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DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity/Oncogenicity Study - rat (83-5)

TOX. CHEM. NO.: 628C

ACCESSION NUMBER/MRID NO.: 407343-01

TEST MATERIAL: Paclobutrazol

SYNONYMS: Clipper 2SC formulation

LABORATORY PROJECT I.D. #'S: CTL/C/1763A-C, F; 72/273

REPORT NUMBER: 5055-72/273

SPONSOR: Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, SK10 4TJ England

TESTING FACILITY: Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, England HG3 1PY

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

AUTHOR(S): D.C. Shaw

REPORT ISSUED: 10/17/86

CONCLUSION:

This was a combined chronic feeding/oncogenicity study in Sprague-Dawley Crl:CD(SD)BR rats. Fifty animals/dose/sex were fed 0, 50, 250 or 1250 ppm technical Paclobutrazol (92.4% pure) in the diet (0, 2.5, 12.5 or 62.5 mg/kg/day) for 2 years. An additional ten animals/sex were placed in the control and high dose groups as microbiological sentinels. Another ten animals/sex were added that were sacrificed at 52 weeks.

At 62.5 mg/kg/day, there was an increase in hypertrophy/steatosis of the liver (both sexes) and increased liver weights (14 and 13% in males and 9 and 24% in females for absolute and relative liver weights over control values, respectively). **The LOEL is 1250 ppm (62.5 mg/kg/day) based upon an increase in hypertrophy/steatosis of the liver (both sexes). The NOEL for chronic effects is 250 ppm (12.5 mg/kg/day).**

There appeared to be a borderline increase in uterine stromal polyps in the high dose and possibly mid-dose females (0/50, 4/50, 5/50 and 7/50 for the control, low dose, mid-dose and high dose groups, respectively). Pairwise comparisons were statistically significant at all dose levels ($p<0.05$, $p<0.01$ and $p<0.01$ for the low dose, mid-dose and high dose groups, respectively), and a positive trend was observed with increasing dose ($p<0.05$). Although the authors stated that this was due to the 0 frequency in the controls, which is unusual, 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 oncogenicity 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 uterine stromal polyps, 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, ICI Test Substance Control Reference #'s Y00001/001/032, 033 and 037
Purity: 92.4% (w/w)
Source: ICI PLC
Vehicle (if applicable): In diet
Positive Control(s) (if applicable): N/A

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

Species and Strain (sexes): Male and female Sprague-Dawley Crl:CD(SD)BR rats
Age: 42 days at start of study
Weight(s): Not given
Source(s): Charles River (UK) Ltd., Manston Road, Margate, England

3. Procedure:

a. Dietary Preparation (if applicable): 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.

Storage conditions: Not stated, but assumed at room temperature because it was stated that the bulk test article was stored at room temperature in the dark, unless the diet itself had to be refrigerated.

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: Samples from each dose group were analysed weekly.

b. Basis For Selection of Dose Levels: Selection was based on 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.	0	50+10*	50+10*	10	10
1	50	50	50	10	10
2	250	50	50	10	10
3	1250	50+10*	50+10*	10	10

* Microbiological sentinels

d. Clinical Observations and Mortality: All animals were examined twice daily for clinical signs of toxicity and mortality. A detailed clinical examination was given once weekly.

e. Body Weight Determinations: Weekly for the first 12 weeks and then every 2 weeks.

f. Food and/or Water Consumption: Food consumption - same as for bodyweights; water consumption - weekly up to 12 weeks, then for 4 week periods at various time intervals.

g. Ophthalmological Examinations (if applicable): The eyes of 20 animals of each sex in the control and high dose groups were examined during weeks 50 and 102.

h. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Samples were collected before start of treatment and again during weeks 4, 12, 26, 39, 52, 78 and 104. Ten animals/sex/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*	x	Prothrombin time (PT)[1]
	Total plasma protein (TP)	x	Activated partial thromboplastin time (APTT)[1]
x	Leukocyte differential count*		

[1] At weeks 52 and 104 only

2) Clinical Chemistry:

Samples were collected at the same time periods as the samples for the hematological analyses. Twenty animals/sex/dose were used for the pre-dose bleed but on all other occasions 12 animals/sex/dose were used. The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
	Calcium*	x	Albumin*
	Chloride*		Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
	Phosphorus*	x	Cholesterol*
	Potassium*		Globulins
	Sodium*	x	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)*		
x	Serum aspartate aminotransferase (also SGOT)*		
	Gamma-glutamyl transpeptidase (GGTP)		

3) Urinalysis:

Collection times for urine (including # of animals): Samples were collected before the start of treatment and again during weeks 4, 12, 26, 39, 52, 78 and 104. Ten animals/sex/group were used.

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen

i. 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: All animals.

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

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

k. Statistical Analyses: Group mean values and standard deviations were calculated. Biological outliers were excluded. The following statistical techniques were used: Kaplan-Meier (survival), one-way analysis of variance, t-test, analysis of covariance, the Kruskal-Wallis test, Wilcoxon rank-sum test, one-directional test for trend, and a pairwise one-directional test.

B. RESULTS:

1. Dietary Preparation: All concentration analyses were within 10% of the nominal concentrations. Most of the results were below 100% of the nominal. The observed concentration ranges were as follows: 50 ppm - 43.6 to 51.2 (87-102%), 250 ppm - 224.5 to 252.0 (90 - 101%), 1250 ppm - 1128.0 to 1311.0 (90-105%). The results from the previously conducted homogeneity and stability tests were not reported.

2. Clinical Observations and Mortality: No treatment-related effects were observed in either mortality or in clinical signs of toxicity. The highest number of putative deaths were due to pituitary tumors in both sexes. Mammary tumors were also the cause of many deaths in females. At week 104, the following number of male survivors/dose group (50 animals) were as follows: 29 (58%), 30 (60%), 33 (66%) and 33 (66%) for the controls, low, mid- and high dose groups, respectively. The numbers for females (50 animals/dose group) were as follows: 21 (42%), 28 (56%), 23 (46%) and 22 (44%) for the controls, low, mid- and high dose groups respectively. The following tables summarize the group survival for males and females.

Group Survival (# Alive for Males)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
0	60 (100)	60 (100)	60 (100)	60 (100)
4	60 (100)	60 (100)	60 (100)	60 (100)
8	60 (100)	60 (100)	60 (100)	60 (100)
12	60 (100)	60 (100)	60 (100)	60 (100)
24	59 (100)	60 (100)	60 (100)	60 (100)
52	55 (95)	57 (97)	59 (98)	60 (100)
78	40 (84)	43 (88)	45 (92)	44 (88)
86	39 (82)	39 (80)	42 (86)	40 (80)
96	35 (76)	34 (57)	38 (78)	35 (70)
104	29 (60)	30 (61)	33 (70)	33 (66)

() = % actuarially adjusted

Group Survival (# Alive for Females)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
0	60 (100)	60 (100)	60 (100)	60 (100)
4	60 (100)	60 (100)	60 (100)	60 (100)
8	60 (100)	60 (100)	60 (100)	59 (98)
12	59 (100)	60 (100)	59 (98)	59 (98)
24	59 (100)	60 (100)	59 (98)	58 (97)
52	57 (98)	58 (98)	58 (98)	57 (95)
78	43 (90)	45 (92)	38 (78)	41 (83)
86	37 (77)	42 (86)	34 (70)	38 (77)
96	29 (48)	34 (57)	32 (66)	33 (55)
104	21 (44)	28 (57)	23 (47)	22 (45)

() = % actuarially adjusted

3. Body Weight Determinations: In males, no treatment-related changes were observed in either body weights or body weight gains for any dose group. In females, body weights in the high dose group fell below 90% of the control values at week 34 and decreased to 84% by week 104. Body weight gains in the high dose group were statistically significantly decreased throughout the study. Body weight gains in this dose group ranged between 87-92% of controls during the first 12 weeks. At week 52, they had dropped to 79% of controls and by week 104, they were 78% of the controls. No treatment-related changes were observed in any of the other dose groups. Although the mean body weight gain in the mid-dose group was 87% of the controls at week 104, this is not considered to be biologically significant because the value was below 90% of the controls only at this time point (it is noted, however, that the values between 52 and 104 weeks were not reported). The NOEL's for body weight and body weight gain are 250 ppm for females and 1250 ppm for males (for further consideration, see discussion in next section on food consumption). The following tables summarize the results:

Mean Body Weights (Males) (g)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
1	215.4	215.8	210.3	214.0
4	319.9	323.4	314.3	320.4
8	413.2	420.0	406.3	414.1
12	456.9	468.4	456.6	463.4
20	537.9	546.3	531.3	537.3
28	571.0	584.6	571.9	575.0
40	618.1	626.0	620.6	622.8
52	633.4	643.4	634.8	637.0
78	711.6	702.6	701.3	709.8
104	704.3	689.5	688.8	716.5

Mean Body Weight Gain (Males) (g)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
1	54.0	52.7	51.2*	50.4**
2	101.3	101.5	96.8*	98.4
3	139.8	141.4	134.2	137.1
4	158.5	160.3	155.1	156.8
5	188.5	191.8	185.6	186.9
6	215.3	218.0	210.1	212.9
7	234.7	239.0	231.5	235.2
8	251.8	256.9	247.1	250.5
9	267.5	272.3	264.0	267.3
10	286.1	290.2	280.8	284.0
11	296.6	305.0	294.5	299.4
12	295.5	305.3	297.4	299.7
52	471.5	479.7	475.6	473.4
104	540.6	529.5	529.5	551.0

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

Mean Body Weights (Females) (g)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
1	153.1	153.9	149.1	148.2
4	200.0	201.8	196.2	190.5
8	236.8	238.5	233.4	220.9
12	250.9	253.7	246.1	233.1
20	284.8	290.5	277.1	258.5
28	297.4	305.4	289.6	269.3
40	333.7	342.0	322.7	293.3
52	378.3	380.5	363.5	322.6
78	431.5	441.6	410.7	378.2
104	482.7	458.3	435.1	403.5

Mean Body Weight Gain (Females) (g)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
1	26.7	27.3	26.2	23.7** (89%)
2	47.0	48.5	48.2	43.2* (92%)
3	65.2	65.9	65.2	58.6** (90%)
4	73.7	75.2	73.3	66.0** (90%)
5	86.5	88.6	87.5	78.7** (91%)
6	95.3	98.2	96.3	84.6** (89%)
7	103.9	105.3	103.9	91.4** (88%)
8	110.5	111.9	110.5	96.6** (87%)
9	116.5	119.2	114.9	101.1** (87%)
10	121.2	125.2	120.2	105.3** (87%)
11	126.9	129.8	126.4	110.5** (87%)

Mean Body Weight Gain (Females) (g)

Week	0 ppm	50 ppm	250 ppm	1250 ppm
12	124.4	127.1	122.7	108.7** (87%)
52	251.7	254.2	239.7	198.8** (79%)
104	358.3	334.3	312.3*	280.4** (78%)

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

4. Food and/or Water Consumption: No treatment related changes in food consumption were observed in males. In females, statistically significant decreases in mean cumulative food consumption were observed at the highest dose level for the first week through the twelfth week. These values were 95-96% of the controls. The mean cumulative food consumption for high dose females was not significantly different from controls for weeks 46-52, 72-78, or 98-104. Mean food conversion efficiency for high dose females was not significantly different from controls at any time. Therefore, as the authors stated, "when food consumption and body weight gain are considered together as food conversion efficiency there was no apparent effect of treatment, at any of the diet concentrations." No significant treatment-related effects were seen in water consumption.

5. Ophthalmological Examinations: No treatment-related effects were observed.

6. Hematology: No treatment-related differences in hematological parameters were observed. There were a number of statistically significant differences noted, however, none of these are considered to be biologically significant. In high dose males, platelet counts appeared to be generally lower than controls but were statistically significantly decreased only at weeks 12 and 39. Platelet counts were occasionally decreased in females as well, but only reached statistical significance at week 52.

7. Clinical Chemistry: In females given 250 and 1250 ppm at week 39 and in females given 1250 ppm at week 52 triglyceride values were significantly decreased when compared to controls. The biological significance of this effect is unknown, since the animals did not

appear to be malnourished. BUN was increased in high dose females at weeks 39 and 52 when compared to controls. Triglycerides were still lower in the high dose females at weeks 78 and 104 but not significantly so. They were not decreased in males and were in fact slightly increased during these time periods. No other treatment-related differences were observed for any other clinical chemistry parameters. The following table summarizes triglyceride values for females.

Triglyceride Levels in Females mg/dl

Week	0 ppm	50 ppm	250 ppm	1250 ppm
Pre-dose	161	183	109**	133
4	42	55	40	44
12	49	59	44	56
26	56	79*	39	55
39	188	167	88**	68**
52	210	255*	195	132*
78	216	319	247	141
104	182	202	148	116

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

8. Urinalysis: No treatment-related differences were observed.

9. Gross Pathology:

Interim Sacrifice: No treatment-related effects were observed. Very little was observed; enlarged pituitary in females was the most common lesion noted.

Sporadic Deaths and Terminal Sacrifice: With the possible exception of enlarged liver in males, no treatment-related lesions were observed. The incidences of enlarged livers were as follows: 3/51, 4/49, 1/50, 7/50 in the controls, low, mid- and high dose animals, respectively. Frequent findings included: enlarged adrenals, hydronephrosis, mottled livers, red lungs and pale foci on the lungs, enlarged pituitaries, masses on the pituitaries, skin masses, fur loss, enlarged spleens, irregular tails, enlarged or diminished testes and enlarged thyroids.

10. Organ Weights: At week 104, statistically significant increases over controls in both absolute (males) and relative (males and females) liver weights were observed in the high dose group. These increases were minimal. No other treatment-related effects were noted. The following table summarizes liver weights at terminal sacrifice.

Mean Absolute Liver Weights and Mean Liver Weights Adjusted for Body Weight (g) at Terminal Sacrifice

Group	Male	Female
0 ppm		
Absolute	14.985	11.023
Adjusted	14.968	10.359
50 ppm		
Absolute	15.144	11.099
Adjusted	15.299	10.862
250 ppm		
Absolute	15.082	10.481
Adjusted	15.161	10.673
1250 ppm		
Absolute	17.111**	12.063
Adjusted	16.899**	12.804**

*p < 0.05; ** p < 0.01

11. Histopathology:

a. Nonneoplastic lesions:

Interim sacrifice: At 1250 ppm, a low grade centrilobular hypertrophy in the liver occasionally accompanied by a minor degree of steatosis was observed in 8/10 males and in 10/10 females. These lesions were not present in either the controls or in the other treated groups. No other treatment-related lesions were observed in any other treated group.

Sporadic deaths and terminal sacrifice: Again, as with the interim sacrifice animals, hypertrophy/steatosis in the liver was observed in the high dose animals. Although steatosis was also present in a few males fed 250 ppm, the authors stated that this probably reflects the tendency for aging animals to become obese, thus increasing their sensitivity to factors influencing lipid storage in hepatocytes. This is

a reasonable explanation. Therefore, the observation of steatosis in the 250 ppm males is probably not biologically significant. The incidences were as follows: 1/51, 0/49, 8/50 and 32/50 in males and 0/50, 0/50, 0/50 and 34/50 in females, for controls, 50 ppm, 250 ppm and 1250 ppm, respectively. Other frequent nonneoplastic lesions observed in these animals included: adrenal telangiectasis and various types of foci and nodules; bone marrow hyperplasia; retinal atrophy; cardiac fibrosis; glomerulonephropathy, mineralization and pyelitis of the kidney; nematodes; hepatic microcystic degeneration, steatosis, biliary proliferation and various types of foci; pulmonary congestion and foamy histiocytes; hyperplasia of the mandibular lymph node; cystic hyperplasia of the mammary gland; neuropathy of the sciatic nerve; ovarian atrophy; pancreatitis; prostatitis; radiculoneuropathy of the sciatic nerve; hemopoiesis of the spleen; testicular atrophy; and c-cell hyperplasia of the thyroid. The NOEL for non-neoplastic lesions is 250 ppm. The following table summarizes the incidences of hypertrophy/steatosis of the liver for the interim sacrifice and for sporadic deaths and the terminal sacrifice.

Hypertrophy/Steatosis of the Liver
Group 1 (0 ppm), Group 2 (50 ppm), Group 3 (250 ppm), Group 4 (1250 ppm)

Group #	1	2	3	4	1	2	3	4
Sex	M	M	M	M	F	F	F	F
				Interim Sacrifice				
	0/10	0/10	0/10	8/10	0/10	0/10	0/10	10/10
				Sporadic Deaths and Terminal Sacrifice				
	1/51	0/49	8/50	32/50	0/50	0/50	0/50	34/50

b. Neoplastic lesions:

Interim Sacrifice: No treatment-related tumors were found. The most frequently observed tumors were found in females and consisted of mammary fibroadenomas (1,1,1,0) and pituitary adenomas (2,5,2,1) in controls, low, mid-, and high dose animals, 10 of each, respectively.

Sporadic Deaths and Terminal Sacrifice: With the possible exception of uterine stromal polyps (benign - the question of which may be cleared up by submission of the mentioned control data from previous studies), 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 Rats Fed Paclobutrazol for 2 Years

Observation	Group Sex	1 M	2 M	3 M	4 M	1 F	2 F	3 F	4 F
# Animals		51	50	50	50	50	50	50	50
Adrenal - # Examined		51	50	50	50	50	50	50	49
Pheochromocytoma - Benign		10	06	12	11	01	05	02	04
Mammary Gland - # Examined		00	00	00	00	48	43	45	48
Fibroadenoma - Benign		-	-	-	-	13	15	14	13
Fibroadenoma - Multiple Benign		-	-	-	-	12	15	05	03
Pituitary - # Examined		51	50	50	50	49	50	49	50
Adenoma - Benign		26	27	28	27	34	41	32	35
Thyroid - # Examined		50	49	49	49	50	50	50	48
C-Cell Adenoma		09	15	08	04	05	09	06	09
Uterus - # Examined		00	00	00	00	50	50	50	50
Stromal Polyp - Benign		-	-	-	-	00	04	05	7*

* Positive trend with increasing dose ($p<0.05$) and for each of the pairwise comparisons with the control ($p<0.05$ for group 2, $p<0.01$ for groups 3 and 4). The authors stated that this was due to the 0 frequency in the controls. The 0 frequency was considered to be unusual, having been seen only once in 10 previous studies, the background incidence normally ranging up to 10 percent.

Using the individual animal data, the incidences of uterine stromal polyps 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 uterine

stromal polyp (both 52 weeks (considered unusual) and 76 weeks). The following table summarizes the results.

Incidences of Uterine Stromal Polyps

Dose (ppm)	Deaths From Wk 52	Deaths From Wk 76	Terminal Sacrifice	Total From Wk 52	Total From Wk 76
0	0/57	0/43	0/21	0/57[3]	0/43[6]
50	3/58	2/46	2/28	5/58[4]	4/46[7]
250	2/58	2/39	3/23	5/58[4]	5/39[8]
1250	4/57[1]	4/43[2]	3/22	7/57[5]	7/43[9]

[1] p = 0.0591 for treated minus animals which died before week 52.
[2] p = 0.058 for treated minus animals which died before week 76.
[3] p = 0.065 for trend (Cochran-Armitage trend test).
[4] p = 0.03 (pairwise comparison).
[5] p = 0.006 (pairwise comparison).
[6] p = 0.037 for trend (Cochran-Armitage trend test).
[7] p = 0.07 (pairwise comparison).
[8] p = 0.021 (pairwise comparison).
[9] p = 0.0105 (pairwise comparison).

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

C. DISCUSSION: This is a combined chronic feeding oncogenicity study. The study design is acceptable according to the EPA Testing Guidelines. The measurement of serum concentration of electrolytes was not part of the protocol. Since the chronic toxicity of this chemical in rats 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 rats. The NOEL for chronic effects is 250 ppm based upon an increase in hypertrophy/steatosis of the liver (both sexes) and increased liver weights (minimal - both sexes). The LOEL is 1250 ppm. As a chronic study, the Core Classification is MINIMUM.

As an oncogenicity study in rats, the Core Classification is SUPPLEMENTARY because the observed chronic effects are 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 effects seen were not considered to be potentially life threatening and included increased liver weights, cholesterol, hepatic aminopyrene-N-demethylase, and alanine transaminase. No supporting microscopic lesions were observed. One of the criteria for considering whether or not the animals were tested at an adequately high dose level is a

significant decrease in body weight and body weight gain. In this study, the females had a significant decrease in body weight and body weight gain. However, as discussed in the food consumption results section, the decrease appears to be related to food consumption because the food conversion efficiency was not affected. Therefore, in this case, the decrease in body weight and body weight gain was not biologically significant.

The tables indicated that the incidence of uterine stromal polyps were increased in the treated animals. A positive trend with increasing dose was observed as well as statistical significance for each of the pairwise comparisons with the control. Although the authors stated that this was due to the zero frequency in the controls, which is considered to be unusual, the response is considered to be a possible positive response.

Due to the low dose levels tested and to the possible positive response for uterine stromal polyps, a repeat study will be needed if the use pattern for the chemical requires it.