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

006797

JUL 28 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPTC - Studies Submitted Under EPA Accession Nos. 404204 and 404423 in Response to Registration Standard
ID No. 476-2140

TB Project No.: 8-0432
Caswell No.: 435

FROM: Irving Mauer, Ph.D. 7-20-88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

THRU: Judith W. Hauswirth, Ph.D., Head 7/21/88
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

7/25/88

Registrant: Stauffer Chemical Company
Farmington, CT

Request

Review and evaluate the following submissions:

1. A Two-Generation Rat Reproduction Study With EPTAM® Technical. Addendum I: Final Report--Histopathology, Study T-10123, originally performed at Stauffer's Environmental Health Center, Farmington, CT. The current Final Report was issued November 2, 1987 (Accession No. 404204-08).

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2. One-Year Chronic Oral Toxicity Study with EPTAM Technical in Dogs, Study T-12723, performed at Stauffer's Environmental Health Center, Farmington, CT, report issued September 8, 1987 (Accession No. 404423-01).

3. A Teratology Study in Rabbits with EPTAM® Technical, Study T-12982, performed at Stauffer's Environmental Health Center, Farmington, CT, report issued August 12, 1987 (Accession No. 404423-02).

Background

Submission 1 (rat reproduction) is an addendum and Final Report of a study (T-10123) originally reported out October 8, 1982 (under Accession No. 249077). The current submission comprises data and clarifications absent in the 1982 report, which caused the study to be judged SUPPLEMENTARY (TB review February 1, 1985, Doc. No. 004277).

Submissions 2 and 3 are new studies submitted in response to data gaps identified in the EPTC Registration Standard (issued in 1983).

TB Conclusions

A summary of our assessment of these three studies is found on the page following (Detailed Reviews are attached).

Although we have upgraded the reproduction study to CORE-MINIMUM, we are concerned that apparently there are no NOELs for nonreproductive effects in the heart and kidneys of animals treated at the LDT, 40 ppm (≡ 2 mg/kg/day), namely, myocardial degeneration in males, and renal tubular degeneration with calcification in females. The registrant is requested to submit historical control data on these lesions from previous recent studies conducted in their laboratories and/or published information from other authorities.

Although we have judged the chronic dog study (submission 2) adequate for regulatory purposes (CORE-MINIMUM DATA), we request submission of:

1. Information on the previous aborted study begun at doses of 0, 5, 25, and 125 mg/kg/day (a completed report, if available), and

2. Background control data from other dog studies conducted by this lab (alluded to in text).

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With respect to the rabbit teratology study (submission 3), we judge the developmental aspects satisfactory, and have therefore graded the study CORE-MINIMUM. However, we are disturbed that EPTC caused maternal effects (inhibition of serum cholinesterase) at the LDT, 5 mg/kg/day, the lowest dose level so far found in toxicology studies submitted to date. The registrant is advised that further studies may be required to define a maternal NOEL.

Submission	Reported Results	Evaluation (CORE)
1. Rat Reproduction Addendum	Doses: 0, 40, 200, 1000 ppm (0, 2, 10, and 50 mg/kg/day) Parental NOEL < 40 ppm (LDT) Parental LOEL = 40 ppm (myocardial degeneration in males) Reproductive NOEL = 1000 ppm Developmental NOEL = 200 ppm Developmental LOEL = 1000 ppm (increased anomalous pups)	MINIMUM
2. Dog Chronic	Doses: 0, 1, 8, and 60 mg/kg/day NOEL = 1 mg/kg/day LOEL = 8 mg/kg/day (erythema; ChEI; thymic atrophy; muscle degeneration; pulmonary changes)	MINIMUM
3. Rabbit Teratology	Doses = 0, 5, 40, and 300 mg/kg/day Maternal NOEL < 5 mg/kg/day Maternal LOEL = 5 mg/kg/day (decreased serum ChE activity) Developmental NOEL = 40 mg/kg/day LEL = 300 mg/kg/day (reduced fetal weight)	MINIMUM

Attachments

Reviewed By: Irving Mauer, Ph.D. Geneticist 7-19-88
Section VI, Toxicology Branch/HED (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D. 7/21/88
Section VI, Toxiclolgy Branch/HED (TS-769C)

DATA EVALUATION REPORT

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I. SUMMARY

Study Type: Two-generation reproduction - rat

TB Project: 8-0432
Caswell No.: 435
MRID No.: N/A
Shaughnessy No.: 041401

Accession No.: 404204-08 (Original: 949077)

Chemical: EPTC

Synonyms: S-ethyl dipropylethiocarbamate, EPTAM®

Sponsor: Stauffer Chemical, Farmington, CT

Testing Facility: (Stauffer) Environmental Health Center, Farmington, CT

Title of Report: A Two-Generation Rat Reproduction Study With EPTAM® Technical (Originally issued October 8, 1982, EPA Accession No. 249007). Addendum 1: Final Report - Histopathology

Authors: G.M. Zwicker and J.L. Minor

Study Number: T-10123

Date of Issue: November 2, 1987 (ADD. I: Final Report)

TB Evaluation/Conclusions:

The original report (issued in 1982) was judged SUPPLEMENTARY because of certain deficiencies. In this Final Report, both the histopathology data submitted (absent in the original report), as well as the clarification of "anomalous pup/observations" are acceptable. In the absence of historical control data or other documentation, the NOEL for parental effects in nonreproductive organs (heart and kidney) cannot be determined. For reproductive organs, however, the NOEL is 1000 ppm (50 mg/kg/day), the HDT, and for developmental effects, 200 ppm (10 mg/kg/day).

Classification (Core-Grade):

Study T-10123 is upgraded to CORE-MINIMUM.

DETAILED REVIEW

II. MATERIALS AND METHODS

A. Test Material: EPTAM® Technical

Description: Amber liquid
Batch (Lot): EHC-0048-01 (CHK0601)
Purity (%): 98.6%
Solvent/carrier/diluent: Mixed with corn oil (1%) and incorporated into Purina Certified Rodent Chow #5002, for dietary administration.

B. Test Organism:

Species: Rat
Strain: Sprague-Dawley [Crl-CD®(SD)BR]
Age: 4 to 6 weeks
Weights--Males: (not available)
Females: (not available)
Source: Charles River, Wilmington, MA

C. Study Design (Protocol):

As given in the original report (1982), groups of 15 males and 30 females were chosen as the parental generation (P_0) and fed test article at levels of 0, 40, 200, and 1000 ppm from 40 days of age, mated during the fourth and sixth months (in a ratio of 1 male: 2 females) to produce the F_1a and F_1b litters of the first generation, respectively. F_1b animals were started on their respective group diets from weaning, then mated in the same fashion as the P_0 (at 3 and 5 months of age) to produce the F_2a and F_2b litters. All treated adults were killed at 7 months of age.

D. Procedure/Methods of Analysis:

Since the original report omitted histopathological examination, no specific methods were therein described. In this addendum and Final Report, the following tissues from control and high-dose (1000 ppm) P_0 and F_1 animals were examined:

Skeletal muscle
Sciatic nerve
Heart
Liver
Kidneys
Brain
Prostate
Bulbourethral gland
Urethra (males only, with prostate)
Ductus deferens/pampiniform plexus (spermatic cord)
Uterus
Cervix

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Seminal vesicles/ Coagulating gland	Vagina
Testes	Ovaries
Epididymides	Gross lesions (apparent at necropsy)

Only the heart and grossly observed masses from intermediate dosed P_0 and F_1 adults (40 and 200 ppm) were examined, but kidneys were also taken from F_1 adults fed at these levels.

The incidence and severity* of heart and kidney lesions were analyzed statistically by the Mantel-Haenszel modification of the Chi-Square Test (no bibliographic reference was given however) only when all dose groups were represented; otherwise, Fisher's Exact Test was used (e.g., when only control and high-dose groups were compared and/or when the incidence of no lesions was compared).

III. RESULTS: HISTOPATHOLOGY

Histological data were presented in this final report in four tabular summaries by sex and generation (Tables A, B, C, and D for P_0 males, F_1 males, P_0 females, and F_1 females, respectively), as well as by individual animal (Appendices A, B, C, and D for P_0 males, F_1 males, P_0 females, and F_1 females, respectively).

A. Parental (P_0) and First Generation (F_1) Males

No pathological changes different in type or incidence from respective controls were recorded in the reproductive organs (bulbourethral glands, epididymides,

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*Histopathological changes were graded by the authors as follows:

1. Minimal (very slight) - ranging from inconspicuous or barely noticeable to noticeable but so minor, small, or infrequent as to warrant no more than the least assignable degree of severity.

2. Mild (slight) - noticeable but not a prominent feature of the tissue.

3. Moderate - prominent but not a dominant feature of the tissue.

4. Moderately-Severe - dominant but not an overwhelming feature of the tissue.

5. Severe (marked) - overwhelming feature of the tissue.

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seminal vesicles, coagulating gland, testes, ductus deferens, prostate) of either high dose (.000 ppm) P_0 or F_1 males (Tables A and B; Appendices A and B, respectively).

The following nonreproductive tissues of these high-dose treated males were also found to be lesion-free in both generations, namely: brain, skeletal muscle, and sciatic (peripheral) nerve. The incidence of liver lesions was low in high-dose P_0 males (one each in control and high-dose groups), but a slight, nonsignificant increase was recorded in the high-dose F_1 (8 affected animals vs. 4 controls), accounted for mainly by minimal-to-slight biliary duct hyperplasia with fibrosis (5 high-dose vs. 1 control).

The incidence and severity of glomerulonephropathy were similar in both treated groups compared to their respective controls (in P_0: 13 minimal vs. 12 minimal-to-slight; in F_1: 14 minimal-to-slight vs. 15). On the other hand, a significant increase in degeneration of the renal tubular epithelium-with-calcification was recorded in high-dose P_0 males (5 vs. 0 in controls), which "was not replicated in P_1 [F_1] males," according to the authors. There was, however, one high-dose F_1 male (#352) described in Appendix B as showing this type of kidney lesion (graded +2), in addition to dilatation (+2) of the right renal pelvis, and glomerulonephropathy (and incidentally, myocardial degeneration). No F_1 male controls were so affected.

A high incidence of myocardial degeneration was recorded among high-dose males of both generations, statistically significant ($p < 0.01$) for F_1 animals (all 15 were affected), and more severe (grade 2) than in the P_0 (13 high dose vs. 8 controls). A statistically significant increase ($p < 0.05$) in this heart lesion was also recorded in 40 ppm F_1 males (11 vs. 5 controls), primarily (10/11) of minimal severity (grade 1). Among mid-dose (200 ppm) F_1 males, 8 of 15 were also affected, with minimal (5) to slight (3) degrees of severity. Increased incidences were also found in both the low-dose and mid-dose P_0 groups (12 and 13 males, respectively, vs. 8 controls).

This cardiac lesion was characterized by the authors as "multifocal hyaline or smooth deeply eosinophilic staining (H&E) myocardial muscle bundles often accompanied by loss of normal structure, proliferation of sarcolemmal cells and/or infiltration by histiocytes and mononuclear inflammatory cells."

A variety of other lesions in other organs and tissues were recorded in high-dose males but at incidences equal to or less than their respective controls.

Three subcutaneous tissue masses were found on necropsy: a thymoma (described as malignant) in a control P_0 male (#3), a mammary fibroadenoma in a low-dose F_1 male (#322), and focal abcessing inflammation of a preputial gland in a second low-dose F_1 animal (#329). The authors did not consider any of these treatment-related, since they were typical of spontaneous lesions seen in male rats of this age and strain. [N.B.: Since no background data on spontaneous masses in this strain of rat were provided, even by reference, there is no way of checking this.]

B. Parental (P_0) and First Generation (F_1) Females

The incidence and severity of lesions in the reproductive organs (cervix/vagina, ovary, uterus) of high-dose females of both generations were not different from their respective controls. An increase in stromal ovarian cysts among F_1 females (5/30 treated animals affected vs. one control) was not significant, and was considered biologically irrelevant by the authors.

No pathological lesions were found in the brains, skeletal muscles, or peripheral nerves of high-dose P_0 and F_1 females. Liver lesions (biliary duct hyperplasia with fibrosis, degenerative changes in hepatocytes, perivascular/portal inflammation, peliosis) were equally distributed between treated and control females.

Kidney changes were comparable in incidence and severity between high-dose P_0 females and their controls. Among F_1 females, however, grade 1/2 glomerulonephropathy was significantly increased over control (6/30) in both low-dose (20/30) and mid-dose (18/30) groups, but not in the high-dose group (10/30). The authors did not consider this alteration either toxicologically significant or treatment-related, because there was no dose-response, and severity was not different from that seen in controls. Degeneration of tubular epithelium with calcification, on the other hand, was increased ($p < 0.01$) in both severity and incidence among high-dose F_1 females (18 with grade 1 and 4 with grade 2, vs. 11 controls with grade 1). The authors considered this lesion to represent a biologically relevant and treatment-related change. [It is to be noted, further, that of 30 low-dose and 30 mid-dose F_1 kidneys examined, the incidence of this degenerative change was also increased, but apparently not reaching the level of significance, i.e., to 19 and 18, respectively.]

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The incidence and severity of myocardial degeneration was also increased significantly ($p < 0.01$) in high-dose females of both generations (P_0 = 26/30 and F_1 = 25/30 vs. 6 and 8 controls, respectively). At the low- and mid-doses the incidence and severity of this lesion was comparable to controls.

Lesions in other organs and tissues of high-dose P_0 and F_1 females were similar in incidence to controls.

Neoplasms were considered spontaneous in origin; the following subcutaneous tissue masses were catalogued at necropsy:

Among P_0 Females

- Focal abcessing inflammation - female #167 (200 ppm)
- Mammary gland adenocarcinoma - female #194 (1000 ppm), female #162 (200), and female #169 (200)

Among F_1 Females

- Mammary gland adenocarcinoma - female #425 (control)
- Mammary gland fibroadenoma - female #435 (40 ppm)
- (Undiagnosed--female #450, 40 ppm)

IV. AUTHORS' CONCLUSIONS

The authors concluded from this histopathological examination of the tissues of rats fed EPTAM-fortified diets for two generation that:

A. These additional data supported the previously drawn NOELs of 200 ppm (10 mg/kg/day) for systemic toxicity, and 1000 ppm (50 mg/kg/day) for reproductive effects (i.e., negative at the HDT).

B. Myocardial degeneration was a treatment-related effect at 1000 ppm in F_1 males and in females of both generations. This lesion was not visible at necropsy, and its ascertainment only under microscopy probably represents the early stages of the "cardiomyopathy" described in untreated Sprague-Dawley rats with increasing frequency and severity with age. However, the overall data suggested to the authors "facilitation" in the expression of this lesion.

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C. Renal tubular degeneration and mineralization was suggestive of a treatment-related effect in high-dose females only, possibly due to less efficient tubular reabsorption of phosphate ions.*

V. AUTHORS' DISCUSSION: "ANOMALOUS" PUPS

In addition to histopathological data on parental and first generation adults requested in its review of the original report, the Agency had requested that the registrant clarify the nature and incidence of "anomalous pups/ observations," as submitted in that report.

In the reply included in this ADDENDUM, the registrant asserted that the specific findings which earned a weanling pup such a label ("anomalous") were stated in the appropriate appendices (individual weanling data of the F_1a through F_2b), from which summary Tables 55 through 58 of the original report were constructed. Thus it is maintained that "the term [anomalous] was appropriately used and defined within the context of its usage."

However, the authors did agree here that discrepancies between summary tables and appendices (as noted by the reviewer of the original report) were inevitable because the summary values included anomalous observations, and not pups with anomalies. Thus a pup with multiple anomalies was counted as that multiple of "pups." Corrected summary tables (listing the numbers of anomalous pups) have been provided in this ADDENDUM, as follows:

Total Anomalous Pups

Mating	From Table	Dose Level (ppm) 0	40	200	1000
P_0 - 1st (F_1a)	55	5	4	1	10
P_0 - 2nd (F_1b)	56	7	6	5	14
F_1 - 1st (F_2a)	57	2	6	2	5
F_1 - 2nd (F_2b)	58	4	13	10	8
Total by Dose Group		18	29	18	37

*As discussed in the referenced paper: H. Nguyen, T. Woodard, and J. Connell: Intranephronic Calcinosis in Rats, An Ultrastructural Study. Amer. J. Pathol. 100:39-56, 1980.

In the original report these anomalies were categorized by the authors as either "malformations" or "growth-related," or were left unclassified." Similar breakdowns of the corrected number of "anomalous" pups in these subcategories were also provided, as follows:

Malformed Pups*

Mating	Dose Level (ppm)			
	[illegible]	40	200	1000
P_0 - 1st (F_1a)	0	2	0	3
P_0 - 2nd (F_1b)	2	1	1	2
F_1 - 1st (F_2a)	0	1	0	2
F_1 - 2nd (F_2b)	0	1	0	1
Total by Dose Group	[illegible]	5	1	8

Growth Related**

Mating	Dose Level (ppm)			
	[illegible]	40	200	1000
P_0 - 1st ((F_1a)	[illegible]	0	0	7
P_0 - 2nd ((F_1b)	[illegible]	3	1	8
F_1 - 1st ((F_2a)	[illegible]	0	0	0
F_1 - 2nd ((F_2b)	0	0	0	0
Total by Dose Group	[illegible]	3	1	15

*Anomalies included: Situs inversus; diaphragmatic hernia; eye missing, protruding or enlarged; corneal opacity; kidney ectopic or without papilla; ectopic testes; irregular cranial or cerebellar shape.

**Anomalies included: Irregular liver lobulations, vagina not open, and small stomach or heart.

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Not Classified***

Mating	Dose Level (ppm) 0	40	200	1000
P_0 - 1st (F_1a)	0	2	1	0
P_0 - 2nd (F_1b)	5	2	3	4
F_1 - 1st (F_2a)	2	5	2	3
F_1 - 2nd (F_2b)	4	12	10	7
Total by Dose Group	11	21	16	14

***Anomalies included: Discoloration in cecum, kidneys or thymus; colored exudate from the eye; irregularities in the border or lobes of the liver; ovarian cyst; small or undescended testes; dilated renal pelves; and ureters enlarged or convoluted.

According to the authors of the current Final Report:

> "The purpose of categorizing anomalous pups is to make sure that malformations haven't been masked by lesser effects. To seize upon the four fold increase in malformed pups over control pups at 1000 ppm as an indication of developmental toxicity does not survive closer scrutiny. Nearly half of these malformed pups had eye defects. The relation between eye defects and EPTAM ingestion was examined in the original report. These numbers are very small and a dose-response was never apparent, with the incidence at the mid dose less than that in the control of low dose" (ADDENDUM I, p. 13).

VI. TB EVALUATION

A. Histopathology

1. This reproduction study (T-10123) was initiated and completed before publication of the 1982 FIFRA Test Guidelines, which specified histopathology requirements for multigeneration studies. The tissues examined and reported in this ADDENDUM I (Final Report) conform to these Guidelines.

2. Although examination of all "gross lesions" in all parental animals is also required, the authors here selected only "masses" at intermediate dose levels, because findings at necropsy in high-dose animals

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(compared to controls) were considered to be incidental. The incidence and description of (non-target) masses provided by the current authors support their conclusion that these were spontaneous in origin, i.e., not treatment-related.

3. Myocardial degeneration was recorded with the following incidences and severity in parental animals:

	P_0				F_1			
	Dose (ppm)				Dose (ppm)			
Grade	0	40	200	1000	0	40	200	1000
				Males (15/Group)				
1	-	10	6	10	5	10	5	5
2	6	2	7	3	-	1	3	10
3	2	-	-	-	-	-	-	-
4/5	-	-	-	-	-	-	-	-
Total by Dose Group	8	12	13	13	5	11*	8	15**
				Females (30/Group)				
1	5	7	7	19	7	7	4	15
2	1	-	1	7	1	1	1	10
3	-	-	-	-	-	-	-	-
4/5	-	-	-	-	-	-	-	-
Total by Dose Group	6	7	11	26**	8	8	5	25**

Significance:
* $p < 0.05$ compared to controls.
** $p < 0.01$ compared to controls.

There was a definitive treatment-related effect ($p < 0.01$) in high-dose F_1 males and females as well as P_0 females, but also increased incidences at the LDT in P_0 and F_1 males (significant, $p < 0.05$ in F_1 males). The registrant's omission in providing background control data makes it difficult to determine whether such increases are related to EPTAM treatment at doses less than 1000 ppm, or are within the range of historical controls. Hence, in the absence of documentation to the contrary, we consider the lowest-effect-level for histological evidence of such an increase in cardiopathy to be

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less than the LDT, 40 ppm, in males (i.e., no NOEL), and to be 200 ppm (10 mg/kg/day) in females (i.e., NOEL = 40 ppm, or 2 mg/kg/day).*

4. While the differential reabsorption of phosphate in treated females vs. males may be a sensitizing trigger for degeneration of renal tubular epithelium with calcification (TEDC), we agree with the authors that its increase is treatment-related, but probably only at the high dose. Here again, historical data would have aided the interpretation of the nonsignificant increases at lesser doses in F_1 females, as tabulated below from the Final Report:

TEDC	P_0				F_1			
	Dose (ppm)				Dose (ppm)			
Grade	0	40	200	1000	0	40	200	1000
	Males (15/Group)							
1				5				1
2								
3								
4/5								
	Females (30/Group)							**
1	17			18	11	16	18	18
2	2					3		4
3								
4/5								

**p < 0.05 compared 0 ppm.

In summary, the lack of background data on spontaneous lesions and/or neoplasms in this Final Report (even by reference) makes it difficult to evaluate some of the histological findings in this study.

B. Incidence and definition of "Anomalous"

We accept the clarification of the terms used in the original report, as well as the corrections to

*At termination of a chronic rat study previously conducted by the registrant, significant increased incidence of chronic myocarditis was found at 125 mg/kg/day (approximately 2500 ppm), but not at 25 mg/kg/day (500 ppm). However, in rat studies by others with the same compound, evidence of such cardiac lesions was found earlier in treatment and at lower dosages, specifically, at about 3 mg/kg/day in 90-day feeding , chronic oral rat and reproduction studies.

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Tables 55 through 58 (inter alia) of that report. We note a consistent (though demonstrably not significant) increase in pups with anomalies at the high dose, whether tabulated by total numbers (doubled, i.e., 37 vs. 18), or as frank malformations (8 vs. 2), or related to growth inhibition (15 vs. 5). If this is a valid trend of EPTAM treatment, the NOEL for developmental effects appears to be 200 ppm (or 10 mg/kg/day). The provision of background or historical control data would have permitted a more definitive NOEL to be drawn.

ATTACHMENTS

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ATTACHMENTS

Tables A, B, C and D of the Final Report

Eptam Science Reviews

Page _____ is not included in this copy.

Pages _17_ through _39_ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ 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.

_____ The document is a duplicate of page(s) _____

_____ The document is not responsive to the request.

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.

eviewed By: Irving Mauer, Ph.D. Geneticist 7-13-88
ection VI, Toxicology Branch/HED (TS-769C) 006797
econdary Reviewer: Judith W. Hauswirth, Ph.D., Head
ection VI, Toxicology Branch/HED (TS-769C) Judith W. Hauswirth 7/14/88

DATA EVALUATION REPORT

tudy Type: Developmental Toxicity - Rabbit (83-3)

TB Project No.: 8-0432
Caswell No.: 435
MRID No.: N/A
Shaughnessy No.: 041401

ccession No.: 404423-02

hemical: EPTC

ynonyms: S-Ethyl Dipropylthiocarbamate; EPTAM®

ponsor: Stauffer Chemical
Farmington, CT

esting Facility: Stauffer Environmental Health Center (EHC), Farmington, CT

tle of Report: A Teratology Study in Rabbits with EPTAM [illegible] Final Report.

thor: Pamela [illegible]

udy Number: [illegible]

te of Issue

Evaluation [illegible]

Doses tested: 0, 5, 40, 300 mg/kg/day by gavage from day 7 to 19 of gestation.

Maternal [illegible]
Maternal [illegible]
Developmental [illegible]
Developmental [illegible]
A/D Ratio: [illegible]

assification (Core-[illegible]

Further studies [illegible]
holinesterase inhibition may [illegible]

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DETAILED REVIEW

MATERIALS AND METHODS

A. Test Material: EPTAM® Technical

Source: de Guigne Technical Center, Richmond, CA
Description: Clear yellow liquid
Batch (Lot): EHC-0751-13 (WRC-4921-4-25)
Purity (%): 97.6%
Solvent/carrier/diluent: Corn oil
Method of administration: Oral gavage

B. Test Organisms:

Species: Rabbit
Strain: New Zealand White (NZW)
Age: 5 months
Weights: Males (not available)
Females (not available)
Source: Hazleton Research Products, Denver, PA.

C. Study Design (Protocol):

A full protocol was provided as Appendix A of the final report (attached to this DER as Attachment I), which also included four (4) minor protocol deviations with no substantive effect on the conduct of the study, nor on the data generated.

Briefly, 66 New Zealand White rabbit females, mated to bucks of proven fertility, were assigned to four treatment groups and given 13 consecutive oral daily doses of the test material from day 7 through 19 of gestation, as follows:

Group	Dose*	No.
1	0 (corn oil)	16
2	5	16
3	40	16
4	300	18

*It was stated that these dose levels were "selected on the basis of preliminary studies," but no further information or data were supplied in this submission.

D. Observations/Procedures:

Animals were observed daily, weighed on gestation days [illegible], and their food consumptions

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recorded daily throughout gestation. Blood was drawn on gestation day 15 for determination of plasma and RBC cholinesterase activities. Animals found dead during the course of the study were examined to ascertain cause of death. All surviving does were killed on day 29 (by sodium pentobarbital injection, followed by axillary exsanguination), the intact reproductive tract (including placental, ovaries, uteri, and cervices) removed, weighed, and examined as follows: Ovaries, for the number of corpora lutea; uteri for the number and distribution of fetuses and resorptions; placentae, for unusual appearance. Thoracic and abdominal organs were examined for macroscopic changes; livers were weighed.

Fully developed fetuses (alive or dead) were weighed and examined for external malformations. Live fetuses were sacrificed (sodium pentobarbital), and examined for both soft-tissue anomalies (by modifications of Wilson's serial cross-section technique of Bouin fixed heads,[1/] and Staples' fresh dissection technique of the torso[2/]) and skeletal variations (by a modification of Kimmel and Trammel's procedure[3/]).

E. Data Analysis/Interpretation:

Enumeration data from treated groups of dams (such as clinical signs, etc.) were evaluated for statistical significance by Fisher's Exact Test, with Bonferroni's correction for multiple comparisons to a single control value, while litter data (fetal anomalies, corpora lutea/doe, etc.) were analyzed by the Mann-Whitney U Two-Sample Rank Test. Continuous data (such as maternal body weight, food consumption) were analyzed by the One-Way Analysis of Variance, and Dunnett's variation of Student's t-Test. The level of significance for all statistical treatments was $p \leq 0.05$.

In addition to simple enumeration, fetal anomalies were ranked (on a scale of 1 to 4) according to their biological relevance and/or severity ("value in predicting teratogenesis"

[1/] J.G. Wilson (1965) Methods for Administering Agents and Detecting Malformations in Experimental Animals. In: Teratology Principles and Techniques (Wilson and Warkany, eds.), Chicago.

[2/] R.E. Staples (1974) Detection of Visceral Alterations in Mammalian Fetuses. Teratology 9:A37-A38.

[3/] C.A. Kimmel and C. Trammel (1981) A Rapid Procedure for Routine Double Staining of Cartilage and Bone in Fetal and Adult Animals. Stain Tech. 56:271-273.

as stated by the author). The ranking system was defined as follows:

Rank 1 - Spontaneous variants that occur regularly, or artifacts (e.g., hematoma, incomplete ossification of the 5th sternebrae).

Rank 2 - Variants which are increased by known teratogens and for which there are no background data (e.g., convoluted ureters, and runting); these should not impair normal life processes.

Rank 3 - Anomalies which impair normal physiological processes, but are not lethal, including those which appear to be minor but which portend more extensive or serious lesions (e.g., umbilical hernia, which might precede defects in ventral wall closure, leading to protrusion of the g.i. tract at higher dosages).

Rank 4 - Frank malformations, which are life-threatening, and usually result in death (e.g., agnathia, diaphragmatic hernia).

QUALITY ASSURANCE MEASURES

A signed Quality Assurance Statement attesting to three dated audits of the data during the course of the study was included in the final report. Further, as testified by the Director of the Quality Assurance Unit, the Study Director and Director of Toxicology, this study was stated to be in compliance with FIFRA's GLPs, as specified in 40 CFR Part 160.

RESULTS

The results of this study were presented in 14 summary tables, derived from 10 appendices of individual animal data.

A. Dose Solution Analyses (Report Table 1):

Dosing solutions were prepared by the weight/volume method, so that the expected concentrations for the three dose levels (5, 40, 300 mg/kg/day) were 10, 80, and 600 mg/mL. Analysis of the solutions revealed measured concentrations of test substances within 6 percent of target (as given in Table 1A of the Report: 10.3 ± 0.2, 75.8 ± 1.3, and 568 ± 21 mg/mL for low, mid and high concentrations, respectively). Test material was not detected in the control solution (corn oil). The author stated there was no loss of test substance after 28 days storage. (This assertion was based on analyses 7, 14, and 28 days after preparation of a made-up solution containing 31.06 mg EPTAM/mL corn oil;

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in fact, Report Table 1B recorded <u>increases</u> of 1.5, 1.2 and 1.8 percent, respectively.]

B. <u>Fertility and Mortality (Report Table 2, Appendix B)</u>:

Three does died during the course of the study: One control, on gestation day 23 (with red lungs and white spots on the heart), and two at 300 mg/kg day, on day 20 (with bloody stool, hairballs in the stomach, clots on the uteri, "colored" foci on the lungs, and hematuria). The two high dose deaths were attributed by the author to EPTAM treatment. Fertility indices in treated groups were comparable to controls. All pregnant survivors had live fetuses, and no does aborted (Report Table 2, appended to this DER in Attachment II).

C. <u>Clinical Findings (Table 3A; Appendix B)</u>:

Cholinergic effects were observed only at the HDT (300 mg/kg), evidenced by an increase in loose stool (9/18 does, a significant change, compared to none affected in the control), hematuria (4/18), salivation (1/18), stained lip or nose (2/18) and wet coat (3/18). Loss of appetite and/or anorexia was also recorded in high dose females more often (12/18 does) and earlier (21.2 days) than in controls (5/16 does, 26.8 days); this effect was also recorded at the mid dose (7/16 does) and at the low dose (8/16 does).

Circulating cholinesterase activities were significantly reduced in high-dose does on gestation day 15, mean plasma value decreasing to 44 percent of controls (310 vs. 709 IU/L), and mean RBC value to 30 percent of controls (536 vs. 1767 IU/pRBC), both considered biologically significant by the author (Table 3B, appended to this DER in Attachment II). Although serum and erythrocyte cholinesterase activities were also statistically reduced at lower doses (at 40 mg/kg/day--both serum at 584 IU/L and RBC at 1518 IU/pRBC; at the 5 mg/kg/day dose, only serum was reduced, at 639 IU/L), the author did not consider these decreases biologically significant, because of the small relative reductions (10 to 17 percent).

D. <u>Body Weights (Tables 4, 5; Appendices C, D)</u>:

Mean body weights and mean corrected body weight (i.e., less the reproductive tract) were recorded to be comparable in all groups, i.e., the author considered EPTAM to have had no effect on these values (Table 4; Appendix C). However, when body weight <u>changes</u> were recorded for the seven sampling periods, a statistically

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significant reduction in mean weight of high-dose does (51 grams) was registered during gestation days 13 and 19 (Table 5), which was mainly accounted for by 6 does in this group. On the other hand, no other body weight changes were found during the remainder of the treatment period, or the posttreatment period, or during the total pregnancy. No significant weight changes were reported in low or mid-dose females at any time.

E. Food Consumption (Table 6; Appendix E):

Mean daily food consumption was recorded as statistically reduced in high-dose females on gestation days 13, 14, 15, 17, 19, and 20 [The authors also included day 10, but no decreased food intake is evident on that day from inspection of Appendix E.] Extended periods of little or no food consumption were recorded for five females of that test group, namely Nos. 361, 369, 370, 375, and 377. [Although not specifically correlated by the authors, these were the same females which lost weight during gestation period day 13 to 19.]

F. Necropsy (Table 7; Appendix F):

Other than the findings in the three pregnant does that died during the course of the study, no gross pathological changes/findings in EPTAM-treated animals were considered "remarkable" by the author. In gravid animals terminated at the end of the study, instances of lungs with colored foci, colored/pale livers and ovarian cysts were found equally among all groups.

The cause of death of the three does found dead was not definitively established. Control female 315 had abruptly stopped eating on gestation day 23, and was found dead the same day. Necropsy findings included "red" discharge from the vagina, "red fluid" in the abdominal cavity, foci of "white discoloration" of the heart, clear fluid in the pericardium, and "red" discoloration of all lobes of the lungs. (It was suggested that death may have been due to either "toxemia" or "cardiovascular failure.")

The two high-dose does (Nos. 369 and 370) were both found dead on gestation day 20, and displayed gross changes which suggested "bleeding problems" to the author. Female 369 had an oesophageal as well as vaginal blood clot, red foci on lungs and liver, and dark clots in both uterine horns; female 370 had a red-stained mouth, red vaginal discharge, red foci on lungs, black foci in the mucosal lining of the stomach, and "dark" uterine clots. Hence, the probable cause of death for these two

does was ascribed to "bleeding within the reproductive tract." Both females also had gastric hairballs, which may have contributed to their weakened condition. [It was not stated here that the bleeding was caused by EPTAM administration, although it is so stated in the Report conclusions.]

G. Organ Weights (Tables 8 and 9; Appendices G and H):

No differences in mean absolute or mean relative weights of liver, ovaries, placenta, reproductive tract, or uterus were recorded between EPTAM-treated groups and control.

H. Intrauterine Data (Tables 10; Appendices I and J):

A statistically significant reduction was recorded in mean body weight of live fetuses from the 300 mg/kg/day treated group of does, 35.3 g vs. a mean control value of 40.3 g (Table 10, appended to this DER in Attachment II). Although percent postimplantation losses at the mid and high doses were more than double the control value (respectively, 7.2 and 6.2 vs. 2.8) and the mean percent malformed live fetuses as well as affected implants at the high dose were approximately tripled (respectively, 7.4 and 13.1 vs. 2.4 and 5.0), none of these differences were statistically significant. Furthermore, these values calculated for this study were within the range of historical control values accumulated by this laboratory in five previous studies conducted between 1984 and 1986 as follows:

Postimplantation losses . .	2 to 13%
Malformed live fetuses . . .	1.4 to 18.1%
Affected implants	4.6 to 25.4%

(Extracted from Report Appendix L, "Intrauterine Data," appended to this DER in Attachment II.)

The increase in fetal malformations and the embryofetal toxicity at 300 mg/kg/day was attributed to maternal cholinergic toxicity (loss of appetite, loose stool, hematuria, etc.) induced at this level in six of the eight does with affected implants. In four does, prolonged periods of anorexia led to body weight losses during the treatment period, spanning gestation days 7 to 19. Severely malformed fetuses were described in two of these does, Nos. 361 and 377, in whom body weight loss during this period (respectively, 450 and 311 g) even exceeded that reported for the other two high-dose does (Nos. 360 and 370) found dead on day 20 (respectively, 32 and 232 g). In addition to five late

resorptions, doe 361 had two runts, one of which also had arthrogryposis (overflexion of limbs) as well as a cleft palate. Doe 377 had four malformed fetuses, three with ocular malformations (convoluted retinal and/or blood-filled eye sockets and vitreous humors), and one had severe skeletal anomalies of ribs and vertebrae (fusion, "crossed," hemi-thoracic, etc.) (Appendix K). Mean fetal weights recorded in these two litters were markedly reduced (to 24.1 and 28.6 g), compared to the overall mean of this high-dose group (35.3 g).

I. Fetal Anomalies (Tables 11 to 14; Appendix K):

Despite the findings in individual litters, no overall statistically significant increases over control were recorded for external (Table 11), soft-tissue (Table 12), or skeletal anomalies (Table 13) in any EPTAM-treated group. Neither were mean rankings of teratogenic potential altered by EPTAM (Table 14).

Malformations graded as Rank 3 and 4 were found in three fetuses from two control litters, in six fetuses from four low-dose litters, in three fetuses from three mid-dose litters, and in nine fetuses from six high-dose litters. The types of malformation observed by the author in this study among the control, low and mid-dose EPTAM groups, as well as those in three fetuses in three litters at 300 mg/kg, have been observed among controls in five previous studies conducted by this laboratory during the prior 2 years (Appendix L, pages 198-219, of the current submission).

The types not heretofore observed by this lab were found only in high-dose litters (361, 375, 377), and included two fetuses with extremely convoluted retinas (retinal folds) and blood-filled eye sockets and/or vitreous bodies, one fetus with forefoot overflexion (arthrogryposis) and cleft palate, and one fetus with malformed centra of the thoracic vertebrae. The author cited other authorities[1] who have reported incidences per 10,000 control rabbits of the same strain of 1.4 for intraocular hemorrhage, 31.5 for arthrogryposis of forelimb, 12.3 for cleft palate, and 1.5 for thoracic vertebra irregular. Hence, the author considered that these "unique" anomalies found in this study were considered secondary to the marked maternal toxicity induced by 300 mg EPTAM/kg in does 361, 375, and 377.

[1]D.C. Woo and R.M. Hoar (1982) Reproductive Performance and Spontaneous Malformations in Control New Zealand White Rabbits: A Joint Study with MARTA. Teratology, 25:32A.

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[It is to be noted that convoluted retinas (retinal folds) without hemorrhage (Ranked "3") were reported to occur only in EPTAM-treated litters in this study, as follows: Four fetuses from two litters at 5 mg/kg, a single fetus at 40 mg/kg, and three fetuses from three litters at 300 mg/kg (Report Table 12). However, such retinal anomalies have been observed in the controls of three of five previous studies from this lab, equally distributed among 12 litters (of 39 litters, APPENDIX L).

Further, although only ranked as "2," absent gallbladder was reported in two fetuses from two high-dose litters (Nos. 363 and 373). "Reduced gallbladder," on the other hand, was equally distributed between control (one fetus) and EPTAM-treated litters (three fetuses in two litters at 5 mg/kg, one fetus at 40 mg/kg, and two fetuses in litter No. 363 of the 300 mg/kg group) (Table 12). As provided in APPENDIX L, "reduced gallbladder" appears to be a common finding in historical controls from this lab, although "absent gallbladder" has been recorded only once before (appended to this DER as ATTACHMENT II).]

REPORT CONCLUSIONS:

The author concluded that the oral administration of EPTAM to pregnant New Zealand White rabbits at doses of 0, 5, 40, and 300 mg/kg from gestation day 7 through 19:

1. Produced maternal toxicity at the highest dose (only), which included death, weight loss, reduced food consumption, cholinergic effects, bleeding problems, and reduced circulating cholinesterase activity (both serum and erythrocyte).

2. Induced embryofetal toxicity, secondary to the maternal toxicity at 300 mg/kg/day, which consisted of reduced mean fetal weight.

3. Was not teratogenic (i.e., for major malformations) at doses up to 300 mg/kg/day, and induced no statistically significant increases in external, soft-tissue or skeletal anomalies or variations.

Therefore, the author considered the no-effect level for both maternal toxicity and embryofetal toxicity to be 40 mg/kg/day EPTAM Technical.

TB EVALUATION:

The study appears to have been conducted with adequate procedures and controls, and with group sizes ensuring valid statistical treatment. The major criticism of this study concerns the dose sequence selected. Although stated to have been selected from "preliminary studies," these were not included in the Final Report nor any other information provided on the conduct or results of these dose-selection practices. This leaves one to wonder why an eightfold interval in the dose schedule was chosen; a tighter schedule might have better defined the NOEL.

Further, we do not agree with the author's choice of 40 mg/kg/day as the NOEL for maternal toxicity. A statistically significant reduction in serum cholinesterase activity was recorded at the LDT, 5 mg/kg/day, while significant decreases in both serum and erythrocyte activities occurred at 40/mg/kg/day, i.e., there appeared to have been no NOEL for reduced cholinesterase activity in this study. The Agency is concerned that such a short course of treatment with EPTC results in inhibition of cholinesterase activity at what appears to be the lowest dose so far recorded in toxicology studies. Further studies may be required in order to more accurately define the NOEL for this effect.

By contrast, observable developmental effects were apparently recorded only at the HDT, 300 mg/kg/day, represented only by decreased mean fetal weight, but not major malformations or increased external, soft-tissue or skeletal anomalies or variations. Although not found in concurrent controls, low incidences of convoluted retinas (retinal folds) and absent gallbladder as described in Eptam-treated litters in this study have been found in controls of this laboratory's previous studies and/or reported in published accounts of spontaneous malformations in this strain of rabbits. Hence, we do not consider these anomalies to be related to Eptam treatment.

TB CONCLUSIONS: CORE-MINIMUM DATA

Maternal NOEL < 5 mg/kg/day
Maternal LOEL = 5 mg/kg/day (serum ChEI)
Developmental NOEL = 40 mg/kg/day
Developmental LOEL = 300 mg/kg/day (reduced mean fetal weight)
A/D Ratio cannot be specifically determined, but probably < 0.125.

Attachments

ATTACHMENT I

PROTOCOL

T-12982: A TERATOLOGY STUDY IN RABBITS WITH EPTAM TECHNICAL

APPENDIX A

STUDY PROTOCOL AND APPROVED PROTOCOL DEVIATIONS

Eptam Science Reviews

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Reviewed By: Irving Mauer, Ph.D., Geneticist 6/29/88
Section VI, Toxicology Branch/HED (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head Judith W. Hauswirth 6/30/88
Section VI, Toxicology Branch/HED (TS-769C)

006797

DATA EVALUATION REPORT

I. SUMMARY

Study Type: Chronic Oral (Capsule)--Dog

TB Project: 8-0432

Caswell No.: 435

Accession No.: 404423-01

MRID No.: N/A

Chemical: EPTC

Shaugh. No.: 041401

Synonyms: S-Ethyl dipropyl thiocarbamate; EPTAM®

Sponsor: Stauffer Chemical Company, Farmington, CT

Testing Facility: (Stauffer) Environmental Health Center (EHC), Farmington. CT

Title of Report: One-Year Chronic Oral Toxicity Study with EPTAM® Technical in Dogs

Authors: G.L. Sprague and D.O.N. Taylor

Study Number: T-12723

Date of Issue: September 8, 1987

TB Evaluation/Conclusions:

This study was conducted in accordance with the FIFRA Test Guidelines for Chronic Studies in Nonrodents (83-1), and the data generated are judged to be valid. The lack of providing historic data on control animals in previous studies from this lab, however, prevents an interpretation of certain findings at the mid-dose level, 3 mg/kg.

Classification (Core-Grade):

CORE-MINIMUM.

Oral doses tested: 0, 1, 3, and 60 mg/kg/day, by capsule.

NOEL = 1 mg/kg/day

LOEL = 3 mg/kg/day: erythema of ears (males), reduced serum ChE (both sexes), thymic atrophy (males), increased pulmonary changes (females).

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II. DETAILED REVIEW

A. Test Material: EPTAM Technical

Source: (Stauffer) de Guigne Technical Center, Richmond, CA
Description: Liquid
Batch (Lot): WRC 4921-4-25 (EHC #0751-13)
Purity (%): 97.6%
Solvent/carrier/diluent: Dispensed neat in No. 000 gelatin capsules for oral administration.

B. Test Organisms:

Species: Dog
Strain: Beagle
Age: 7 months
Weights--Males: 8.9 to 11.2 kg
Females: 6.9 to 8.9 kg
Source: Marshall Research, North Rose, NY

C. Study Design (Protocol):

A protocol (dated January 8, 1986) was included in the Final Report (as Appendix A), attesting that FIFRA Test Guideline No. 83-1 was to be followed. Several addenda and deviations were also incorporated here. The addenda extended descriptions of some procedures to be performed (by technicians), for example: I. Safety Procedures (in handling EPTAM); II. Neurologic Examination of Treated Animals (i.e., physical tests in-life); III. EKG Procedures and Techniques (details). None of the protocol deviations (isolated, missed collections of samples; one dosing error; personnel changes) appeared to have any impact on the conduct of the study or on the results generated.

D. Procedure/Methods of Analysis:

Capsules of the test material were administered daily (7 days/week) to groups of beagle males and females at levels of 0 (empty gelatin caps only, 5/sex), 1 (4/sex), 8 (4/sex), and 60 mg/kg (5/sex). Dose selection was based upon a previous chronic study at dose levels of 0, 5, 25, and 125 mg/kg/day that had to be aborted because of severe toxicity and mortality in the high-dose group. (More information is requested on this aborted study.)

Animals were observed twice daily, body weights recorded weekly, and food consumption measured weekly during the first 3 months; monthly thereafter. Complete physicals were performed on all dogs prior to beginning treatment and quarterly thereafter. Ophthalmologic examinations were performed twice, before treatment and at terminus.

Neurologic examinations by a board-certified veterinary neurologist were conducted during quarantine (before treatment), and at 3, 6, and 12 months. The method used (DeLahunta 1983)* assessed functional integrity of the peripheral and central nervous systems, the details of which are found in Appendix D o the Report.

Electrocardiograms, as described in Report Appendix E, were also recorded for all dogs during the pretest quarantine period, as well as after 3, 6, and 12 months of treatment. Bipolar, augmented unipolar and chest leads were monitored.

The schedules for the routine analysis of biochemical parameters from blood, urine, and feces during the study were as follows:

Hematology (Quarantine, 3, 6, 12 months):

Hematocrit
Hemoglobin
Erythrocyte count
Total leukocyte count
Differential leukocyte count
Platelet count
Reticulocyte count (determined when hematocrit < 32.0 percent)
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration

Clinical Chemistry (Quarantine, 1 week, 1 month, 3 months, 6 months, and/or 12 months):

Prothrombin time
Activated partial thromboplastin time
Factor VII analysis

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*DeLahunta, A. (1983) Veterinary Neuropathology and Clinical Neurology, 2nd Ed., pp. 365-387, Phila.: W.B. Saunders.

(Quarantine, 3, 6, 12 months):

Serum aspartate aminotransferase (Serum glutamic oxaloacetic transaminase)
Serum alanine aminotransferase (Serum glutamic pyruvic transaminase)
Serum alkaline phosphatase
Creatine phosphokinase and isozyme distribution
Gamma glutamyl transpeptidase
Blood urea nitrogen
Cholesterol
Triglycerides
Total bilirubin
Direct bilirubin (when total bilirubin > 0.4 mg/dl)
Total protein
Albumin
Globulin
Albumin/globulin ratio
Glucose
Calcium
Inorganic phosphate
Sodium
Potassium
Chloride

(Quarantine, 1 week, 1, 3, 6, 12 months):

Serum cholinesterase
Red blood cell cholinesterase

(12 months):

Brain cholinesterase

Urinalysis (Quarantine, 3, 6, 12 months):

Volume
Color and appearance
Specific gravity
pH
Protein
Glucose
Ketone
Bilirubin
Urobilinogen
Occult blood
Microscopic examination

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Occult blood in feces (Quarantine and 12 months)

In addition, blood was also collected at 1 week and 1 month for "selected" coagulation (PT and APTT) and cholinesterase assays.

Necropsy:

At the termination of the study, dogs were sacrificed after an overnight fast, by injection with sodium pentobarbital, and exsanguination. The following organs and tissues were fixed in 10% neutral formalin (asterisked organs were also weighed):

Inguinal skin and mammary gland
Deep digital flexor muscle
Femur
Nasal passage
Trachea
Lung (right middle and left caudal lobes)
Heart*
Thoracic aorta
Thymus
Spleen
Medial retropharyngeal lymph node
Bone marrow (sternum)
Mandibular salivary gland
Oesophagus
Stomach (body, antrum)
Duodenum
Jejunum
Ileum
Caecum
Colon
Rectum
Liver* (right and left lateral lobes)
Gallbladder
Pancreas
Kidney*
Urinary bladder
Prostate
Uterus (horns, body, and cervix)
Vagina
Brain*
Spinal cord (cervical, thoracic, sacral, lumbar)
Peripheral nerves (sciatic, tibial, caudal, cutaneous, sural)
Gross lesions

The following organs were fixed in 2.5% buffered glutaraldehyde:

Ovaries*
Testes* and epididymides
Pituitary*
Thyroids* + parathyroids
Adrenals*
Eyes

Tissues were examined microscopically, and lesions graded for degree of severity according to the following scheme:

Grade	Definition
1:	Minimal (very slight). Histopathologic changes ranging from inconspicuous or barely noticeable to noticeable, but so small or infrequent as to warrant no more than the least assignable degree of severity.
2:	Mild (slight). Histopathologic changes that were noticeable, but not prominent features of the tissue.
3:	Moderate. Histopathologic changes that were prominent but not dominant features of the tissue.
4:	Severe (moderate). Histopathologic changes that were dominant but not overwhelming features of the tissue.
5:	Severe (marked). Histopathologic changes that were an overwhelming feature of the tissue.

Statistics:

Body weight and clinical chemistry values were analyzed by a one-way ANOVA, and Dunnett's version of the t-test. Selected clinical observations were analyzed for significance by Fisher's Exact Test. The Mandel-Haenszel modification of chi-square was used for selected histopathological observations, in order to ascertain any indication of the relation of dose to severity of lesion ("grade"). Severity grade distribution in controls was employed as the basis for any treatment-related effects at each EPTAM dose level. The significance level was set at minimally 0.05, but test results which reached the 0.01 level were duly noted.

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Quality Assurance Measures:

Testimonials were included to the effect that: 1) The study was conducted in compliance with EPA's GLP Standards (40 CFR 160); and 2) the reported results were periodically audited by the Quality Assurance Unit.

III. RESULTS

A. Test Material Analysis (Appendix B, page 125 ff of Final Report)

Test material was dispensed volumetrically (neat) into gelatin capsules, from approximately 0.01 mL for the 1 mg/kg dosage, 0.08 mL for the 8 mg/kg dosage, and 0.50 mL for the 60 mg/kg dosage. Capsules were prepared weekly, because the test material is stable at room temperature for only 2 weeks. An error in dosing was discovered for the week beginning July 16, 1986: pipette calibration for the low-dose was 75 percent greater than expected; therefore, the low-dose animals actually received 1.75 mg/kg/day, instead of 1.0 mg/kg/day.

B. Clinical Observations (Report Table 3 and Appendix C):

One high-dose male had to be sacrificed in poor condition on study day 84, following a 19-day aggravated course of effects including one or more of the following (all considered treatment-related): anorexia (reduced food consumption) and progressive weight loss; stiff gait/weakness, ataxia/fasciculations of both hindlegs; reduced activity; and emaciation. All other animals survived the 1-year treatment period.

Other clinical signs the authors considered treatment-related included erythema of the ears, "dull coat," and unilateral lameness in rear legs (see tabulation on page following). Except for male No. 27 (terminated in extremis on study day 84), scheduled (3, 6, and 12 months) neurologic examinations (for general behavior; gait and posture; postural reactions; spinal reflexes; "sensation," muscle tone; and cranial nerve function) revealed no differences between treated and control groups for either males (Report Table 3) or females (Report Table 5 and 6, respectively; Appendix D). An unscheduled neurologic examination (on day 80) of two control males (Dog Nos. 1 and 2) and three high-dose male (Dog Nos. 27, 28, and 29) males were essentially normal for all dogs except male No. 27, which was

unable to support its weight on the rear legs, had abnormal postural reactions, reduced sensate reactions, and moderate muscular atrophy in both hind legs (Report Table 5).

Selected* Clinical Signs in Dogs
Given EPTAM for 1 Year**

Sign	Dose (mg/kg):	Males 0	1	8	60	Females 0	1	8	60
~~Anoxoria~~ (ANOREXIA)		-	-	-	1 1/	-	-	-	-
Ataxia		-	-	-	1 1/	-	-	-	-
Dull coat		-	-	-	2	-	-	-	-
Dull coat (general)		-	-	-	1 1/	-	-	1	1
Emaciation		-	-	-	1 1/	-	-		-
Erythema (ears)		-	-	3	3	3	-	3	2
Fasciculations		-	-	-	1 1/	-	-	-	-
"Lameness"		-	-	1	1	-	-	-	-
Reduced activity		-	-	-	1 1/	-	-	-	-
Stiff gait (hind legs)		-	-	-	1 1/	-	-	-	2
Weakness (forelegs)		-	-	-	1 1/	-	-	-	-

* Acknowledged as treatment-related by authors (or, if in quotes, possibly indicative).
** Extracted from Table 3 and Appendix C.
1/ Same animal (male No. 27).

C. Body Weight/Food Consumption (Report Tables 6 and 7; and Appendix F)

Weekly mean body weights of control and treatment groups were found to be comparable throughout the study for both males and females. However, body weight gain in high-dose males over the entire 52-week study was 0.1 kg, significantly reduced compared to the 1.7 kg gain for controls. Body weight gains for low-dose and mid-dose males, or all female groups, were similar to controls.

No differences in food consumption were recorded between controls and EPTAM-treated dogs in any dose group.

D. Electrocardiograms (Report Tables 8 and 9; Appendix E)

EKG tracings in high-dose groups taken at 3, 6, and 12 months were considered generally similar to their respective pretest (quarantine) values, and not indicative of any "significant" treatment effect.

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QRS intervals for two high-dose males were increased: 0.08 second for dog 29 at 6 months, and 0.09 second for dog 28 at termination, compared to the greatest pretest control value of 0.7 second. However, two controls (Nos. 3 and 4) exhibited values of 0.08 second at termination. Male 30 (60 mg/kg) exhibited both P and T wave amplitudes outside pretest values at 12 months: 0.35 vs. 0.30 mv, which the authors acknowledged as "potentially" treatment-related.

EKG values for high-dose females were either within pretest ranges or considered not unusual for beagles at the ages and weights of sampling.

E. Clinical Laboratory Values (Tables 10 and 11; Appendix G)

Slight but significantly reduced values were found at termination among high-dose males for hemoglobin (15.1 vs. control 16.9 G/DL) and hematocrit (40.9% vs. control 45.9%), both of which the authors considered treatment-related. No other hematological parameter was stated to have been affected.

[N.B.: Table 10, however, records a significant reduction in RBCs in high-dose females at termination, 6.15 x 10^6/cu mm compared to 7.22 x 10^6 for controls.]

Acknowledged treatment-related clinical chemistry changes were recorded for alkaline phosphatase (AlkP), serum cholinesterase (SChE), blood urea nitrogen (BUN), and serum albumen (Alb), more severe in affected males (Table 11). Statistically significant increases in AlkP were measured only in high-dose males after 6 and 12 months' treatment (respectively, 73 and 91 IU/L, vs. control values of 38 and 32 IU/L). Two high-dose females with values > 100 IU/L at 6 and 12 months contributed to that group's increased mean activities (78 and 90 IU/L, vs. control values of 51 and 35 IU/L), which apparently did not quite reach the level of significance.

[N.B.: It should be also be noted that increased AlkP activities were also recorded after 3 months' treatment for both high-dose males, 69 vs. 45 IU/L, and females, 74 vs. 52 IU/L (Table 11).]

While other measures of liver function in EPTAM-treated groups (SGOT, SGPT, gamma-GT, CP) were found to be comparable to control values, serum cholesterol was acknowledged to have increased in high dose

males [presumably] at all sampling times (by 35 to 45 percent, which nonsignificant changes).

[N.B.: Not mentioned in text was a 30 to 35 percent increase in serum cholesterol (again not reaching the level of significance) in high-dose females at 6 and 12 months (Table 11). Also not mentioned were consistent decreases in blood urea nitrogen at all sampling times in both high-dose males and females, reaching significance at term in males (12 mg/dL vs. the control value of 16).]

Serum cholinesterase activity was reduced significantly at all sampling intervals in high-dose males, (consistently by some 30 to 40%), but only at 1 week and at termination in high-dose females (as shown in the tabulation on the following page). Neither RBC nor brain cholinesterase was apparently affected by EPTAM treatment.

[N.B.: Although apparently not reaching the 5-percent level of significance, a consistent inhibition of serum-ChE activity (10 to 12%) was recorded in mid-dose (8 mg/kg) males at all study intervals except 1 week, and in mid-dose females (ranging from 10 to 26%) at all intervals, including a statistically significant 18 percent reduction at 1 week (Table 11).]

Increased serum globulin levels (by approximately 30%) were reported in high-dose males at 3, 6, and 12 months, with a corresponding small decrease (10%) in serum albumen in this group at 6 months. Increased total bilirubin was recorded in high-dose males at term (0.2 mg/dL vs. 0.1 in controls).

[N.B.: Other isolated and slight (but statistically significant) changes not mentioned in text by the authors, but recorded in the Report's tabulations, were unrelated to dose: Increased serum sodium in mid-dose females at 6 months only (158 MEQ/L vs. control 143); decreased serum calcium in high-dose males at 3 months (10.2 vs. 10.9 mg/dL) and at 6 months (10.3 vs. 10.9 mg/dL). No changes in prothrombin time (PT) or activated partial thromboplastin time (APTT) were recorded.]

F. Gross Pathology (Tables 12 and 13; Appendix H)

Other than muscle atrophy in the one high-dose male (#27) which had to be terminated early, the type and incidence of necropsy observations in surviving

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Cholinesterase Activities in Dogs
Administered EPTAM Technical for 1 Year*

Component	Males					Females				
	Dose: (mg/kg/day)	0	1	8	60	Dose: (mg/kg/day	0	1	8	60
	INT					INT				
Serum (IU/L)	01	1926	2172	1945	1116[a]	01	2215	2284	1817[a]	1695[a]
	02	2029	2175	1814	1251[a]	02	2271	2200	1834	1865
	03	2113	2390	1909	1350[a]	03	2470	2600	2225	2052
	06	2016	2246	1779	1361[a]	06	2519	2400	2099	1855
	12	2149	2307	1887	1449[a]	12	2703	2477	1995	2008[a]
Erythrocyte (IU/P RBC)	01	5120	5230	5190	4984	01	5468	5230	5375	4888
	02	5448	5525	5250	5121	02	5208	5340	5680	5132
	03	4964	5095	5175	4785	03	5024	5030	5700	4652
	06	1728	1520	1575	1495	06	1336	1235	1580	1128
	12	1800	1360	1460	1365	12	1352	1295	1730	1068
Brain (IU/g tissue)	12 (term)	1.60	1.65	1.60	1.69	12 (term)	1.63	1.68	1.77	1.89

*Extracted from Final Report Table 11, pp. 66-72.
[a]Significantly different ($p < 0.05$) from the control (0 mg/kg) value.
INT, sampling interval (01 = 1 week, 02 = 1 month, 03 = 3 months, 06 = 6 months, 12 = 12 months).

EPTAM-treated animals were similar to concurrent controls, as well as considered comparable to historic controls from this laboratory. [No background data, however, were provided in this Report.]

G. Organ Weight (Table 14; Appendix H)

Livers of high-dose females weighed significantly more than controls (by 20%, 304.9 g vs. 238.4 g). While absolute liver weights of high-dose males were not significantly greater (mean 300.9 g vs. a control value of 250.2 g), relative weights were reported as significantly increased: 3.8, 3.0, 2.9, and 3.0 percent compared to the control values of 1.8, 2.2, 2.1, 2.6, and 2.5 percent. The authors considered this weight increase as treatment-related. No changes in either absolute or relative weight were recorded in any other organ (brain, heart, kidney, prostate, testes, ovaries, thyroid, adrenals, pituitary--Table 14).

H. Histopathology (Tables 15 and 16; Appendix I)

Wallerian (fatty) degeneration in the spinal cords and various peripheral nerves (sciatic, sacral, and tibial)* was recorded as a prominent neuropathological lesion in high-dose dogs of both sexes, but more severe (Grades 2 to 4) and more extensive in the four of five affected males (three survivors to term plus dog 27, sacrificed on study day 84). The four of five affected high-dose females were reported to exhibit minimal (Grade 1) to mild (Grade 2) degrees of such degeneration. A mild lesion was also observed in the cervical cord of one 8 mg/kg female, but a comparable change was found at the thoracic level of the spinal cord of one control. No neuropathological changes were described for the low-dose group.

Male 27 (60 mg/kg, terminated at day 84) had widespread and very severe degenerative lesions in peripheral nerves as well as at all levels of the spinal cord sampled (cervical, thoracic, lumbar, and sacral), extending into the medulla (brain stem) as well as the brain itself (cerebellar peduncles). Degenerative changes in skeletal muscles were also described, ranging in severity from mild to moderate, including atrophy of fibres, increased eosinophilia, and calcification

*Described in the Report as swelling and/or degeneration of axons and/or myelin sheaths, plus the presence of lipid-laden macrophages and/or proliferating Schwann (neurilemmal) cells.

and/or fibrosis (at advanced sites). Grade 2 myocardial degeneration was also evident in the interatrial septum of dog 27, while mild-to-moderate degeneration (Grade 3) was observed in atria, and severe changes in the right ventricle.

Moderate (Grade 3) atrophy in the deep digital flexor muscle of a surviving high-dose male (#28) was also recorded.

[N.B.: Not mentioned in the text but listed in the tabulations, however, was a minimal degree of degeneration in the skeletal musculature of dog 19, a mid-dose (8 mg/kg) male.]

A mild degree (Grade 2) of atrophy of the thymus was found in one mid-dose male and one low-dose female. Grade 4/5 (i.e., severe) thymic atrophy was found in two high-dose males (#27 and 28). The authors ascribed thymic atrophy to stress related to treatment, rather than a direct effect of EPTAM.

Mild bile stasis was recorded in the livers of four high-dose males and one high-dose female, in two low-dose males as well as in controls (one male, one female), but not at the mid-dose. The authors considered these hepatic changes as "potentially treatment related." Affected high-dose males also had elevated alkaline phosphatase values and alterations in serum albumen levels.

Small foci of alveolar fibrosis accompanied by mild chronic inflammation ~~was~~ were found in the lungs of high-dose males (2 of 5), and in all female dose groups (1 low-dose, 3 mid-dose, and 2 high) as well as one control female. The authors discounted this finding as "biologically insignificant," since such lesions have been commonly observed in dogs acquired for their laboratory. [But again, no background data were presented.]

A single neoplasm was found, a grossly visible mass on the right hindleg of a mid-dose male, diagnosed histologically as a benign papilloma.

I. Authors' Conclusions

EPTAM administered to beagle dogs for 1 year caused reduced body weight gain, neurotoxicity (and consequent skeletal muscle degeneration), (serum) cholinesterase inhibition, thymic atrophy and liver effects, but only

at the highest dose tested (60 mg/kg/day). The no-observed-effect level for the study was considered by the authors to be 8 mg/kg/day.

TB Evaluation/Conclusions:

1. The study appears to have been conducted in accordance with sound scientific procedures, and in accord with EPA Test Guidelines, such that the data generated are judged to be valid. Since the authors did not provide historical data on controls from other dog studies performed in their laboratory, however, a meaningful interpretation of certain findings at the mid-dose level cannot be made. Therefore at this juncture, TB considers the mid-dose level, 8 mg/kg, to be an effect level because of the following:

   - o Erythema of the ears in males;
   - o A dose-related trend in reduced serum cholinesterase in both males and females; and
   - o Histological changes, such as:
     - Thymic atrophy in males, and
     - Increased pulmonary changes in females.

The study is graded CORE-MINIMUM, with the following parameters:

Doses tested: 0, 1, 8, and 60 mg/kg/day orally by capsule

NOEL = 1 mg/kg/day

LOEL = 8 mg/kg/day (Otic erythema in males; reduced serum ChE in both sexes; thymic atrophy in males; increased pulmonary changes in females).

At the highest dose (60 mg/kg/day) the following additional treatment-related effects were described:

- clinical signs of neurotoxicity in one male (sacrified in extremis);
- nerve degeneration in both sexes;
- reduced body weight gain in males;

- liver pathology in males;
- relative liver weight increase in females;
- EKG changes in one male;
- reduced hemoglobin and hematocrit in males;
- increased alkaline phosphatase;
- decreased blood urea nitrogen in males; and
- increased serum globulin and total bilirubin.

2. It is requested that information be submitted on the previous aborted study begun at doses of 0, 5, 25 and 125 mg/kg/day (a completed report, if available).

3. Further, it is requested that background control data from other dog studies conducted by this lab (alluded to in text) also be submitted.

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ATTACHMENTS

Tables 3, 4,
5, 6, 7, 8,
and 9 of the
Final Report.

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