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

009828

NOV 18 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Phosmet (Imidan®) - Submission of a Series 83-5 Combined Chronic/Carcinogenicity Study in Rats

Tox Chem No.:	543
Project No.:	1-1683
PC No.:	059201
Submission No.:	S398754
DP Barcode:	D166028

FROM: William B. Greear, M.P.H. William B. Greear 11/12/92
Review Section IV, Toxicology Branch I
Health Evaluation Division (H7509C)

TO: Brigid Lowery/Larry Schnaubelt, PM Team # 72
Reregistration Branch
Special Review and Reregistration Division (H7509W)

THRU: Marion P. Copley, D.V.M., Section Head Marion Copley 11/13/92
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

I. CONCLUSIONS:

The combined chronic/carcinogenicity study in rats (T-13241, 4/15/91) is classified as Minimum Data for carcinogenicity (83-2) and as Supplementary for chronic toxicity (83-1).

II. REQUESTED ACTION:

SRRD has requested that TOX-I evaluated the following study that was submitted by Andrew A. Davidson of ICI Americas, Inc.

- "2-Year Chronic Toxicity/Oncogenicity Study with R-1504 in Rats", Ciba Geigy Corp. #T-1341, April 15, 1991.

III. DISCUSSION:

The results of the 2-Year chronic/carcinogenicity are provided below:

NOEL (ChE) < 20 ppm (1.1 mg/kg/day)

LEL (ChE) = 20 ppm (decrease in RBC ChE in males)

NOEL (systemic) < 20 ppm

LEL (systemic) $\leq$ 20 ppm (increase in the incidence of fatty change in the liver of males)

In addition, at 40 ppm (M=1.8 mg/kg/day; F=2.1 mg/kg/day) and above RBC and serum ChE were decreased in females. At 200 ppm (M=9.4 mg/kg/day; F=10.9 mg/kg/day) serum and brain ChE were decreased in males and females; there were increases in the incidences of depressed foci in the liver of males, fatty change in the liver of females, hyperkeratosis of the stomach in males, and mineralization of the thyroid in females. At 400 ppm (M=23 mg/kg/day; F=27 mg/kg/day), body weight and body weight gain were decreased. Decreased kidney weight and an increase in BUN occurred in females in the 400 ppm group. There was no treatment related increase in cancer.

Strain: Sprague-Dawley Crl:CD® SD BR
Route: oral in diet
Dose levels: 0, 20, 40, 200, and 400 ppm (400 ppm terminated at 12 months)

Core Classification: This study is classified as Minimum Data for carcinogenicity (83-2) and as Supplemenmtary for chronic toxicity (83-1) because no NOEL was obtained.

Acceptability: The study satisfies the requirement for a Guideline Series 83-2 carcinogenicity study, but does not satisfy the requirement for a Guideline Series 83-1 chronic toxicity study. The study is potentially upgradeable to Minimum for chronic toxicity with the submission of an additional study using lower doses to more clearly define the NOEL.

The DER is attached.

DOC920038

FINAL

DATA EVALUATION REPORT

R-1504 TECHNICAL

Study Type: Combined Chronic Toxicity/Oncogenicity in Rats

009828

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

July 13, 1992

Principal Author Carrie Rabe Date 7/7/92

Reviewer Annette Shipp Date 7/13/92

QA/QC Manager Sharon Segal Date 7/15/92

Contract Number: 68D10075
Work Assignment Number: 1-10
Clement Number: 91-58
Project Officer: James E. Scott

EPA Review by: William Greear, M.P.H. Signature: William B. Greear
Review Section IV, Toxicology Branch I, Date: 10/28/92
Health Effects Division

Secondary EPA Review by: Marion Copley, D.V.M. Signature: Marion Copley
Section Head, Review Section IV, Date: 10/28/92
Toxicology Branch I, Health Effects Division

DATA EVALUATION REPORT

STUDY TYPE: Combined chronic toxicity/oncogenicity in rats

TEST MATERIAL: R-1504 Technical

Tox Chem. Number: 543

SYNONYMS: Phosmet, Imidan

MRID Number: 419164-01

STUDY NUMBER: T-13241

P.C. Number: 059201

SPONSOR: ICI Americas, Inc.

TESTING FACILITY: CIBA-GEIGY Corp.
Agricultural Division
Environmental Health Center
400 Farmington Ave.
Farmington, CT 06032

TITLE OF REPORT: 2-Year Chronic Toxicity/Oncogenicity Study with R-1504 in Rats

AUTHORS: J.C.F. Chang, Ph.D.
R.L. Morrissey, D.V.M., Ph.D.
Stuart Wyand, D.V.M.

REPORT ISSUED: Study completed on 4/15/91

CONCLUSIONS:

NOEL (ChE) < 20 ppm (1.1 mg/kg/day)

LEL (ChE) $\leq$ 20 ppm (decrease in RBC ChE in males)

NOEL (systemic) < 20 ppm

LEL (systemic) $\leq$ 20 ppm (increase in the incidence of fatty change in the liver of males)

In addition, at 40 ppm (M=1.8 mg/kg/day; F=2.1 mg/kg/day) and above RBC and serum ChE were decreased in females. At 200 ppm (M=9.4 mg/kg/day; F=10.9 mg/kg/day), serum and brain ChE were decreased in males and females; there were increases in the incidences of depressed foci in the liver of males,

fatty change in the liver of females, hyperkeratosis of the stomach in males, and mineralization of the thyroid in females. At 400 ppm (M=23 mg/kg/day; F=27 mg/kg/day), body weight and body weight gain were decreased. Decreased kidney weight and an increase in BUN occurred in females in the 400 ppm group. there was no treatment related increase in cancer
Strain: Sprague-Dawley Crl:CD® SD BR
Route: oral in diet
Dose levels: 0, 20, 40, 200, and 400 ppm (400 ppm terminated at 12 months)

Core Classification: This study is classified as Minimum Data for carcinogenicity (83-2) and as Supplementary for chronic toxicity (83-1) because no NOEL was obtained.

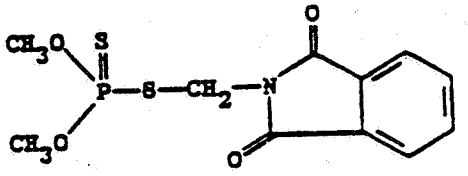
Acceptability: The study satisfies the requirement for a Guideline Series 83-2 carcinogenicity study but does not satisfy the requirement for a Guideline Series 83-1 chronic toxicity study. The study is potentially upgradeable to Minimum for chronic toxicity with the submission of an additional study using lower doses to more clearly define the NOEL.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: R-1504 technical

Formula: Phosphorodithioic acid S-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl] O,O-dimethyl ester



Lot Number: EHC-0866-24, WRC-4921-42-8

Purity: Reported to be 94.3%;
subsequent analysis showed a purity of 95.2%;
impurities were not identified

Physical Property: Off-white crystalline solid

Stability: Stable at room temperature

2. Test Article Analyses for Purity and Stability

Appropriate amounts of R-1504 were thoroughly mixed with basal diet in a Hobart mixer. The resulting mixture was mixed with additional basal diet in a Patterson-Kelly twin shell blender to obtain the appropriate concentrations. The diets were prepared approximately every 2-4 weeks on an as-needed basis. All prepared diets were refrigerated until presented to the rats.

The stability of R-1504 in dietary mixtures was tested by EHC Analytical Chemistry Laboratory. R-1504 was shown to be stable in

dietary mixtures for up to 30 days at 4°C or room temperature. In addition, no loss of test material was observed when kept in an open feed jar for 7 days.

Both basal and test diets were analyzed for test material concentration and homogeneity at the first two blends and then monthly by EHC Analytical Chemistry Laboratory. For the first 6 weeks of the study, the animals at 40 ppm inadvertently received diet containing 100 ppm. This resulted in a time-weighted average dose of 44.2 ppm (calculated by reviewer). Excluding that error, the mean concentrations in the diets at dietary levels of 20, 40, 200, and 400 ppm were 19.3 ± 1.1, 39.4 ± 2.9, 194.1 ± 5.6, and 389.5 ± 17.9, respectively (calculated by reviewer; data extracted from Study T-13214, Table 1, which presents the results of analysis of 53/83 blends). All but two of the blends were within acceptable limits for homogeneity. The unacceptable blends included a blend of the 20- and 200-ppm test levels prepared 11 days after the start of the study and a blend of the 20-ppm blend prepared 16 weeks after the initiation of the study. The relative standard deviation percentages for homogeneity of these doses were 27.2, 12.1, and 18.3, respectively. These two blends were fed to the animals as prepared. The lack of homogeneity in these blends should not have affected hematology, clinical chemistry, or urinalysis determinations done at 6 months.

3. Animals

Rats (312 males and 337 females, Sprague-Dawley Crl:CD® SD BR Strain) were received from Charles River Laboratories, Kingston, N.Y. The rats were 26 days old upon arrival and were randomly caged (two of the same sex/cage). Rats were randomized by body weight and assigned to study groups such that all groups of the same sex had similar mean body weights. Dietary levels were selected based on the results of a 6-week range-finding study in rats (T-13209). The doses used and the results observed in the range-finding study were not presented. At assignment (day 32 after birth), the male body weight ranged from 53 to 74 grams and the female body weight ranged from 61 to 90 grams. The number of rats assigned to each group and the number scheduled for sacrifice at the end of 12 and 24 months were as follows:

Dietary Level (ppm)	Number/Sex (at start)	Number/Sex Terminated	
		At 12 Months	At 24 Months
0	70	20	50
20	60	10	50
40	60	10	50
200	60	10	50
400	20	20	

Rats were maintained in quarantine for a total of 12 or 14 days. During the quarantine/acclimation period, rats were examined by a veterinarian for their suitability as test animals. During this

period and during the study, rats were housed in a single room individually in suspended polycarbonate cages (19 x 21 x 20 cm) with hardwood chip bedding. During the last 3 months of the study, all surviving males were housed individually in cages measuring 48 x 27 x 20 cm. The rack position in the animal room was rotated during the last 6 months of the study. The animal room was operated on a 12-hour light/dark cycle and temperature and humidity were maintained at 19-24°C and 40-60%, respectively, with at least 15 room air changes per hour. Water and food (Purina Certified Rodent Chow #5002 ground meal) were provided ad libitum.

4. Statistical Methods

Body weights, body weight gains, food consumption, feed efficiency, hematology values, clinical chemistry values, and organ weights were analyzed using a one-way analysis of variance followed by Dunnett's "t" test. Urinalysis results were analyzed using either Duncan's Multiple Range test or the Chi-Square test. Treatment-related neoplastic and nonneoplastic lesions were analyzed using Fischer's Exact test with Bonferroni correction. All tests were two-tailed and the probability of α-error was set at 0.05.

5. General Observations

(a) Mortality/moribundity/survival

All animals were observed twice daily for general appearance, behavior, signs of toxicity, and mortality.

Survival was not adversely affected during the study (see Table 1).

(b) Clinical observations

In addition to the twice daily observations noted above, animals were given a detailed physical examination each week.

Neither the incidence nor the time of onset of any clinical observations made were treatment related. It is noteworthy that diarrhea, excessive urination, lacrimation, and salivation, symptoms of cholinesterase inhibition, were not significantly increased in treated animals with respect to incidence or time of onset.

The incidence of palpable masses in male and female rats also was not increased above controls at any dose level.

(c) Body weights/food consumption/test material intake

Body weights--Individual body weights were determined weekly during the first 13 weeks and once every 4 weeks thereafter.

Tables 2 presents data on body weights at selected intervals. Although the body weights of males given diet containing 400 ppm

TABLE 1. Cumulative Mortality and Percent Survival in Rats Fed Diets Containing R-1504 for 104 Weeks[a]

Dietary Level (ppm)	Cumulative Mortality and (Percent Survival)[b] at Week: 26	52	78	At Termination[c]	Weeks to Death (Mean ± S.D.)[d]
			Males		
0	0 (100%)	5 (93%)	14 (72%)	40 (20%)	82.86±19.76
20	3 (95%)	9 (84%)	21 (58%)	38 (24%)	79.32±26.39
40	0 (100%)	6 (90%)	15 (70%)	34 (32%)	86.70±19.99
200	0 (100%)	4 (93%)	9 (81%)	30 (38%)	91.17±18.40
400	0 (100%)	2 (90%)	--[e]	--	--
			Females		
0	0 (100%)	3 (96%)	14 (72%)	34 (34%)	89.78±18.23
20	0 (100%)	4 (93%)	17 (65%)	32 (35%)	86.47±21.26
40	0 (100%)	3 (95%)	14 (72%)	34 (34%)	88.34±19.86
200	0 (100%)	2 (97%)	6 (88%)	29 (42%)	94.04±17.16
400	0 (100%)	0 (100%)	--	--	--

[a]Data were extracted from the Survival Report, Appendix C, Vol. 2, p. 469.

[b]Cumulative mortality and percent survival values exclude accidental deaths (2 males in the 200-ppm group and 1 female in the 20-ppm group) and scheduled sacrifices at 12 months. Percent survival was calculated by dividing the total live animals by the sum of the live and dead animals (excluding accidental deaths and scheduled sacrifices).

[c]Termination at week 105.

[d]Calculated by reviewer; excludes accidental deaths and scheduled sacrifices at 12 months.

[e]All of the 400-ppm animals were sacrificed at week 53.

TABLE 2. Mean Body Weight and Body Weight Gains at Representative Intervals in Rats Given Diets Containing R-1504[a,b]

Dietary Level (ppm)	Mean Body Weight at Week: 0	1	7	13	25	53	77	104	Mean Body Weight Gain at Week: 0-13	0-104
					Males					
0	114±9	183±14	459±37	567±54	672±70	784±98	838±124	777±148	453±51	659±148
20	115±10	184±18	465±39	576±53	684±74	818±95	865±113	858±64	461±47	747±59
40	117±9	187±15	464±39	576±54	691±75	796±93	851±95	811±98	459±52	694±102
200	119±10*	184±17	456±41	566±49	679±60	796±77	843±88	810±112	447±45	690±113
400	118±13	176±15	446±32	543±44	647±60	743±75	--[c]	--	425±43	--
					Females					
0	123±11	162±14	275±26	320±37	372±53	476±70	525±76	560±120	196±33	437±117
20	124±10	164±12	277±26	321±33	376±48	491±80	556±106	570±130	197±29	447±126
40	123±9	159±10	270±18	306±23	352±36	448±59	511±72	515±64	184±22*	392±60
200	123±10	153±11*	272±24	318±36	377±55	481±74	532±86	570±146	195±32	449±145
400	122±10	145±12*	250±21**	298±30**	355±50	434±66	--	--	176±26	--

[a]Data extracted from Study #T-13241, Tables 5-8.

[b]Value in grams; Mean ± S.D.

[c]All of the surviving 400-ppm animals were sacrificed at week 53.

*Significantly different from control; $p \leq 0.05$.

*Significantly different from control; $p < 0.01$

R-1504 appeared to be lower than controls, no statistically significant decreases in body weights in males were obtained. Females consuming diet containing 400 ppm R-1504 had significantly ($p \leq 0.05$) decreased body weights throughout the first quarter of the study (weeks 1-10, 12, 13, and 17) and lower (but not statistically significantly lower) weights until their sacrifice at week 53. During the first 6 weeks of the study, the 400 ppm female body weights were 10-14% lower than controls. During the next 11 weeks, body weights of 400 ppm females were 5-10% lower than controls.

Significantly ($p \leq 0.05$ or $p \leq 0.01$) depressed cumulative body weight gains were observed in males at 200 ppm at week 2 and in males at 400 ppm repeatedly during the first quarter of the study (weeks 1-5, 10, 29). The body weight gains of the males at 400 ppm remained below the controls (but not statistically significantly) throughout their duration on study. Cumulative body weight gains were significantly ($p \leq 0.05$ or 0.01) depressed in females at 400 ppm during weeks 1-10, 12, 13, and 17 (cumulative body weight gains were not determined at weeks 14, 15, or 16) and remained lower than controls (but not statistically significantly lower) until their sacrifice at week 53. In females at 200 ppm, cumulative body weight gains were significantly ($p \leq 0.01$) decreased only at weeks 1-4. Thereafter, body weight gains of this group returned to control levels.

A MTD was not reached on a weight basis. Males in the 200 ppm and 400 ppm groups had decreases of 1.3% and 6.2% in weight gain, respectively, when compared to controls (Table 2). Females had 0.5% and 10.2% decreases in weight gain, respectively, at 200 and 400 ppm. Body weight gain data do not support a MTD based on changes up to week 13 at 200 ppm.

Food consumption--Individual food consumption was determined weekly during the first 13 weeks and once every 4 weeks thereafter.

Mean weekly food consumption was not affected by inclusion of R-1504 in the diet at any dose.

Feed efficiency--Individual feed efficiency was determined weekly during the first 13 weeks and once every 4 weeks thereafter. Feed efficiency was calculated by dividing the mean daily body weight gain by the mean daily food consumption and multiplying the result by 100.

During the first 6 weeks of the study, all treated females had intermittent significantly decreased feed efficiency (11%-36% decrease). Otherwise, only sporadic decreases in feed efficiency were observed in both treated males and females. The decreases in feed efficiency were probably not treatment related since they were not mirrored by significant decreases in body weight gain.

Test article intake--Test article intake (mg/kg/day) was calculated based on nominal R-1504 concentration, and individual food consumption and body weight data. The nominal concentration was closely approximated by the actual concentration for most of the study. However, the 40 ppm animals received diet containing 100 ppm of test material for approximately the first 6 weeks of the study. This is not reflected in the test material intake calculations. Therefore, the actual R-1504 concentration in the diet should have been used to calculate test article intake. This information should be provided by the submitter.

The study authors calculated that intake of R-1504 for male rats receiving diets containing 20, 40, 200, and 400 ppm were 1.1, 1.8, 9.4, and 23 mg/kg/day. The intake for female rats receiving diets containing 20, 40, 200, and 400 ppm were 1.1, 2.1, 10.9, and 27 mg/kg/day, respectively. These values do not reflect "actual" concentrations of R-1504 in the diet.

(d) Ophthalmoscopic examination

Ophthalmoscopic examinations were performed pretest on all animals, at 12 months on all animals scheduled for interim sacrifice, and at 24 months in all surviving animals.

No statistically significant changes were observed in treated animals at either the 12- or 24-month exam.

6. Clinical Pathology

Hematology, clinical chemistry, and urinalysis were performed every 6 months on samples obtained from rats (10/sex/group) that had been fasted overnight. Blood samples were obtained from the intraorbital sinus or, at the 12- and 24-month sacrifices, from aortic punch. Urine samples were obtained from metabolism cages in which animals had been housed overnight. The parameters under each study category that have been checked were examined.

(a) Hematology

X Hematocrit (HCT)*	X Leukocyte differential count**
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	X Prothrombin time***
X Reticulocyte count (RETIC)	X Activated partial thromboplastin time (PT)***

* Recommended by Subdivision F (November 1984) Guidelines
** Conducted on 10 rats/sex/group (0 and 400 ppm at 6 and 12 months and 0 and 200 ppm at 18 and 24 months).
*** Conducted on 10 rats/sex/group (0, 200, and 400 ppm groups) at 12 months and on 10 rats/sex/group (0 and 200 ppm groups) at termination.

Several statistically significant changes were only observed at the 6-month test. However, no hematologic values of treated animals were significantly different than control values at 12, 18, or 24 months. It is unlikely that the effects observed at 6 months were treatment related because consistent dose- and time-related changes were not observed.

(b) Blood (clinical) chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium*	X Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	X Cholesterol*
X Sodium*	X Globulins
	X Glucose*
Enzymes	X Total bilirubin*
	X Direct bilirubin
X Alkaline phosphatase (ALP)	X Total protein*
X Serum cholinesterase	X Triglycerides
X Red Blood Cell cholinesterase	
X Creatinine phosphokinase	
Lactic acid dehydrogenase	
X Serum alanine aminotransferase (SGPT)*	
X Serum aspartate aminotransferase (SGOT)*	
X Gamma glutamyltransferase (GGT)	
X 5'-Nucleotidase	
X Sorbitol dehydrogenase	
X Brain cholinesterase**	

* Recommended by Subdivision F (November 1984) Guidelines
** Measured in brain homogenates from 10 rats/sex/group at the 12 month sacrifice and from animals designated for laboratory tests at 24 months.

Table 3 presents the data on cholinesterase activities obtained from rats on the study. At 400 ppm, serum, RBC, and brain cholinesterase activities in both males and females were significantly ($p \leq 0.01$) inhibited by approximately 40-75%, 75-90%, and 35-45%, respectively, at all test intervals. At 200 ppm, serum cholinesterase was significantly ($p \leq 0.05$) inhibited by 54% in males at the 18-month test and RBC and brain cholinesterase were significantly ($p \leq 0.01$) inhibited by 67-74% and 12-20%, respectively, at all test intervals. In females at 200 ppm, serum, RBC, and brain cholinesterase were significantly ($p \leq 0.01$) inhibited by 45-49%, 70-77%, and 19-27%, respectively, at all test intervals. In males at 40 ppm, RBC cholinesterase was significantly ($p \leq 0.05$ or $p \leq 0.01$) inhibited by 16-19% at the 6-, 18-, and 24-month intervals. At 40 ppm in females, serum cholinesterase was significantly ($p \leq 0.05$) inhibited by 26% at the 6-month interval and RBC cholinesterase was significantly ($p \leq 0.05$ or $p \leq 0.01$) inhibited by 15-19% at the 6-, 18-, and 24-month

TABLE 3. Cholinesterase Activities in Rats Given Diets Containing R-1504[a,b,c]

Parameter/ Interval	Dietary Level (ppm) 0	20	40	200	400
			Males		
Serum Cholinesterase (I.U./L)					
6-Month	654±192	669±214 (-2%)	621±260 (5%)	498±93 (24%)	393±62** (40%)
12-Month	705±196	704±255 (0%)	636±161 (10%)	511±140 (28%)	358±56** (49%)
18-Month	1352±923	1002±276 (26%)	860±311 (36%)	625±171* (54%)	--[d]
24-Month	886±276	873±207 (1%)	935±241 (-6%)	729±352 (18%)	--
RBC Cholinesterase (I.U./P RBC)					
6-Month	1706±267	1431±215* (16%)	1388±131** (19%)	507±188** (70%)	452±159** (74%)
12-Month	1344±203	1154±193 (14%)	1206±291 (10%)	447±153** (67%)	310±103** (77%)
18-Month	142..40	1286±187 (10%)	1198±204* (16%)	416±143** (71%)	--
24-Month	1838±418	1616±221 (12%)	1536±156* (16%)	658±187** (74%)	--
Brain Cholinesterase (I.U./G Tissue)					
12-Month	1.54±0.25	1.59±0.20 (-3%)	1.53±0.23 (1%)	1.23±0.17** (20%)	1.01±0.07** (34%)
24-Month	1.85±0.11	2.02±0.11* (-9%)	1.87±0.09 (-1%)	1.62±0.11** (12%)	--

TABLE 3 (Continued)

Parameter/ Interval	Dietary Level (ppm) 0	20	40	200	400
			Females		
Serum Cholinesterase (I.U./L)					
6-Month	3690±1292	3524±909 (4%)	2744±762* (26%)	1891±571** (49%)	947±236** (74%)
12-Month	2138±469	1957±531 (8%)	1834±498 (14%)	1130±302** (47%)	692±172** (68%)
18-Month	3054±1206	2401±729 (21%)	2455±695 (20%)	1677±445** (45%)	--
24-Month	2288±1077	1795±576 (22%)	1967±481 (14%)	1197±623* (48%)	--
RBC Cholinesterase (I.U./P RBC)					
6-Month	1452±208	1424±165 (2%)	1180±194** (19%)	338±116** (77%)	127±152** (91%)
12-Month	1180±131	1250±168 (-6%)	1080±248 (8%)	288±127** (76%)	264±142** (78%)
18-Month	1470±135	1307±271 (11%)	1246±193* (15%)	348±175** (76%)	--
24-Month	1662±371	1484±74 (11%)	1410±158* (15%)	492±159** (70%)	--
Brain Cholinesterase (I.U./G Tissue)					
12-Month	1.36±0.13	1.41±0.19 (-4%)	1.37±0.17 (-1%)	0.99±0.12** (27%)	0.78±0.08** (43%)
24-Month	1.77±0.12	1.86±0.13 (-5%)	1.74±0.11 (2%)	1.43±0.12** (19%)	--

[a]Data extracted from Study #T-13214, Tables 17 and 18.

[b]Mean ± standard deviation.

[c]Percent decrease from control, calculated by reviewer presented in parenthesis.

[d]All of the surviving 400-ppm animals were sacrificed at week 53.

*Significantly different from controls; $p \leq 0.05$.

**Significantly different from controls; $p \leq 0.01$.

intervals. Significantly depressed RBC cholinesterase activity (16% decrease) was observed in males at 20 ppm.

A significant increase in blood urea nitrogen at 12 months was observed in 400-ppm females. This effect was small, but may have been treatment related given the decreases in kidney weight observed in the 400-ppm females. A number of other clinical chemistry values were significantly ($p \leq 0.05$ or $p \leq 0.01$) different than controls but appeared to be spurious findings because either the direction of the change had no biological significance or there was no dose response.

(c) Urinalysis

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

* Recommended by Subdivision F (November 1984) Guidelines.

No remarkable effects were observed.

7. Sacrifice and Pathology

All animals that died, were sacrificed in extremis, or were sacrificed as scheduled, received a complete gross examination. The tissues marked with an X were examined histologically and those marked with XX were also weighed at necropsy. All indicated tissues were fixed in 10% neutral buffered formalin, except the testes, epididymides, ovaries, pituitary gland, adrenal glands, eyes, and Harderian glands, which were fixed in 2.5% buffered glutaraldehyde.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain
X Salivary glands*	X Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen	X Eyes (Optic nerve)*
X Jejunum*	X Thymus	
X Ileum*		
X Cecum*	Urogenital	
X Colon*		Glandular
X Rectum	XX Kidneys*	
XX Liver*	X Urinary bladder*	XX Adrenals*
Gallbladder*	XX Testes*	X Lacrimal gland
X Pancreas*	X Epididymides	X Mammary gland
	X Prostate	X Thyroids*
Respiratory	X Seminal vesicle	X Parathyroids*
	XX Ovaries	X Harderian glands
X Trachea*	X Uterus	
X Lung*	X Vagina	
X Nasal turbinates	X Cervix	

Other

Bone (sternum and femur)*
X Skeletal muscle*
X Skin
X All gross lesions and masses
X Joint (tibial-femoral)

* Recommended by Subdivision F (November 1984) Guidelines.

(a) Macroscopic

The incidence of depressed foci in the liver was significantly increased in 200-ppm females but not in 200-ppm males (Table 4).

(b) Organ weights and body weight ratios

The mean organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios were calculated for the adrenals, brain, kidneys, liver, ovaries, and testes.

A statistically significant decrease in the absolute kidney weight and the kidney-to-brain weight ratio was observed at the 12-month interim sacrifice in females receiving diets containing 400 ppm R-1504 (Table 5).

(c) Microscopic

All collected tissues were examined histologically.

Nonneoplastic--At the end of 12 months of exposure, a significantly ($p \leq 0.05$) increased incidence of fatty liver was observed in 200 and 400 ppm males (Table 6). At the end of 2 years, a significantly ($p \leq 0.05$) increased incidence of fatty change of the liver was observed in males at all dose levels and in females given diets containing 200 ppm. In addition, a significantly increased incidence of hyperkeratosis of the stomach in 200-ppm males and a significantly increased incidence of mineralization of the thyroids in 200-ppm females were observed. The incidences at the end of 2 years were calculated after subtracting animals sacrificed at the interim sacrifice.

The study authors reported only a statistically significant increase in the incidence of fatty changes in the livers of males and females at 200 ppm and males at 400 ppm. However, the study authors' statistical comparison was made using all animals (including those sacrificed at 12 months). This is an inappropriate comparison because data from animals on study for only 1 year were combined with data from animals on study for up to 2 years. Lesions may have appeared in animals sacrificed at 12 months if they had been allowed to remain on study for the full two years. The appropriate comparison should have separated results obtained at the end of 1 year (all animals dying 0-12 months plus the data from the 12-month sacrifice) from data

TABLE 4. Incidence of Gross Pathological Lesions Observed by the End of 2 Years in Rats Given Diets Containing R-1504[a,b]

	Dietary Level (ppm)			
	0	20	40	200
# Examined	50	50	50	50
Females				
Depressed Foci in Liver	2 (4)	3 (6)	3 (6)	10* (20)
Males				
Depressed Foci in Liver	3 (6)	3 (6)	4 (8)	7 (14)

[a]Results extracted from Study # T-13214, Tables 31 and 32 (excluding data from the 12-month interim sacrifice [Tables 27 and 28]; numbers calculated by reviewer).

[b]Percent incidence, calculated by the reviewer, presented in parenthesis

*Significantly different from control, $p \leq 0.05$; Fischer's exact test.

TABLE 5. Selected Organ Weight Data from Female Rats Given Diets Containing R-1504[a,b]

Parameter Interval	Dietary Level (ppm) 0	20	40	200	400
			(absolute wt-grams)		
Kidney					
12-Month	2.45±0.51	2.62±0.19	2.48±0.44	2.40±0.28	2.15±0.28*
24-Month	2.67±0.44	2.84±0.60	2.89±0.51	2.82±0.55	--[c]
			(organ-to-body weight ratio x 100)		
Kidney					
12-Month	0.55±0.12	0.56±0.06	0.50±0.13	0.51±0.09	0.52±0.05
24-Month	0.54±0.09	0.55±0.12	0.60±0.09	0.55±0.20	--
			(organ-to-brain weight ratio x 100)		
Kidney					
12-Month	119±23	130±13	125±20	115±14	104±12*
24-Month	132±22	137±30	139±24	134±22	--

[a]Data extracted from Study #T-13214, Tables 21 and 22

[b]Mean ± standard deviation

[c]All of the surviving 400-ppm animals were sacrificed at week 53.

*Significantly different from controls; $p \leq 0.05$

TABLE 6. Incidence of Nonneoplastic Lesions in Rats Given Diets Containing R-1504[a,b]

Parameter/ Interval	Dietary Level (ppm) 0	20	40	200	400
			Males		
Fatty change in liver					
12-Month	10/25 (40)	10/19 (52)	8/16 (50)	13/16* (81)	15/20* (75)
24-Month	16/50 (32)	28/50* (56)	28/50* (56)	34/50* (68)	--[c]
Hyperkeratosis of Stomach					
12-Month	not observed				
24-Month	0/50 (0)	0/50 (0)	2/50 (4)	5/50* (10)	--
			Females		
Fatty Change in Liver					
12-Month	1/23 (4)	2/15 (13)	0/13 (0)	1/12 (8)	4/20 (20)
24-Month	10/50 (20)	11/50 (22)	14/50 (28)	20/50* (40)	--
Mineralization of the Thyroid					
12-Month	not observed				
24-Month	0/50 (0)	0/50 (0)	0/50 (0)	5/50* (10)	--

[a]Data for 12-Month combined from Tables 33 and 37, and 34 and 38.
Data for 24-Month from Tables 41 and 42 with data from Tables 37 and 38 subtracted.
[b]Incidence shown over the number examined. Percent incidence calculated by reviewer is presented in parentheses.
[c]All of the surviving 400-ppm animals were sacrificed at week 53.
*Significantly different from controls; $p \leq 0.05$

obtained at the end of 2 years (all animals dying 0-24 months, excluding the data from the 12 month sacrifice, plus data from the 24-month sacrifice).

Neoplastic--Tumors occurred with similar frequencies in all dose groups, and were not considered to be treatment related.

The statistical test used to analyze for differences in nonneoplastic and neoplastic lesions did not consider differences in survival.

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The reviewer has no other comments regarding the materials and methods sections.

A description of the statistical analysis employed was included in the report.

A Good Laboratory Practice Compliance Statement was signed by James Craig (Quality Assurance Unit), Jane C.F. Chang (Study Director), Donald R. Saunders (Director, Environmental Health Center), Ann Manley (Sponsor), and Andrew A. Davidson (Applicant). A Quality Assurance Statement including a list of Quality Assurance Inspections was signed by Kathleen F. Gallant (Quality Assurance Inspector). Neither of these documents was dated.

B. DISCUSSION

The study design was, for the most part, complete and adequate. A few minor errors in the summary tables were noted by the reviewer (i.e., Table 46, the number of 200 ppm animals with neoplasms was 26, not 29) but none of the errors affected the statistics or interpretation of the study. In general, the data were well reported and the statistics used were appropriate with some exceptions. The major exceptions were (1) the histopathological data from all animals (Tables 41, 42, 51, and 52) was statistically analyzed without excluding data from animals on the study for only 1 year (by virtue of the interim sacrifice at 53 weeks) and (2) the statistical tests used by the study authors to analyze for differences in macroscopic and histopathological findings did not take into account differences in the survival of the control and test groups.

The fact that histopathological data from all animals (satellite animals plus main study animals) were combined and statistically analyzed caused the study authors to reach a few erroneous conclusions (i,e., statistically significant increases in fatty liver in the males at 20 and 40 ppm, mineralization of the thyroids of 200-ppm females, hyperkeratosis of the stomachs of 200-ppm males were not identified). Such an analysis is inappropriate because the satellite animals were on study for only 1 year and the majority of the animals were on the study for 2 years. It is unknown what lesions may have appeared in the sacrificed animals if they had been allowed to continue on study. No other erroneous conclusions were detected by the reviewer as a result of the inclusion of all animals in the statistical analysis of histopathological data.

The methods of statistical analysis of histopathological effects did not take into consideration differences in survival between the various treatment groups. A Tyrone test or a Kaplan-Meyer test to determine differences in survival among groups and an incidental analysis to analyze whether the difference in survival affected the incidence of lesions would have been more appropriate given the differences in survival between control animals and treated animals. Survival among controls was less than the survival of the mid- (40 ppm) and high-dose (200 ppm) animals. This could affect the incidence of age-related lesions in controls and cause the identification of increased incidences of age-related findings in mid- or high-dose animals versus the controls. No increase in tumor incidence in either the mid- or high-dose animals was observed when compared to controls using the Fischer's exact test. It is unlikely that an incidental analysis would uncover a difference that was masked by lower survival of the controls because the incidence of tumors is expected to increase with age. However, the significant differences that were identified by the reviewer (i.e., fatty change in the liver, mineralization of the thyroids, and hyperkeratosis of the stomach) may have been due to differences in the survival of the various groups. Thus, an incidental analysis of the data may have revealed that no significant difference in this finding exists.

The dietary levels selected were based on a 6-week range-finding study in rats (# T-13209). The details and results of the range-finding study were not given. Thus, it is difficult to know what end point was used as the rationale for the choice of doses used in this study that would assure that adequate toxicity was achieved to assess carcinogenic potential.

The reviewer agrees with the study authors' conclusion that under the conditions of this study, there was no evidence of carcinogenicity. The slightly higher death rate in controls than in test animals would only have biased the results such that there would be an increased likelihood of observation of tumors in test animals (due to their greater longevity). The reviewer also agrees with the study authors' conclusions that survival, food consumption, hematology, and urinalysis were not affected by dietary exposure to R-1504.

The results of the clinical chemistry determinations clearly show that R-1504 inhibits cholinesterase activity. The reviewer does not agree with the assertion of the study authors that the NOEL for this study is 40 ppm based on the absence of biologically significant inhibition of cholinesterase activity at 40 ppm and below. The LOEL should be 20 ppm because this is the first dose at which a dose-related statistically significant decrease in cholinesterase activity was observed over the duration of the study. The NOEL for cholinesterase inhibition should be <20 ppm.

The cholinesterase inhibition was apparently well tolerated by the rats because no clinical signs normally associated with cholinesterase inhibition (diarrhea, excessive urination, lacrimation, and salivation) were significantly increased in treated animals. Up to 80% inhibition of cholinesterase may be tolerated by animals without clinical signs if the inhibition increases gradually.

Treatment of rats with R-1504 was also associated with decreased body weights in females at 400 ppm when compared to controls and decreased body weight gains in males and females at 400 ppm.

A significant increase in fatty change in the liver was observed in males at 200 and 400 ppm at the end of 1 year of exposure and in males at all dose levels and in females at 200 ppm at the end of 2 years of exposure. A significant increase in hyperkeratosis of the stomach was observed in 200-ppm males and mineralization of the thyroids was observed in 200-ppm females. This effect was not observed in the 400-ppm group sacrificed at 1 year.

The results of organ weight determinations suggest that the kidney may be a target organ of R-1504 in female rats. At the highest dose of the satellite study (400 ppm), a significant decrease in kidney weight (both absolute and kidney-to-brain weight ratio) and a slight increase in BUN were observed in females at 12 months, indicating a measure of renal dysfunction. However, no significant effects were observed in renal histopathology to support these changes.

Adequate toxicity to assess carcinogenic potential was achieved based on the inhibition of red blood cell, plasma, and brain cholinesterase at 200 ppm in both sexes. According to Farber (EPA 1982), the significant depression of at least two of the assayed cholinesterase enzyme measurements can be used as an indication that adequate toxicity to assess carcinogenic potential has been achieved. The LOEL for cholinesterase inhibition was 20 ppm based on inhibition of RBC cholinesterase in male and female rats. The NOEL for cholinesterase inhibition was <20 ppm. The LOEL for other systemic effects was also 20 ppm based on the increased incidence of fatty liver in males at 20 ppm. The LOEL for systemic effects was <20 ppm.

Reference

EPA. 1982. Environmental Protection Agency. Pesticide Assessment Guidelines, Subdivision F, Position Document: Selection of a Maximum Tolerated Dose (MTD) in oncogenicity studies. Washington, DC. EPA/540/09-88-003.