (88)	48 (92)	44 (87)	44 (86)
84	48 (95)	42 (84)	46 (88)	42 (83)	38 (74)
92	43 (85)	37 (74)	42 (81)	39 (77)	35 (68)
104	28 (55)	23 (46)	29 (56)	28 (56)	27 (52)

() = % actuarially adjusted.

3. Body Weight Determinations: There were no treatment-related effects on body weight in either sex. A few mean values were statistically significantly different from the control values, however, these values were always greater than the controls.

4. Food and/or Water Consumption: No treatment-related effects on either food or water consumption were observed in either sex.

5. Hematology: No consistent treatment-related effects on hematological parameters were observed. In females, significant decreases in group means of total WBC and monocytes were observed at week 52 in several dose groups. These were non-dose related. There was also a significant increase in platelets at week 104 in the high dose females. Neither of these observations are considered to be biologically significant.

6. Clinical Chemistry: In males, at week 52, triglycerides were significantly decreased for the low and high dose groups and at week 104, triglycerides and cholesterol were significantly decreased in the high dose group. It is doubtful that these decreases were biologically significant because there were no supporting microscopic lesions and the animals did not appear to be malnourished. No consistent treatment-

related effects on any other clinical chemistry parameters were observed. The following table summarizes triglyceride and cholesterol for males and females.

Mean Triglyceride and Cholesterol Values for Males and Females

Group (ppm)	Males		Females	
	Cholesterol mg/dl	Triglycerides mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
		52 Weeks		
0	162	98	106	61
0	154	89	103	63
25	149	66*	104	51
125	147	74	127	61
750	126	61**	96	34*
		104 Weeks		
0	215	60	106	70
0	175	59	116	78
25	217	58	113	59
125	154	54	121	76
750	119**	41**	104	58

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

7. Gross Pathology:

Interim Sacrifice: No treatment-related lesions were observed. Frequent findings included ovarian cysts, uterine distension, tail lesions and various lesions in the liver and lung.

Animals Which Died or Were Sacrificed Moribund: Again, no treatment-related lesions were observed. Frequent findings included bladder distension; protruding and opacity of the eyes; enlarged heart, preputial glands, thymus, seminal vesicles, spleen, lymph nodes, liver and kidney; pale, mottled, irregular surfaces, cysts, and hydronephrosis of the kidney; irregular surface, pale, dark or mottled and masses or multiple masses in the liver; pale, red or dark, and nodules and masses in the lung; ovarian cysts; gelatinous or masses and sores

of the skin; fur loss; tail lesions; red fluid in the thoracic cavity; and distension and masses in the uterus.

Terminal Sacrifice: No treatment-related lesions were observed. Frequent findings included fat deposition in the abdominal cavity; and most of the lesions mentioned above.

8. Organ Weights: At the highest dose level, statistically significant increases in both absolute and adjusted (adjusted for body weights) liver weights were observed in males at 52 weeks and in both sexes at study termination when compared to controls. These increases were minor (18 - 27% increase over controls). In addition, a small but statistically significant increase in kidney weights of the females in the mid- (relative only) and high dose groups (absolute and adjusted) at 104 weeks were observed. No other treatment-related differences in organ weights were observed. The following tables summarize absolute and adjusted liver and kidney weights in males and females.

Absolute and Adjusted Liver and Kidney Weights

	Males		Females	
	Week 52			
	Absolute			
Dose (ppm)	Liver	Kidney	Liver	Kidney
0	1.814	.670	1.354	.378
0	1.588	.609	1.247	.370
25	1.605	.638	1.227	.349
125	1.655	.592	1.353	.366
750	1.979*	.640	1.419	.385
	Adjusted for Body Weights			
0	1.770	.661	1.334	.378
0	1.570	.606	1.305	.372
25	1.635	.644	1.231	.350
125	1.698	.601	1.333	.365
750	1.961	.636	1.398	.384

Absolute and Adjusted Liver and Kidney Weights

Dose (ppm)	Males		Females	
	Week 104			
	Absolute			
	Liver	Kidney	Liver	Kidney
0	1.874	.666	1.360	.383
0	1.671	.662	1.243	.384
25	2.164	.672	1.409	.381
125	1.892	.712	1.297	.401
750	2.388**	.696	1.609**	.417**
	Adjusted for Body Weights			
0	1.869	.656	1.315	.380
0	1.674	.668	1.268	.386
25	2.174	.690	1.389*	.378
125	1.899	.725	1.371	.408*[1]
750	2.377**	.676	1.564**	.414**

[1]Excluding the extreme value for animal 482 (group 3), the comparison with controls would be significant ($p<0.05$).

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

9. Histopathology:

a. Nonneoplastic lesions:

Interim Sacrifice: The following were the most commonly observed microscopic findings: foci of subcapsular cell proliferation in the adrenal; arthrosis in the knee joint of males; vacuolation, tubular regeneration and leucocyte foci in the kidney; ovarian cysts; distended (pro-estrus) uteri and leucocyte foci and steatosis in the liver. The severity of the steatosis was dose-related in the male liver.

Main Study: The microscopic findings were consistent with those which would be expected in aging mice. Some of the more common lesions included adrenal subcapsular focus; bone marrow hyperplasia; cerebral vacuolation; lenticular degeneration and retinal atrophy; cardiac fibrosis; joint arthrosis; renal cysts and

nephropathy; hepatic focal necrosis and steatosis; pulmonary congestion and the presence of several types of histiocytes; sciatic nerve neuropathy; ovarian cysts and atrophy; seminal vesicle distension; splenic hematopoiesis; testicular atrophy; thymus hyperplasia; thyroid follicular distension; and uterine cystic hyperplasia. Again, the only obvious treatment-related observation was that of severity of steatosis in the male liver. There were some other observations that may possibly have been treatment-related. These are summarized in the following table. The data include both interim animals as well as the animals in the main study.

Non-Neoplastic Lesions Observed in
Mice Fed Paclobutrazol for 2 Years

Observation Interim Animals	Group Sex	1 M	2 M	3 M	4 M	5 M	1 F	2 F	3 F	4 F	5 F
# Animals		10	11	12	11	11	11	12	10	12	10
Liver - # Examined		10	11	12	11	11	11	12	10	12	10
Steatosis (Gr 1)		1	1	1	0	0	0	0	3	0	0
Steatosis (Gr 2)		2	2	2	5	0	2	2	0	0	1
Steatosis (Gr 3)		2	5	5	4	1	3	4	4	5	1
Steatosis (Gr 4)		5	1	3	2	3	4	5	2	4	5
Steatosis (Gr 5)		0	2	1	0	7	2	1	1	3	3
Sporadic Deaths and Terminal Sacrifice											
# Animals*		53	52	51	52	52	52	51	53	51	53
Liver - #*		53	51	50	51	52	50	50	53	51	53
Steatosis (Gr 1)		32	23	28	30	18	18	24	19	25	19
Steatosis (Gr 2)		06	16	11	03	07	07	06	07	03	06
Steatosis (Gr 3)		13	09	10	10	12	09	13	13	13	16
Steatosis (Gr 4)		01	02	01	04	09	14	06	14	10	09
Steatosis (Gr 5)		01	01	00	00	05	02	01	00	00	03
Kidney - # Examined		53	51	51	52	52	50	50	53	51	53
Focal nephropathy		09	13	15	16	18	18	15	18	17	13
Lung - #		53	51	51	52	52	52	50	53	51	53
Congestion		12	10	09	12	10	06	08	05	02	12
Mandibular Lymph Nodes - # Examined		50	50	45	51	49	46	46	48	49	50
Hyperplasia		10	10	04	06	10	02	05	03	06	11
Mesenteric Lymph Lodes - # Examined		50	48	46	51	49	50	47	50	49	52
Hyperplasia		06	06	04	05	03	02	05	06	03	10

* These numbers include those animals that were designated for interim sacrifice but either died or were killed *in extremis*.

Neoplastic Lesions Observed in Mice (Cont.)

Observation	Group Sex	1 M	2 M	3 M	4 M	5 M	1 F	2 F	3 F	4 F	5 F
# Animals Examined		53	52	51	52	52	52	51	53	51	53
Lung - # Examined		53	51	51	52	52	52	50	53	51	53
Adenoma		23	16	10	10	13	13	09	17	08	06
Adenoma Multiple		01	06	02	03	06	01	01	00	01	03
Carcinoma		02	01	03	02	03	01	01	00	00	03
Carcino./Adenoma		00	00	00	00	00	00	00	00	01	00
Mammary Gland - # Examined		00	00	00	00	00	47	41	46	42	41
Adenoma		00	00	00	00	00	00	00	02	00	00
Carcinoma		00	00	00	00	00	03	03	02	03	02
Pituitary - # Examined		49	48	43	49	49	51	48	50	51	50
Adenoma		00	00	01	01	00	03	01	03	00	05
Testis - # Examined		53	50	51	52	52	00	00	00	00	00
Leydig Cell Tumor		02	00	02	01	5*	00	00	00	00	00
Uterus - # Examined		00	00	00	00	00	51	49	53	51	53
Leiomyoma		00	00	00	00	00	03	01	03	00	01
Stromal Polyp		00	00	00	00	00	01	06	03	05	03
Stromal Sarcoma		00	00	00	00	00	05	01	03	03	01

* $p<0.05$; test for increasing trend also significant. The authors stated that this was not considered to be biologically significant because variations from 0 to 5 cases have been seen in previous control groups, and in a concurrent study one of the 2 control groups had zero and the second control had 4 cases of Leydig cell tumors.

b. Neoplastic lesions:

Interim Sacrifice: There was no evidence of any dose-related increase in any tumor type in any of the tissues examined. The most common tumors seen at this interim period were adenomas of the lung (both sexes) and liver (males).

Main Study: With the possible exception of Leydig cell tumors (the question of which may be cleared up by submission of the mentioned historical control data), there was no evidence of any dose-related effect on the frequency of tumor-bearing animals. The following table summarizes the incidences of the most frequently found tumors in these animals. The numbers also include any animals that were designated for interim sacrifice but either died or were sacrificed in extremis prior to the interim sacrifice.

Neoplastic Lesions Observed in Mice Fed Paclobutrazol for 2 Years

Observation	Group No.	1	2	3	4	5	1	2	3	4	5
	Sex	M	M	M	M	M	F	F	F	F	F
# Animals		53	52	51	52	52	52	51	53	51	53
Hemolymphoreticular											
Lymphoma Lymphocytic		03	02	01	05	01	06	09	07	09	07
Hardarian Gland - Number Examined		52	50	49	52	51	51	50	52	51	51
Adenoma		05	03	03	11	02	02	04	09	02	07
Liver - # Examined		53	51	50	51	52	50	50	53	51	53
Adenoma		10	14	11	11	15	01	01	01	02	02
Adenoma Multiple		07	03	08	05	09	00	00	00	00	00
Carcinoma		02	00	05	01	01	00	00	00	00	00
Carcinoma Multiple		00	00	00	01	00	00	00	00	00	00
Carcino./Adenoma		00	01	04	02	02	00	00	00	00	00

Using the individual animal data, the incidences of Leydig cell tumors were examined for decreases in time-to-tumor development. There was no treatment-related indication of any decreases in time-to tumor development. In addition, statistical analyses were conducted on incidences after eliminating animals which either died or were sacrificed prior to the finding of the first Leydig cell tumor (82 weeks). The following table summarizes the results.

Incidences of Leydig Cell Tumors

Dose (ppm)	Group	Sporadic Deaths	Terminal Sacrifice	Total Animals
0	Control A	2/37	0/20	2/37[3]
0	Control B	0/40	0/22	0/40
25	Low Dose	1/38	1/18	2/38
125	Mid Dose	1/38	0/23	1/38
750	High Dose	2/42[1]	3/27[2]	5/42[4]

[1] For sporadic deaths, there were no differences between treated and controls.
[2] At terminal sacrifice, there were no differences between treated and controls (pairwise comparison with each control group separately).
[3] $p = 0.027$ for trend (Cochran-Armitage trend test).
[4] $p = 0.27$ compared to control A; $p = 0.03$ compared to control B; $p = 0.052$ compared to combined controls (pairwise comparisons).

10. Quality Assurance Measures: Signed Good Laboratory Practice and Quality Assurance statements were provided.

C. DISCUSSION: This was a combined chronic feeding/oncogenicity study in mice. The NOEL for chronic effects is 125 ppm based upon an increase in the severity of steatosis of the liver (males) and increased relative and absolute liver weights (minor effects, both sexes). The LOEL is 750 ppm. As a chronic study, the Core Classification is MINIMUM because the following tests were not conducted: urinalysis, ophthalmological examinations and some significant clinical chemistry parameters, particularly electrolytes. Since the chronic toxicity of this chemical in mice appears to be minimal, it is not likely that any effects would have been observed in these tests. Therefore, in this particular case, these tests are not considered to be critical to the acceptance of the study as a chronic toxicity feeding study in mice.

As an oncogenicity study in mice, the Core Classification is SUPPLEMENTARY because the observed chronic effects were not considered to be severe enough to indicate that the animals were tested at a sufficiently high dose level for an adequate oncogenicity study.

The tables indicated that the incidence of Leydig cell tumors of the testis were significantly increased in high dose animals. Although the authors of the report stated that the increase was not biologically significant because of the wide variation in the incidence of this particular tumor type seen in the historical controls, it is still considered to be indicative of a positive response.