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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 20 1986

MEMORANDUM

SUBJECT: EPTC (EPTAM) - Review and Evaluation of Thirteen-Week Subchronic Rat Study, Submitted as 6(a)2 Data by PPG EPA Accession Nos. 255614, 255615, 255616, 255617

Registration No. 748-233 Caswell No. 435

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Action Requested:

Review and evaluate the following study, submitted as a Section 6(a)2 Report, November 8, 1984:

Thirteen-Week Subchronic Study in Rats with EPTC, Study No. 6100-105, dated September 14, 1984, conducted for PPG by Hazleton Laboratories, America.

Toxicology Branch Conclusions:

The study was judged CORE-MINIMUM DATA.

Doses tested: 0, 3, 15, 72, and 120 mg/kg/day.

Effects noted:

1. Dose-related decreases in body weight gains and food consumption at all levels but the LDT.

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2. Increased aspartate aminotransferase (AST) at the two higher dose levels (72 and 120 mg/kg/day), correlated with a treatment-related increase in cardiomyopathy found at 15, 72, and 120 mg/kg/day.

3. Depressed brain cholinesterase activity in high-dose females.

NOEL = 3 mg/kg/day

LEL = 15 mg/kg/day (decreased body weight and/or weight gain; mild chronic myocarditis).

TOXICOLOGY BRANCH: DATA REVIEW

Compound: Technical EPTC

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

Study Type: Subchronic (13-week) feeding - rat

Citation: Final Report, Thirteen-Week Subchronic Study in Rats with EPTC.

Accession Nos.: 255614, 255615, 255616, 255617

Sponsor/Testing Lab: PPG Industries/Hazleton Laboratories America (HLA)

Study No./Date: HLA No. 6100-105/September 14, 1984

Test Material: EPTC technical 518-996 (BR 83-64), 98.4% ai

Procedures:

Groups of 20 male and 20 female weanling Charles River albino rats (CD-Crl:CD(SD)BR) were fed diets providing 0 (Purina Certified Rodent Chow), 18, 36, 72, and 120 mg/kg test material (calculated weekly from body weights and feed consumption) for the first 6 weeks of the 13-week study. From week 7 through 13 the two lowest dose groups, 18 and 36 mg EPTC/kg body weight, were reduced to 3 and 15 mg EPTC/kg body weight,* respectively, whereas the other groups continued to receive the same amount given in the first 6 weeks, as shown below.

Dose Schedule (mg EPTC/kg body weight)

Group	Week	
	0 - 6	7 - 13
1	0	0
2	18	3
3	36	15
4	72	72
5	120	120

Observations of the animals were made twice daily for toxic signs and mortality. Dead animals were immediately removed from the cages and preserved. Once a week the animals were removed from their cages and examined for behavioral and clinical signs of toxicity. Body weights and feed consumption were recorded weekly.

*Because it became apparent to the authors that a no-effect level for body weight may not be achieved.

Before assignment to test groups, baseline hematological and blood chemical values (including brain cholinesterase) were determined in a pretest group of males and females from the same shipment of animals. At study week 6, one-half of each test group was bled for hematology evaluation, and at 13 weeks, the same animals sampled for electrolytes, blood chemistry, urinalysis, and cholinesterase.

At study termination (13 weeks), selected organs (heart, liver, kidneys, spleen, brain, and gonads) were weighed. All test animals were subjected to complete necropsy (see APPENDIX A of this review). Histopathology examination was performed on all of the above tissues in the 120 and 72 mg/kg groups, including animals which died during the test. Heart, liver, kidneys, spleen, and gross lesions were examined in the 18/3 and 36/15 mg/kg dose groups.

Statistical analysis was performed to evaluate changes in body weight, feed consumption, organ weight, organ weight to body weight ratios, blood chemistry, and hematology for each sex; the level of significance was chosen at $p \leq 0.05$. Analysis of variance was used to determine whether group means differed significantly, followed by Dunnett's t-test to compare treatment means with the control mean. Chi-Square analysis was used to evaluate "nonparametric" data, according to the test report.

According to the test report, the study was conducted according to FDA Good Laboratory Practice Regulations, and was monitored by the Hazleton Laboratory America Quality Assurance unit. The test report further states the study conformed to EPA Guidelines for Testing Chemicals.

Full details of the procedure used to test the homogeneity and stability of the feed mixed with EPTC were reported. Analyses of the feed were made weekly; fresh diet was prepared once a week.

Results:

The calculated average consumptions of EPTC were reported as 95 to 105 percent of theoretical amounts for each test group throughout the study (Table 1 and pp. 60-85 of the Report), except for week 13. Analysis of feed samples for the terminal week revealed that diets for the 15 mg/kg males and females had been switched, so that males received only 73 percent of targeted dose, and females 138 percent.

All animals survived the entire 13-week period of the study, except for one control male (animal #C-7701), which died at the end of week 7 during orbital bleeding. The pathology report did not indicate any other contributory

factors. Other than incidental clinical signs normally found in a colony of rats this size (alopecia, malocclusions, skin lesions, reactions to ear tagging, and lacrimation), no treatment-related effects were noted at any dose level, as indicated in Table 2 of the Report. Animals with abnormal ophthalmic lesions prior to initiation were not used in this study; ocular discharge, phthisis bulbi, ectopic pupil, and chloroidal atrophy unrelated to dose or treatment were found sporadically in all dose groups (Table 3 of the Report).

Statistically significant, dose-related decreases in body weight gains (87% to 72% of control males and 83% to 74% of control females) were reported for the three highest test groups, beginning at week 1 for males and week 3 for females, carrying through to termination (Tables 4 through 7, figures 1 through 4, and Appendix A of the Report). At the two lowest doses, a decreased body weight gain compared with control was observed for weeks 1 to 6, but not for weeks 7 to 13 after dose reduction. Reduced food consumption followed a similar pattern to decreased weight gain at the three highest doses; after dose level reduction at week 7 (from 36 to 15 mg/kg), the trend in reduced feed consumption in the next to lowest dose group was no longer evident.

Although slight but statistically significant differences compared to controls were recorded in a few hematological values (Tables 10, 11, and Appendix B) for high dose males after 6 weeks' treatment (slightly lower mean hematocrits and mean corpuscular volumes), as well as in all female test groups at both the 6- and 13-week sampling (but only in MCV), these were not considered of toxicological importance.

No effects on clinical chemistry values were reported at the two lower dose levels, as recorded in Tables 12 and 13 and Appendix B of the Report. Blood urea nitrogen (BUN), however, was significantly elevated in males and females at the 72 and 120 mg/kg levels, as shown below:

mg EPTC/kg Body Weight/Day	BUN level in Males (mg/dl)	Females (mg/dl)
0	14.2 ± 3.06	13.5 ± 2.15
3	14.2 ± 2.84	13.6 ± 2.82
15	16.1 ± 5.22	14.9 ± 2.94
72	19.1 ± 2.88	20.0 ± 3.62
120	19.3 ± 6.08	20.6 ± 4.59

Blood glucose (GLU) was decreased at 13 weeks in females at 72 and at 120 mg/kg. There was no significant change noted in males.

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mg EPTC/kg Body Weight/Day	GLU level in Females (mg/dl)
0	114 ± 14.7
3	115 ± 10.5
15	111 ± 8.1
72	100 ± 7.6
120	87.9 ± 9.61

Significantly elevated levels of aspartate aminotransferase (AST) were found at 13 weeks in males and females fed 72 and 120 mg/kg, in some cases twice control levels (as shown below). The AST levels in males at 72 and 120 mg/kg were, respectively, 2.1 and 2.4-fold and in females, 1.5 and 2.0-fold greater than controls. The authors ascribe these increased AST levels to the chronic myocarditis observed microscopically in these two dose groups (see pages 5 and 6 of this review).

mg EPTC/kg Body Weight/Day	AST level in Males (IU/l)	in Females (IU/l)
0	107 ± 25.6	109 ± 20.3
3	109 ± 31.9	102 ± 26.1
15	112 ± 18.3	108 ± 19.9
72	221 ± 106	162 ± 39.6
120	254 ± 124	214 ± 60.8

Although alanine aminotransferase (ALT) was slightly elevated in males at 72 and at 120 mg/kg and in females at 120 mg/kg/day, these increases were not significant.

Among animals treated at the two higher doses, urine specific gravity was greater and urine volume less than controls (Table 14). Higher values for urinary ketone (Appendix B) in females fed 72 and 120 mg/kg/day were reportedly related to decreased glucose values in these animals.

Plasma cholinesterase values in males were decreased, but not statistically (87% of control) at the two highest doses, and slightly above control values in females. Erythrocyte cholinesterase was not significantly reduced at any dose level in males or females. Brain cholinesterase in 120 mg/kg females, however, was significantly lower (86%) than the control group value.

Since terminal body weight was significantly lowered in both males and females in the 15, 72, and 120 mg/kg dose groups, Dunnett's test was used to compare control to treatment means at the 0.05 significance level for body weight, individual

organ weights, and the ratio of organ weight to body weight (Tables 15, 16, and Appendix C). As recorded in the Report, differences in absolute and/or relative heart, brain, testes, and spleen weights reflected these differences in terminal body weights. Lowered heart weights in males were determined to be significant at 15 and 120 mg/kg. The ratio of heart weight to body weight was significantly increased at 72 and 120 mg/kg, as shown in the following table.

mg EPTC/ kg Body Weight/Day	Weight of Heart in Males	
	Mean Organ Weight (g)	Mean Organ Weight(g)/Body Weight
0	1.47 ± 0.148	0.2957 ± 0.0215
3	1.41 ± 0.145	0.3041 ± 0.0250
15	1.36 ± 0.142*	0.3054 ± 0.0251
72	1.37 ± 0.112	0.3275 ± 0.0265*
120	1.34 ± 0.116*	0.3358 ± 0.0308*

No significant changes in heart weight were found in females. Other significantly lowered mean organ weights were recorded at the two higher dose levels in males for spleen and brain, and in females for brain at 72 and 120 mg/kg. Females also showed significantly elevated (about 10%) mean liver weight at 120 mg/kg, and increased liver-to-body weight ratios at the two highest doses.

Except for an increased incidence of "accentuated lobular pattern" in the livers of high-dose males and females (120 mg/kg/day) and "diffusely light" livers in females fed this level, no other treatment-related lesions were observed at gross necropsy (Tables 18, 19, 20, and Appendix C). On the other hand, EPTC dose-related histopathological findings were recorded in the heart (more severe in males), characterized as "chronic inflammation," and consisting of focal to multifocal myocardial degeneration, infiltrating mononuclear cells, fibroplasia, and occasional fatty changes (as summarized below from page 13 and Table 19 of the Report):

Incidence of Chronic Inflammation

Treatment (mg/kg)	Males					Females				
	0	18/3	36/15	72	120	0	18/3	36/15	72	120
Number Examined	19	20	20	20	20	20	20	20	20	20

Incidence of Chronic Inflammation (Cont'd)

Treatment (mg/kg)	Males 0	18/3	36/15	72	120	Females 0	18/3	36/15	72	120
Lesion Grade										
-	19	19	14	3	0	20	20	18	5	11
1	0	1	5	5	1	0	0	2	3	4
2	0	0	1	5	6	0	0	0	10	2
3	0	0	0	7	9	0	0	0	2	3
4	0	0	0	0	4	0	0	0	0	0
Totals	0	1	6	17	20	0	0	2	15	9

The authors suggested that this type of cardiomyopathy may be stress related rather than a direct cardiotoxic effect of EPTC, resulting from its anticholinesterase activity (as referenced in the Pathology Report, p. 17, for isoproterenol, cardiac ischemia, and acetycholine).

Conclusions:

EPTC caused dose-related decreases in body weight gains at the three highest dose levels. Increased AST levels were recorded in animals on 72 and 120 mg/kg/day, reportedly related to the cardiomyopathy evident in these groups. Depression in brain cholinesterase activity (86% of control) was also recorded in highest-dosed females (120 mg/kg). Although there was a trend for increased liver weight in the two higher dose groups, accompanied by hepatic color (females) and pattern (both sexes) changes at the HDT, histopathological examinations did not reveal any contributory treatment-related lesions.

The most significant clinical finding was treatment-related (chronic) myocarditis in all but the lowest dose level, the incidence and severity of which were dose-related and slightly greater in males.

TB Evaluation/Core:

CORE-MINIMUM DATA. Conservatively, the lowest observable effect level for the cardiopathy, and decreased weight and food consumption is 15 mg/kg. Hence, the NOEL may be set at 3 mg/kg.

As noted, the dose levels were changed 6 weeks into the study. The two lowest dose levels, 36 and 18, were lowered to

15 and 3, respectively. Effects seen at these doses at the end of the study are assigned to the lower level (e.g., 15, not 36), even though their occurrence may have been initiated in the first 6 weeks of the study at the higher dose level. This is the most conservative and advisable approach.

Among other minor deficiencies found in reviewing this study, the test report does not indicate how soon the animals were sacrificed after stopping administration of the test compound. This could be critical for determination of the cholinesterase levels.

APPENDIX A

MATERIALS AND METHODS

(pp 3 through 9 of FINAL
REPORT # HLA 6100-105,
September 14, 1984)

Eptam Science Reviews

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