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

005740

FEB 25 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPTC - Review of Toxicology Data Submitted Under Accession Nos. 263691, 263692, 263693, 263694, 263695, 263696, and 263697
EPA Registration No. 748-223

TB Project: 2123
Caswell No.: 435

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor/Joanne I. Miller, PM Team 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Edith W. Hauswirth, Ph.D., Acting Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: PPG Industries, Inc., Pittsburgh, PA.

Action Requested:

Review and evaluate eight (8) toxicology studies submitted by the registrant as required by the EPTC Registration Standard dated September 30, 1983.

TB Conclusions/Recommendations:

A summary of the reported results and TB evaluations of these studies is as follows (detailed reviews are appended to this memorandum).

Study	Reported Results	TB Evaluation
1-Year Oral Feeding Study of the Chronic Toxicity of EPTC in Dogs, Hazleton Labs, #6100-109, June 10, 1986 (EPA Accession No. 263691)	Syst. NOEL = 600 ppm (Equiv. to 17.27 mg/kg/day) Syst. LOEL = 1800 ppm (HDT) (Equiv. to 48.51 mg/kg/day), based on slight but significant decreased testes weight, not accompanied by any histopathological changes.	MINIMUM - Higher dosages should have been used in the study.
Oncogenicity Study in Mice with EPTC, Hazleton Labs, #6100-104, May 15, 1986 (EPA Accession Nos. 263692 and 263693)	Syst. NOEL = 200 ppm (30 mg/kg/day) Syst. LOEL = 600 ppm (90 mg/kg/day), based on decreased body weight and food consumption in females. Not oncogenic at the HDT (1800 ppm, 270 mg/kg/day).	MINIMUM
Effect of EPTC on Pregnancy of the Rat, Huntingdon Res. Centre, #PPC 19/851002, November 6, 1985 (EPA Accession No. 263694)	Mat. NOEL = 300 mg/kg/day Fetal NOEL = 300 mg/kg/day Terat. NOEL > 300 mg/kg/day (HDT)	SUPPLEMENTARY - The study should be repeated at higher dosages.
Effect of EPTC on Pregnancy of the Rabbit, Huntingdon Res. Centre, #PPC 14 & 18/b5601, October 10, 1985 (EPA Accession No. 263694)	Mat. NOEL ≥ 300 mg/kg/day (HDT) Fetal NOEL ≥ 300 mg/kg/day Terat. NOEL ≥ 300 mg/kg/day	SUPPLEMENTARY - The study should be repeated at higher dosages.
Two-Generation Reproduction Study with EPTC in Rats, Hazleton Labs, #6100/108, June 9, 1986 (EPA Accession Nos. 263695 and 263696)	Parental NOEL = 50 ppm (3.75 mg/kg/day) Parental LOEL = 200 ppm (15 mg/kg/day), based on degenerative cardiomyopathy Repro. NOEL = 200 ppm Repro. LOEL = 800 ppm, based on reduced pup weight.*	MINIMUM
Mouse Lymphoma Mutagenesis Assay (TK+/- TK-/-) of EPTC, SRI International #LSC-8644-1, February 1986 (EPA Accession No. 263697)	Positive at all doses (42-250 ug/mL) with activation; negative without activation up to toxic level (250 ug/mL).	ACCEPTABLE
Clastogenic Evaluation of EPTC Technical, 518-996 BR-85-40, in an In vitro Cytogenic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells, Stauffer Biomettics #20990, November 1985 (EPA Accession No. 263697)	Negative with/without activation up to cytotoxic levels (150-225 ug/mL).	ACCEPTABLE
Evaluation of the Potential of Ethyl (N,N Dipropyl)Thiocarbamate to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures, SRI International #LSC-8644, January 1986 (EPA Accession No. 263697)	Negative up to toxic level (250 ug/mL); procedural and reporting deficiencies.	UNACCEPTABLE

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* Although not specifically stated in the Contractor review, TB does not consider the reduced pup weight at 7 days in the F1 generation of the 200 ppm group to be biologically significant or compound related, since this was the only time point at which statistical significance was achieved (i.e., not seen at earlier or later time points).

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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copy 1

EPA: 68-02-4225
DYNAMAC No. 248A
February 4, 1987

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DATA EVALUATION RECORD

EPTC

Chronic Feeding Study in Dogs

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-4-87

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EPA: 68-02-4225
DYNAMAC No. 248A
February 4, 1987

[redacted]

005740

DATA EVALUATION RECORD

EPTC

Chronic Feeding Study in Dogs

REVIEWED BY:

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Principal Reviewer
Dynamac Corporation

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Date: 2-4-87

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Judith Hauswirth, Ph.D.
EPA Section Head, Section VI
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Signature: Judith A. Hauswirth
Date: 2/6/87

DATA EVALUATION REPORT

00574C

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic feeding study in dogs.

ACCESSION NUMBER: 263691

TEST MATERIAL: EPTC, S-ethyl dipropylthiocarbamate.

SYNONYMS: Eptam.

STUDY NUMBER(S): 6100-109.

SPONSOR: PPG Industries, Inc., Barberton, Ohio.

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI.

TITLE OF REPORT: One-Year Oral Feeding Study of the Chronic Toxicity of EPTC in Dogs.

AUTHOR(S): Giesler, P., Dickie, B., and Tisdel, M.

REPORT ISSUED: June 10, 1986.

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CONCLUSIONS:

Under conditions of the study, EPTC was not toxic when fed to male and female beagle dogs for 1 year at levels of 200, 600, or 1800 ppm, representing mean compound consumption for males of, respectively, 5.57, 17.27, and 48.51 mg/kg/day and for females, 6.08, 17.39, and 54.66 mg/kg/day. Mean body weights of males fed 600 ppm were significantly ($p < 0.05$) decreased from week 32 (10% decrease) to week 52 (12% decrease). The mean body weights of males fed 200 and 1800 ppm and all dosed females were similar to controls. There were no compound-related effects on mortality, clinical observations, food consumption, hematology, urinalysis, or pathology. The values of various clinical chemistry parameters in dosed animals varied sporadically from controls. At 1800 ppm there was a statistically significant decrease in absolute testes weights as well as testes/body weight (right only) and testes/brain weight (right only) ratios; however, these changes were not accompanied by any histopathological changes.

Although the NOEL could be set at 600 ppm based on the weight changes in testes at the HDT, the biological significance of this finding is questionable, and this reviewer feels that EPTC should have been tested at higher dosages.

Classification: Core Minimum.

A. MATERIALS:

1. Test Compound: EPTC, technical, lot No. 518-996 (BR83-64); description: not reported; purity: 98.4%.

2. Test Animals: Species: Canis familaris; strain: Beagle; age: 5 months; mean weights: males--7.0-9.7 kg and females--6.2-8.1 kg; source: Hazleton Research Animals, Cumberland, VA.

B. STUDY DESIGN:

1. Animal Assignment: After 20 days of acclimation, animals were assigned to the following test groups with a computerized randomization procedure:

Test Group	Dose in Diet (mg/kg/day)	Main Study (12 months) Male	Main Study (12 months) Female
1 Control	0	5	5
2 Low (LDT)	200	5	5
3 Mid (MDT)	600	5	5
4 High (HDT)	1800	5	5

During the acclimation period, a complete physical examination and fecal examination for parasites were performed.

Rationale for dose selection: The dose levels selected for this study were based on a 3-month subchronic toxicity study in dogs in which compound-related decreases in body weight gains (34% of control) and plasma cholinesterase (CHEP) activities (71% to 76%) were observed in males receiving 1800 ppm.

2. Diet preparation: Diet was prepared weekly and stored at 5°C. Samples of test diet taken for analysis were stored at -6°C. The purity of the test compound was analyzed by the sponsor at pretest, at 4-month intervals during the study, and at study termination. Samples of treated food were analyzed by the sponsor for concentration at weekly intervals, homogeneity at study initiation, and stability at study initiation and on day 48.

   Results: The test compound was found to be stable (±0.1-±0.2%) throughout the study period. The test compound was homogeneously mixed with the diet; recovery values of the diets were within acceptable limits, e.g., 98-101% of nominal values. The mean concentrations of four separate sample sites were 200±2.16 (200 ppm), 596±5.35 (600 ppm), and 1789.25±25.82 ppm (1800 ppm). The mean concentration recovery values for all dose levels of diet for 53 weeks were ≥97% of the nominal values; the mean target levels for the study period were 195.09±7.44 (200 ppm), 582.2±10.48 (600 ppm), and 1743.43±48.24 ppm (1800 ppm). The diets were reported to be stable for the duration of the study; stability recovery values ranged from ≥98% for samples shipped frozen to ≥95% for samples shipped at ambient temperature.

   The test diets were warmed to ambient temperature for 1 day during month 10 of the study due to technical error. The samples, which were stored in sealed containers, were analyzed prior to feeding on the following day; test diets were reported to be unaffected.

3. Animals received food, Purina Certified Canine Diet #5007, 2 hours/day, 7 days per week, and water ad libitum.

4. Statistics: Body weights, food consumption, clinical chemistry, organ weights, organ-to-body weight ratios, and appropriate hematologic data were analyzed using analysis of variance followed by Dunnett's test for comparison of group means.

5. A quality assurance statement was signed and dated June 11, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality. An electrocardiogram (EKG) was performed on all animals at study initiation and at 3, 7, and 12 months. Immediately prior to study termination, neurological evaluations were performed on control and high-dose males and females.

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Results: It was reported that there were no overt signs of toxicity. Clinical changes observed (e.g., occasional emesis, fecal condition--texture and amount, alopecia, and erythema) were considered to be consistent with strain-matched historical laboratory controls. However, three high-dose males and three high-dose females and one mid-dose male and two mid-dose females exhibited little or no feces during the first week of testing even though food consumption was considered to be comparable to that of the controls. Control animals did not display this same pattern until 3-5 months later and then in only one dog/sex. There were no compound-related effects on EKG or neurologic parameters. Two high-dose males exhibited some abnormal EKG conduction at 3, 7, and 12 months; however, this abnormality has been recognized in strain-matched historical laboratory controls, and cardiac lesions were not found in these animals at necropsy. This finding is therefore not considered toxicologically significant.

2. Body Weight: Dogs were weighed weekly through 14 weeks, at week 16, and then every 4th week through week 52.

   Results: Mean body weights of males fed 600 ppm were significantly ($p < 0.05$) decreased relative to controls from week 32 (90% of controls) to week 52 (88% of controls). The mean body weight gain for this group was also significantly ($p < 0.05$) decreased when compared to controls from weeks 16 to 52. The mean body weights of males fed 200 and 1800 ppm and all dosed females were similar to controls. Table 1 presents mean body weight data at selected intervals.

3. Food Consumption and Compound Intake: Consumption was determined daily from study initiation to week 14, then at the same intervals as weighings. Compound intake was calculated from the consumption and body weight gain data.

   Results: Food consumption was similar in dosed and control groups of males and females. Food efficiency was not calculated. Mean compound consumption for males fed 200, 600, and 1800 ppm was 5.57, 17.27, and 48.51 mg/kg/day, respectively, and for females, 6.08, 17.39, and 54.66 mg/kg/day for the same dosed groups. Table 2 presents food and compound consumption data at selected intervals.

4. Ophthalmological examinations were performed pretest on all animals and at study termination.

   Results: Two high-dose females had prolapsed Harderian glands of the left eye; following surgical removal of the prolapsed glands the animals appeared normal. This was not considered to be compound related; there were no other changes reported.

5. Blood was collected from the jugular vein of unanesthetized animals before treatment and at 3, 6, and 12 months for hematology and clinical analysis from 5 animals/sex/dose. The CHECKED (X) parameters were examined. Animals were fasted prior to blood collection.

TABLE 1. Representative Results of Mean Body Weights (±SD) of Dogs Fed EPTC for 1 Year[a]

Dose Group (ppm)	Mean Body Weights (kg) at Week					
	0	13	32	40	48	52
			Males			
0	8.0±0.52	10.9±0.72	11.5±0.84	12.0±0.79	11.8±0.71	11.9±0.87
200	8.9±0.66*	12.0±1.16	12.6±1.73	13.1±1.69	13.1±2.05	13.1±2.14
600	7.9±0.53	10.3±0.77	10.4±0.47*	10.7±0.34*	10.6±0.45*	10.5±0.37*
1800	8.4±0.77	11.2±1.01	11.6±0.96	12.0±1.07	11.7±1.14	11.8±1.08
			Females			
0	7.1±0.59	9.4±0.58	10.0±0.43	10.1±0.43	10.3±0.54	10.0±0.49
200	6.9±0.50	9.3±0.85	9.7±0.91	9.6±1.01	9.9±0.84	9.6±0.89
600	7.4±0.30	9.8±0.63	10.0±0.65	10.0±0.60	10.3±0.92	10.5±0.84
1800	7.0±0.68	9.4±0.97	9.8±1.27	9.9±1.06	10.0±1.02	9.9±0.91

sed on six dogs/sex/group.

gnificantly different from control value ($\alpha = 0.05$) as evaluated by the study authors ing Dunnett's test.

TABLE 2. Representative Food and Compound Consumption by Dogs Fed EPTC for 1 Year[a]

Dose Group (ppm)	Mean Food Consumption (kg±SD) at Week						Mean Compound Consumption (mg/kg/day)
	1	13	32	40	48	52	
				Males			
0	1.8±0.19	2.2±0.27	2.4±0.28	2.6±0.25	2.5±0.18	2.2±0.20	—
200	1.7±0.36	2.3±0.20	2.3±0.25	2.6±0.34	2.6±0.17	2.2±0.32	5.57
600	1.4±0.49	2.0±0.25	1.9±0.24	2.2±0.44	2.1±0.38*	1.8±0.37	17.27
1800	1.5±0.32	2.0±0.18	2.1±0.36	2.3±0.24	2.1±0.17*	1.8±0.19	48.51
				Females			
0	1.5±0.18	2.0±0.24	2.0±0.39	2.2±0.30	1.9±0.13	1.4±0.36	—
200	1.6±0.29	2.0±0.25	1.8±0.37	2.2±0.28	1.9±0.51	1.7±0.41	6.08
600	1.5±0.20	1.9±0.23	1.9±0.48	1.9±0.46	2.1±0.20	1.8±0.33	17.39
1800	0.9±0.70* [b]	1.9±0.37	1.9±0.29	2.3±0.47	2.0±0.34	1.7±0.30	54.66

[a]Based on six dogs/sex/group.

[b]Reevaluated by our reviewers and found significant ($p < 0.05$) using ANOVA followed by Duncan's test for multiple comparisons.

*Significantly different from control value ($\alpha = 0.05$) as evaluated by the study authors using Dunnett's test.

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a. Hematology

X	Hematocrit (HCT)†		Total plasma protein (TP)
X	Hemoglobin (HGB)†	X	Leukocyte differential count
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Platelet count†	X	Mean corpuscular volume (MCV)
X	Reticulocyte count (if signs of anemia were present)	X	Prothrombin time
		X	Partial thromboplastin time (PTT)

†Recommended by Subdivision F (October 1982) Guidelines.

Results: There were no toxicologically important effects on hematology parameters. Significant ($p < 0.05$) increases were found in the monocyte count at 3 months in males fed 600 ppm and the eosinophil count at 6 and 12 months in males fed 1800 ppm; however, the values were within the range of those found for strain- and age-matched historical laboratory controls and were not considered to be compound related. The hematologic parameters of females were similar to controls.

b. Clinical Chemistry

	Electrolytes
X	Calcium†
X	Chloride†
	Magnesium†
X	Phosphorus†
X	Potassium†
X	Sodium†

	Enzymes
X	Alkaline phosphatase (ALP)
X	Cholinesterase
X	Creatine phosphokinase*
X	Lactic acid dehydrogenase*
X	Serum alanine aminotransferase (also SGPT)
X	Serum aspartate aminotransferase (also SGOT)

	Other
X	Albumin†
X	Blood creatinine†
X	Blood urea nitrogen† (BUN)
X	Cholesterol†
X	Globulins
X	Glucose
X	Total bilirubin
X	Total protein
	Triglycerides
X	Gamma glutamyl transferase

†Recommended by Subdivision F (October 1982) Guidelines.

*Performed pretest and at 3, 6, 8 (control and high dose only), and 12 months; the 8- and 12-month tests were performed in an attempt to assess potential cardiac toxicity.

Acetylcholinesterase activity was determined in plasma (CHEP) and erythrocytes (CHER) before dosing and at 3 and 12 months; brain cholinesterase (CHEB) activity was determined at 12 months. The blood and plasma samples were obtained and analyzed immediately following feeding. At 12 months, creatine phosphokinase (CK) and lactic acid dehydrogenase (LDH) values in males receiving 1800 ppm were found to be significantly different from controls. Therefore, blood for isoenzyme determinations was collected from 200- and 600-ppm animals, although not on the same day. Since fresh samples indicated some variation, total CK and CK isoenzymes were conducted on frozen serum samples.

Results: Various blood chemistry parameters in dosed males and females were reported to be significantly different from their respective control values; however, these values were within the range of those found for strain- and age-matched historical laboratory controls and were not considered to be compound related by the study authors. Selected clinical biochemistry parameters are listed in Table 3.

Mean values for glucose [significant ($p < 0.05$) at 12 months] and cholesterol [significant ($p < 0.05$) at 6 months] were found to be increased throughout the study in males receiving 1800 ppm; albumin was found to be slightly but significantly ($p < 0.05$) decreased at 3 months in this same group. Calcium was found to be significantly ($p < 0.05$) decreased at 6 and 12 months in males receiving 600 and 1800 ppm and potassium at 12 months in high-dose males. Gamma glutamyl transferase (GGT) was slightly increased at 6 and 12 months in all dosed males.

There was no toxicologically important inhibition of cholinesterase activity in red cell, plasma or brain. CHER values were found to be slightly but significantly ($p < 0.05$) decreased at 3 months in males receiving 600 ppm (85% of control) (98% of pretest); however, pretest CHER values for this group were slightly decreased from the respective control values. CHER activity was also found to be significantly increased at 12 months in males receiving 1800 ppm; however, the CHER values of controls and low-dose animals were found to be reduced at this time compared to pretest values. CHEP activities were slightly but nonsignificantly decreased at 3 months in males and females receiving 600 and 1800 ppm; no differences in CHEB activities were found in males or females at study termination.

Mean values of plasma cholesterol were found to be significantly ($p < 0.05$) increased throughout the study in females receiving 600 and 1800 ppm; however, pretest values of these groups were also significantly increased. Large individual variations between alkaline phosphatase levels in females receiving 600 and 1800 ppm produced large standard deviations and increased mean values for these groups at 3, 6, and 12 months. Potassium levels were found to be significantly ($p < 0.05$) decreased in all dosed groups at 6

TABLE 3. Selected Clinical Chemistry Values (±SD) for Dogs Dosed Orally with EPTC for 1 Year[a]

Parameter/Month	Males/Dose (ppm) 0	200	600	1800	Females/Dose (ppm) 0	200	600	1800
Glucose (mg/dl)								
Pretest	109.0±7.61	109.4±2.98	108.9±7.85	104.2±6.75	109.8±5.49	113.3±2.21	113.5±5.89	114.0±5.11
3	104.0±1.99	104.8±6.18	107.2±4.19	108.3±5.31	96.9±9.83	103.3±3.23	97.3±6.56	101.5±5.86
6	104.0±4.66	105.2±3.16	103.9±4.65	105.5±4.74	98.3±4.50	103.8±8.24	103.8±5.16	103.5±3.55
12	97.4±4.51	103.6±6.07	107.0±9.28	110.8±8.36*	97.7±6.23	100.6±5.91	98.7±4.27	105.5±3.86
Cholesterol (mg/dl)								
Pretest	155±34.8	153±29.0	154±25.9	131±20.4	124±16.0	134± 4.2	162±16.7*	146±17.7*
3	143±20.5	143±17.3	162±12.5	170±35.7	122±14.0	138±19.3	166± 7.7*	159±19.8*
6	140±16.1	143±15.6	156±13.3	170±27.9*	146±22.6	162±42.2	184±26.0	179±36.3
12	122±20.0	130±22.6	138±11.0	156±26.2	130±29.7	168±45.7	188±24.1*	188±25.2*
Calcium (mg/dl)								
Pretest	11.3±0.29	11.3±0.21	11.0±0.22	11.0±0.17	11.3±0.23	11.5±0.23	11.7±0.24	11.5±0.21
3	10.4±0.24	10.4±0.19	10.3±0.20	10.2±0.27	10.5±0.17	10.9±0.29	10.8±0.22	10.2±0.36
6	11.0±0.26	10.8±0.08	10.6±0.10*	10.5±0.33*	11.2±0.23	11.3±0.36	11.1±0.22	10.8±0.48
12	11.0±0.45	10.7±0.20	10.4±0.10*	10.4±0.18*	10.4±0.37	10.5±0.24	10.4±0.23	10.2±0.42
Potassium (mmol/l)								
Pretest	5.0±0.34	5.1±0.10	5.1±0.32	5.0±0.44	5.0±0.31	5.0±0.25	4.6±0.44	4.8±0.39
3	4.4±0.15	4.6±0.29	4.5±0.36	4.2±0.26	4.3±0.10	4.6±0.31	4.2±0.12	4.2±0.25
6	4.5±0.37	4.6±0.11	4.5±0.26	4.4±0.19	4.9±0.44	4.5±0.12*	4.4±0.10*	4.5±0.16*
12	4.7±0.26	4.4±0.14	4.4±0.28	4.3±0.14*	4.5±0.26	4.4±0.29	4.3±0.14	4.4±0.15
Creatine phosphokinase (IU/l)								
Pretest	119± 18.0	125± 19.7	190±151.1	149± 41.5	162± 53.6	126± 39.5	149± 29.5	128± 25.7
3	94± 11.3	135±121.9	89± 17.3	71± 13.0	132± 60.5	104± 27.2	110± 34.2	113± 29.5
6	79± 23.6	62± 14.9	77± 10.4	50± 7.1*	84± 16.6	78± 24.2	72± 21.0	89± 18.8
8	171±148.5[b]			213±207.9	104± 30.7			87± 21.2
12	98± 24.3	50± 12.8*	47± 7.7*	51± 17.7*	96± 25.1	49± 14.4	58± 24.5	85± 60.2
Lactic dehydrogenase (IU/l)								
Pretest	46±14.9	52±28.0	58±27.7	66±36.5	80±57.4	70±63.4	57±11.2	42±12.2
3	46±20.1	51±28.8	38±14.2	28±10.4	72±23.6	60±33.4	60±25.6	77±35.8
6	59±29.5	38±22.4	38±14.3	29± 8.1	63±18.6	68±43.5	48±17.5	64±18.1
8	97±47.2			86±62.3	71±27.6			57±18.9
12	101±44.6	40±22.9*	37±14.0*	34±18.5*	100±35.0	38±24.3*	40±15.0*	56±44.8*[c]
Red cell cholinesterase (mmol/mL)								
Pretest	1723±235.6	1712±190.1	1614±225.0	1762±108.3	1598±192.4	1544±164.9	1452±174.0	1606± 99.1
3	1871±211.3	1663±134.4	1581±178.1*	1957±140.2	1492±166.7	1486±102.0	1447±179.5	1532± 82.2
12	1534±217.2	1492±108.5	1575±175.9	1904±167.3*	1417±220.2	1361±117.0	1364±120.1	1496±147.0
Plasma cholinesterase (mmol/mL)								
Pretest	1621±388.1	1641±237.5	1644±180.8	1732±192.3	1480±141.8	1604±334.2	1425±196.6	1670±311.9
3	1573±394.7	1434±215.8	1350±234.6	1351± 84.4	1379±102.8	1360±213.8	1190±137.7	1198±206.7
12	1395±300.9	1297±183.7	1316±209.4	1323±119.9	1439±125.9	1420±258.0	1204±179.5	1237±182.5

[a] Based on six animals/sex/group with exception of 8-week male-control value of creatine phosphokinase, which was recalculated for five animals.

[b] Mean value recalculated for five animals on basis of technical error reported for sixth animal.

[c] Recalculated by our reviewers and found to be significant ($p < 0.05$) using ANOVA followed by Duncan's test for multiple comparisons.

* Significantly different from control value ($p < 0.05$).

months. These differences were not considered to be of toxicologic significance. Many differences in clinical biochemistry parameters in males and females were sporadic and not consistent over time.

Levels of CK and LDH were found to be decreased in control and dosed males at 6 months. The CK value of a control male (animal #H00384) taken at 8 months was found to be abnormally high. This may have been an inaccuracy in reporting (CBI pages 76 and C-124). Since the validity of this value was in question, the mean CK value for control males at 8 months was recalculated by the reviewers for only the five remaining males. Even so, large individual variations exist between mean CK values for males at 8 months (only control and high-dose animals measured). CK and LDH values were reported to be significantly decreased ($p < 0.05$) relative to controls in all groups of dosed males at 12 months. LDH values were also significantly ($p < 0.05$) decreased in females receiving 200, 600, or 1800 ppm at 12 months; CK values were nonsignificantly decreased. Blood levels of CK and LDH isoenzymes were determined to detect evidence of cardiotoxicity. Since results were obtained from samples collected and analyzed under varying conditions, the results were reported to be inconclusive. In the absence of any increase in total CK and LDH levels normally associated with muscle degeneration, the differences observed in these values as compared to controls were not considered to be of toxicologic importance by the study authors.

6. Urinalyses: Urine was collected from fasted animals at 8 and 12 months. The CHECKED (X) parameters were examined.

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

†Recommended by Subdivision F Guidelines (October 1982).

Results: Parameters for urinalyses were not statistically evaluated with the exception of pH. Urinalyses parameters were similar in control and dosed groups.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerves†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 level)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	X	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	X	Thymus†		Glandular
X	Ileum†		Urogenital	XX	Adrenals†
X	Cecum†	XX	Kidneys†		Lacrimal gland
X	Colon†	X	Urinary bladder†	X	Mammary gland†
X	Rectum†	XX	Testes†	X	Parathyroids†
XX	Liver†		Epididymides	XX	Thyroids†
X	Gall bladder†	X	Prostate		Other
X	Pancreas†		Seminal vesicle	X	Bone (femur)†
	Respiratory	XX	Ovaries	X	Skeletal muscle†
X	Trachea†	X	Uterus†	X	Skin
X	Lung†	X	Vagina	X	All gross lesions and masses

†Recommended by Subdivision F Guidelines (October 1982).

Results:

a. Organ Weight: It was reported that no changes of toxicologic significance were evident in organ weights. Absolute and relative testical weights and testis-to-brain weight ratios were significantly ($p < 0.05$) decreased in males fed 1800 ppm; absolute left thyroid weights were slightly decreased in this group. Absolute and relative pituitary weights and pituitary-to-brain weight ratios were significantly ($p < 0.05$) decreased in females fed 1800 ppm. However, there were no histologic changes to correspond to these organ weight changes; these weight changes were considered to be unrelated to treatment.

b. Gross Pathology: There were no increases in any gross lesion in dosed groups when compared to controls. All findings were those normally found in age- and strain-matched laboratory controls.

c. Microscopic Pathology: There were no microscopic findings that were considered to be compound related. All findings were those normally found in age- and strain-matched laboratory controls.

D. STUDY AUTHORS' CONCLUSIONS:

Male dogs treated with 600 ppm had decreased body weights from week 32 to study termination. There were no other apparent treatment-related toxicologic or pathologic changes when EPTC was fed at 200, 600, or 1800 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete and the conduct of the study and reporting of data were acceptable with the exception of the clinical biochemistry data. The results of many clinical biochemistry parameters had large individual variations and standard deviations. There was some difficulty in the sampling of blood for isoenzyme profiles; results are reported to be inconclusive as a result, but not considered to be of toxicologic importance since CK and LDH values in dosed animals were not elevated. The CK value of a control male (animal #H00384) taken at 8 months was found to be abnormally high. This may have been an inaccuracy in reporting. Therefore, the mean CK value for control males at 8 months was recalculated for only the five remaining males. Mean food consumption values for females receiving 1800 ppm at week 1 and the LDH values taken at 12 months for females receiving 1800 ppm were reevaluated by our reviewers and found to be significant ($p < 0.05$) using ANOVA followed by Duncan's test for multiple comparisons.

The 3-month dog study used as rationale for dose selection was not available for review. The information reported on this preliminary study in the present report (C3I p. 4) did not clearly state if CHEP activities in males receiving 1800 ppm were 71%-76% of control values or were decreased 71%-76% from controls.* Although the rationale for dose selection appeared adequate, there was no inhibition of cholinesterase activity at the highest dose and no dose-related decrease in mean body weight gain in the present study. At 52 weeks, the mean body weight gains for 0, 200, 600, and 1800 ppm males was +3.9, +4.2, +2.6, and +3.4, respectively. There was no effect on body weight in females. At 1800 ppm there was a statistically significant decrease in absolute testes weights as well as testes/body weight (right only) and testes/brain weight (right only) ratios; however, these changes were not accompanied by any histopathological changes.

Although the NOEL could be set at 600 ppm based on the weight changes in testes at the HDT, the biological significance of this finding is questionable, and this reviewer feels that EPTC should have been tested at higher dosages.

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*This preliminary study has been reviewed in-house (Mauer to R. Taylor, dated June 9, 1986, TB Document No. 005190), and a 25% reduction (compared to controls) was reported in high-dose males only (i.e., 71% to 76% of control values), and only for CHEP.

005740

EPA: 68-02-4225
DYNAMAC No. 2488
January 28, 1987

DATA EVALUATION RECORD

EPTC

Oncogenicity Study in Mice

APPROVED BY:

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Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-28-87

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CC5740

EPA: 68-02-4225
DYNAMAC No. 248B
January 28, 1987

DATA EVALUATION RECORD

EPTC

Oncogenicity Study in Mice

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Date: January 28, 1987

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Date: 2/9/87

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005740

DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Oncogenicity study in mice.

ACCESSION NUMBER: 263692-263693.

TEST MATERIAL: EPTC.

SYNONYMS: R-1608; Eptam; s-ethyl dipropylthiocarbamate.

STUDY NUMBER(S): 6100-104.

SPONSOR: PPG Industries, Inc., Barberton, Ohio.

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI.

TITLE OF REPORT: Oncogenicity study in mice with EPTC.

AUTHOR(S): Kehoe, S., Tisdell, M., and Carter, J. L.

REPORT ISSUED: May 15, 1986.

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CONCLUSIONS:

Under the conditions of the study, 200, 600, or 1800 ppm EPTC was not oncogenic when fed to CD-1 mice for 78 weeks. There were no compound-related effects on mortality, hematologic parameters, and gross or histopathologic findings. Malignant neoplasms, benign tumors, and other histopathologic lesions generally occurred to the same extent in the control and dosed animals. Some types of tumors were more prevalent in one sex (e.g., lymphomas in females and hepatocellular carcinomas/adenomas in males), but were comparable between dietary groups. High-dose females showed a consistent ($p < 0.05$) decrease in body weights and food consumption throughout the study. Mid-dose females showed a slight but significant ($p < 0.05$) decrease in body weights at most measurement intervals up to week 68 and a significant decrease in food consumption up to week 20, but sporadically thereafter. High-dose males showed a decreased ($p \leq 0.05$) body weight during the first 60 weeks of the study and decreased food consumption up to week 32 and sporadically thereafter.

A LOEL for systemic toxicity, based on a moderate decrease in food consumption and a decrease in weight gain in females, is 600 ppm and the NOEL is 200 ppm.

Classification: Core Minimum.

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A. MATERIALS:

1. Test Compound: EPTC, batch No. 518-996; purity: 98.5 percent; contaminants: information not provided in the CBI report.

2. Test Animals: Species: mice; strain: CRL:CD-1(ICR)BR; age: about 5 weeks; weight: males--23.3-23.5 g, females--19.6-19.8 g; source: Charles River Laboratories, Wilmington, MA.

B. STUDY DESIGN:

1. Animal Assignment: After 2 weeks of acclimation, the animals were distributed into the following treatment groups using a computer-generated randomization scheme:

Test Group	Dose in Diet (ppm)	Main Study (78 Weeks)		Interim Sacrifice (65 Weeks)	
		Male	Female	Male	Female
1 Control	0	60	60	5	5
2 Low (LDT)	200	60	60	5	5
3 Mid (MDT)	600	60	60	5	5
4 High (HDT)	1800	60	60	5	5

The animals were individually caged in an environmentally controlled room with a 12-hour light/12-hour dark cycle.

2. Diet Preparation: Diets were prepared weekly and stored refrigerated. Samples of treated food were analyzed for homogeneity at the start of the study and for the test material concentration at each week. A single batch of EPTC concentrate (lot No. 518-996), with a determined purity of 98.5 percent, was used to prepare the test diets throughout the study.

Results: The mean analyzed dietary levels of the test compound were acceptable throughout the study. The mean analyzed levels (±SD) were 187±8, 567±27, and 1719±89 ppm, which were 93.5, 94.5, and 95.5 percent of nominal levels of 200, 600, and 1800 ppm, respectively. The stability of EPTC in the test diets was not determined in this study. It was inferred from the stability tests performed for another study that no appreciable loss of EPTC occurred during the study period. The test material purity was determined to be 98.4 to 98.6 percent pure at six intervals during the study and it was stable throughout the study.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics: Analysis of variance (ANOVA) was performed on all continuous data (body weight, food consumption, and hematology) measurements. Intergroup differences were analyzed by Dunnett's t-test, and the differences were considered statistically significant at $p \leq 0.05$. Survival data were analyzed using the Kaplan-Meir method and Cox's test for linear trends.

5. A quality assurance statement was dated May 15, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were observed twice daily for moribundity, mortality, and signs of toxicity, and they also received detailed examinations weekly.

   Results: None of the reported clinical lesions or behavioral observations were considered related to dosing (Table 1). Among the most commonly observed conditions in all dietary groups were convulsions and other behavioral conditions; tremors and pale bodies; urine-stained fur, cyanosis, and other dermal lesions; and lacrimation/chromodacryorrhea and other ocular lesions. These lesions and excessive salivation were more prevalent in males than females. Twenty-five percent of males (61/240) had palpable tissue masses, including multiple masses among 13 percent (32/240) of them. Only one mid-dose female had a palpable tissue mass.

   The number of cumulative deaths at 81 weeks ranged between 45-51 percent among males and between 25-49 percent among females (Table 2). The intergroup differences were not significant in either sex.

2. Body Weight: Mice were weighed weekly for 14 weeks, at week 16, then every 4 weeks until week 76, and finally at week 78.

   Results: The mean body weight of males receiving 1800 ppm EPTC was moderately (5.6-9.3 percent) but significantly ($p \leq 0.05$) lower than controls between weeks 1 and 60 (Table 3). No other dosed male group showed any significant change in body weight. Females in the 1800-ppm group showed a significant decrease (8.9-13.6 percent, $p \leq 0.05$) in mean body weight throughout the study. Females receiving 600 ppm EPTC showed a significant ($p \leq 0.05$) decrease (3.5-5.9 percent) between weeks 2 and 68, excluding weeks 7 and 8. A dose-related trend ($p < 0.05$) in decreased mean body weights was observed in females at all time intervals.

3. Food Consumption and Compound Intake: Individual food consumptions were determined once a week through week 14, at week 16, then every fourth week through week 76, and finally at week 78. The daily compound intake was calculated as milligrams of EPTC consumed per kilogram body weight.

TABLE 1. Summary of Selected Frequent Clinical and Behavioral Observations in Mice Fed EPTC

Observation	Males/EPTC (ppm)				Females/EPTC (ppm)			
	0	200	600	1800	0	200	600	1800
No. animals examined	60	60	60	60	60	60	60	60
Palpable tissue masses (multiple)	16(13)	15(8)	13(3)	17(8)	0(0)	1(0)	1(0)	0(0)
Behavioral								
Convulsions	30	19	23	19	15	11	16	12
Other conditions (hunched posture, ataxia, prostrate, anorexia, languid)	14	24	19	18	12	14	15	12
Appearance								
Tremors	9	15	11	7	7	5	8	6
Swollen genital	20	18	20	25	--	--	--	--
Pale--whole body	6	12	7	14	10	6	10	9
Low body temperature	16	20	21	15	11	11	15	7
Secretory								
Excess salivation	9	7	5	6	1	0	1	0
Skin and Hair								
Alopecia, thin hair coat	25	28	18	18	11	6	13	9
Urine stained	34	39	36	24	13	10	14	5
Cyanosis	3	5	6	6	7	12	14	8
Other lesions (necrotic, swollen, infected, blistered, ulcerated skin with pus-like discharge; exposed penis in males)	34	38	31	28	14	23	20	12
Eyes								
Lacrimation, chromodacryorrhea	20	23	19	13	8	1	4	6
Other ocular conditions (opaque, squinting, pupil offset, red, swollen, ulcerated, exophthalmus)	25	31	27	21	11	14	6	17

TABLE 2. Mortality (Percent Survival) in Mice Fed EPTC

Dose Level (ppm)	Mortality[a] (Percent Survival) From Weeks		
	0-52	53-65	66-81[b]
	MALES		
0	4 (93)	6 (83)	18 (49)
200	0(100)	5 (92)	23 (49)
600	2 (97)	7 (85)	18 (51)
1800	4 (93)	9 (78)	12 (55)
	FEMALES		
0	2 (97)	6 (87)	11 (65)
200	2 (97)	6 (87)	19 (51)
600	2 (97)	6 (87)	16 (56)
1800	2 (97)	3 (92)	9 (75)

[a]Includes all animals that died during the study or were sacrificed moribund.

[b]Does not include five animals withdrawn for interim sacrifice.

EPTC (ppm)	Mean Body Weight (g) ± SD at Week							
	1	12	24	36	48	60	72	78
				MALES				
Control	25.8±1.74 (60)[a]	34.2±2.44 (60)	37.1±3.32 (60)	38.3±4.31 (59)	39.2±4.65 (57)	39.7±6.79 (55)	38.7±5.00 (34)	39.5±5.22 (28)
200	25.9±1.52 (60)	34.1±2.54 (60)	36.8±3.80 (60)	37.3±4.13 (60)	37.9±4.53 (60)	38.0±4.24 (58)	38.0±4.15 (36)	38.7±5.23 (28)
600	25.3±1.73 (60)	33.4±2.64 (60)	35.8±3.26 (60)	37.1±3.80 (59)	37.9±4.03 (58)	37.4±4.20 (56)	38.3±4.68 (36)	38.6±5.09 (30)
1800	22.4±2.20* (60)	31.9±2.48* (59)	34.4±2.89* (60)	35.6±3.19* (58)	36.3±3.61* (56)	36.0±3.49* (50)	37.4±4.10 (35)	36.9±4.14 (32)
				FEMALES				
Control	21.7±1.34 (60)	27.9±2.44 (59)	30.1±2.70 (59)	31.9±3.41 (59)	32.7±3.43 (58)	33.6±3.44 (55)	34.6±3.63 (44)	34.8±4.12 (42)
200	21.3±1.45 (60)	27.6±2.07 (60)	29.7±2.35 (59)	31.1±2.82 (59)	32.2±3.18 (58)	33.2±4.24 (57)	34.4±5.63 (40)	34.7±6.79 (33)
600	21.1±1.64 (60)	26.6±2.21* (60)	28.8±2.27* (60)	30.0±2.56* (59)	30.8±2.80* (58)	31.9±3.33* (55)	32.8±3.65 (42)	33.3±3.65 (34)
1800	18.9±1.56*[b] (60)	25.0±2.12*[b] (60)	27.0±2.21[b] (60)	28.3±2.31*[b] (59)	29.1±2.59*[b] (58)	29.5±2.77*[b] (56)	30.4±3.43*[b] (47)	30.8±3.44*[b] (43)

*Significantly different from control, values ($p \leq 0.05$).

[a]Numbers in parentheses are numbers of animals weighed.

[b]Significant dose-related trend by regression analysis at all weeks (1, 12, 24, 36, 48, 60, 72, and 78) ($p \leq 0.05$).

Results: A selective summary of weekly food consumption is given in Table 4. Food consumption in high-dose females was significantly ($p \leq 0.05$) depressed (13.6-42 percent) throughout the study, excluding week 78, when compared to controls. In mid-dose females, a significant ($p \leq 0.05$) decrease (9.8-18.0 percent) was observed during the first 20 weeks of the study, excluding week 11. In addition, a few sporadic but statistically significant ($p \leq 0.05$) decreases were also observed in this group at later time points in this study. A dose-related trend ($p \leq 0.05$) in decreased mean food consumption was observed in females at all time intervals, except week 78 (Table 4). In high-dose males, food consumption was significantly ($p \leq 0.05$) depressed (8.3-43.6 percent) between weeks 1 and 52, excluding week 36. In mid-dose males, significant ($p \leq 0.05$) depression in food consumption was observed only between weeks 1 and 4 and during week 40.

In general, increased compound intake (mg/kg/day) was similar to dietary concentration increments in low- and mid-dose groups. The reduced compound intake in the high-dose animals was attributed to lower food consumption values.

4. Ophthalmologic examinations were not performed.

5. Blood smears were prepared from samples collected from orbital sinus puncture at weeks 52 and 78. At week 52, differential blood counts were determined from 10 animals/sex in the high-dose and control groups. At week 78, the CHECKED (X) parameters were examined in all the treatment groups.

a. Hematology

X	Hematocrit (HCT)†		Total plasma protein (TP)
X	Hemoglobin (HGB)†	X	Leukocyte differential count
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Platelet count†	X	Mean corpuscular volume (MCV)
X	Nucleated RBC	X	Prothrombin time
X	Reticulocytes		

Results: There were no intergroup differences in hematologic parameters at any interval of analysis. The observed values were within the normally expected range for CD-1 mice.

b. Clinical chemistry parameters were not determined.

6. Urinalysis was not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subjected to gross pathological

Exhibit 4. Selected Mean Food Consumption (g/animal/week) in Mice Fed EPTC

EPTC (ppm)	Mean Food Consumption ± SD at Week							
	1	12	24	36	48	60	72	78
MALES								
Control	44.3±5.71 (57)[a]	32.5±4.28 (59)	30.8±5.74 (58)	30.3±4.86 (59)	29.0±4.50 (57)	30.1±9.17 (55)	29.4±7.05 (34)	29.5±7.52 (28)
200	43.6±5.74 (57)	33.0±4.87 (58)	30.8±4.90 (57)	30.6±6.98 (60)	28.8±6.32 (60)	29.0±6.73 (55)	28.4±6.69 (36)	29.0±5.62 (27)
600	36.4±5.74* (58)	31.2±4.35 (60)	28.9±4.07 (59)	29.6±4.77 (59)	28.1±3.83 (58)	28.5±4.92 (55)	31.7±10.58 (35)	27.1±3.39 (30)
1800	25.0±4.81* (60)	28.1±7.17* (58)	27.3±3.91* (57)	29.1±6.86 (58)	26.6±3.87* (56)	27.3±4.10 (49)	25.8±3.96 (34)	26.6±4.40 (31)
FEMALES								
Control	41.2±5.60 (58)	37.7±8.73 (57)	35.4±8.22 (55)	33.5±6.05 (55)	30.0±6.42 (56)	26.1±5.94 (51)	28.9±5.30 (44)	26.1±5.78 (42)
200	43.8±5.69 (55)	39.0±8.08 (54)	36.5±7.45 (54)	34.8±7.61 (57)	30.8±5.52 (57)	26.4±4.95 (52)	27.5±6.67 (38)	27.7±9.96 (29)
600	37.0±6.66* (60)	32.3±5.99* (55)	32.4±5.31 (56)	30.2±5.55* (57)	28.6±4.55 (54)	24.4±4.48 (52)	26.7±6.65 (42)	26.4±4.70 (33)
1800	25.9±4.02*[b] (60)	26.0±4.71*[b] (52)	26.6±6.45*[b] (57)	26.2±4.79*[b] (52)	25.8±5.23*[b] (57)	21.6±5.28*[b] (54)	24.6±4.68*[b] (39)	27.7±6.45 (39)

*Significantly different from the control values $p \leq 0.05$.

[a]Numbers in parentheses are numbers of animals examined.

[b]Significant dose-related trend by regression analysis at all weeks except week 78 ($p \leq 0.05$).

examination and the CHECKED (X) tissues were collected for histological examination:

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

The gross pathologic examination covered external surface and orifices; visceras of abdominal, thoracic, and pelvic cavities; cranial cavity; carcass; external and cut surfaces of the brain and spinal cord; and nasal cavity and paranasal sinuses.

All organs listed above were subjected to histopathologic examinations in all high-dose and control mice and in low- and mid-dose animals that died on test or were sacrificed moribund. Only heart, lungs, liver, kidneys, and gross lesions were examined in animals at interim sacrifice and in the low- and mid-dose animals sacrificed at the end of study.

Results:

a. Organ Weight: Organ weights were not determined.

b. Gross Pathology: Macroscopic lesions were not considered unusual in type, frequency, or severity and were not considered related to dosing. Table 5 summarizes gross findings. Lesions observed at the interim sacrifice were similar to those observed at terminal necropsy. Tissue masses in the liver and lungs were more frequent in males (25/239) than females (7/240). Tissue masses were also present in the uterus and ovaries of all female treatment groups. In addition, female treatment groups also showed a high incidence of uterine (91/240) and ovarian (102/240) cysts. A high incidence of thrombus was observed in the hearts of mid-dose males. In a substantial number of females and males, organs such as uterus, seminal vesicles, urinary bladder, spleen, and lymph nodes (submandibular and mesentric) were larger than normal.

†Recommended by Subdivision F (October, 1982) Guidelines for chronic studies.

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TABLE 5. Distribution of Macroscopic Lesions in Mice Found Dead, Sacrificed Moribund, and Sacrificed at 65 and 78 Weeks

	Males/EPTC (ppm)				Females/EPTC (ppm)			
Organs/Lesions[a]	0	200	600	1800	0	200	600	1800
No. of mice necropsied	60	60	60	59	60	60	60	60
General Condition/ Lesions								
Stains--perineum/ perianal	14	16	10	9	11	12	11	3
Lungs								
Masses	1	4	2	1	1	2	2	0
Kidneys								
Diffusely light	8	11	6	6	6	8	9	5
Large pelvis	13	15	9	3	3	5	10	4
Rough surface, diffuse	3	5	3	4	6	6	10	3
Cyst(s)	12	5	3	6	4	3	6	2
Liver								
Accentuated lobular pattern	4	0	1	4	1	0	1	2
Mass(es)	5	6	6	1	0	1	1	0
Gallbladder								
Enlarged	2	0	1	3	3	2	4	3
Heart								
Thrombus	1	1	5	0	0	0	0	0
Spleen								
Enlarged	10	7	4	7	8	9	11	5
Mottled	6	5	1	1	1	3	4	1
Eyes								
Opaque cornea	5	3	7	4	0	2	0	1
Pancreas								
Gelatinous	3	2	1	4	2	0	2	1

(Continued)

[a]Only frequently occurring lesions were included.

TABLE 5. Distribution of Macroscopic Lesions in Mice Found Dead, Sacrificed Moribund, and Sacrificed at 65 and 78 Weeks[a] (Cont.)

Organs/Lesions	Males/EPTC (ppm)				Females/EPTC (ppm)			
	0	200	600	1800	0	200	600	1800
Stomach								
Dark contents	11	7	9	7	8	4	7	4
Dark focal areas--glandular	6	3	6	0	3	3	1	2
Skin								
Subcutaneous edema	15	13	11	10	4	6	6	3
Abrasion/ulceration	3	7	2	3	0	0	2	0
Alopecia--focal	3	1	3	3	0	0	0	1
Submandibular Lymph Node								
Large	8	8	3	7	2	5	4	1
Mesenteric Lymph Node								
Large	9	8	8	5	12	10	10	6
Mottled	17	14	21	16	26	22	18	16
Diffusely red	6	6	1	1	4	3	2	2
Urinary Bladder								
Large	3	6	5	2	0	1	1	0
Seminal Vesicles								
Large	14	13	16	15	0	0	0	0
Testes								
Small	0	2	1	4	0	0	0	0
Soft	3	4	6	1	0	0	0	0
Uterus								
Large, diffuse	0	0	0	0	18	23	21	16
Thickened walls	0	0	0	0	25	29	29	23
Cyst(s)	0	0	0	0	26	20	24	21
Masses	0	0	0	0	1	5	3	4
Large, segmented	0	0	0	0	24	16	17	12
Ovaries								
Cyst(s)	0	0	0	0	28	20	26	28
Masses	0	0	0	0	1	3	1	1

(Concluded)

[a]Only frequently occurring lesions were included.

c. Microscopic Pathology

1. Nonneoplastic: A variety of nonneoplastic lesions in high frequency was found in animals that died during the study, were sacrificed moribund, or were sacrificed at 65 and 78 weeks (Table 6). These lesions were randomly distributed in the male and female dosage groups and were not considered compound related. Lesions frequently observed included chronic progressive nephropathy; cysts in the kidneys, thyroids, and ovaries; atrophic thymuses and testes; mineralization of testes and brain; edema of skin and cecum; focal mononuclear infiltration, necrosis, and vacuolation in the liver; enlarged lumen of the uterus; a high incidence of erosion/ulceration in males; and thrombus in hearts of control males and low- and mid-dose males and females. In addition to the lesions shown in Table 6, high incidences of amyloidosis and chronic inflammation were observed in multiple organs of all groups including controls.

2. Neoplastic: Table 7 summarizes the incidence of neoplastic lesions. There was no significant compound- or dose-related increase in neoplasms at any site when dosed males or females were compared with controls. Lymphomas in multiple organs represented the largest number of malignant tumors in both the sexes. Hepatocellular carcinomas/adenomas were more prevalent in males than in females; however, this is expected in this strain of mouse and no increased incidence was found in dosed animals. Endometrial stromal polyps and hemangiomas were the main tumor types found in the uterus.

D. STUDY AUTHORS' CONCLUSIONS:

The study authors concluded that EPTC, when fed to CD-1 mice for 78 weeks, did not cause any compound-related antemortem effects in either sex; however, it produced a significant decrease in body weights and food consumption in mid-dose females and high-dose males and females. There were no observable compound-related effects in low-dose animals.

Gross pathologic and histopathologic examinations performed during the course of this study indicated that there were no compound-related lesions. The incidences of neoplastic and nonneoplastic lesions observed were typical for aging individuals of this mouse strain. These lesions showed no intergroup differences in frequency or severity.

Under the conditions of this study EPTC was not considered to be carcinogenic in this strain of mouse.

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TABLE 6. Histopathologic Distribution of Nonneoplastic Lesions in Mice Found Dead, Sacrificed Moribund, and Sacrificed at 65 and 78 Weeks

	Males/EPTC (ppm)				Females/EPTC (ppm)			
Organs/Lesions[a]	0	200	600	1800	0	200	600	1800
Kidneys	(60)[b]	(60)	(60)	(59)	(60)	(60)	(60)	(60)
Chronic progressive nephropathy	45	49	46	35	51	42	49	42
Cyst(s)	3	4	1	1	2	2	5	5
Infarct(s)	2	7	3	2	2	3	6	4
Pelvic dilatation	8	9	7	3	1	1	4	3
Liver	(60)	(60)	(60)	(59)	(60)	(60)	(59)	(60)
Focal mononuclear infiltration	18	25	23	14	38	28	25	30
Necrosis	4	4	4	7	7	2	5	6
Vacuolation, hepatocytes	4	10	7	4	2	3	7	5
Brain	(60)	(29)	(27)	(59)	(60)	(27)	(24)	(60)
Mineralization	11	2	0	6	4	1	0	3
Heart	(60)	(60)	(60)	(59)	(60)	(60)	(58)	(60)
Thrombus	4	5	7	0	3	1	0	0
Spleen	(60)	(30)	(27)	(59)	(60)	(29)	(28)	(60)
Extramedullary hematopoiesis, increased	10	6	3	7	4	5	6	4
Thyroid	(58)	(28)	(27)	(58)	(60)	(28)	(24)	(60)
Follicular cyst(s)	6	3	1	6	5	0	2	4
Adrenals	(60)	(28)	(28)	(59)	(60)	(27)	(24)	(60)
Pigmentation	6	4	2	5	11	4	2	8
Cecum	(59)	(26)	(26)	(57)	(59)	(26)	(23)	(58)
Edema	3	8	8	3	8	4	5	6
Skin	(60)	(33)	(30)	(59)	(60)	(26)	(26)	(60)
Edema	13	10	9	8	3	2	5	4
Erosion/ulceration	7	6	5	6	0	0	2	0
Thymus	(57)	(24)	(27)	(51)	(55)	(26)	(22)	(56)
Atrophy	25	18	15	15	7	11	10	3

(Continued)

[a] Only frequently occurring lesions were included.

[b] Numbers in parentheses are the numbers of tissues examined.

TABLE C. Histopathologic Distribution of Nonneoplastic Lesions in Mice Found Dead, Sacrificed Moribund, and Sacrificed at 65 and 78 Weeks[a] (Continued)

	Males/EPTC (ppm)				Females/EPTC (ppm)			
Organs/Lesions[a]	0	200	600	1800	0	200	600	1800
Mesenteric Lymph Node	(58)[b]	(42)	(40)	(57)	(59)	(40)	(38)	(59)
Erythrocytes in sinuses	14	15	15	10	29	24	13	13
Seminal Vesicles	(60)	(34)	(33)	(59)	--	--	--	--
Distended with secretions	18	10	11	12	--	--	--	--
Testes	(60)	(28)	(28)	(59)	--	--	--	--
Atrophy	15	8	13	14	--	--	--	--
Mineralization	10	2	7	12	--	--	--	--
Uterus	--	--	--	--	(60)	(52)	(50)	(60)
Enlarged lumen	--	--	--	--	27	18	15	10
Ovaries	--	--	--	--	(60)	(41)	(40)	(60)
Cyst(s)					22	19	23	32

(Concluded)

nly frequently occurring lesions were included.

umbers in parentheses are the numbers of tissues examined.

TABLE 7. Histopathologic Distribution of Neoplastic Lesions in Mice Found Dead, Sacrificed Moribund, and Sacrificed at 65 and 78 Weeks

Organs/Lesions[a]	Males/EPTC (ppm) 0	200	600	1800	Females/EPTC (ppm) 0	200	600	1800
Lungs	(60)	(60)	(60)	(59)	(60)	(60)	(59)	(60)
Alveolar/bronchiolar adenoma	2	1	0	2	2	1	0	0
Alveolar/bronchiolar carcinoma	1	2	2	1	0	0	1	0
Liver	(60)	(60)	(60)	(59)	(60)	(60)	(59)	(60)
Hepatocellular adenoma	5	6	4	1	0	2	0	0
Hepatocellular carcinoma	2	1	3	0	0	0	0	0
Spleen	(60)	(30)	(27)	(59)	(60)	(29)	(28)	(60)
Hemangioma	2	1	0	0	1	0	0	0
Uterus	--	--	--	--	(60)	(52)	(50)	(60)
Endometrial stromal polyp	--	--	--	--	2	3	3	0
Hemangioma	--	--	--	--	1	0	0	5
Multiple Organ	[4][c]	[0]	[1]	[1]	[2]	[2]	[6]	[4]
Malignant lymphoma[d]	52	0	31	31	29	39	101	42

Only frequently occurring lesions were included.

Numbers in parentheses are the numbers of tissues examined.

[ ] Numbers in brackets are the numbers of animals with multiple organ malignant lymphomas.

Number of lymphomas.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The experimental methods were complete and adequate for assessing the oncogenicity of EPTC in mice. The summary data presented with the report were supported by individual animal data; the report was well organized. Under the conditions of the study, the test compound was clearly nononcogenic.

Many nonspecific clinical conditions, including convulsions, tremors, cyanosis, lacrimation/chromodacryorrhea, and pale bodies, were observed throughout the study equally distributed between groups.

There were no compound-related effects on mortality, hematologic parameters, and gross or histopathologic findings. Malignant neoplasms, benign tumors, and other histopathologic lesions generally occurred to the same extent in the control and dosed animals. Some of these lesions were more prevalent in one sex (e.g., lymphoma in females and hepatocellular carcinomas/adenomas in males), but were comparable between the dietary groups. Historical laboratory data on this strain of mice were available in the literature for comparison.

A consistent ($p \leq 0.05$) decrease in weight gain and food consumption in high-dose females was sufficient to establish that a maximum tolerated dose was administered. At study termination the mean body weight for high-dose females was 11.5 percent less than controls. High-dose males showed a modest loss (5.6-9.3 percent) in weight gain during the first 60 weeks of the study. A dose-related trend ($p \leq 0.05$) in decreased mean body weight and food consumption was observed in females at most intervals (Tables 3 and 4 in this report).

We assess that the LOEL, based on decreased food consumption and weight gain in females, should be established at 600 ppm. The NOEL is 200 ppm.

A major deficiency in this study was that organ weights (as recommended by EPA Pesticide Guidelines, 1982) were not determined. However, this deficiency had no impact on the outcome of the study.

The number and chemical nature of contaminants, if any, in the test material were not reported in the CBI report.

In CBI Vol. 1, p. 55, Table 9, mean Weekly Food Consumption, the mean value for 1800-ppm females is 27.7±6.45 with N (number of animals) = 39, not 25.5±6.20 with N = 37.

EPA: 68-02-4225
DYNAMAC No. 248C-1
February 18, 1987

DATA EVALUATION RECORD

EPTC

Teratogenicity Study in Rats

STUDY IDENTIFICATION: James, P., Smith, J. A., and John, D. M. Effect of EPTC on pregnancy of the rat. (Unpublished report No. PPG 19/851002 by Huntingdon Research Centre Ltd., England, for PPG Industries, Inc.; dated November 6, 1985.) Accession No. 263694.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-17-87

1. CHEMICAL: EPTC; S-ethyl dipropylthiocarbamate.

2. TEST MATERIAL: EPTC was described as a clear amber liquid from batch No. 518 996 BR 84-13.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: James, P., Smith, J. A., and John, D. M. Effect of EPTC on pregnancy of the rat. (Unpublished report No. PPG 19/851002 by Huntingdon Research Centre Ltd., England, for PPG Industries, Inc.; dated November 6, 1985.) Accession No. 263694.

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Date: 2-20-87

Judith Hauswirth, Ph.D.
Acting EPA Section Head

Signature: Judith D Hauswirth
Date: 2/20/87

7. CONCLUSIONS:

A. Pregnant Sprague-Dawley rats were administered EPTC by oral gavage at doses of 0 (control), 30, 100 or 300 mg/kg/day. Although slight, nonsignificant changes occurred in treated dams, none are considered evidence of frank, compound-related maternal toxicity (i.e., the maternal NOEL ≥300 mg/kg).

No significant developmental toxicity and no compound-related malformations were induced at any level of EPTC; other findings (pregnancy rates and post-implantation losses) at all dose levels were considered to be inconclusive. Hence the reproductive/fetal NOEL ≥ the HDT.

The A/D ratio could not be determined.

B. The study is classifed Core Supplementary (see Recommendation below).

8. RECOMMENDATIONS: We recommend that thi study be repeated using higher dosages that produce frank maternal toxicity.

9. BACKGROUND:

A. Dosage levels for this study were based on a preliminary study in pregnant rats dosed at 0, 160, 240, or 360 mg/kg/day; the testing laboratory also conducted a pilot study in nonpregnant rats at dosages up to 500 mg/kg/day. Treatments with 360 mg/kg/day were associated with increased salivation and hair loss in all animals. Food consumption was decreased, water consumption was increased and body weight gain was retarded during the treatment period; slight increases in postimplantation loss were noted at this dose level. At 240 and 160 mg/kg/day the authors noted increased salivation and slight effects in food and water consumption.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix A for details.)

A. Test Material:

EPTC was described as a clear amber liquid from batch No. 518-996 8R 84-13 with a purity of 98.4%. Dosing preparations consisted of suspensions of the test material in 1% aqueous methylcellulose

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[1]Only items appropriate for this DER have been included.

(this vehicle was also used as control material). Dosage levels for this study were 0 (control), 30, 100, and 300 mg/kg/day; dose volumes were adjusted to 1 mL/100 g body weight. Dosing suspensions were prepared daily. Samples of dosing suspensions were obtained at the beginning, middle, and end of the dosing period; these samples were frozen and sent to the sponsor for analyses.

B. Methods:

One hundred and five time-mated female rats (CrL:COBS CD (SD) BR strain, specific pathogen free) were obtained from Charles River UK, Ltd. Upon arrival, animals were examined and weighed; rats within the weight range of 157-243 g were randomly assigned to four study groups consisting of 25 animals each.

Animals were individually identified and housed five per cage in a room maintained at 21 ± 3°C and 55 ± 10% relative humidity with a 12-hour light cycle. Food and water were available ad libitum.

The dose preparations were administered by gavage on gestation days (GD) 6-15. Dose volumes were based on body weights obtained on GD 6, 10, and 14.

Animals were observed daily for clinical signs of reaction to treatment. Individual body weights were recorded on GD 1, 3, 6, 10, 14, 17, and 20. Food consumption for animals in each cage was measured for the intervals between the above weigh-in days. Water consumption for each cage was measured daily from GD 6-20.

On GD 20, animals were killed by carbon dioxide asphyxiation and necropsied. The number of corpora lutea was recorded; uterine contents were examined to determine the number, location, and status of implantations. The pregnancy status of animals appearing to be nonpregnant was determined after immersing the uteri in 10% ammonium sulfide. Fetuses were weighed and examined externally. One half of the fetuses in each litter was fixed in Bouin's solution and examined for visceral abnormalities by the methods described by Wilson. The remaining fetuses were fixed in "industrial methylated spirits," eviscerated, cleared, stained (using a modification of the technique described by Dawson), and examined for skeletal abnormalities.

Statistical analyses were conducted using the litters as the basic units. Jonckheere and Kruskal-Wallis tests were used to analyze mean values for litter size, litter weight, implantation losses, fetal weight, and incidences of fetal findings. Fisher's exact test was used where 75% or more tied values occurred.

C. Protocol: A protocol was not included with the study report.

12. REPORTED RESULTS:

A. Test Material: Analyses of dosing suspension samples (conducted by PPG Industries, Inc.) indicate that the concentration, homogeneity, and stability of the test material in the dosing preparations were acceptable.

B. Maternal Effects: No mortalities were reported for this study. Increased salivation was noted in all dosed groups; brown staining, hair loss, and/or unkempt coat were reported for all of the females dosed with 300 mg/kg/day.

Body weight gains were not affected at 30 and 100 mg/kg/day; slight (nonsignificant) reductions were noted at 300 mg/kg/day when compared with controls (Table 1). A slight dose-related reduction in food consumption was noted during the dosing period (Table 2). Water consumption was increased in a dose-related manner during GD 6-9; increased water consumption persisted in the 300-mg/kg/day group even in the postdosing period (Table 2).

C. Reproductive and Developmental Effects: The pregnancy rate was 84, 76, 76, and 72% for the 0-, 30-, 100- and 300-mg/kg/day groups, respectively. The authors reported that litter size was slightly reduced (as a consequence of increased postimplantation loss) at 100 and 300 mg/kg/day when compared with controls (Table 3). The increased postimplantation loss was not significant by Kruskal-Wallis analysis of variance; however, a significant ($p \leq 0.05$) increase was reported at this dose level using multiple comparison procedures. A significant dose-related trend was observed for the reduced fetal body weights; although the values at [illegible] mg/kg/day were reduced, they were not significantly different from controls. Fetal sex ratios were comparable [illegible] (Table 3). No teratogenic effects were noted [illegible] the incidence of reduced skeletal ossification [illegible] study, authors [illegible] skeletal anomalies [illegible] incidence was slightly increased at 300 mg/kg/day. The authors attributed this effect on skeletal ossifications to the reduction in fetal body weight seen in this group. No other compound-related effects were noted in fetal development.

13. STUDY AUTHOR'S [illegible]

A. The study [illegible] levels and [illegible] mg/kg/day were compound [illegible] consumption [illegible] Maternal body weight [illegible]

TABLE 1. Effects of EPTC on Mean Maternal Body Weight Changes (g) in Rats

	Dosage (mg/kg/day)			
Gestation Days	0	30	100	300
1-6	40.2	37.2	41.9	41.4
6-10	24.5	23.9	24.3	20.0
6-14	49.8	48.5	47.6	43.6
6-17	83.3	82.5	81.7	75.4
6-20	131.0	128.2	124.0	119.3

TABLE 2. Effects of EPTC on Mean Maternal Food and Water Consumption in Rats

	Dosage (mg/kg/day)			
Gestation Days	0	30	100	300
	Food Consumption (g/rat/day)			
6-9	25.1	24.5	24.8	23.1
10-13	26.7	25.3	25.7	24.3
14-16	27.3	26.7	26.5	25.3
17-19	28.7	27.2	25.7	28.0
	Water Consumption (g/rat/day)			
6-9	33.7	34.4	36.0	42.4
10-13	39.2	39.1	41.7	57.9
14-16	41.1	38.3	41.5	53.9
17-19	43.7	39.5	40.7	46.3

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TABLE 3. Effects of EPTC on Developmental Parameters in Rats

Parameter	Dosage (mg/kg/day) 0	30	100	300
No. females mated	25	25	25	25
No. females pregnant	21	19	19	18
% Females pregnant	84	76	76	72
No. corpora lutea per female	14.4	14.3	14.4	13.9
No. implantations per female	12.8	13.6	12.6	12.6
No. resorptions per female	0.3	0.7	0.8*	0.7
% Postimplantation loss	2.5	5.4	6.8*	5.3
No. live fetuses per litter	12.4	12.8	11.8	11.9
Mean fetal body weight (g)	3.30	3.31	3.26	3.17
% Male fetuses per litter	52.0	52.2	50.2	49.0
% Fetuses with malformations[a]	0.3	0.4	0.0	0.0
% Fetuses with skeletal anomalies[b]	17.3	15.9	15.6	25.0
% Fetuses with visceral anomalies[b]	6.5	5.8	8.3	6.4

[a]Malformations in this study were defined by the authors as structural deviations that were rare and/or probably lethal, e.g., exencephaly, anury.

[b]Anomalies in this study were defined by the authors as minor differences from 'normal' that were detected relatively frequently either by free-hand sectioning, e.g., increased renal pelvic dilatation or at skeletal examination, e.g., bipartite centrum.

*Significantly different from control value ($p \leq 0.05$), but not significant when all groups were analyzed together.

Slight reductions in the number and weight of fetuses were noted at 100 and 300 mg/kg/day; in addition, fetuses in the 300-mg/kg/day group had reductions in the degree of vertebral ossification when compared with controls. No teratogenic effects were noted.

B. A quality assurance statement was signed and dated October 15, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material: Analyses of samples of dosing suspensions obtained at three times during the dosing period indicated that the test material was stable in methylcellulose and that the homogeneity and concentration of the suspensions were acceptable.

B. Maternal Effects: No mortalities were reported at any dose level in this study. Although increased salivation was noted for all groups dosed with EPTC (but not for controls), the authors did not indicate if this finding was observed following gavage administration (in which case, salivation may have been associated with the taste of the dosing preparations and may not have necessarily been indicative of maternal toxicity). The presence of brown staining, hair loss, and/or unkempt coat in most animals dosed with 300 mg/kg/day suggest that EPTC was toxic at this dose level. Body weight gains for females in the 300-mg/kg/day group were moderately reduced after the initiation of dosing and remained lower than controls throughout gestation. Slight dose-related decreases in food consumption and increases in water consumption were noted for all dose levels when compared with controls; however, none of the differences were statistically significant.

C. Reproductive and Developmental Effects: Of the 25 females mated for each group, 21, 19, 19, and 18 were pregnant at 0, 30, 100, and 300 mg/kg/day; the calculated pregnancy rates were 84, 76, 76, and 72%, respectively (Table 3). Embryonic/fetal lethal effects were suggested by slight reductions in the number of live fetuses per litter at 100 and 300 mg/kg/day. We assess that the mild increases in postimplantation loss at all dose levels when compared with controls were not statistically significant and biologically inconclusive.

A total of three malformed fetuses were reported in this study. One control fetus (from litter No. 4) had multiple craniofacial malformations, and another control fetus (from litter No. 21) had an interventricular septal defect and other vascular malformations. One fetus in the 30-mg/kg/day group (from litter No. 35) had a diaphragmatic hernia. We assess that these findings were incidental.

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Although apparently consistent with the slight (nonsignificant) reduction in fetal body weight at 300 mg/kg/day, we assess that the reductions in ossification of the sacral and caudal vertebrae were not compound related.

All other reproductive and developmental parameters were comparable among dosed groups and controls.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-8.

APPENDIX A

Materials and Methods

Eptam Science Reviews

Page _____ is not included in this copy.

Pages _46_ through _52_ are not included in this copy.

The material not included contains the following type of information:

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_____ Identity of product inert impurities.

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_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

__X__ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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EPA: 68-02-4225
DYNAMAC No. 248-C2
February 13, 1987

DATA EVALUATION RECORD

EPTC

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: James, P., Smith, J. A., Masters, R. E., and Offer, J. M. Effect of EPTC on pregnancy of the rabbit. (Unpublished report No. PPG 14&18/85601 by Huntingdon Research Centre Ltd., England, for PPG Industries Inc., Barrerton, OH; dated May 28, 1985.) Accession No. 263694.

APPROVED BY:

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Date: 2-13-87

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1. CHEMICAL: EPTC; S-ethyl dipropylthiocarbamate. 005740

2. TEST MATERIAL: EPTC was described as a clear amber liquid from batch No. 518-996 BR 84-13.

3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.

4. STUDY IDENTIFICATION: James, P., Smith, J. A., Masters, R. E., and Offer, J. M. Effect of EPTC on pregnancy of the rabbit. (Unpublished report No. PPG 14&18/85601 by Huntingdon Research Centre Ltd., England for PPG Industries Inc., Barrerton, OH; dated May 28, 1985.) Accession No. 263694.

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7. CONCLUSIONS:

A. Pregnant New Zealand White rabbits were dosed with 0 (control), 30, 100, or 300 mg EPTC/kg/day. The NOEL for frank maternal toxicity of EPTC in this study was 300 mg/kg/day, the highest dose level tested; the LOEL for maternal toxicity could not be determined.

Although increased incidences of fetuses and litters with malformations were noted in all groups dosed with EPTC, they occurred only sporadically in each dosage group, were unrelated, and are represented in the control background of this strain of rabbits.

The A/D ratio could not be determined.

B. In addition to the absence of frank maternal and fetal toxicity or any compound related defects, several deficiencies were noted in the study methodology (see section 14.D. of this DER for details); therefore, this study was considered Core Supplementary.

8. RECOMMENDATIONS: We recommend that the study authors address the deficiencies noted in this report (section 14.D.) and that this study be repeated, at higher doses in order to produce frank maternal toxicity.

9. BACKGROUND: Dosage levels for this study were based on a preliminary study in which pregnant rabbits were dosed at 0, 130, 200, and 300 mg/kg/day. In that study, dams dosed with 300 mg/kg/day had a slightly increased incidence of cold ears, reduced fecal output, reduced food consumption, and reduced body weight gain. The authors reported that litters at this dose level had slightly increased post-implantation loss and decreased fetal body weight. One fetus in the 130 mg/kg/day group had anury, situs inversus, and no spleen.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix A for details.)

A. Test Material: The test material was described as a clear amber liquid from batch No. 518-996 BR 84-13 with a purity of 38.1%. Dosing preparations consisted of suspensions of the test material in 1% aqueous methylcellulose (this vehicle was also used as the control material). Dosage levels for this study were 0 (control), 30, 100, and 300 mg/kg/day; dose volumes were adjusted to 1 mg/kg

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[1]Only items appropriate to this DER have been included.

body weight. Dosing suspensions were prepared daily. Samples of dosing suspensions were obtained at the beginning, middle, and end of the dosing period; these samples were frozen and sent to the sponsor for analyses.

B. Methods: Sexually mature, nonpregnant female rabbits (New Zealand White strain) were obtained from three different sources (Cheshire Rabbit Farms, Ranch Rabbits, and Buxted Rabbit Company, Ltd.) These animals were individually caged and acclimated for at least 10 days. The animal room was maintained at 18 ± 3°C and 50 ± 10% relative humidity. The room was illuminated for 14 hours daily. Females within a body weight range of 2.8-4.1 kg were mated with proven males and then injected with luteinizing hormone. The day of copulation was designated gestation day (GD) 0. A total of 17, 16, 18, and 16 mated females were assigned to the 0-, 30-, 100-, and 300-mg/kg/day groups, respectively.

The dose preparations were administered by gavage on GD 6-18. Dose volumes were based on body weights obtained on GD 6, 10, and 14.

Animals were observed daily for clinical signs of reaction to treatment. Body weights were recorded on GD 1, 6, 10, 14, 19, 23, and 29. Food consumption was measured for the intervals between the above weigh-in days. Water consumption was measured daily from GD 6-20.

On GD 29, animals were killed by cervical dislocation and necropsied. The number of corpora lutea was recorded; uterine contents were examined to determine the number, location, and status of implantations. Fetuses were weighed, examined externally, and killed by intrathoracic injection of sodium pentobarbitone. After these procedures, fetuses were dissected and examined for visceral abnormalities and sexed. Further examinations (microdissection, histopathology, etc.) were performed for fetuses with suspected abnormalities. Dissected fetuses were eviscerated and fixed in "industrial methylated spirit." Fixed brains were examined through a slice along the frontoparietal suture. Carcasses were cleared, stained (using a modification of the technique described by Dawson), and examined for skeletal abnormalities.

Statistical analyses of litter data, fetal sex distribution, and incidences of skeletal variations were conducted using the Kruskal-Wallis test and Jonckheeres's test; the litter was used as the experimental unit. The incidences of malformations were analyzed using a generalized linear model with binomial error structure and a logit link function.

C. Protocol: A protocol was not included with the study report.

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12. REPORTED RESULTS:

A. Test Material: Analyses of dosing suspension samples (conducted by PPG Industries, Inc.) indicate that the concentration, homogeneity, and stability of the test material in the dosing preparations were acceptable.

B. Maternal Effects: A total of one, one, two, and one animal died or were killed after dosing started in the 0-, 30-, 100- and 300-mg/kg/day groups. Most of these deaths were associated with anorexia and body weight loss; no compound relationship was suggested. The study authors reported an increased incidence of cold ears and reduced feces at 30 and 300 mg/kg/day; however, the authors reported that this transitory effect was not seen at 100 mg/kg/day.

Slight increases in water and food consumption were noted at 100 and 300 mg/kg/day when compared with controls. Body weight gains for animals in the 100- and 300-mg/kg/day groups were slightly larger than that of controls from the initiation of dosing (GD 6) until GD 29 (Table 1). Body weight gains for the 30-mg/kg/day group were less than that of controls for the first four days of dosing (GD 6-9), but were comparable to controls later in gestation.

TABLE 1. Effects of EPTC on Mean Maternal Body Weight Gains (g) in Rabbits

Gestation Days	Dosage (mg/kg/day)			
	0	30	100	300
1-6	171	188	176	145
6-19	298	261	432	360
6-29	485	474	625	512

Findings at terminal necropsy did not suggest adverse compound-related effects.

C. Reproductive and Developmental Effects: Only one nonpregnant animal (in the 100-mg/kg/day group) was reported in this study. Pregnancy rates were 100, 100, 92.9, and 100% for the 0-, 30-, 100- and 300-mg/kg/day groups, respectively. No abortions were reported at any dose level. No compound-related effects were noted on the number of corpora lutea, implantations, pre- and postimplantation losses, litter size, and litter and fetal weight or sex ratios (Table 2).

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TABLE 2. Effects of EPTC on Mean Litter Data in Rabbits

Parameter	Dosage (mg/kg/day)			
	0	30	100	300
No. Corpora Lutea	10.1	9.7	9.4	10.4
No. Implantations	8.8	8.4	8.4	9.0
No. Resorptions	1.3	1.8	0.6	1.5
% Preimplantation Loss	12.7	14.8	10.8	12.4
% Postimplantation Loss	15.2	23.8	7.3	16.5
No. Live Fetuses	7.5	6.6	7.8	7.5
Fetal Weight (g)	43.7	45.8	43.8	42.8
Litter Weight (g)	316.8	296.1	337.9	312.9
% Male Fetuses	44.2	47.9	48.4	47.0

The incidences (and percentages) of fetuses and litters with malformations are summarized below:

Affected	Dosage (mg/kg/day) 0	30	100	300
Fetuses				
No.	1/112	4/99	5/101	5/112
%[a]	0.8	6.2	5.3	6.6
Litters				
No.	1/15	3/15	4/13	5/14
%[b]	6.7	20.0	30.7	35.7

[a]Mean percent per litter.

[b]Values calculated by the reviewers based on litter incidences presented in this data summary.

The study authors reported that the elevated incidences of malformations noted in the groups dosed with the test material were due to the use of certain males (Nos. 27 and 30, in particular) for matings (Table 3). The incidences of skeletal variations (13th rib and variations in sternebrae) were comparable for all groups.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that in this study 300 mg/kg/day was associated with an increase in the incidence of transitory, nonspecific clinical signs during gestation; in addition, animals dosed with 100 and 300 mg/kg/day had body weight gains larger than controls. At 30 mg/kg/day, the authors noted an increase in transitory, nonspecific signs and a decrease in body weight gain at the initiation of dosing.

No adverse compound-related effects on any reproductive or developmental parameters were reported; the authors stated that the increase in malformed fetuses noted in the dosed groups resulted from the use of several males used for mating and that the malformations were, therefore, not considered related to EPTC.

B. A quality assurance statement was signed and dated 29 August 1985. This study was periodically inspected by the Quality Assurance Unit. The study director stated that this study was in compliance with Good Laboratory Practice standards.

TABLE 3. Effects of EPTC on Fetal Malformations in Rabbits

Dosage (mg/kg/day)	Sire No.	Dam No.	Summary of Malformations[a]
0	30	109	Retroesophageal subclavian artery
30	12	208	Total situs inversus, nonseptated cardiac outlet, interventricular septal defect, hydrocephaly, brachygnathia
	27	210	Meckel's diverticulum
	27	210	Absent interparietal bone
	30	215	Gastroschisis, malrotated hindlimbs
100	43	302	Left forelimb flexure
	15	305	Hydrocephaly, agnathia, cyclopia, malrotated heart, abnormal cardiac outlet, interventricular septal defect, left forelimb flexure
	17	311	Hydrocephaly
	17	311	Encephalocele, rhinencephaly, anophthalmia
	27	317	Absent interparietal bone
300	30	402	Brachydactyly
	37	405	Retroesophageal subclavian artery
	14	412	Cleft palate
	27	415	Retroesophageal subclavian artery, interventricular septal defect
	43	416	Malformed cardiac outlet, interventricular septal defect

[a]As defined by the study authors.

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14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material: Analyses of test material samples obtained three times during the dosing period suggest that the test material was stable in the methylcellulose vehicle; however, the homogeneity assays indicated that the 300-mg/kg preparations had unacceptably low concentrations of the test material (average recovery was 46±11%). Since assays of the 30-mg/kg preparation (which were prepared from the 300-mg/kg suspensions by serial dilution) were acceptable, we attribute the the low assay results tc sampling error. Repeat analysis for homogeneity and stability at this dose level gave acceptable results.

B. Maternal Effects: No adverse effects were noted on maternal survival. Increased incidences of reduced fecal output and cold ears were reported for the 300- and 30-mg/kg/day groups; these effects were transitory and were not dose related. Therefore, we assess that they are not indicative of frank maternal toxicity. We consider the mild increases in body weight gains (and food and water consumption) reported for the 100- and 300-mg/kg/day dose groups and the decreases in body weignt gains noted at 30 mg/kg/day to be inconclusive. Necropsy findings suggest that EPTC did not adversely affect maternal health under the conditions of this study.

C. Reproductive and Developmental Effects: The test material did not adversely affect the pregnancy rates or incidences of abortions at any dose level. Similarly, no effects were noted on the number of corpora lutea, implantations, resorptions, live fetuses, or on fetal weights, and sex ratios.

Although apparent increased incidences of malformed fetuses (and litters) were noted in EPTC-treated groups (see Table 3), they occurred only sporadically, were unrelated to each other, and each has been observed in the control background of this strain. Therefore, we agree with the authors that no compound-related fetotoxicity or other developmental defects occurred in this study.

D. Several deficiencies were noted in the study methods and report:

1. The testing laboratory obtained the females for this study from three sources; this may have caused the study to have genetically heterogeneous animals. The study authors did not report the source of each individual female. This deficiency precludes our evaluation of the acceptability of group assignment procedures (by animal source) and our assessment of possible associations between the reported fetal malformations and animal supplier.

2. The study authors reported that the females were not pregnant at the initiation of the study; the reproductive history of these females, however, was not described. In the absence of this information it was not possible for us to determine if the animals used in this study were all nulligravid.

3. The procedure of intrathoracic injection for sacrifice of the fetuses is considered unacceptable due to the possible physical perforation of cardiac structures and the distortion of cardiac and major vessel anatomy produced by the volume of fluid injected into the cardiac chambers. The anatomic disruptions resulting from these procedures could have negatively affected the reliability of cardiovascular examinations, and perhaps confounded the visualization of cardiac septal defects, valve malformations, pericardial hemorrhages, and various other malformations or lesions in the mediastinum of fetuses.

4. The methods used for examining of the thoracic and abdominal cavities were not described or referenced in the study report nor was it stated whether these examinations were conducted with the aid of magnifying equipment. The study authors stated that microdissection was used when a fetus was suspected to have a malformation. Magnifying equipment should be used routinely to examine fetal viscera, particularly the hearts and kidneys. This is of particular concern since cardiac structures may have been perforated during fetal sacrifices prior to examination for intrathoracic abnormalities. In addition, the method of intracranial examination, as described in the report, was too limited. The authors stated that fixed "heads were sliced through the line of the frontoparietal suture" to examine the fetal brains for possible abnormalities. It would have been more acceptable for the testing laboratory to examine the intracranial structures in serial coronal planes; this method would have provided sectional views of the nasal cavities and septum, olfactory lobes of the brain, eyes, lateral, third, and fourth ventricles, vestibulocochlear apparatus, and cerebellum. The inherent deficiencies of the single coronal section method described by the authors may have precluded the visualization of various malformations and variations.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-9.

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Appendix A

Materials and Methods

Eptam Science Reviews

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Pages _64_ through _71_ are not included in this copy.

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EPA: 68-02-4225
DYNAMAC No. 2480
February 17, 1987

005740

DATA EVALUATION RECORD

EPTC

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Giesler, P.J., Tisdel, M., and MacKenzie, K.M. Two-generation reproduction study with EPTC technical in rats. (Unpublished study No. 6100-108 by Hazleton Laboratories America, Inc., Madison, WI, for PPG Industries, Inc., Barberton, OH; dated June 9, 1986.) Accession Nos. 263695-263696.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-19-87

1. CHEMICAL: EPTC; S-ethyl dipropylthiocarbamate.

005740

2. TEST MATERIAL: EPTC technical, from lot No. 518-996, was reported to be 98.4% pure.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Giesler, P.J., Tisdel, M., and MacKenzie, K.M. Two-generation reproduction study with EPTC technical in rats. (Unpublished study No. 6100-108 by Hazleton Laboratories America, Inc., Madison, WI, for PPG Industries, Inc., Barberton, OH; dated June 9, 1986.) Accession Nos. 263695-263696.

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7. CONCLUSIONS:

A. The NOEL and LOEL for parental toxicity of EPTC in Crl:CD(SD)Br rats were 50 and 200 ppm, respectively, based on reduced body weights, weight gains, and dose-related degenerative cardiomyopathy at 200 and 800 ppm.

The NOEL and LOEL for reproductive/developmental toxicity were 200 and 800 ppm, respectively, based on reduced pup weights at 800 ppm (the HDT).

Based on a theoretical conversion ratio of 1 ppm to 0.075 mg/kg/day, the dose levels of 50, 200, and 800 ppm correspond to 3.75, 15, and 60 mg/kg/day.

B. This study is classified Core Minimum.

Item 8--See footnote 1.

9. BACKGROUND: The study authors reported that in a subchronic study with EPTC, depressed body weights and liver and heart effects occurred at daily doses of 72 and 120 mg/kg. Body weights were also depressed at 36 mg/kg; the dose was reduced to 15 mg/kg after 6 weeks.

In a concurrent chronic toxicity/oncogenicity study, cardiomyopathies were observed after 12 months on test. Due to these findings, the protocol of the present reproduction study was amended to include cardiac histological examinations in the F_1 generation. A 10% reduction in body weights was noted at 36 mg/kg.

Item 10--See footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: EPTC technical, lot No. 518-996, HLA sample No. 797569, was reported to be 98.4% pure. Characteristics of the compound were not described. Appropriate amounts of the test material were added to corn oil and mixed with the basal diet (Purina Certified Rodent Chow #5002) to produce concentrations of 0, 50, 200, and 800 ppm. Diets were prepared weekly and refrigerated until use. Diet samples were analyzed to verify the concentration of the test material. Week-1 preparations were also assayed for homogeneity of the diets by analyzing samples from different locations of the mixer. Stability assays were not performed in conjunction with this study; however, the study authors indicated that no appreciable loss of test material concentration was noted when canine diets were assayed.

[1]Only items appropriate to this DER are included.

2. Animals and Experimental Design: Weanling albino Crl:CD(SD)Br rats were obtained from the Kingston facility of Charles River Laboratories, Stoneridge, NY, and acclimated for 16 days; none of these animals were from the same litter. The animals were stratified by weight and 30 males and 30 females were randomly assigned to each of the four study groups; these rats were designated F_0 parental animals. Animals received their respective diets starting at approximately 5 weeks of age and throughout the study.

After 10 weeks of treatment, females were randomly paired with males of the same group for up to 21 days to produce F_{1a} litters. Thirty male and 30 female F_{1a} weanlings from each group were randomly selected to continue treatment and were designated F_1 parental animals. After at least 10 weeks of treatment, F_1 adults were bred to produce F_{2a} litters using the same procedures as those described for their parents; sibling pairings were avoided.

3. Observations and Measurements: Animals were observed twice daily for survival and obvious signs of toxicity. Animals were removed from their cages at least once each week and examined for clinical abnormalities. Body weights of parental males were recorded at study initiation, weekly during the study, and at termination. Females were weighed at study initiation, weekly prior to mating, on gestation days (GD) 0, 7, 14, and 20, and on lactation days 0, 4, 7, 14, and 21. Food consumption was measured weekly for both males and females prior to mating and for mated females on GD 7, 14, and 20.

Vaginal smears were examined daily during mating; the day that sperm or a plug was observed was designated GD 0. During gestation, females were observed for abortion, excessive bleeding, premature delivery, and difficult or prolonged parturition. As soon as possible after parturition, the numbers of live and dead pups were recorded. The number of live pups was also recorded on days 4, 7, 14, and 21. On day 4, F_{1a} and F_{2a} litters were reduced by random selection to a maximum of 8 and 10 pups, respectively, leaving equal numbers of males and females when possible. The sex and weight of each pup were determined on days 4 and 21. Pups in each litter were weighed collectively on days 0, 7, and 14.

Live pups were examined for external abnormalities at each weighing interval. Pups found dead were grossly necropsied; grossly abnormal pups on day 0 were preserved for possible future examination.

Ten F_{1a} weanlings/sex/group were randomly selected for complete necropsy; "unusual" lesions and liver, kidney, spleen, and reproductive tissues were preserved for histological examination.

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After 10 weeks of treatment, five F_1 adults/sex/group were randomly selected for an interim necropsy. The remaining F_1 adults and all F_0 adults were killed and necropsied after their progeny were weaned. Prior to termination, orbital sinus blood samples were collected from 10 randomly selected adults/sex/group. Hematological and clinical chemistry parameters were measured, and brain, plasma, and erythrocyte cholinesterase activity levels were determined.

Histological examinations were performed for the gonads of all F_0 and F_1 adults and the hearts of all F_1 adults. Other collected tissues were examined histologically only for control and high-dose adults killed after litters were weaned.

4. Statistical Methods: Body weight, food consumption, litter population, and pup weight data were evaluated using analysis of variance (ANOVA). Significant differences between groups were identified using Dunnett's t-test. Reproductive indices were analyzed using contingency tables to determine whether the probability of successful mating or gestation was independent of the treatment group.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Test Material Analyses: Except for one sample from the week-17 low-dose preparation, all diet samples were within 16% of target values. The mean concentrations of assays at 1-5 week intervals were 47.37, 188.6, and 766.13 ppm for the 50, 200, and 800 ppm preparations, respectively. Homogeneity samples of the week-1 diets yielded 89-100, 91-96, and 86-95% recoveries of the target concentration for the low-, mid-, and high-dose diets, respectively.

B. Parental Data: Mortality occurred sporadically in all groups; necropsy findings indicated that the deaths were incidental. In general, body weights of F_0 high-dose males and females were significantly reduced when compared to controls after week 5 (Tables 1 and 2). In addition, mid-dose females weighed significantly less than controls on weeks 8 and 9. Significant reductions in cumulative body weight gain were generally evident in both mid- and high-dose adults of the F_0 generation. For the F_1 parental animals, high-dose male and female body weights and weight gains were significantly reduced throughout most of the generation.

Food consumption of mid- and high-dose F_0 males and females were generally reduced when compared to controls, but significant reductions occurred only in the high-dose males during weeks 4, 5, and 9 and in mid-dose males during week 4 (Table 3). In the F_1 generation, significant reductions were evident at all EPTC dose levels for both males and females.

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TABLE 1. Mean Body Weights (g) of Rats Fed EPTC[a]

	Dose Level (ppm)	Week 0	1	4	8	12	23
F_0 Males	0	187	244	385	476	542	647
	50	187	243	377	465	521	624
	200	189	243	372	461	515	609
	800	189	242	371	445*	494*	569*
F_0 Females	0	142	172	234	273	328	
	50	146	173	235	272	327	
	200	144	171	228	259*	321	
	800	143	170	224	248*	300*	
F_1 Males	0	169	231	388	493	549	673
	50	170	232	382	489	541	648
	200	175	233	367*	478	531	637
	800	145*	201*	330*	419*	469*	542*
F_1 Females	0	137	164	228	270	319	
	50	139	167	227	269	328	
	200	140	165	225	266	318	
	800	125*	152*	202*	237*	283*	

[a]The data presented here are abstracted from Tables 1, 2, 11, and 12 of the study report.

*Significantly different from control value ($p < 0.05$), as determined by the study authors.

TABLE 2. Mean Maternal Body Weights and Food Consumption of Rats Fed EPTC[a]

	Dose Level (ppm)	Body Weights (g)				
		Gestation Day		Lactation Day		
		0	20	0	14	21
F_0 Females	0	286	398	312	328	310
	50	287	395	318	334	315
	200	278	405	307	334	309
	800	260*	373*	278*	312	301
F_1 Females	0	281	388	316	329	322
	50	278	400	314	330	320
	200	277	393	304	330	314
	800	245*	346*	271*	300*	292*

	Dose Level (ppm)	Food Consumption (g/animal/interval) on Gestation Days		
		0-7	7-14	14-20
F_0 Females	0	167	173	157
	50	159	171	155
	200	163	176	160
	800	155	168	151
F_1 Females	0	164	167	133
	50	159	163	140
	200	158	159	145
	800	133*	147	127

[a]The data presented here are abstracted from Tables 8, 9, 18, and 19 of the study report.

*Significantly different from control value ($p < 0.05$), as determined by the study authors.

TABLE 3. Mean Food Consumption (g/animal/week) of Rats Fed EPTC[a]

	Dose Level (ppm)	Week 1	2	4	8	10
F_0 Males	0	180	200	212	205	200
	50	175	193	207	205	194
	200	172	188	194*	195	191
	800	172	193	192*	192	191
F_0 Females	0	134	144	163	155	163
	50	133	143	154	169	150
	200	129	147	149	148	150
	800	126	141	151	149	141
F_1 Males	0	179	202	232	215	218
	50	173	187*	214*	207	199*
	200	169	187*	214*	192*	193*
	800	153	169*	179*	177*	172*
F_1 Females	0	125	137	167	163	142
	50	129	134	149*	140*	132
	200	131	137	144*	140	131
	800	129	134	130*	125*	120*

[a]The data presented here are abstracted from Tables 5, 6, 15, and 16 of the study report.

*Significantly different from control value ($p < 0.05$), as determined by the study authors.

Antemortem observations and gross necropsy findings of adult animals did not suggest parental toxicity. Histological examinations of F_0 tissues revealed only incidental findings; however, the study authors indicated that hearts were not examined histologically in the F_0 generation. Evidence of degenerative cardiomyopathy was present in F_1 adults of the mid- and high-dose groups (Table 4).

The only significant changes noted by the study authors in hematological, clinical chemistry, and cholinesterase data were minimally increased erythrocyte count in mid-dose males and reduced mean corpuscular volume and mean corpuscular hemoglobin values in mid- and high-dose females.

Reproductive and Developmental Data: Precoital intervals, the numbers of females mating, pregnancy rates (Table 5), and male fertility rates were comparable for all groups in both generations. Except for one low-dose F_0 female that died during parturition, all pregnant females successfully maintained pregnancy and delivered without complications.

Litter sizes of F_{1a} low-dose litters were significantly reduced at birth and day 4 (Table 6). Mid- and high-dose litter sizes, however, were comparable to controls. F_{2a} litter sizes were comparable for all groups throughout lactation.

Body weights of F_{1a} high-dose progeny were significantly reduced throughout lactation when compared to controls; body weights of F_{2a} high-dose pups were reduced from days 4-21 (Table 6). Mid-dose pup weights were significantly reduced on day 7 of the F_{2a} lactation. Low-dose pup weights were comparable to controls for both generations.

Antemortem observations of progeny revealed one high-dose F_{1a} pup with a small palpable mass that was noted on day 14 but was no longer evident at approximately 5 weeks postpartum. No other antemortem observations were noted in progeny of either generation. Necropsies of F_{1a} weanlings revealed only incidental findings.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that the NOEL for body weight effects was 200 ppm and the NOEL for reproductive parameters was 300 ppm EPTC technical.

B. A quality assurance statement was signed and dated June 11, 1986. Inspections were conducted periodically.

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TABLE 4. Incidences of Degenerative Cardiomyopathy in Rats Fed EPTC[a]

	Dose Level (ppm)	After 10 Weeks of Treatment (5 examined/sex/group)	After Weaning of F_{2a} Litters (25 examined/sex/group)
F_1 Males	0	1	4
	50	0	3
	200	5	15
	800	5	25
F_1 Females	0	0	1
	50	1	0
	200	3	5
	800	5	25

[a]The data presented here are abstracted from Tables 29 and 32 of the study report.

TABLE 5. Summary of Breeding Results of Female Rats Fed EPTC[a]

	Dose Level (ppm)	No. Paired	Mated		Pregnant	
			No.	%	No.	%
F_{1a} Interval	0	30	30	100	26	87
	50	30	30	100	25	83
	200	30	30	100	26	87
	800	30	30	100	27	90
F_{2a} Interval	0	25	24	96	23	92
	50	24	23	96	21	88
	200	25	23	92	23	92
	800	25	25	100	24	96

[a]The data presented here are abstracted from Tables 10 and 20 of the study report.

TABLE 6. Mean Litter Data of Rats Fed EPTC[a]

	Dose Level (ppm)	No. Live Pups on Day			Pup Weight (g) on Day			
		0	4	21	0	4	7	21/14
F_{1a} Litters	0	12.1	11.9	7.3	6.5	11.0	17.3	56
	50	10.0*	9.8*	6.8	6.9	11.7	18.0	56
	200	13.3	13.0	8.0	6.2	10.0	16.4	55
	800	12.7	11.7	7.6	5.9*	8.8*	13.7*	47*
F_{2a} Litters	0	11.6	11.3	8.9	6.9	11.5	18.1	56
	50	13.1	13.0	10.0	6.4	10.4	16.5	53
	200	12.4	12.4	9.5	6.3	10.3	16.1*	52
	800	12.2	12.0	9.0	6.2	9.1*	14.4*	47*

[a]The data presented here are abstracted from Tables 10 and 20 of the study report.

*Significantly different from control value ($p < 0.05$), as determined by the study authors.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: 005740

A. The reviewers validated the text and summary tables of the study report by comparing them against the individual animal data; no major discrepancies were found.

B. Test Material Analyses: Chemical analyses of the test diets indicated that the preparations were acceptable. We assess that the single sample with a low assay result (i.e., less than 84% of the nominal concentration) did not adversely affect the integrity of the study.

Parental Data: We assess that the deaths, antemortem observations, and gross necropsy findings were not indicative of any compound effects.

We consider the reduced body weights, weight gains, and food consumption of F_0 males and females to reflect parental toxicity at 200 and 800 ppm. In the F_1 generation, significantly reduced food consumption was consistent with significantly reduced body weights at 800 ppm only. We do not consider the F_1 low- and mid-dose reductions in food consumption to reflect compound toxicity. We regard the degenerative cardiomyopathy seen in F_1 males and females at 200 and 800 ppm to reflect a more significant parental toxicity, thus setting the study NOEL at 50 ppm (the LDT).

Although there were significant hematological changes in F_1 adults, these changes were minimal; we do not consider these findings to be evidence of overt parental toxicity.

Reproductive and Developmental Data: We assess that compound administration had no effect on the reproductive parameters of precoital time, mating incidence, pregnancy rate, parturition, or male fertility.

We regard the reduction in F_{1a} litter size in the low-dose group to be incidental since no adverse changes were noted in the mid- or high-dose groups. Litter sizes throughout the F_{2a} interval also did not indicate adverse compound effects. We consider the reductions in pup weights, however, to indicate toxicity at 800 ppm in both generations. Although the study authors did not cite reduced pup weights in their conclusions, they did note this finding as a compound effect at 200 and 800 ppm.

Antemortem observations and necropsy data of the progeny did not suggest any compound effects. We consider these findings to be incidental.

C. Therefore, we conclude that the NOEL for parental toxicity was 50 ppm, based on reduced body weights and food consumption and dose-related degenerative cardiomyopathy at 200 and 800 ppm.

We also conclude that the NOEL and LOEL for reproductive/developmental toxicity were 200 and 800 ppm, respectively, based on reduced pup weights at 800 ppm.

Item 15--See footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3-12; Appendix B, Protocol, CBI pp. A-1 to A-26.

APPENDIX A

Eptam Science Reviews

Page _____ is not included in this copy.

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005740

TOXICOLOGY BRANCH: DATA REVIEW

Reviewed by: Irving Mauer, Ph.D. TB Project No.: 2123
Toxicology Branch
Hazard Evaluation Division Date: January 16, 1987

Through: Judith W. Hauswirth, Ph.D., Acting Head
Section VI, Toxicology Branch
Hazard Evaluation Division

CHEMICAL: EPTC Caswell No.: 435
EPA Chem No.: 041401

STUDY TYPE: Mutagenicity - Gene mutation in mammalian cells in vitro (L5178Y/TK).

CITATION: Mouse Lymphoma Cell Mutagenesis Assay ($TK^{+/-} \rightarrow TK^{-/-}$) of EPTC. Final Report.

ACCESSION NO.: 263697

MRID: N/A

SPONSOR: PPG Industries, Inc., Pittsburgh, PA.

TESTING LAB: SRI International, Inc., Menlo Park, CA.

STUDY NO.: SRI Project No. LSU-8644-1

STUDY DATE: February 13, 1986

TB CONCLUSIONS/EVALUATION:

ACCEPTABLE in demonstrating a positive response in the presence of metabolic activation, negative results in nonactivated cultures. A NOEL was not determined.

STUDY DETAILS

TEST MATERIAL:

EPTC technical (lot #518-996), 98.5% ai, supplied by the sponsor as a clear liquid, dissolved in ethanol for testing.

STUDY PROCEDURES:

Following preliminary cytotoxicity testing, duplicate cultures of heterozygous ($TK^{+/-}$) L5178Y mouse lymphoma cells (clone 3.7.2C) were exposed for 4 hours to 1% ethanol or six selected concentrations of test article, in both the absence and presence of a metabolic

activation system (MA) provided by Aroclor 1254-induced rat liver microsomes (S9) plus NADPH-generating cofactors, according to published procedures (referenced in the Final Report). The cells, washed free of treatment solutions, were resuspended in fresh tissue culture medium, and incubated a further 2 days to allow for expression of mutations, following which they were cloned in medium containing trifluorothymidine (TFT) for selection of mutant colonies ($TK^{-/-}$). After 11 to 12 days in the cloning medium, colonies were counted electronically, sized, and mutation frequency (MF) of each culture (ratio of number of mutant cells to the number of viable cells) calculated, averaged for each treatment concentration, and expressed as mutants per 10^6 cells. Positive controls (reference mutagens) were run concurrently with each type of assay: ethylmethanesulfonate (EMS) for the nonactivated series, and 3-methylcholanthrene (MCA) for activation.

Standard criteria for acceptability and evaluation, as well as a QA/GLP statement, were included in the Final Report.

STUDY RESULTS:

Preliminary cytotoxicity testing with and without MA revealed precipitation of test article at concentration of 389 ug/mL and above. In nonactivated cultures, the next lower dose, 233 ug/mL, was cytotoxic (only 1% cell growth relative to ethanol control), but six doses lower than that (ranging from 140 down to 11 ug/mL) were not cytotoxic (92 to 107% of control). Under activation conditions, there was a shallow but definitive dose-related increase in cytotoxicity in the same dose range, from 72 percent relative cell growth at 50 ug/mL to 36 percent at 233 ug/mL, and then a sharp increase at the next two higher concentrations, 389 ug/mL (2%) and 648 ug/mL (1%); no cell growth was observed at higher doses (1080 to 5000 ug/mL). Hence, six concentrations of EPTC ranging from 118 to 200 ug/mL were selected for the mutagenesis assay without activation, and six from 42 to 250 ug/mL, for the series with S9.

In the absence of activation, no increase in mutant colonies over the solvent control value (average mutant frequency/10^6 cells = 80) was found at any level of EPTC (range, 73 to 103, with a value of 81 for the highest dose). In activated cultures, on the other hand, there was a flat three- to fourfold increase over control (= 86) in mutants in the dose range 42 to 123 ug/mL (average MF = 316 to 424), with a steeper increase at the two highest doses (5.6-fold at 174 ug/mL, and 6.1X at 250 ug/mL). Both positive controls responded as expected, EMS inducing a tenfold increase in nonactivated cultures, and MCA a sevenfold increase with activation.

Colony sizing measurements in the activation assay revealed the majority of colonies induced by EPTC were small, indicating to the investigators a mechanism of mutation ". . . not yet known."

STUDY CONCLUSIONS:

The authors concluded that EPTC induces a positive response in activated cultures, but not in the absence of activation.

TB EVALUATION:

ACCEPTABLE. The study was conducted with procedures adequate to generate valid results, demonstrating EPTC was positive in inducing an increase in mutant (TFT-resistant) colonies at all concentrations tested (42 to 250 ug/mL) under activation conditions, but negative in the absence of MA. The positive response was induced at levels of cytotoxicity ranging from approximately 20 percent at the lowest dose tested (79% relative cell growth) to 75 percent (25% RCG) at the HDT. The investigators should have sought to obtain a NOEL at a level of cell survival more closely approximating the negative control, to completely fulfill the Agency's Guidelines for this type of study (although this does not invalidate the study).

Because of the predominance of small (over large) colony induction, the authors suggest the positive response obtained in this study may not reflect gene mutation at the thymidine kinase (TK) locus (i.e., alteration of its DNA sequence), but by an as yet unknown mechanism. Current research activity in this type of mutagenesis assay* (by EPA investigators at the Agency's laboratories in RTP, North Carolina, as well as others) indicates the production of large colonies may result from true point mutation at the TK locus, whereas small colonies result from cytogenetic changes involving a large segment of the chromosome (assigned as No. 11 of the mouse karyotype, the known location of the TK gene) which includes a large number of genes on either side of TK. A definitive evaluation of this colony size discrimination could have been

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* e.g. (1) Sawyer, J.; Moore, M.M.; Clive, D.; Hozier, J. Cytogenetic characterization of the L5178Y $TK^{+/-}$ 3.7.2C mouse lymphoma cell line. Mutation Research. 147 (1985) 243-253.

(2) Blazak, W.F.; Stewart, B.E.; Galperin, I.; Allen, K.L.; Rudd, C.J.; Mitchell, A.D.; Caspary, W.J. Chromosome Analysis of Trifluorothymidine-Resistant L5178Y Mouse Lymphoma Cell Colonies. Environmental Mutagenesis 8:229-240 (1986).

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provided by the study authors if they had tested to lower (nontoxic, no-effect) levels, as well as performed cytogenetic analysis of chromosome preparations from both observably large and small colonies. If small colonies were to reveal alterations in chromosome No. 11 (and thus the more likely mechanism of mutant induction in mouse L5178Y cells by EPTC), this would be at variance with the negative cytogenetic response reported by another lab (LBI Project 20990, Genetic Assay 8031, dated November 1985) in CHO cells treated with this chemical under the same conditions (e.g., with activation) up to concentrations causing comparable levels of cytotoxicity (150-225 ug/mL).

005740

TOXICOLOGY BRANCH: DATA REVIEW

Reviewed by: Irving Mauer, Ph.D. TB Project No.: 2123
Toxicology Branch
Hazard Evaluation Division Date: January 16, 1987

Through: Judith W. Hauswirth, Ph.D., Acting Head
Section VI, Toxicology Branch
Hazard Evaluation Division
Judith W. Hauswirth 1/28/87

CHEMICAL: EPTC

Caswell No.: 435
EPA Chem No.: 041401

STUDY TYPE: Mutagenicity - Chromosome aberrations in vitro (CHO cells).

CITATION: Clastogenic Evaluation of EPTC Technical, 518-996 BR85-40 in an in vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells. Final Report.

ACCESSION NO.: 263697

MRID: N/A

SPONSOR: PPG Industries, Inc., Pittsburgh, PA.

TESTING LAB: Litton Bionetics, Inc., Kensington, MD.

STUDY NO.: LBI Project No. 20990 (Genetics Assay No. 8031)

STUDY DATE: November 1985.

TB CONCLUSIONS/EVALUATION:

ACCEPTABLE in demonstrating negative results for inducing chromosome aberrations in CHO cells treated with and without metabolic activation up to cytotoxic levels (150 to 225 ug/mL).

STUDY DETAILS

TEST MATERIAL:

EPTC technical, 518-996 BR85-40, supplied by the sponsor as a clear liquid (% purity not stated here), dissolved in ethanol for testing.

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STUDY PROCEDURES:

Following range-finding tests, to select both the doses (based on toxicity, as determined by monolayer confluency and counts of mitotic cells) and optimal harvest times (to insure analysis of first metaphases postdose), duplicate cultures of Chinese hamster cells (CHO-WBL, originally obtained from Dr. S. Wolff, University of California, SF) were exposed (in a single trial) to a range of four doses of test article for 17.5 hours in the absence of metabolic activation, or for 2 hours in the presence of an Aroclor 1254-induced rat liver S9 activation system (MA), then prepared for cytogenetic analysis of 100 metaphase cells per culture per dose level by established (referenced) procedures. Overall chromosome aberration frequencies and percent cells with one or more than one aberration at each dose level were compared to pooled solvent (ethanol) and negative (untreated) controls by Fisher's Exact Test (with appropriate adjustment for multiple comparisons). Evidence of any dose response as well as the estimated number of breaks involved in the production of different types of aberrations were also considered. Cultures exposed to appropriate positive control substances for each series were also run in parallel: mitomycin-C (MMC) for non-activation, and cyclophosphamide (CP) for activation.

STUDY RESULTS:

In range-finding assays with/without MA, no cells survived concentrations of 333 ug/mL EPTC or higher. At the next lower dose, 100 ug/mL, there was 50 percent reduction in monolayer confluency with few cells in mitosis in nonactivated cultures, but no toxicity and no apparent cell cycle delay with activation. Therefore, a delayed harvest time (20 hours) with a dose range 30 to 200 ug/mL EPTC was selected for the nonactivated aberration assay, the usual harvest (10 hours) and a dose range of 15 through 300 ug/mL with activation.

In the absence of activation, no dividing cells were observed at the two highest EPTC doses selected, 150 and 200 ug/mL, and 20 percent reduction in monolayer confluency at the two next lower concentrations, 90 and 120 ug/mL, combined with observable decreases in mitoses. However, at neither of these two latter concentrations, nor at lower doses (30 to 60 ug/mL) were significant increases in chromosomally aberrant cells over controls found (range, 1.5 to 4.0% for EPTC, 2.5% in controls). In contrast, the positive control, MMC, induced large increases in aberrant cells (26%), number of aberrations per cell (> 0.32 vs. 0.03 for controls), and multiply aberrant cells (4.0% vs. 0.5%).

In S9-activated cultures, the top EPTC concentration, 300 ug/mL, was lethal, as was the next lower dose, 225 ug/mL. At 150 ug/mL there was a moderate reduction in confluency (25%), and

slightly reduced number of observable mitotic cells, but no significant increase in chromosome aberrations at this dose (4.0%), nor at any of the lower doses (2.5%, 1.0%, and 1%, respectively at 15, 30, and 75 ug/mL), compared to the pooled control value (3.0%). Cultures treated with the mutagen (CP) responded as expected, 36.0% of cells with aberrations overall, at a rate of 0.48 aberrations per cell, and 8.0% with more than one aberration.

STUDY CONCLUSIONS:

The authors concluded that technical EPTC was negative for the induction of chromosome aberrations in CHO cells, under both activation and nonactivation conditions.

TB EVALUATION:

ACCEPTABLE. The assay was performed with adequate procedures to demonstrate the negative results obtained.

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005740

TOXICOLOGY BRANCH: DATA REVIEW

Reviewed by: Irving Mauer, Ph.D. TB Project No.: 2123/2344
Toxicology Branch
Hazard Evaluation Division Date: January 26, 1987

Through: Judith W. Hauswirth, Ph.D., Acting Head
Section VI, Toxicology Branch, Judith W. Hauswirth 1/28/87
Hazard Evaluation Division

CHEMICAL: EPTC

Caswell No.: 435
EPA Chem No.: 041401

STUDY TYPE: Mutagenicity - Primary DNA damage/repair (HPC/UDS)

CITATION: Evaluation of the Potential of Ethyl-(N,N-dipropyl) thiocarbamate to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures. Final Report.

ACCESSION NO.: 263697

MRID: N/A

SPONSOR: PPG Industries, Pittsburgh, PA.

TESTING LAB: SRI International, Inc., Menlo Park, CA.

STUDY NO.: SRI Project LSC-8644

STUDY DATE: January 1986

TB CONCLUSIONS/EVALUATION: Although reporting negative results for UDS in this in vitro rat hepatocyte assay up to cytotoxic concentrations of EPTC (250 ug/mL), this study is UNACCEPTABLE because of procedural and reporting deficiencies (as detailed in the TB EVALUATION).

STUDY DETAILS

TEST MATERIAL:

Ethyl-(N,N-dipropyl)thiocarbamate (EPTC technical, Lot No. 518-996), 98.5% ai, supplied by the sponsor as a clear, yellow liquid, dissolved in 95% ethanol for testing.

PROCEDURES:

Coverslip cultures of primary rat hepatocytes, isolated from two adult male Fischer-344 rats, were exposed in repeat experiments to graded concentrations of test article (three cultures per concentration), and simultaneously to 10 uCi/mL ^{3}H-thymidine (sp. act., 80 Ci/mmole), for 19 to 21 hours at 37 °C, following

which they were washed, swelled in hypotonic saline, fixed, and prepared for autoradiographic grain counting indicative of unscheduled DNA synthesis (UDS), according to standard (referenced) procedures. For the preliminary experiment, 10 concentrations up to 5000 ug/mL (the limit of solubility) were tested; in the repeat, 6 concentrations up to 500 ug/mL.

Grains from 50 normal-appearing cells per slide were counted electronically, the highest count from two nuclear-sized areas over the heaviest-labeled cytoplasmic area adjacent to the nucleus being subtracted from the nuclear grain count to give net grains per nucleus (NG); the percentage of cells in UDS was calculated as percentage of cells with 5 NG or more. Thus, 150 cells were scored for each concentration for each experiment, and frequency distributions of grain counts for each test concentration, as well as average and median grain counts, calculated and compared with control values by a computer data analysis program (VAX 11/782). No other statistical methods were used to analyze data.

Three controls were run concurrently with each assay: a positive control, 2-acetylaminofluorene (2-AAF) at two concentrations (but only one scored), a solvent control (95% ethanol), and an untreated medium control. Although stated to have been included (as page ii, so indicated in the Table of Contents), a QA statement was inadvertently missing from this review copy of the Final Report.

STUDY RESULTS:

As presented in a summary tabulation for both assays, EPTC was cytotoxic at concentrations of 250 ug/mL and above, and precipitation was observed at levels of 1000 and 5000 ug/mL.

In contrast to the positive UDS response in the majority of cells treated with the mutagen, 2-AAF (39.5 NG in 95% of cells in the first experiment and 5.7 NG in 52% in the second assay*), test plates were negative at all useful concentrations of EPTC up to the nontoxic level, 100 ug/mL, registering negative NG values in the few cells with any grains (not different from negative controls).

STUDY CONCLUSIONS:

EPTC was negative (for UDS induction) in this *in vitro* rat hepatocyte DNA repair assay.

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* Stated as "18.7 NG" in text (Results and Discussion section), but 5.7 in tabular summary (Table 1 of Report).

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TB EVALUATION:

This study appears to have been conducted according to procedures adequate to generate and support the negative results obtained with EPTC. However, the study is not acceptable because of significant deficiencies in procedure and/or reporting:

1. The subtraction of the "two highest" (presumably) cytoplasmic grain counts to determine "net nuclear grain counts" is at variance with the customary practice of subtracting the average count from several (commonly, three) cytoplasmic areas to arrive at NG per nucleus, and produces unreasonably high negative values (even for the negative controls, as reported in the Summary tabulation).

2. The investigators' procedures probably also contributed to the disparately low mean NG value reported for the positive control (2-AAF) in the replicate assay (Summary tabulation).

3. The discrepancy in reporting the level of response to 2-AAF (18.7 NG in the Results and Discussion section, but 5.7 in Table 1) is noted by us.

4. Individual counts for each of the triplicate cultures per treatment are not presented.

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TABLE 1

ASSAY FOR INDUCTION OF UNSCHEDULED DNA SYNTHESIS BY EPTC
IN THE IN VITRO HEPATOCYTE DNA REPAIR ASSAY

Treatment	Concentration (µg/ml)	Preliminary Assay				Replicate Assay			
		N.G.	S.E.	Median	% IR	N.G.	S.E.	Median	% IR
CONTROL/MEDIUM	---	-13.4	1.0	-14.0	0	-18.9	2.1	-18.6	1
CONTROL/EtOH	1%	-14.9	0.9	-14.0	1	-18.1	2.9	-17.7	1
2-ACETYLAMINOFLUORENE	0.5	39.5	7.2	39.1	95	5.7	2.4	5.6	52
EPTC	.1	N.S.				N.T.			
	.5	-14.5	4.3	-15.0	4	N.T.			
	1	-11.1	2.0	-11.2	1	N.T.			
	3	N.T.				-17.5	3.1	-16.7	1
	5	-12.3	2.3	-12.1	1	N.T.			
	10	-18.9	2.9	-17.6	1	-17.0	1.7	-16.3	1
	30	N.T.				-20.5	2.1	-19.5	1
	50	-18.6	3.1	-18.2	3	N.T.			
	100	-17.1	4.4	-16.7	3	-17.6	1.1	-17.7	1
	250	N.T.				toxic			
	500	toxic				toxic			
	1000	toxic				N.T.			
	5000	toxic				N.T.			

N.G. = Net grains/nucleus (> +5).
Standard errors (S.E.) represent slide-to-slide variation.
% IR = Percentage of cells in repair.
toxic = Cytotoxicity observed; slides unscorable.
N.T. = Not tested at this concentration.
N.S. = Not scored at this concentration.