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

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MEMORANDUM

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SUBJECT: Review of the 2 Year Chronic Toxicity Study in Rats (Captafol) Technical (SX-945). EPA Reg. No. 239-22
TOX. Chem. No. 328

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Attached is the review of the Captafol 2 year chronic ra performed by Hazleton Labs., Vienna, Va., June 15, 1983, #210 The EPA accession numbers are 250921 - 250924.

We are concerned about the neoplastic nodules/ hepatocel carcinomas in the liver and the fibroadenomas in the female m glands. This study, at the low and middle dose levels, submi only the rats with gross lesions for histopathology. We will a histopathological examination of the remainder of the anima For example, at the 75ppm dose level for females, 23 fibroade were reported for only 33 (of 50 rats).

We will be performing an oncogenic risk assessment on th and need the following information:

1. A histopathology examination of all the livers at the lo mid dose levels.

2. A histopathology examination of all female mammary glands the low and mid doses.

Although this contract review classifies this study as g lines, it has been decided to reclassify it as supplementary pending receipt of the above information. The experimental da will be retained in Tox Branch since it may need to be reanal if time-to-tumor information is needed for risk assessment.

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MRID: Not specified

Study Type: Chronic Toxicity Study In Rats

Accession Number: 250921 - 250924

MRID Number: Not specified

Sponsor: Chevron Chemical Co. Richmond, CA

Contracting Lab: Hazleton Laboratories America, Inc. Vienna, VA (P.

Date: June 15, 1983

Reviewed by: _____ Date: 7/11/87
The MITRE Corporation

Approved by: _____ Date: 7/23/87
EPA

Test Material: Difolatan

Protocol:

The following descriptions of the materials and methods used for this study were abstracted and paraphrased from the original report.

1. Test substance and purity: Difolatan, Lot No. SX-945, Technical, White Powder. Three samples were analyzed and their purities ranged from 91.6 to 98.6%. For calculations of the test material in the diet, Difolatan was assumed to be 100% pure.

2. Species of animals: Charles River Crl:CD Sprague Dawley BR albino rats were quarantined three weeks and initiated on treatment at approximately seven weeks of age when body weight ranges were 132.6 to 313.6 gms for males and 145.6

to 230.5 gms for females. The animals were individually housed in stainless steel cages in rooms with 12 hours light and 12 hours dark, 63 to 84°F temperature, and 26 to 71% relative humidity.

3. Dosing Schedule: Four groups of 50 males and 50 females were administered dietary levels of 0, 75, 300 and 1200 ppm Difolatan in Purina Rodent Laboratory Chow. The diets were prepared fresh weekly and stored frozen. On days 1, 3, and 5 the animals were provided feed from the frozen reserves. Food and water were available ad libitum until terminal sacrifice after 121 weeks of feeding.

4. Parameters To Be Examined:

- Control and test diet analyses weekly in weeks 1-4 and monthly thereafter.
- Viability, general appearance, and deaths twice daily.
- Individual body weights and food consumption one week before initiation of treatment, weekly for thirteen weeks, and biweekly thereafter.
- Clinical examinations weekly (not recorded for weeks 14, 16, 18, 20, or 22 due to a technical error).
- Ophthalmologic examinations at weeks 52, 104, and 122.

- Hematology, clinical chemistry and urinalysis on ten rats/sex/group prior to initiation of treatment and at weeks 26, 52, 78, 104 and 122.
- Gross and microscopic pathology and selected organ weights and organ-to-body weight ratios at necropsy.

5. Statistics Used (The Statistical Analyses section is directly quoted from the chronic study report. MITRE confirmed statistical analyses provided for all tables in this report and for randomly selected data provided in the chronic study report.)

Statistical Analysis

"Absolute body weights, growth rates, total food consumption, clinical pathology data (except leukocyte differentials, erythrocyte morphology, and urinalysis), and organ weight data of the control group were compared statistically to the data of the treated groups of the same sex. (Absolute body weights and food consumption were recorded at weeks 1 to 121 and individual clinical pathology data were recorded at weeks 26, 52, 78, 104, and 122.) Absolute body weights were evaluated at initiation and at Weeks 13, 25, 39, 51, 65, and 77; and total food consumption (excluding pretreatment values) was evaluated from initiation through Weeks 13, 25, 39, and 51.

Individual growth rates (rates of body weight gain) were compiled using body weight values from the following intervals:

Analysis Interval[a]	Intervals Used (Week)[b]	
	Males	Females
0 - 13	0, 1, 3, 5, 8, 13	0, 1, 3, 5, 8, 13
0 - 25	0, 2, 4, 7, 12, 25	0, 2, 4, 8 15, 25
0 - 39	0, 2, 5, 9, 19, 39	0, 2, 6, [illegible], 23, 39
0 - 51	0, 2, 5, 11, 25, 51	0, 2, 7, 15, 33, 51

[a]The body weight and all organ weights of any animal having one or more tissue masses that weighed greater than 10% of their body weight were excluded from statistical analysis. In addition, individual organ weights were excluded if the organ was damaged at necropsy.

[b]Week 0 refers to initiation of study."

"Analyses of the above data were performed in the following order. Bartlett's test for homogeneity of variance was performed and if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA). If the variances proved to be heterogeneous, a $\log_{10}$ transformation was performed, which was followed by Bartlett's test. If the $\log_{10}$ transformation was ineffective in removing variance heterogeneity, a $\log_e$ transformation of the original data was performed, which was followed by Bartlett's test. If homogeneity could not be achieved by transformation, ANOVA of the nontransformed data was completed. In the case of significant overall variation as indicated by ANOVA of

homogeneous data, the Scheffe multiple comparison procedure was used to compare the group means. In the case of significant overall variances as indicated by ANOVA of heterogenous data, Games and Howell's modification of the Tukey-Kramer multiple pairwise comparison procedure was used to compare the group means. All analyses were evaluated at the 5.0% probability (one-tailed) level."

"In addition to the above data, cumulative survival (through study termination), was analyzed by the National Cancer Institute Package."

"Unadjusted tumor incidence was analysed using the Cochran-Armitage test for linear trend of proportions. All tumor analyses were evaluated at the 5.0% one-tailed probability level."

Results

Diet Analyses

Table 1 summarizes the diet analyses for Difolatan carried out between weeks 16 and 120. The means of assays of freshly prepared diets for all doses were within 10% of the target levels. One week after the diets were prepared, the percentages of target values were reduced from 91 to 61% at 75 ppm, from 93 to 72% at 300 ppm and from 94 to 89% at 1200 ppm. Because of instability of the test material/diet mixtures the calculated mean concentrations of Difolatan in the test diets over the 16 to 120 week period were 56

TABLE 1

SUMMARY OF DIFENZOQUAT ANALYSES IN DIETS PREPARED BETWEEN WEEKS 16 AND 120 OF THE CHRONIC TOXICITY STUDY IN RATS

Sample Assayed	Target Level		
	75 ppm	300 ppm	1200 ppm
		Percent of Target Value	
1. Fresh Mix[a]:			
Mean (%) ± SD[b] (%)	91 ± 11	93 ± 7	94 ± 5
Range (%)	57 - 106	66 - 105	83 - 104
N[c]	76	74	74
2. End of First Feeding[d]			
Mean (%) ± SD (%)	72 ± 20	76 ± 6	91 ± 7
Range (%)	49 - 95	58 - 84	82 - 105
N	54	50	50
3. End of Week:			
Mean (%) ± SD (%)	61 ± 11	72 ± 9	89 ± 7
Range (%)	37 - 85	55 - 99	77 - 104
N	54	52	54
Mean of 1 + 2 + 3 (%)	75	80	91
Calculated Mean Concentration	56 ppm	241 ppm	1096 ppm

[a] Fresh Mix = freshly prepared diet
[b] ± standard deviation
[c] N = number of samples analyzed between weeks 16 and 120
[d] End of First Feeding = end of first feeding provided day 1 and before second feeding provided day 3.

ppm (75% x 75 ppm), 241 ppm (80% x 300 ppm) and 1096 ppm (91% x 1200 ppm). Technical problems encountered in the diet analyses yielded data in weeks 1 to 16 that could not be verified and that were not included in the report. The diet samples were not reanalyzed because they were stored for a longer time than stability could be guaranteed.

Mortality

The survival rates did not differ significantly between control and Difolatan treated rats.

General Appearance

Alopecia and weeping lesions appeared in or near week 83 in three males and two females receiving 1200 ppm Difolatan. Skin scrapings revealed secondary Staphylococcus aureus, Proteus mirabilis, Bacillus spp, and/or Escherichia coli infections, but no parasites. The lesions appeared to be spontaneous and not related to treatment.

The incidences of palpable tissue masses noted in control and treatment groups of both sexes are reported for selected weeks in Table 2. The increases occurred primarily after week 65. Higher incidences were observed in females than males. In Table 2 significant increases in the number of females with palpable tissue masses were observed only in the 1200 ppm group at week 78 ($p<0.05$), week 91 ($p<0.001$), and week 120 ($p<0.05$). The LEL is 1200 ppm and the NOEL is 300 ppm for palpable tissue masses.

TABLE 2

INCIDENCES OF RATS WITH ONE OR MORE PALPABLE TISSUE MASSES AT SELECTED TREATMENT WEEKS

Treatment Week	No. animals with masses / No. animals examined							
	Males				Females			
	0 ppm	75 ppm	300 ppm	1200 ppm	0 ppm	75 ppm	300 ppm	1200 ppm
65	0/48	1/47	0/46	1/49	13/49	11/48	8/48	20/49
78	0/47	3/44	0/45	4/48	12/44	17/46	20/47	25/48*
91	1/42	2/39	3/41	2/45	5/38	14/39	14/43	23/46***
104	2/35	1/35	2/33	5/37	9/33	18/34	14/37	17/35
120	2/21	0/27	2/22	6/27	6/27	15/21	14/21	19/26*

* Significantly Different From Control Value ($p<0.05$)
*** Significantly Different From Control Value ($p<0.001$)

Body Weight and Food Consumption

Table 3 shows the group mean body weights for control and treated groups by sex for selected intervals. Significant ($p<0.05$) decreases in mean body weights of male and female high dose groups compared with their respective controls were observed in weeks 13 to 77. Significantly lower mean body weight gains ($p<0.05$) were also observed in high dose groups of both sexes compared with untreated control groups in weeks 13 to 51. The LOEL is 1200 ppm and the NOEL is 300 ppm for body weight effects of Difolatan. Note that the initial mean body weight for the high dose males was significantly lower than the male control. Lower food consumption by high dose males, but not females, in weeks 13 to 39 may contribute to the reduced body weight gain in males. There were no significant differences in food consumption in female control and Difolatan treated groups.

Opthalmologic Examination

Ophthalmologic examinations conducted in weeks 52, 104, and 121 revealed a few findings. Cataracts were observed with greatest but equal frequency in treated and control animals. No treatment related effects were observed.

Hematology

Hematologic findings reported in weeks 26 to 122 were comparable in control and dosed groups for each sex with no significant differences.

TABLE 3

MEAN BODY WEIGHTS OF CONTROL AND DIFOLATAN TREATED GROUPS OF RATS AT SELECTED INTERVALS

Weeks On Study	Mean Body Weight 1)							
	Males				Females			
	0 ppm	75 ppm	300 ppm	1200 ppm	0 ppm	75 ppm	300 ppm	1200 ppm
-1	221	214	211	207	153	157	159	155
Start	270	267	266	257[a]	176	177	182	179
1	306	310	305	282	193	193	198	189
13	502	509	496	455[a,b]	276	274	280	259[a,b]
25	556	557	555	506[a,b]	304	299	311	282[a,b]
39	599	595	593	546[a,b]	331	326	344	302[a,b]
51	631	628	620	569[a,b]	355	347	365	318[a,b]
65	637	628	620	579[a]	376	367	385	331[a]
77	659	644	639	603[a]	403	397	412	355[a]
121	601	529	569	542	356	419	399	364

[a] Statistically significant decrease in mean body weight compared with the control value ($p<0.05$).
[b] Statistically significant decrease in mean growth rate (weight gain) compared with the control value ($p<0.05$).

Clinical Chemistry

Clinical chemistry included analyses for total protein, albumin, globulin, albumin:globulin ratio, sodium, potassium, alkaline phosphatase, total bilirubin, BUN, glucose; SGOT, SGPT, calcium, direct bilirubin, creatinine, LDH, inorganic phosphorus, and total cholesterol. With few exceptions the mean group values for weeks 26 to 122 for both sexes were comparable for control and treated groups and were within the expected baseline range for Charles River rats (Bulletin 1984. The Charles River Breeding Laboratories, Inc. Wilmington, Mass.). The exceptions were (1) significantly ($p < 0.05$) reduced globulin in 1200 ppm males in weeks 52 to 122 (2) increase in albumin: globulin ratios in 1200 ppm males, and (3) significantly ($p<0.05$) decreased SGPT in 1200 ppm females in weeks 52 to 104. Other sporadic but not treatment related findings were noted through the chronic study. For clinical chemistry effects the LFT is 1200 ppm and the NOEL is 300 ppm Difolatan.

Urinalysis data were comparable for control and treated animals of both sexes for the duration of the study.

Organ Weights And Organ-To-Body Weight Ratios

Brain, heart, liver, kidney, testes, and ovaries from 10 to 25 animals per group were weighed at necropsy and the organ-to-body weight ratios were calculated. The only significant finding was an

increase in the mean absolute brain weight in 300 ppm females.

Gross Pathology

The incidences of selected diagnoses made at necropsy are list for control and treated rats in Table 4. Significant increases in discolored livers in 75 and 1200 ppm males ($p<0.05$) and 300 and 1200 ppm females ($p<0.01$) were observed. There were higher incidences pitted mucosa in stomach for 1200 ppm ($p< 0.05$) males and subcutaneous tissue masses in 1200 ppm ($p < 0.05$) females compared controls. Other gross pathological findings were not significantly different in control and treated groups. For gross pathology in females the LEL is 300 ppm and NOEL is 75 ppm Difolatan and in mal the LEL is 75 ppm and NOEL is not established.

Microscopic Histopathology

The incidences of major histopathologic anomalies affecting liver, kidney, stomach and mammary gland are listed in Table 5.

According to the detailed study protocol, "the preserved tissu from all control and high dose animals, and the kidneys, stomach, any unusual lesions and/or suspected neoplasms from low- and mid-d animals were embedded in Paraplast®, sectioned, stained with hematoxylin and eosin, and examined microscopically." Therefore, "number examined" in Table 5 is 50 or near 50 in all tissues liste for the control (0 ppm) and high-dose (1200 ppm) groups. In the

TABLE 4

INCIDENCE OF SELECTED GROSS PATHOLOGICAL FINDINGS IN CONTROL AND DIFOLATAN TREATED RATS

	Incidence							
	Male (ppm)				Female (ppm)			
	0	75	300	1200	0	75	300	1200
Liver (Number Examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Cystic Foci	6	3	5	4	7	5	4	13
Discolored Focal Areas	20	31*	26	32*	16	25	33**	30**
Mammary Gland (Number Examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Thickened	1	2	2	2	13	12	10	23
Stomach (Number Examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Thickened	6	5	12	12	5	6	7	10
Mucosa, Pitted	6	9	5	19*	4	6	8	12
Subcutaneous Tissue (Number Examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
One Mass	2	3	4	7	17	25	26	30*

* Significantly Different From Control Value ($p<0.05$).
** Significantly Different From Control Value ($p<0.01$).

TABLE 5

INCIDENCE OF SELECTED MICROSCOPIC HISTOPATHOLOGICAL FINDINGS IN CONTROL AND DIFOLATAN TREATED RATS[a]

Tissue Finding	Incidence						
	Male (ppm)				Female (ppm)		
	0	75	300	1200	0	75	300
Liver (Number Examined)	(50)	(34)	(32)	(50)	(50)	(29)	(37)
Cholangiectasis	2	3	4	1	6	6	6
Hepatocellular Carcinoma (M)[b]	1	1	3	2	0	0	1
Neoplastic Nodule (B)[b]	4	5	1	2	4	1	2
Kidney (Number Examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Chronic Progressive Nephropathy	49	48	49	49	45	40	42
Hyperplasia of Tubule Epithelium	2	1	1	13**	1	1	2
Megalocytic Cells	0	0	0	47***	0	0	0
Microcalculi	27	18	11**	3***	46	41	40
Pigment in Tubule Epithelium	11	7	9	12	14	16	18
Transitional Epithelia Hyperplasia	30	21	15**	10***	44	35	37
Stomach (Number Examined)	(48)	(49)	(48)	(50)	(50)	(50)	(50)
Congestion/Hemorrhage	4	7	11	4*	2	1	3
Erosion/Ulceration	15	18	15	28*	15	14	23
Hyperkeratosis/Acanthosis	10	8	15	34***	10	9	4
Increased Ground Substance In Mucosa-Glandular Region	0	1	40***	49***	0	0	17***
Increased Incidence of Dilated Gastric Pits	2	0	7	15**	0	0	1
Mammary Gland (Number Examined)	(48)	(5)	(3)	(42)	(49)	(33)	(35)
Fibroadenoma, benign[b]	0	1	0	0	22	23	25

* Significantly Different From Control Value ($p<0.05$).
** Significantly Different From Control Value ($p<0.01$).
*** Significantly Different From Control Value ($p<0.001$).

a According to the protocol, "The preserved tissues from all control and high dose animals, and the kidn and any unusual lesions and/or suspected neoplasms from low- and mid-dose animals were embedded in Par sectioned, stained with hematoxylin and eosin, and examined microscopically."

b Because all animals were examined in 0 and 1200 ppm groups and only animals with unusual lesions and/o neoplasms were examined in 75 and 300 ppm groups, statistical comparisons are not appropriate (see tes 12, 15 and 16).

low-dose (75 ppm) and mid-dose (300 ppm) groups, 50 or near 50 kidneys and stomachs are examined per group. In low- and mid-dose groups, only mammary glands and livers with unusual lesions and/or suspect neoplasms were examined microscopically; therefore the "number examined" in Table 5 is substantially below 50 for these tissues in low- and mid-dose groups.

In Table 5 increases in cholangiectasis ($p<0.05$) and neoplastic nodules ($p<0.01$) in liver in 1200 ppm Difolatan treated females were observed. Very high incidences of megalocytic cells in kidney were observed in 1200 ppm males (47/50; $p<0.001$) and females (48/50; $p<0.001$) compared with no occurance in all other treated and control animals. Microcalculi and transitional epithelial hyperplasia in kidney were evident in all groups, but were significantly decreased in 1200 ppm groups of both sexes ($p < 0.001$) and in 300 ppm males ($p < 0.01$). An increased incidence ($p<0.01$) of hyperplasia of tubule epithelium was observed in 1200 ppm Difolatan treated males. Chronic progressive nephropathy was evident in nearly all treated and control animals. Other histopathological findings involving kidney that are not listed in Table 5 were not treatment related.

Significant increases in several microscopic histopathological findings involving stomach were observed in Difolatan treated animals, particularly in high dose males and females (Table 5). The

incidence of erosion/ulceration was increased in 1200 ppm males ($p<0.05$). Significant increases in hyperkeratosis/acanthosis were observed in high dose males ($p< 0.001$) and females ($p< 0.05$). Treatment related increases ($p<0.001$) in ground substance in the glandular region of the stomach mucosa appeared in both 300 and 1200 ppm Difolatan treated groups of both sexes ($p< 0.001$). An increased incidence of dilated gastric pits was evident in 1200 ppm males ($p<0.01$). Congestion/hemorrhage and other findings not listed in Table 5 occurred sporadically in control and treated groups and were not treatment related.

The fibroadenoma incidence was significantly increased in the 1200 ppm group compared with the control (Table 5), where all animals were examined in each of these groups. In the low- and mid-dose, (75 and 300 ppm groups, respectively) only the animals with unusual lesions and/or suspect neoplasms of the mammary gland were examined microscopically. In contrast, all control and 1200 ppm animal mammary tissues were examined. Because of this difference in the composition of control versus low- and mid-dose groups, there is inherent bias in the data, and statistical comparisons of the control with these treatment groups are not appropriate. Because all animals were examined in the control and high-dose group the statistically increased mammary gland fibroadenoma incidence ($p<0.001$) is

biologically significant and the LEL for fibroadenomas is established to be 1200 ppm.

For microscopic histopathological effects overall excluding fibroadenoma, the LEL is 300 ppm and NOEL is 75 ppm Difolatan.

Conclusions

Groups of 50 male and 50 female Sprague Dawley rats were fed nominal dietary levels of 0, 75, 300, and 1200 ppm Difolatan for 121 weeks. Observations included, but were not limited to, mortality, body weight, food consumption, hematology, clinical chemistry, and pathology. The MTD was exceeded because of the observation in one or more treatment groups of reduced bodyweight, increased palpable tissue masses, changes in clinical chemistry parameters and gross and microscopic pathology.

- Because of the instability of Difolatan, the calculated mean concentrations of Difolatan in the test diets were 56, 241, and 1096 ppm instead of the nominal concentrations of 75, 300, and 1200 ppm, respectively.
- Mortality did not differ for control and Difolatan treated rats.
- A significant increase in palpable tissue masses was observed in 1200 ppm Difolatan treated females (LEL = 1200 ppm; NOEL = 300 ppm).

- Both sexes treated with 1200 ppm Difolatan had lower body weights than their controls (LEL = 1200 ppm; NOEL = 300 ppm). The reduced food consumption by 1200 ppm males during weeks 13 to 39 may have contributed to the lower body weight in males.
- The clinical chemistry examinations revealed reduced globulin in 1200 ppm males, increased albumin:globulin ratios in 1200 ppm males, and decreased SGPT in 1200 ppm females (LEL = 1200 ppm; NOEL = 300 ppm).
- Gross pathological findings included significant increases in the incidence of disclored livers, pitted stomach mucosa, and subcutaneous tissue masses (LEL = 300 ppm and NOEL = 75 ppm for females; LEL = 75 ppm and NOEL = not established for males).
- The primary microscopic histopathological diagnoses involved liver, kidney, stomach, and mammary gland anomalies. These were increases in cholangiectasis in liver, increases in hyperplasia of tubule epithelium, megalocytic cells and transitional cell hyperplasia in kidney, increases in erosion/ulceration, hyperkertosis/acanthosis, ground substance in glandular mucosa, and dilated pits in stomach. With respect to tumorigenesis an increase in fibroadenoma in mammary gland was observed. Overall, for microscopic pathology, excluding fibroadenomas of mammary gland in females, the LEL is 300 ppm and NOEL is 75 ppm Difolatan (nominal concentrations).