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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SEP 23 1992

MEMORANDUM
Subject: Isofenphos--Evaluations of Toxicity Studies

To: Ruby Whiters, Chemical Review Manager
Accelerated Reregistration Branch, SRRD H7508W

From: Patricia McLaughlin, Ph.D. P McLaughlin 9/9/92
Toxicology II Branch, HED

Thru: Elizabeth Doyle, Ph.D., Head E. A. Doyle 9/9/
Section IV, Toxicology II Branch, HED

Marcia van Gemert, Ph.D., Chief M. van Gemert 9/9/92
Toxicology II Branch, HED

Chemical: Isofenphos
Case/chemical number: 2345/109401

Attached are review memoranda for some toxicity studies on technical isofenphos. These reviews should fill some of the gaps in the FIFRA 88 requirements. The dog chronic study (guideline 83-1) may be upgraded with the submission of the information that is lacking, as stated on p. 22 of the DER.

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EPA No.: 68D80056
DYNAMAC No.: 359-C
TASK No.: 3-59C
October 30, 1991

SEP 23 1992

DATA EVALUATION RECORD

ISOFENPHOS

Chronic Oral Toxicity Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L. McLellan for

Date: Oct. 30, 1991

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EPA No.: 68D80056
DYNAMAC No.: 359-C
TASK No.: 3-59C
October 30, 1991

DATA EVALUATION RECORD

ISOFENPHOS

Chronic Oral Toxicity Study in Dogs

REVIEWED BY:

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Date: 10/30/91

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Date: 9/9/92

DATA EVALUATION RECORD

CUIDELINE § 83-1

STUDY TYPE: Chronic oral toxicity study in dogs.

MRID NUMBER: 92085-016.

TEST MATERIAL: SRA 12869.

SYNONYM(S): Isofenphos; oftanol: O-ethyl-O-(2-isopropoxy-carbonyl) phenyl isopropylphosphoramidothioate.

REPORT NUMBER(S): 7072.

SPONSOR: Mobay Corporation, Stilwell, KS.

TESTING FACILITY: Bayer Ag, Dept. of Toxicology, Wuppertal, West Germany.

TITLE OF REPORT: Phase 3 Reformat of MRID 00083067: SRA 12869 Chronic toxicity study on Dogs (Two-year Feeding Experiment); Project Number 54410.

AUTHORS: Hoffmann, K. and Kaliner, G.

REPORT ISSUED: October 25, 1977; reformat date April 24, 1990.

CONCLUSIONS:

The 104-week dietary administration of Isofenphos to male and female beagle dogs at dose levels of 0, 3/2, 15, or 75/150/300 ppm (corresponding to an approximate intake of 0, 0.09, 0.45, and 4.24 mg/kg/day in males and 0, 0.10, 0.53, and 3.43 mg/kg/day in females) resulted in marked depression in cholinesterase activity at the high dose. Two high-dose males exhibited anorexia, weakness of the hind extremities, and drowsiness in the final weeks of dosing. The condition of the dogs deteriorated following the increase in dosing from 150 to 300 ppm; one dog was found dead at week 100 and one dog was sacrificed moribund at study termination. The cause of death in these animals was attributed to the severe depression of erythrocyte cholinesterase activity. Plasma and erythrocyte cholinesterase activity were depressed in mid- and high-dose dogs throughout the study; brain cholinesterase was also depressed in high-dose males and females. Alkaline phosphatase levels were increased and slight changes occurred in the albumin and globulin levels of high-dose animals. Hematocrit, hemoglobin, and erythrocyte counts of high-dose males were slightly but consistently depressed and reticulocyte counts were increased. Body weights of high-dose males and females were depressed following the increase in the test diet concentration to 300 ppm. Microscopic changes included erosions of the esophageal mucosa, inflammatory cell infiltrations of the lungs, and brainstem vacuoles at the high-dose. Based on the depression of plasma and erythrocyte cholinesterase activity, the NOEL is 3 ppm for females and 2 ppm for males and the LOEL is 15 ppm for both sexes.

Classification: Core Supplementary. This study does not satisfy the requirements for Guideline 83-1, chronic toxicity in a non-rodent. Several additional study deficiencies are also described in Reviewers' Discussion and Interpretation of Results.

A. MATERIALS:

1. Test Compound: SRA 12869; description: colorless oil; batch No.: not reported; purity: 89.3%.

2. Test Animals: Species: Dog; strain: Beagle; age: 22 to 29 weeks at study initiation; weight: 5.9 to 11.0 kg at study initiation; source: Schering AG, Bergkamen, West Germany.

B. STUDY DESIGN:

1. Animal Assignment: Animals were randomly assigned to the following test groups based on ranges of plasma cholinesterase activity. The period of acclimation was not reported.

Test group	Dose in diet (ppm)	Main study (104 weeks) Males	Main study (104 weeks) Females
1 Control	0	4	4
2 Low (LDT)	3/2[a]	4	4
3 Mid (MDT)	15	4	4
4 High (HDT)	75/150/300[a]	4	4

[a]Low-dose males were administered 3 ppm from weeks 1 to 83, and 2 ppm from weeks 84 to 104. Low-dose females were administered 3 ppm from weeks 1 to 104. High-dose males and females were administered 75 ppm from weeks 1 to 53, 150 ppm from weeks 54 to 99, and 300 ppm from weeks 100 to 104. Doses were increased to obtain a clinically recognizable toxic effect.

Dogs were housed individually at a temperature of approximately 21°C, and a 12-hour light/dark cycle. Conditions of humidity were not specified. Prior to study initiation, the dogs were immunized against distemper, infectious hepatitis, and leptospirosis and treated with Uvilon for ascarid infestations. Worming was repeated on all dogs at 3-month intervals.

2. Diet Preparation and Dosing: A 50% premix of the test material (oily liquid) was mixed with Wessalon S and refrigerated at 4°C. Mixtures of the test premix and pulverized basal diet at appropriate dietary concentrations were mixed with tapwater to form a mash and administered to test animals daily in 300-g portions during weeks 1 to 4 and in 350-g portions from weeks 5 to 104. Mixtures of test material premix and basal diet were prepared weekly. The amount of ration not consumed within 24 hours was weighed prior to feeding the following day to measure the daily intake of the test material. The time required for each dog to consume its ration was noted.

Results: Analyses of homogeneity, stability, and test material concentration in the test diets were not provided in the study report.

3. Food and Water Consumption: Animals received food (Altromin H-Diet No. 4021) and water ad libitum.

4. Statistics: Methods of statistical analysis were not reported with the exception of the use of Wilcoxon's rank test for differences between control and dosed animals. Although the study text reported statistical differences for clinical chemistry parameters, these differences were not indicated on the summary tables. Other data also did not appear to be statistically documented. The study authors did not report standard deviations for many parameters.

5. Quality Assurance: The study was performed prior to Good Laboratory Practices requirements. No quality assurance statement was included in the study report.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of morbidity and mortality. The pupillary reflex, patellar reflex, flexor reflex, extensor thrust, and body temperature of each dog were tested at study initiation, weekly for 52 weeks, and biweekly thereafter.

Results: One high-dose male (animal No. 537) died following administration of 300 ppm isofenphos in the feed for 4 weeks (study week 100); one high-dose male was sacrificed moribund (animal No. 530) at autopsy. Deaths were attributed to severe depression of erythrocyte cholinesterase activity. Reflex testing of the dogs was normal. Slight body temperature increases were observed in 5/32 control and dosed animals; these changes were not considered to be related to dosing by the study authors.

Clinical findings were not tabulated. Occasional vomiting and loose stool were reported sporadically in control and dosed animals; two control dogs exhibited epileptic attacks in association with clinical tests. Two high-dose males (animal Nos. 535 and 537) exhibited anorexia in the final weeks of dosing; the anorexia was considered to be compound related and was initially observed in animal No. 537 at week 28. At study week 88, animal No. 537 exhibited weakness of the hind extremities; the condition of this dog deteriorated, and the dog became unsteady by week 93. Additional signs of drowsiness, salivation, and immobility were apparent at this time. High-dose male No. 431 exhibited weakness and unsteady gait by week 98. Following the increase in dietary concentration of the test material from 150 to 300 ppm at week 100, vomiting and loose feces were exhibited in high-dose dogs, and clinical signs

observed in males 537 and 431 intensified. At the end of week 100, dog 431 exhibited paresis of the hind extremities, trembling, sticky coat, salivation, and protruding tongue; the condition of this dog improved slightly in the following 2 weeks. Dog 537 was unable to stand during week 100 and stopped eating; this dog was found dead at the end of week 100. During study week 101, high-dose male Nos. 425 and 535 developed similar signs of weakness of hind extremities, trembling, salivation, and protruding tongue. At week 104, dog 535 was unable to stand and exhibited mild spasms after feeding; the dog was reported to be in a moribund condition at study termination. Females of this group exhibited vomiting, and loose feces; mild transient signs of weakness were also apparent in 2/4 animals. Males appeared to be more sensitive than females to the effects of Isofenphos.

2. Body Weight: Dogs were weighed prior to study initiation, weekly for 52 weeks, and biweekly thereafter.

   Results: Body weights of dosed and control animals were not equal at study initiation; the study authors reported that dogs were randomly allocated to study groups on the basis of plasma cholinesterase activity rather than initial body weights. Representative body weight and body weight gain data are summarized in Tables 1 and 2, respectively. The mean body weight of high-dose males was 14% less than the weight of concurrent controls at study week 1; this difference in body weight remained consistent through study week 102. Following the increase in dietary concentration of the test material from 150 to 300 ppm at week 100, body weights of high-dose males decreased progressively from week 100 to week 104. An additional 5% depression in the body weight of these animals was exhibited at week 102 as compared to week 100, and an additional 7% depression was exhibited at week 104. The body weight loss was most pronounced in male dog Nos. 535 and 537. The mean body weight of these high-dose animals was 23% less than concurrent controls at week 104. The body weight loss in this group from weeks 78 to 104 was -1.6 ± 5.4 kg (Tables 1 and 2). In addition, 3/4 high-dose females exhibited depression in body weights at weeks 102 and 104 following the increase in dietary concentration of the test material. Prior to this increase in dosing, body weights of high-dose females appeared to increase over the duration of the study. The depression in body weights of high-dose males and females from study week 102 to study termination was considered to be related to dosing.

3. Food Consumption and Compound Intake: Consumption was determined weekly for each dog for 54 weeks and biweekly thereafter.

TABLE 1. Mean Body Weights at Selected Intervals in Dogs Administered Isofenphos for 104 Weeks[a,b]

Dose Group (ppm)	Mean Body Weight (kg ± S.D.) at Study Week:				
	1	26	52	78	104
			Males		
0	8.8 ± 0.66	10.7 ± 0.88	10.8 ± 0.87	11.4 ± 1.15	11.4 ± 1.02
3/2	8.7 ± 1.44	10.6 ± 0.94	10.9 ± 0.74	11.5 ± 0.76	11.2 ± 1.02
15	9.1 ± 1.26	11.1 ± 0.91	11.7 ± 1.39	12.9 ± 1.85	13.0 ± 1.77
75/150/300	7.6 ± 1.06	9.4 ± 0.87	9.5 ± 1.33	10.5 ± 1.17	8.8 ± 1.22
			Females		
0	7.2 ± 0.47	9.5 ± 0.54	9.7 ± 0.92	10.8 ± 1.15	11.0 ± 1.36
3/2	6.7 ± 0.73	9.7 ± 1.25	10.3 ± 1.21	11.6 ± 1.41	12.0 ± 1.40
15	6.8 ± 1.00	9.7 ± 0.75	9.9 ± 0.52	10.6 ± 0.79	10.9 ± 0.99
75/150/300	7.3 ± 0.96	10.5 ± 0.74	11.3 ± 0.94	12.9 ± 1.70	12.5 ± 1.96

[a]Based on four dogs/sex/dose.

[b]Means and standard deviations recalculated by reviewers using individual data reported by study authors.

TABLE 2. Mean Body Weight Gains at Selected Intervals in Dogs Administered Isofenphos for 104 Weeks[a,b]

Dose Group (ppm)	Mean Body Weight Gains (kg ± S.D.) at Study Weeks:						
	0-26	26-52	0-52	52-78	0-78	78-104	0-104
				Males			
0	1.8 ± 0.62	0.1 ± 0.59	1.9 ± 1.08	0.6 ± 0.32	2.5 ± 1.21	-0.03 ± 0.21	2.5 ± 1.20
3/2	2.1 ± 0.83	0.4 ± 0.48	2.4 ± 0.86	0.6 ± 0.96	3.0 ± 0.93	-0.30 ± 0.36	2.7 ± 1.05
15	1.9 ± 1.23	0.6 ± 0.83	2.4 ± 2.00	1.3 ± 0.68	3.7 ± 2.60	0.05 ± 0.19	3.7 ± 2.57
75/150/300	1.7 ± 1.31	0.1 ± 0.54	1.8 ± 1.34	1.0 ± 0.21	2.7 ± 1.15	-1.6 ± 0.54	1.1 ± 1.39
				Females			
0	2.4 ± 0.52	0.2 ± 0.55	2.6 ± 0.70	1.0 ± 0.25	3.6 ± 0.88	0.3 ± 0.31	3.9 ± 0.97
3/2	3.2 ± 1.10	0.6 ± 0.56	3.3 ± 1.01	1.3 ± 0.33	5.1 ± 1.20	0.4 ± 0.18	5.5 ± 1.25
15	2.6 ± 0.83	0.2 ± 0.50	2.8 ± 1.12	0.7 ± 0.29	3.5 ± 1.07	0.3 ± 0.31	3.8 ± 0.88
75/150/300	3.0 ± 0.30	0.9 ± 0.24	3.8 ± 0.40	1.6 ± 0.79	5.4 ± 0.88	-0.4 ± 0.42	4.8 ± 0.74

ıed on four dogs/sex/dose.

ıns and standard deviations recalculated by reviewers using recalculated mean body weight data.

Results: Representative results of mean food consumption and test compound intake are presented in Table 3. Food consumption was slightly depressed in high-dose males and females during the last half of the study; however, differences were slight and were considered to be of little importance by the study authors. The mean test compound intake in low- and mid-dose females was slightly higher than that found in males; however, the compound intake in high-dose females was less than that found in males. This latter effect was due to the depressed food consumption of two high-dose females. The mean corresponding consumptions at dietary levels of 3/2, 15, and 75/150/300 ppm were 0.09, 0.45, and 4.24 mg/kg/day, respectively, in males and 0.10, 0.53, and 3.43 mg/kg/day, respectively, in females. The study authors noted that the two high-dose males that died or that were sacrificed moribund at study termination (animal Nos. 535 and 537) had the highest test compound intake in relation to body weight (4.44 and 4.75 mg/kg/day, respectively).

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to study initiation and at study weeks 14, 27, 39, 53, 66, 79, 92, and 104. In addition, the ocular fundus of each dog was photographed prior to study initiation, and at weeks 52 and 104.

   Results: There were no compound-related ophthalmological findings. Individual ophthalmological data were not provided. The study authors indicated that dogs were treated for a transient condition of conjunctivitis. However, the number of dogs affected and the duration of the problem were not reported. This finding was not considered to be associated with dosing.

5. Hematology and Clinical Chemistry: Blood was collected from the jugular vein of all dogs for hematology and clinical analysis. The samples were collected prior to study initiation and at study weeks 14, 27, 39, 53, 66, 79, 92, and 104. Erythrocyte and plasma cholinesterase activities were measured at weeks 1, 3, 7, 10, 14, 27, 39, 52, 66, 79, 92, and 103. Brain cholinesterase activity was measured at week 104. The CHECKED (X) parameters were examined:

   a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count (RETIC)
Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
Mean corpuscular HGB concentration (MCHC)
Mean corpuscular volume (MCV)
X Coagulation: thromboplastin time (PT)
X Sedimentation rate

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Representative Results of Mean Food Consumption and Test Compound Intake in Dogs Administered Isofenphos for 104 Weeks[a,b]

Dose Group (ppm)	Mean Food Consumption (kg ± S.D.) at Study Weeks:					Mean Test Compound Intake (Weeks 1 to 104)	
	1	26	52	78	104	mg/kg/week	mg/kg/day
				Males			
0	2.10 ± 0.00	2.45 ± 0.00	2.45 ± 0.00	4.68 ± 0.43	4.85 ± 0.11	0	0
3/2	2.10 ± 0.00	2.45 ± 0.00	2.45 ± 0.00	4.90 ± 0.00	4.90 ± 0.00	0.633	0.09
15	2.08 ± 0.05	2.45 ± 0.00	2.45 ± 0.00	4.78 ± 0.22	4.78 ± 0.23	3.13	0.45
75/150/300	2.10 ± 0.00	2.45 ± 0.00	2.33 ± 0.24	4.75 ± 0.21	4.20 ± 1.00	29.66	4.24
				Females			
0	2.10 ± 0.00	2.45 ± 0.00	2.45 ± 0.00	4.90 ± 0.00	4.90 ± 0.00	0	0
3/2	2.10 ± 0.00	2.45 ± 0.00	2.35 ± 0.21	4.68 ± 0.45	4.81 ± 0.18	0.708	0.10
15	2.10 ± 0.00	2.45 ± 0.00	2.45 ± 0.00	4.90 ± 0.00	4.90 ± 0.00	3.7[illegible]	0.53
75/150/300	2.10 ± 0.00	2.45 ± 0.00	2.37 ± 0.17	4.51 ± 0.45	4.59 ± 0.63	24.04	3.43

[a]Based on four dogs/sex/dose.

[b]Standard deviations of food consumption calculated by reviewers from individual data.

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Results: Table 4 summarizes data on selected hematology parameters. The study authors reported that no compound-related changes were observed in any hematological parameter. However, means and standard deviations of hematocrit, hemoglobin concentrations, erythrocyte, and reticulocyte counts of control and dosed animals were recalculated by the reviewers because incorrect group means and standard deviations were presented in the study report for males, and female group data were not reported. Hematocrit (8 to 13% depression), hemoglobin concentrations (6 to 12% depression), and erythrocyte counts (8 to 14% depression) of high-dose males were slightly but consistently depressed throughout the study; these depressions were significant ($p < 0.05$) at week 27 for hemoglobin and hematocrit and at weeks 39, 53, 66, and 104 for erythrocyte counts. Reticulocyte counts of high-dose males were increased from 19 to 167% throughout the study; these increases were significant at 66 ($p < 0.01$) and 104 ($p < 0.05$) weeks.

The reviewers question the accuracy of the tabulation of individual data for females. According to the recalculated individual data, the hematocrit, hemoglobin concentrations, and erythrocyte counts of mid-dose females appear to be slightly but consistently depressed throughout the study; the levels of these parameters in high-dose females are similar to controls. This appears to be inconsistent with the changes in these parameters in dosed males. Perhaps these data were incorrectly recorded for mid- and high-dose females.

The platelet count and sedimentation rate of male No. 537 were increased immediately prior to the death of this animal at week 104. These increases were reported by the study authors to be a result of accelerated blood decomposition.

Parameter/ Interval (Week)	Dietary Level (ppm) 0	3/2	15	75/150/300
			Males	
Hematocrit (%)				
14	42.00 ± 2.45	42.00 ± 2.71	39.50 ± 3.11	38.25 ± 3.77
39	43.00 ± 1.63	43.50 ± 1.73	40.00 ± 3.37	37.25 ± 5.74
66	44.25 ± 2.63	42.50 ± 2.52	42.25 ± 2.99	40.75 ± 5.32
92	44.25 ± 0.50	42.25 ± 2.75	44.80 ± 6.40	41.50 ± 2.65
104	46.00 ± 2.16	43.00 ± 3.65	43.80 ± 5.0	40.50 ± 2.38
Hemoglobin (g/100 mL)				
14	14.78 ± 0.95	14.58 ± 0.83	14.05 ± 0.66	13.88 ± 0.55
39	15.20 ± 0.67	15.25 ± 0.81	14.18 ± 1.05	13.40 ± 1.64
66	15.15 ± 1.03	14.95 ± 0.70	14.68 ± 0.95	13.90 ± 1.43
92	15.15 ± 0.37	14.55 ± 0.82	15.63 ± 1.86	14.18 ± 0.57
104	15.55 ± 0.38	14.98 ± 1.08	15.28 ± 1.57	13.80 ± 0.80
Erythrocyte Count ($10^6/mm^3$)				
14	6.21 ± 0.36	6.21 ± 0.41	5.98 ± 0.68	5.69 ± 0.32
39	6.36 ± 0.25	6.30 ± 0.54	5.57 ± 0.52	5.20 ± 0.58*
66	6.17 ± 0.35	5.96 ± 0.37	5.89 ± 0.30	5.34 ± 0.59*
92	6.27 ± 0.18	6.11 ± 0.39	6.34 ± 0.76	5.65 ± 0.23
104	6.51 ± 0.21	6.25 ± 0.59	6.29 ± 0.63	5.58 ± 0.35*
Reticulocyte Count (%)				
14	6.75 ± 3.20	4.00 ± 0.82	8.75 ± 2.06	5.50 ± 3.11
39	6.75 ± 0.5	7.50 ± 2.08	9.25 ± 3.86	8.00 ± 2.94
66	3.50 ± 0.58	4.50 ± 1.91	6.25 ± 3.20	9.00 ± 1.83**
92	5.50 ± 1.00	4.00 ± 0.82	6.25 ± 3.86	8.00 ± 7.53
104	4.50 ± 2.89	5.75 ± 2.50	7.25 ± 5.91	12.00 ± 2.16*

(continued)

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TABLE 4. (continued)

Parameter/ Interval (Week)	Dietary Level (ppm) 0	3/2	15	75/150/300
		Females		
Hematocrit (%)				
14	43.50 ± 1.00	43.75 ± 0.96	40.25 ± 3.66	43.00 ± 2.00
39	44.50 ± 2.40	42.50 ± 2.08	39.25 ± 2.22*	42.00 ± 2.94
66	42.25 ± 4.11	47.00 ± 2.24	38.25 ± 4.63	39.50 ± 1.91
92	47.0 ± 2.16	47.00 ± 6.73	36.75 ± 5.12*	43.25 ± 5.06
104	43.[illegible] ± 2.00	49.50 ± 3.11	38.50 ± 2.89	45.00 ± 5.16
Hemoglobin (g/100 mL)				
14	[illegible].05 ± 0.37	5.13 ± 0.63	14.40 ± 1.20	15.05 ± 0.57
39	[illegible].28 ± 0.96	14.68 ± 0.78	14.03 ± 0.28	14.76 ± 1.15
66	14.95 ± 1.40	16.55 ± 1.19	13.13 ± 0.95	13.88 ± 0.69
92	15.85 ± 0.53	15.88 ± 1.48	12.85 ± 1.63*	15.05 ± 1.32
104	15.00 ± 0.29	16.63 ± 1.13*	12.98 ± 1.13*	15.30 ± 1.16
Erythrocyte Count ($10^6/mm^3$)				
14	6.20 ± 0.10	6.35 ± 0.56	6.12 ± 0.57	6.33 ± 0.44
39	6.24 ± 0.43	5.86 ± 0.45	5.60 ± 0.33	5.89 ± 0.50
66	6.04 ± 0.57	6.47 ± 0.55	5.12 ± 0.46	5.42 ± 0.33
92	6.52 ± 0.31	6.49 ± 0.66	5.17 ± 0.64*	6.19 ± 0.46
104	6.28 ± 0.15	6.90 ± 0.16	5.38 ± 0.49*	6.25 ± 0.51
Reticulocyte Count (%)				
14	6.25 ± 1.90	6.00 ± 1.41	5.75 ± 2.36	5.00 ± 2.16
39	7.50 ± 1.91	4.75 ± 0.50	10.25 ± 2.22	11.25 ± 2.63
66	6.00 ± 3.16	4.50 ± 1.00	4.75 ± 1.71	7.00 ± 1.83
92	3.50 ± 0.58	5.75 ± 1.50	4.75 ± 0.96	6.25 ± 1.71*
104	4.25 ± 1.71	3.75 ± 1.71	5.00 ± 2.45	4.50 ± 2.38

[a]Means and standard deviations recalculated by reviewers; data statistically analyzed by reviewers using Dunnett's test.

*Significantly different from control values at $p < 0.05$.

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b. Clinical Chemistry:

	Electrolytes		Other
	Calcium†	X	Albumin†*
	Chloride†	X	Albumin/globulin ratio*
	Magnesium†	X	Blood creatinine†
	Phosphorus†	X	Blood urea nitrogen†
	Potassium†	X	Cholesterol†
	Sodium†	X	Globulins*
		X	Glucose†
	Enzymes		Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
X	Cholinesterase (erythrocyte, plasma, brain)	X	Total protein†
	Creatine phosphokinase†		Triglycerides
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

Results: Tables 5 and 6 summarize results of alkaline phosphatase and cholinesterase activity data, respectively. Initial alkaline phosphatase levels measured at study initiation varied widely among individual animals. The alkaline phosphatase levels of high-dose males and females were increased throughout the study; increases were significant in high-dose males at weeks 27, 39 ($p < 0.05$), 66, 79, 92, and 104 ($p < 0.01$) and in high-dose females at week 92 ($p < 0.05$). Increases were highest in males when compared to females and highest in both sexes at week 104. Slight changes in albumin, globulin, and albumin/globulin ratios were observed in high-dose animals following an increase in test diet concentration to 300 ppm; these changes (depressed albumin, elevated globulin, and depressed albumin/globulin ratio) were especially apparent in animal Nos. 535 and 537 at week 104.

The plasma and erythrocyte cholinesterase activities of mid- and high-dose males and females were depressed from study weeks 1 to 103. The depression in plasma cholinesterase ranged from 24 to 64% in mid-dose animals ($p < 0.05$) and from 46 to 88% in high-dose animals ($p < 0.01$). The depression in erythrocyte cholinesterase ranged from 4 to 17% in mid-dose animals and from 23 to 50% in high-dose animals; depressions were significant ($p < 0.01$) in males at weeks 39, 79, and 103 and in females at week 103. The inhibitory effect on erythrocyte cholinesterase activity was more pronounced in males.

*Measured by serum protein electrophoresis.
†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 5. Mean Alkaline Phosphatase Levels (mU/mL) in Dogs Administered Isofenphos for 104 Weeks[a]

	Dietary Level (ppm)							
	Males				Females			
Week	0	3/2	15	75/150/300	0	3/2	15	75/150/300
0	166.5 ± 52.37	157.0 ± 35.80	148.8 ± 22.59	190.0 ± 38.30	174.8 ± 25.47	193.8 ± 44.17	209.0 ± 68.75[b]	228.0 ± 34.52
14	96.8 ± 20.85	102.0 ± 28.44	91.0 ± 14.88	137.0 ± 19.15	98.8 ± 30.10	137.3 ± 26.23	115.8 ± 24.17	128.8 ± 30.53
39	63.0 ± 11.34	71.8 ± 20.52	57.8 ± 12.66	95.5 ± 8.58*	78.8 ± 26.70	107.3 ± 18.17	92.5 ± 24.31	108.8 ± 26.13
66	46.5 ± 12.18	55.8 ± 18.73	50.3 ± 16.78	123.5 ± 36.08**	66.8 ± 22.10	79.8 ± 18.01	76.0 ± 25.32	113.5 ± 46.09
92	47.3 ± 8.66	57.3 ± 24.13	41.3 ± 8.06	173.3 ± 57.05**	64.5 ± 22.75	95.5 ± 16.82	80.0 ± 23.40	131.5 ± 46.29*

[a]Based on four animals/sex/group.

[b]Individual data of one animal not clearly indicated in study report.

*Significantly different from controls at $p < 0.05$ as analyzed by the reviewers using Dunnett's test.

**Significantly different from controls at $p < 0.01$ as analyzed by the reviewers using Dunnett's test.

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TABLE 6. Representative Results of Cholinesterase Activity (± S.D.) in Dogs Administered Isofenphos for 104 Weeks[a]

Parameter/ Week	Dose Groups (ppm)							
	Males				Females			
	0	3/2	15	75/150/300	0	3/2	15	75/150/300
Erythrocyte Cholinesterase (µM/mL)								
0	8.4 ± 1.72	8.9 ± 0.67	7.8 ± 0.97	9.6 ± 1.35	9.8 ± 1.18	8.9 ± 2.35	10.2 ± 1.37	9.0 ± 0.76
14	7.5 ± 1.25	8.7 ± 1.46	6.8 ± 1.35	4.9 ± 1.00*	8.2 ± 0.95	8.5 ± 1.36	8.3 ± 1.49	5.6 ± 0.90*
39	9.3 ± 2.18	9.1 ± 1.28	7.8 ± 0.75	3.7 ± 1.49**	8.6 ± 1.84	8.9 ± 0.78	9.1 ± 2.64	5.7 ± 1.54
79	9.2 ± 2.13	8.9 ± 2.09	7.5 ± 1.36	2.6 ± 0.67**	9.1 ± 1.99	9.1 ± 1.99	7.6 ± 1.81	5.7 ± 1.16
103	8.7 ± 1.94	8.5 ± 2.02	7.1 ± 1.09	1.0 ± 0.73**	8.5 ± 1.13	8.4 ± 0.89	9.1 ± 1.17	1.3 ± 0.34**
Plasma Cholinesterase (µM/mL)								
0	7.8 ± 1.51	8.0 ± 0.62	8.1 ± 1.09	7.7 ± 0.83	7.2 ± 1.44	8.1 ± 0.65	7.8 ± 0.36	7.7 ± 0.93
14	7.2 ± 0.94	7.6 ± 1.01	5.1 ± 2.15	2.6 ± 0.52**	6.8 ± 1.34	6.6 ± 0.50	5.7 ± 0.66	5.08 ± 1.45[b]
39	8.2 ± 1.55	6.5 ± 0.42	6.7 ± 0.54*	2.1 ± 0.29**	6.8 ± 0.97	6.2 ± 0.51	4.7 ± 1.07*	3.7 ± 0.94**
79	8.5 ± 1.19	6.5 ± 0.53	4.4 ± 0.80**	0.6 ± 0.15**	7.5 ± 1.87	6.7 ± 1.92	4.1 ± 0.50*	1.8 ± 0.84**
103	8.5 ± 1.75	7.8 ± 0.50	4.4 ± 1.58**	0.9 ± 0.25**	6.8 ± 1.33	6.2 ± 0.40	4.8 ± 1.14*	0.9 ± 0.85**
Brain Cholinesterase (µM/g)								
104	0.67 ± 0.107	0.64 ± 0.139	0.63 ± 0.143	0.23 ± 0.252**	0.69 ± 0.185	0.74 ± 0.206	0.62 ± 0.170	0.23 ± 0.073**

[a]Means ± S.D. recalculated by reviewers; data statistically analyzed by reviewers using Dunnett's test.

[b]Mean value reported as 5.07; reviewer was unable to read one individual value because of a poor copy of the study report.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

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Following the increase in dosing from 150 to 300 ppm at week 100, the plasma and erythrocyte cholinesterase activities were depressed by 88 and 90% in high-dose males and 88 and 86% in high-dose females, respectively. Brain cholinesterase activity was depressed 66% and 67% ($p < 0.01$) in high-dose males and females, respectively, at week 104.

The study authors indicated that the dietary concentration of the test material in low-dose males was reduced from 3 to 2 ppm at study week 84 as a result of the reduction of plasma cholinesterase activity more than 20%; this reduction was not observed in low-dose males when the data were reanalyzed by the reviewers.

6. Urinalysis: Urine was collected from fasted animals at 6-hour periods prior to study initiation and at weeks 14, 27, 39, 53, 66, 79, 92, and 104. The CHECKED (X) parameters were examined:

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

Results: There was no effect of dosing on urinary parameters.

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

†Recommended by Subdivision F (November 1984) Guidelines.

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

Results:

a. Organ Weights: There were no effects of dosing on organ weights. Lung, liver, and kidney weights of one high-dose male (animal No. 537) which was found dead at week 104, were increased; the study authors reported these increases were due to the nonexsanguinated state of the organs at the time of weighing. In addition, the organs of the high-dose male sacrificed moribund (animal No. 535) at study termination were nonexsanguinated when weighed. When the organ weights of the high-dose males are recalculated without the weights of these outliers, the weights are similar to those of concurrent controls.

[a]Two bone marrow smears were prepared for each dog.

†Recommended by Subdivision F (November 1984) Guidelines.

b. Gross Pathology: The gross lesions reported by the study authors included increased blood in the gastrointestinal tract and spleen, focal stratifications on the spleen, and focal areas of pulmonary tissue consolidation. The severity of the lesions was not described; the incidence was sporadic among dosed and control animals. Individual data were not reported.

c. Microscopic Pathology:

1) Nonneoplastic: The study report did not present a histopathologic tissue inventory or summary of histopathologic lesions. Erosions of the esophageal mucosa were found in 3/4 high-dose males, severe inflammatory cell infiltrations of the lung were found in one high-dose male that died at study week 100, and vacuoles of minimal severity were found on the brainstem of 2/4 high-dose males (Table 7). These lesions were considered by the study authors to be related to dosing. Sproradic thyroid lesions were considered to be spontaneous.

2) Neoplastic: There were no neoplastic lesions reported.

D. STUDY AUTHORS' CONCLUSIONS:

Groups of four male and four female Beagle dogs were administered isofenphos in the diet at doses of 0, 3/2, 15, or 75/150/300 for 104 weeks. To obtain a toxic effect, the dietary concentration of high-dose animals was raised from 75 to 150 ppm at week 54. Cholinesterase activities of these animals were severely inhibited by the end of the second year of dosing, and clinical signs of trembling, salivation, vomiting, loose stool and uncoordinated movements were observed as a result. To intensify the effect, the dose level of high-dose animals was again raised to 300 ppm. One high-dose male died at study week 100 and one high-dose male was sacrificed moribund at week 104. The cause of death of these animals was attributed to the severe depression of erythrocyte cholinesterase activity. Body weights of 7/8 high-dose animals were depressed following the increase in the test diet concentration to 300 ppm. There were no other changes in body weights. Alkaline phosphatase activity was increased in high-dose animals following the increase in dietary concentration from 75 to 150 ppm. Changes in albumin, globulin, and albumin/globulin ratios were observed in high-dose males (animal Nos. 535 and

TABLE 7. Representative Nonneoplastic Histopathological Findings of Dogs Administered Isofenphos for 104 Weeks

Organ/Parameter	Dose Group (ppm)							
	Males				Females			
	0	2/3	15	75/150/300	0	2/3	15	75/150
Thyroid	(4)[a]	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Collections of lymphocytic cells or lymph follicles								
Moderate	0	0	0	1	0	0	0	0
Severe	0	0	0	0	0	0	0	1
	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Esophagus								
Erosion of esophageal mucosa, cellular infiltration, and proliferation of propria mucosa								
Moderate	0	0	0	2	0	0	0	0
Severe	0	0	0	1	0	0	0	0
	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Lung								
Inflammatory cell infiltrates								
Very minimal	0	1	1	1	0	1	1	2
Severe	0	0	0	1	0	0	0	0
	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Brainstem								
Vacuoles	1	0	0	2	0	0	0	0

[a]Numbers in parentheses equal number of tissues examined.

and 537) that died or were sacrificed moribund. Microscopic lesions of the esophagus and brainstem were considered to be possibly related to dosing. Splenic and thyroid lesions were considered to be spontaneous. The NOEL for cholinesterase depression is 3 ppm in females and 2 ppm in males, and the NOEL for somatic damage is 15 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate; however, many deficiencies in study conduct and data reporting were noted. Analyses of homogeneity, stability, and test material concentration in the test diets were not provided in the study report. The period of acclimation for the test animals was not reported. Individual data were not provided for clinical observations, ophthalmology, or gross pathology. Summary data group tabulation for body weights, body weight gains, hematology, and clinical chemistry parameters were incorrect when means were calculated from individual data provided. Other summary data were not provided (i.e., food consumption standard deviations, hematology data for females, histopathologic tissue inventory or summary of histopathologic lesions). The reviewers recalculated means and standard deviations of individual data and statistically analyzed differences between control and test animals using Dunnett's test. The study was performed prior to Good Laboratory Practices requirements; a quality assurance statement was not provided. Methods of statistical analysis were not reported.

Test animals were randomized based on the range of plasma cholinesterase activity. As a result, body weights of dogs in control and test groups were not similar at study initiation. The study authors' description of histopathological findings of the thyroid contradicted the individual data tabulated for the thyroid.

The reviewers question the accuracy of the tabulation of individual hematology data for females. Using these data to recalculate means and standard deviations, the results indicated that the hematocrit, hemoglobin concentrations, and erythrocyte counts of mid-dose females were consistently depressed throughout the study while the levels of these parameters in high-dose females were similar to controls. The opposite was indicated for high-dose males. The study authors considered the slight food consumption and hematological changes to be of no importance; the reviewers believe these changes should be recognized as compound-related changes.

Pesticide Assessment Guidelines (1984) for chronic nonrodent studies indicate that the highest dose level should produce no fatalities. Cholinesterase activities of high-dose animals

were severely inhibited when these animals were administered 150 ppm isofenphos; debilitating clinical signs were apparent in three high-dose males. However, the high-dose level was elevated to 300 ppm at study week 100 to obtain a clinically recognizable toxic effect. Two high-dose males died or were sacrificed moribund as a result. Based on the depression of plasma and erythrocyte cholinesterase activity, the NOEL is 3 ppm for females and 2 ppm for males. The LOAEL for systemic toxicity was between 3 and 15 ppm.

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

009749

EPA No.: 68D80056
DYNAMAC No.: 359-F
TASK No.: 3-59F
November 5, 1991

SEP 2 3 1992

DATA EVALUATION RECORD

ISOFENPHOS

Mutagenicity--*Salmonella typhimurium*/Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 11/4/91

00035

Guideline Series 84: Mutagenicity

EPA No.: 68D80056
DYNAMAC No.: 359-F
TASK No.: 3-35F
November 5, 1991

DATA EVALUATION RECORD

ISOFENPHOS

Mutagenicity--*Salmonella typhimurium*/Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

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(H-7509C)

Signature: E A Doyle
Date: 9/9/92

Salmonella

DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: Isofenphos.

STUDY TYPE: Salmonella/mammalian activation gene mutation assay.

MRID NUMBER: 416099-12.

SYNONYM(S)/CAS NUMBER: None listed/25311-71-1.

SPONSOR: Mobay Corp., Stilwell, KS.

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD.

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test).

AUTHOR: San, R.H.C., and Wagner, V.O.

STUDY NUMBER: T9341.501.

REPORT ISSUED: August 17, 1990.

CONCLUSIONS - Executive Summary: Isofenphos was evaluated for the potential to cause gene mutations in the *Salmonella typhimurium*/mammalian microsome plate incorporation mutagenicity assay. The five nonactivated or five S9-activated doses ranged from 667 to 10,000 µg/plate; doses ≥3333 µg/plate +/-S9 precipitated. Results indicated that isofenphos was neither cytotoxic nor mutagenic in any strain either with or without S9 activation in a well-controlled study. We assess, therefore, that an appropriate range of test material doses was evaluated and that isofenphos was not mutagenic in this test system. The study, therefore, fulfills Guideline requirements for Category I, Gene Mutations.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material:

Name:	Isofenphos technical.
Description:	Clear, colorless liquid.
Batch No.:	8005258/8030052.
Purity:	92.3%.
Contaminants:	None listed.
Solvent used:	Dimethyl sulfoxide (DMSO).
Other comments:	The test material was stored at -20°C. Dosin solutions were prepared immediately prior t use. Aliquots of the highest and lowes dosing solutions were held at -20°C an shipped to the sponsor for analytica determinations. Analytical data accompanyin the study report indicated that the actua concentration of the high and low doses wa ≈110% of the nominal concentrations.

2. Control Materials:

Negative: DMSO

Solvent/final concentration: 50 µL/plate

Positive: Nonactivation:

Sodium azide	1.0 µg/plate	TA100, TA1535
2-Nitrofluorene	1.0 µg/plate	TA98, TA1538
ICR-191	2.0 µg/plate	TA1537

Other:

Activation:

2-Aminoanthracene (2-anthramine) 0.5 µg/plate all strains.

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

If other, describe below. Describe S9 composition (i purchased, give details). The S9 liver homogenate was prepare by the performing laboratory and was characterized for it ability to metabolize 7,12-dimethylbenzanthracene and 2 aminoanthracene to mutagenic forms prior to use.

S9 mix composition:

H_2O	0.56 mL
1.00 M NaH_2PO_4/K_2HPO_4 (pH 7.4)	0.10 mL
0.05 M Glucose 6-phosphate	0.10 mL
0.04 M NADP	0.10 mL
0.20 M $MgCl_2$/0.825 M KCL	0.04 mL
S9	0.10 mL
TOTAL	1.00 mL

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538; list any others:

Test organisms were properly maintained?: Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)?: Yes.

5. Test Compound Concentrations Used:

Preliminary cytotoxicity assay: Ten doses (10, 33, 67, 100, 333, 667, 1,000, 3,333, 6,667, and 10,000 µg/plate) were evaluated with or without S9 activation in S. typhimurium strain TA100. Single plates were used per dose per condition.

Mutation assay: Five doses (667, 1,000, 3,333, 6,667 and 10,000 µg/plate) were evaluated in all tester strains in both the presence and the absence of S9 activation. Triplicate plates were prepared per dose per strain per condition.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x__ Standard plate test
_____ Pre-incubation (___) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

a. Protocol:

1) Plating procedures: In general, similar procedures were used for the preliminary cytotoxicity and the mutation assays.

To tubes containing 2.5-mL volumes of molten top agar, 100 µL of an overnight broth culture of the appropriate tester strain and 50 µL of the appropriate test material dose, solvent, or

positive controls were added. For the S9-activated test, 0.5 mL of the S9 mix was added to tubes containing 2.0 mL of top agar; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37° ± 2°C for ≈48 hours. At the end of incubation, plates either were immediately scored for revertant colonies or were refrigerated and subsequently counted. Means and standard deviations were determined for the mutation assay.

2) Sterility controls: A sterility test was performed on the highest dose of the test material and 0.5 mL of the S9 mix as described for the mutation assay.

b. Evaluation Criteria:

1). Assay validity: The assay was considered valid if the following criteria were met: (1) the presence of the appropriate genetic markers was verified for each strain; (2) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; (3) cell densities were ≥6.0 x 10^8 cells/mL; and (4) all positive controls caused at least a 3-fold increase in revertants per plate compared to the respective solvent control.

2) Positive response: The test material was considered positive if it caused a ≥2-fold increase in mean revertant colonies of strains TA98 and TA100, or if it caused a ≥3-fold increase in mean revertant colonies of strains TA1535, TA1537, or TA1538 and the increase was accompanied by a dose-response to increasing concentrations of the test material.

2. Preliminary Assay: Ten doses ranging from 10 to 10,000 µg/plate +/-S9 were assayed for cytotoxic effects on strain TA100. Slight compound precipitation was observed on plates containing the two highest nonactivated doses (6,667 and 10,000 µg/plate) and the three highest S9-activated doses (3,333, 6,667, and 10,000 µg/plate). There was, however, no evidence of a cytotoxic effect at any test material level either with or without S9 activation. Based on these findings, the five doses selected for the mutation assay ranged from 667 to 10,000 µg/plate +/-S9.

3. Mutagenicity Assay: Representative results from the mutation assay are presented in Table 1. As shown, isofenphos was neither cytotoxic nor mutagenic at any assayed level either in the presence or absence of S9 activation. Although slightly lower than control revertant colony counts were observed for the majority of strains over the nonactivated and S9-activated dose ranges, these reductions were not considered sufficient to conclude a cytotoxic effect. Also noted at doses ≥3333 µg/plate +/- S9 was slight precipitation of the test material.

   In contrast to the uniformly negative test material results, all strains responded to the appropriate nonactivated or S9-activated positive control.

   Based on the overall findings, the study authors concluded that isofenphos was not mutagenic in this test system.

4. Reviewers' Discussion/Conclusions: We assess that the study was properly conducted and that the study authors interpreted the data correctly. Isofenphos was assayed over a dose range that included precipitating levels but failed to induce either a cytotoxic or mutagenic effect.

   The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results with the nonactivated and S9-activated positive controls. We conclude, therefore, that Isofenphos was assayed over an appropriate range of test material concentrations with no indication of a mutagenic effect in a well-controlled study.

5. Quality Assurance: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated August 17, 1990.)

6. CBI Appendix: Appendix A, Materials and Methods, CBI pp. 10-18; Appendix B, Protocol, CBI pp. 39-50.

TABLE 1. Representative Results of the *Salmonella typhimurium* Mutagenicity Assay with Isofenphos

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	--	10 ± 2	7 ± 3	10 ± 5	16 ± 6	140 ± 23
	+	--	14 ± 6	8 ± 3	17 ± 5	22 ± 6	179 ± 14
Positive Control							
Sodium azide	-	1.0	402 ± 11	--	--	--	435 ± 20
2-Nitrofluorene	-	1.0	--	--	393 ± 37	183 ± 109	--
ICR-191	-	2.0	--	88 ± 10	--	--	--
2-Anthramine	+	0.5	185 ± 3	304 ± 9	1955 ± 132	1739 ± 99	1610 ± 159
Test Material							
Isofenphos	-	1000[b]	6 ± 2	5 ± 1	6 ± 3	11 ± 4	150 ± 12
	-	10,000[c]	8 ± 4	4 ± 2	4 ± 1	13 ± 2	156 ± 21
	+	1000	16 ± 3	6 ± 3	16 ± 2	22 ± 2	179 ± 6
	+	10,000[c]	10 ± 4	8 ± 4	12 ± 1	22 ± 4	160 ± 10

[a]Means and standard deviations of counts from triplicate plates.

[b]Highest nonprecipitating level; results for the lowest concentration (667 µg/plate +/-S9) did not suggest a mutagenic effect.

[c]Highest assayed level; slight compound precipitation observed at this concentration and also at 3333 and 6667 µg/plate +/-S9. The revertant colony counts for the intermediate doses (3333 and 6667 µg/plate +/-S9) did not suggest a mutagenic effect.

APPENDIX A
Materials and Methods
(CBI pp. 10-18)

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~~APPENDIX B~~

Protocol

(CBI pp. 39-50)

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