CASWELL FILE

005008

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

MAR 28 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

SUBJECT: Chronic Feeding Study in Hamsters

FROM: Thomas Edwards, Pharmacologist W Thomas Edwards 3-24-86
Hazard Evaluation Division (TS-769)

TO: Robert Taylor
Registration Division (TS-767)

THRU: Clint Skinner, Section Chief Alan C. Kat for CS 3/26/86
Review Section III

and

Theodore Farber, Chief N/A 3/28/86
Toxicology Branch, HED (TS-769)

Chemical: diallate

Caswell No.: 299

EPA Registration No.: 524-306

Accession Nos.: 258000

Requested Actions: Review. (RS)

Comments:

This reply is for response to Monsanto's correspondence of 4-26-85, however also see Monsanto's letter of 5-7-84 and Toxicology Branch comments of 10-10-84, attached.

The Chronic Hamster Study report adds little of value to the preliminary information in the letter of 5-7-84. In several respects needed histopathology is lacking. This study does not meet EPA guideline requirements. See attached DER for more details. It does not fulfill its stated intention to further support the registration of diallate and Avadex herbacide.

Regarding Oncogenic effects, the data give evidence that diallate can present risk to forestomach and skin. Melanomas of skin were included in considerations included in EPA PD 4.

No additional tolerances or lessening of restrictions should be permitted without other information.

Reguarding a NOEL, a NOEL was not determined. We do not have a NOEL from a valid Chronic or a valid Subchronic feeding study which can be used for future registration.

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EPA: 68-02-4225
DYNAMAC No. 1-015-A
March 24, 1986

DATA EVALUATION RECORD

DIALLATE

Chronic Feeding Study in Hamsters

STUDY IDENTIFICATION: Adams, R. A. Lifetime chronic toxicity study of diallate technical administered orally to Syrian golden hamsters. (Unpublished study No. C-258, project No. BR-81-391, prepared by Bio-Research Consultants, Inc., Cambridge, MA, for Monsanto Co., St. Louis, MO; dated April 16, 1985.) Accession Nos. 257999-258002.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 3-24-86

1. CHEMICAL: Diallate; S-(2,3-dichloroallyl)diisopropylthiolcarbamate.

2. TEST MATERIAL: Diallate technical, from lot No. LBDD-07-28, contained 98.14 percent active ingredient.

3. STUDY/ACTION TYPE: Chronic feeding study in hamsters.

4. STUDY IDENTIFICATION: Adams, R. A. Lifetime chronic toxicity study of diallate technical administered orally to Syrian golden hamsters. (Unpublished study No. C-258, project No. BR-81-391, prepared by Bio-Research Consultants, Inc., Cambridge, MA, for Monsanto Co., St. Louis, MO; dated April 16, 1985.) Accession Nos. 257999-258002.

5. REVIEWED BY:

Robert J. Weir, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: James R. Blaut for RJW
Date: March 24, 1986

Charles E. Rothwell, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Charles E. Rothwell
Date: 3-24-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Chronic Toxicology
Technical Quality Control
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Signature: I. Cecil Felkner
Date: 3-24-86

W. Thomas Edwards
EPA Reviewer

Signature: W. Thomas Edwards
Date: 3-24-86

Clint Skinner, Ph.D.
EPA Section Head

Signature: Alan Kat for CS
Date: 3/26/86

7. CONCLUSIONS:

A. A NOEL was not demonstrated in this study. The triglyceride levels were significantly lower at all doses when compared to the controls. Furthermore, gross changes in skin pigmentation appear to be prevalent at all doses in the females. In the high-dose males, the mortality and food consumption were increased. In the high-dose females, body weight was increased whereas food consumption was decreased. Organ weight effects were seen in liver and adrenals of the high-dose males. There were no gross pathologic correlates to the organ weight changes. Gross findings in addition to skin pigmentation possibly indicated neoplasia, including melanoma and papillomatous growths in the forestomach. The results of this study cannot be completely evaluated without histopathologic data, which were not reported in full.

B. This study is Core Supplemental because data on pathology, urinalysis, and test material homogeneity and stability in the diet were not presented. In addition, a NOEL was not demonstrated. Mortality was increased and resulted in early termination; life table analysis should be conducted on mortality, but was not done by the reviewers because the study had other deficiencies.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The hamsters used in this study were first-generation hybrids of B10 15.16 sires and B10 87.20 dams. This hybrid strain was designated B10 F_1D Alexander and was maintained by Bio-Research Consultants, Inc., Cambridge, MA. The study was started with 70 males and 70 females for the dose groups and 120 males and 120 females for the control population. An additional control group of 10 hamsters per sex was sacrificed before study initiation to obtain pretest hematologic and blood chemistry values. Six days prior to the start of the study, the animals were between 32 and 36 days of age with body weights ranging from 63 to 84 g for the males and from 59 to 75 g for the females.

2. The animals were housed individually in plastic box-type cages with stainless steel tops. The animal room conditions were controlled to 68-76°F, 40-60% relative humidity, and a 12 hour light, 12 hour dark cycle of illumination. The facility was a barrier-type facility with 10-15 air changes/hour.

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

3. Diets containing test material were prepared once a week. The test material was mixed with Purina Certified Rodent Chow 5002 to provide nominal diallate concentrations of 20, 200, and 1000 ppm. Prior to study initiation, duplicate samples of the diet from the top, middle, and bottom of a freshly mixed batch were sent to the sponsor for determination of the homogeneity of mixing. Samples of all three dose levels were submitted throughout the study for determination of diallate concentration and stability in the test diet. Male hamsters were fed the test material-treated diets for 24 months. Females were maintained on study for 20 months. Tapwater was provided to all animals ad libitum.

4. Animals were observed twice daily for mortality and for signs of toxicity. Physical examinations were made weekly. Body weights were recorded weekly through 4 months and monthly thereafter. Food and water consumption were measured weekly through week 4, every 2 weeks through month 4, and then monthly until termination.

5. Hematologic evaluation consisting of white blood cell, red blood cell, and differential white blood cell counts and clinical chemistry determinations consisting of glucose, blood urea nitrogen, alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase (GPT), lactic dehydrogenase, creatinine, uric acid, cholesterol, triglycerides, total protein, albumin (A), globulin (G), A/G, total bilirubin, calcium, phosphorus, hemoglobin determinations, mean corpuscular volume, and mean corpuscular hemoglobin (MCH) concentration were conducted at 6 and 12 months and at termination on blood samples taken from 10 randomly selected animals/sex/dose in addition to the pretest control group.

6. Ten randomly selected hamsters of each sex from each group and 17 control animals of each sex were killed at 12 months for an interim evaluation. At termination, necropsies were performed on all surviving animals. At both scheduled sacrifices weights were recorded for brain, heart, spleen, thymus, adrenals, liver, gonads, uterus, and kidneys, and approximately 41 tissues were retained but not examined histologically. The skin of all animals with pigment lesions, along with lungs showing possible foci of metastatic formation, were also examined histopathologically.

7. Statistical significance of the differences between test and control mean values were evaluated by the Dunnett test. Mortality was evaluated using the Natrella[2] method.

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[2] Natrella, M. G. Experimental Statistics. National Bureau of Standards, Handbook 91, Government Printing Office, August 1, 1963, Table A28, pp. T-55 to T-58.

B. Protocol: See Appendix A for protocol and amendments.

12. REPORTED RESULTS:

A. Dietary Analysis: The diallate content of the diets was generally in agreement with the nominal dose. However, due to formulation errors during weeks 56 and 61, the low-dose animals were fed <5 ppm diallate rather than the intended 20 ppm. The data on the stability of the test material in the diet and the homogeneity of mixing were not included in this report.

B. Mortality and Signs of Toxicity: Mortality just prior to termination was slightly increased in the high-dose male hamsters (Table 1). Mortality for all other test groups was comparable to the control animals. The study was to last 24 months; however, because of frequent deaths, an early termination was implemented. There was a slightly elevated incidence of pigmented lesions (Table 2) of the skin of the high-dose males. There were no other clinical signs of toxicity or behavioral changes that could be associated with the administration of the test material.

C. Body Weight and Food and Water Consumption: Mean body weights at various times are presented in Table 3. The female 1000-ppm dose group had significantly ($p \leq 0.05$) increased body weights at 1, 26, and 54 weeks. Selected mean food consumption values are presented in Table 4. At week 2, food consumption was increased for the high-dose males and decreased for the high-dose females. A statistically significant increase ($p \leq 0.05$) was also seen in the mid-dose males at 54 weeks, but this was not part of a dose-related pattern. In the female high-dose group, all values were lower than the control, and they were statistically significant ($p \leq 0.05$) at 2 and 54 weeks. Nevertheless, the authors did not consider this trend in reduced food consumption to be biologically significant in either sex as a dose effect was not consistent with time. Water consumption was variable in both sexes.

D. Hematology: Aside from MCH, there were no hematological differences between the control and dosed females at any dose or interval of evaluation. The study author indicates certain significant differences for MCH (Table 5) at various intervals when compared to their controls. The study author concludes that these are not biologically meaningful differences.

E. Clinical Chemistry: The triglyceride values (Table 6) of hamsters fed diallate in the diet were significantly ($p < 0.01$) lower than those of the controls at 6 months. The males were significantly lower ($p \leq 0.01$) in all dose groups whereas the females were significantly lower ($p \leq 0.01$) only in the 1000-ppm group. At 1 year

TABLE 1. Percent Mortality by Dose Groups Prior to Terminal Kill

Dose (ppm)	Males[a]	Females[b]
0	52	64
20	53	73
200	47	78
1000	70	63

[a]After 96 weeks on study.

[b]After 80 weeks on study.

TABLE 2. Incidence [observed/dosed (%)] of Abnormal Skin Pigmentation[a] in Male and Female Hamsters Fed Diallate for 80 to 90 Weeks

Dose (ppm)	Males	Females
Control	5/120 (4%)	4/120 (3%)
20	2/70 (3%)	4/70 (6%)
200	3/70 (4%)	6/70 (9%)
1000	7/70 (10%)	3/70 (4%)

[a]The data were recorded as two findings. However, increased black pigmentation around the eyes and whiskers and increased black spots and pigmentation of the skin are combined for the purpose of this table.

TABLE 3. Selected Mean Body Weights[a] of Hamsters Fed Diallate for 20 Months (Females) or 24 Months (Males)

Dose (ppm)	Body Weights (g) at Week 1	26	54	78	94
			MALES		
0	85.8 ±5.9	144.6 ±9.2	146.7 ±10.4	136.0 ±11.3	126.6 ±13.4
20	85.3 ±6.3	144.3 ±7.8	148.0 ±7.3	137.7 ±11.4	131.0 ±12.4
200	84.9 ±5.9	144.0 ±7.6	147.9 ±8.6	138.3 ±9.2	128.3 ±13.4
1000	85.0 ±6.5	143.5 ±8.4	147.2 ±9.6	137.2 ±13.5	132.8 ±11.5
			FEMALES		
0	77.6 ±4.8	131.5 ±12.1	132.3 ±12.6	132.0 ±12.9	-- --
20	78.9 ±5.4	133.4 ±10.6	134.9 ±10.9	134.2 ±12.0	-- --
200	76.7 ±5.4	132.6 ±11.9	136.4 ±10.7	129.6 ±14.9	-- --
1000	81.3*/*a ±4.2	136.0* ±11.9	137.2* ±11.2	137.7 ±11.6	-- --

[a]Group mean body weight ± standard deviation.

*Significantly different from control value ($p \leq 0.05$) as calculated by the study author.

*[a]significantly different from control value ($p < 0.05$) as calculated by our reviewers using ANOVA and Dunnett's test for significance.

TABLE 4. Selected Mean Food Consumption Values[a] for Hamsters Fed Diallate for 20 Months (Females) or 24 Months (Males)

Dose (ppm)	Food Consumption (g/kg/day) at Week				
	2	26	54	78	94
			MALES		
0	87.5 ±12.5	49.4 ±4.5	49.5 ±5.9	66.2 ±15.2	60.4 ±15.4
20	86.4 ±9.7	48.8 ±5.3	48.3 ±3.8	65.7 ±11.5	55.2 ±14.9
200	86.7 ±12.9	48.7 ±5.2	52.1* ±6.8	68.7 ±9.9	57.0 ±17.2
1000	92.1* ±12.5	49.5 ±5.6	50.0 ±6.6	66.1 ±12.5	56.4 ±14.9
			FEMALES		
0	97.4 ±11.7	54.1 ±5.8	61.1 ±7.9	56.1 ±15.0	-- --
20	97.0 ±14.9	54.1 ±5.7	60.9 ±7.5	52.3 ±11.0	-- --
200	94.8 ±8.4	52.1 ±5.1	57.1 ±7.4	58.0 ±13.2	-- --
1000	91.5* ±6.6	52.8 ±5.6	55.1* ±8.1	49.7 ±11.5	-- --

[a]Mean food consumption ± standard deviation.

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

TABLE 5. Mean Corpuscular Hemoglobin[a] for Male Hamsters Fed Diallate for 24 Months

Dose (ppm)	6 Months			1 Year			Termination		
	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.
0	10	21	0	17	22	1	47	22	1
20	10	21	0	10	22*	0	28	22	1
200	10	21	1	10	22**	1	27	22	1
1000	10	20**	1	10	22	1	15	22	1

[a]Values are expressed as picograms.

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

**Significantly different from control value ($p < 0.01$).

TABLE 6. Selected Mean Clinical Chemistry Values[a] for Male and Female Hamsters

Dose (ppm)	6 Months		1 Year		Terminal	
	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Triglyercide (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)
Males						
0	137±25	144±14	89±27	124±12	125± 51	131±44
20	101±34**	135±14	81±23	127±33	112± 39	113±43
200	84±17**	132±15	57±16**	115±10	154±137	138±49
1000	76±18**	127±11*	52±14**	119±19	103± 50	138±50
Females						
0	101±22	116±17	144±62	133±38	146± 63	139±48
20	99±29	112±15	132±45	122±47	152± 56	146±49
200	81±30	130±41	85±16**	108±13	118± 62	156±43
1000	60± 8**	105±10	78±25**	112±21	88± 27**	128±47

[a]Values represent means ± S.D. of 10 hamsters/group at 6 months and 1 year (except for controls at 1 year, 17/group) and 13–47 animals/group at sacrifice.

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

**Significantly different from the control value ($p < 0.01$).

the two highest doses of both sexes showed significantly ($p < 0.01$) decreased triglyceride values. At termination the effect was not as severe, being significantly decreased ($p < 0.01$) only in the high-dose females. Cholesterol was also significantly decreased ($p \leq 0.05$) in the high-dose males at the 6-month interval. Globulin was significantly depressed in the high-dose males at 1 year. Serum GPT was also depressed in the high-dose females at 1 year. In neither case were they affected at 6 months or at termination.

F. Organ Weights: The report author provided individual and mean data on absolute and relative organ weights but no statistical significance is provided in the tables. However, the report author did provide information on significance in the text. The liver weights of high-dose males were significantly elevated ($p \leq 0.05$) at the 1-year interval. The relative liver weight was also significant in this group and interval. The kidney weight of the mid-dose females at the 1-year interim was significantly different ($p \leq 0.05$) from the controls. Depression of the absolute heart and liver weights at the mid dose in the females was not confirmed when relative organ-to-body weight was examined nor were there gross lesions in these two organs. At the terminal evaluation, there was no significant liver weight (absolute or relative) change evident in any group but there was a trend to dose-related increases in liver weight in both sexes. The adrenal weights were significantly different ($p \leq 0.05$) from the control values in the high-dose males. There was also a dose-related increase in the adrenal weights in the males at lower doses.

Due to the absence of statistical data the reviewers provided analysis as indicated in Table 7.

Mean kidney weights were decreased in the mid-dose female group at the interim sacrifice, but not in higher dose groups or in the males at any dose level. At termination, no effect on kidney weights was observed at any dose. At the interim sacrifice, the liver weights were significantly ($p < 0.05$) increased in the high-dose males but not in the females at any dose. At termination, the liver weight was unaffected at all doses, but the adrenal weight in the high-dose males was significantly ($p < 0.05$) heavier.

In the relative organ weight data (Table 8), it can be seen that the relative liver weight was significantly ($p < 0.05$) different from the control value in the high-dose males at the interim sacrifice. The females were not affected at the interim sacrifice and neither sex was different from the control at termination. The relative adrenal weights were significantly ($p < 0.05$) different from the control values in the high-dose males at termination. Relative kidney weights were significantly different ($p \leq 0.05$) from the controls in the mid-dose females at the interim sacrifice. This was not considered biologically significant, as a dose-effect relationship was absent.

TABLE 7. Selected Mean Organ and Body Weights[a] ± Standard Deviation for Male and Female Hamsters Fed Diallate for 2 Years

	Control	Low Dose	Mid Dose	High Dose
		Interim Males[b]		
Body	143.1 ± 7.2	137.0 ±17.0	143.5 ± 7.4	140.6 ±10.2
Kidney	1.306± 0.080	1.262± 0.116	1.308± 0.090	1.309± 0.120
Adrenal	25.6 ±10.1	24.5 ± 2.6	25.2 ± 2.5	22.5 ± 2.8
Liver	4.669± 0.404	4.623± 0.678	4.710± 0.448	5.271± 0.431*
		Interim Females[b]		
Body	136.9 ±13.6	135.3 ±15.4	128.8 ±11.0	134.7 ±11.1
Kidney	1.610± 0.167	1.578± 0.214	1.399± 0.127*	1.521± 0.123
Adrenal	15.8 ± 3.4	16.0 ± 5.8	14.0 ± 1.8	14.0 ± 4.0
Liver	5.364± 0.889	5.344± 0.742	4.627± 0.414	5.191± 0.468
		Terminal Males		
Body	125.0 ±14.1	124.8 ±14.5	124.2 ±12.8	129.3 ±11.3
Kidney	1.768± 0.294	1.758± 0.162	1.786± 0.197	1.950± 0.317
Adrenal	41.3 ±42.5	100.8 ±255.4	187.0 ±441.2	263.9 ±420.0*
Liver	5.42 ± 1.75	5.31 ± 1.50	5.87 ± 2.48	6.51 ± 1.88
		Terminal Females		
Body	131.2 ±13.2	129.8 ± 8.7	129.2 ± 8.5	133.5 ± 9.8
Kidney	1.858± 0.207	1.847± 0.144	1.777± 0.171	2.003± 0.702
Adrenal	26.9 ±15.4	28.5 ±16.5	22.8 ± 9.0	35.1 ±28.9
Liver	5.65 ± 1.16	5.61 ± 0.99	5.36 ± 0.80	5.85 ± 1.10

[a] Adrenals are recorded in milligrams; all other tissues and body weights are in grams.

[b] 12-month interval sacrifice.

*Significantly different from control value ($p < 0.05$) using Dunnett's t-test, as calculated by our reviewers.

TABLE 8. Selected Mean Relative Organ Weights (mg/g Body Weight) for Male and Female Hamsters Fed Diallate for 2 Years

	Control	Low Dose	Mid Dose	High Dose
		Interim Males[a]		
Kidney	9.15± 0.51	8.92± 0.81	9.13± 0.45	9.20± 0.52
Adrenal	0.1782±0.0675	0.1824±0.0355	0.1759±0.0205	0.1593±0.0258
Liver	32.75± 1.74	33.71± 1.92	32.78± 1.63	37.50± 1.82*
		Interim Females[a]		
Kidney	11.79± 0.68	11.67± 0.92	10.87± 0.58*	11.50± 0.89
Adrenal	0.1159±0.0212	0.1185±0.0417	0.1088±0.0147	0.1046±0.0262
Liver	39.02± 3.70	39.52± 3.77	36.00± 2.52	38.59± 2.50
		Terminal Males		
Kidney	14.31± 2.64	14.00± 1.60	14.48± 0.17	15.20± 2.92
Adrenal	0.330 ±0.325	0.790 ±2.004	1.472 ±3.410	2.222 ±3.633*
Liver	43.19± 11.64	41.68± 9.10	47.04± 17.56	49.93± 11.52
		Terminal Females		
Kidney	14.20± 1.20	14.26± 0.89	13.74± 1.04	14.96± 4.80
Adrenal	0.2068±0.1187	0.2234±0.1350	0.1740±0.0651	0.2617±0.2064
Liver	42.96± 7.06	43.24± 7.04	41.26± 4.45	43.86± 7.39

[a]12-month interval sacrifice.

*Significantly different from control value ($p < 0.05$) using Dunnett's t-test, as calculated by our reviewers.

G. Gross Necropsy Findings: The nonneoplastic gross lesions that occurred with greater frequency in the treated animals when compared to controls consisted of skin changes such as dry, leathery, flaky, thickened, ulcerated, infected, or reddened skin. The incidence was approximately twice as high in the high-dose males as in the controls. Abnormal skin pigmentation occurred at a higher frequency in high-dose males and in all dose groups of the females when compared to controls (Table 2). Whether the latter lesions were neoplastic or nonneoplastic cannot be determined with certainty without histopathologic evaluation. There was also an increased incidence of forestomach papilliform lesions (Table 9) in the high- and mid-dose groups for both sexes. No histologic confirmation was made on these lesions with regard to their neoplastic state. The incidence of nonneoplastic lesions of the spleen was greater in the high-dose group of both sexes than in the control groups.

H. Histopathology: The protocol did not require that histopathologic evaluation be performed on the tissues. No histopathologic examination was performed on the lesions of the forestomach in compliance with the protocol. However, some histopathologic examination of melanotic skin lesions selected on the basis of those lesions thought to be malignant melanoma by gross appearance was performed. As expected, microscopic findings did not always confirm the presence of malignant melanoma.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "The oral (dietary) administration of diallate to hamsters at dosage levels of 20, 200, and 1000 ppm for 20 months (females) and 24 months (males) resulted in increases in mortality, abnormal skin condition and pigmentation and liver weight in the high dose (1000 ppm) males. Dose-related depression of serum triglycerides was observed in both sexes and a dose related increase in adrenal weight was seen in the dosed males. An increased incidence of forestomach papilliform lesions was observed in high-dose males and mid- and high-dose females A dose-related increase in pre-malignant and/or malignant melanocytic lesions was observed for both sexes. A complete evaluation of this study is not possible without a complete microscopic analysis of tissues."

B. An undated but signed quality assurance certificate was included with the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Body weights were generally normal in the males, but lower than the controls in the high-dose females. During the first 66 weeks of the study, food consumption appeared elevated in the high-dose female group. The latter effect was inconsistent with the body weight effect unless the test compound affected energy metabolism. Water

TABLE 9. Selected Macroscopic Findings[a] at Autopsy from Hamsters Fed Diallate for a Lifetime

Organ	Males				Females			
	High Dose [60][b]	Mid Dose [60]	Low Dose [60]	Control [103]	High Dose [60]	Mid Dose [60]	Low Dose [60]	Control [103]
Forestomach[c]	7 (7)	5 (5)	3 (1)	8 (2)	2 (5)	(2)	1	2
Adrenal	17(12)	16(10)	18(10)	40(14)	18 (2)	12 (4)	17 (7)	27 (3)
Skin[d]	14 (8)	8 (5)	7 (5)	19 (3)	8 (3)	3 (1)	1 (1)	5 (2)
Spleen	13	8	7	11	19 (2)	8 (1)	9 (1)	14 (1)
Forestomach[e]	12(12)	8 (8)	5 (2)	8 (2)	3 (8)	(3)	2	3
Adrenal[e]	28(20)	26(16)	30(16)	39(14)	30 (3)	20 (6)	28(12)	26 (3)
Skin[e]	23(13)	13 (8)	12 (8)	18 (3)	13 (3)	3 (2)	2 (2)	8 (3)
Spleen[e]	21	13	12 (2)	11	31 (3)	13 (2)	15 (2)	14 (1)

[a] Intercurrent deaths and terminally killed animals. Values are numbers of hamsters with one or more nonneoplastic lesion. Numbers in parentheses are the numbers of hamsters with tumor.

[b] Number of hamsters examined.

[c] "Papillomatous growth" regarded as tumor.

[d] Excludes irregular, pigmented areas of the skin (IPAS) and melanocyte accumulation.

[e] Data expressed as percent of numbers of hamsters tested.

consumption was too erratic to conclude if it was affected by the test substance. Mortality was increased for the high-dose males just prior to termination. The cause of death was not indicated by the study author. Clinical signs were limited to the skin where dry, reddened, irritated, leathery, flaky, or thickened lesions were seen together with increased pigment accumulation in the high-dose males. The study author indicated that there were statistical differences in MCH for high-dose males when compared to the controls. We do not consider these changes either statistically or biologically significant. There were no hematological alterations in this study. Triglyceride levels were significantly reduced ($p < 0.01$) in the males at all dose levels at the 6-month interval, in the mid- and high-dose male groups at the 1-year interval, and were normal at all levels at termination. In females, the triglyceride levels were lower than the controls in a dose-related fashion at all intervals, but they were only significantly different from the control levels ($p < 0.01$) at the high dose (all sampling intervals) and in the 200-ppm group at 1-year. In addition, the cholesterol level was reduced for the high-dose males at the 6-month interval. Globulin and serum GPT were significantly depressed in the high-dose males at the 1-year interval. Since there was no change in these values at 6 months or at termination and since reduced serum GPT has no biological meaning, these findings may not have been biological significant.

At necropsy, the skin changes noted above were seen in high-dose males. Melanotic alterations were observed upon gross examination in the high-dose male animals and in all test groups in the females. In addition, papilliform lesions were found upon gross examination in the forestomach. None of the gross lesions cited above could account for the changes in organ weights, which are noted as follows:

a) Increased liver weight in the high-dose male group at the 1-year interim kill.

b) Increased adrenal weight in the high-dose male group at the 1-year interim kill.

c) Decreased kidney weights in the mid-dose female group at the 1-year interim had no biological significance; it did not follow a dose regression.

The relative organ weights for the liver in the high-dose males at the interim kill was different from the control as was the adrenal weight for the terminal high-dose male group.

Since little histopathologic evaluation was conducted on these tissues, no conclusions can be made on the real meaning of these data.

This study does not meet EPA guideline requirements; no data were provided to demonstrate the homogeneity and stability of the test material in the diet. No urinalysis data were provided. Gross necropsy findings were reported, but no histopathology was conducted

on the tissues that were taken, as required in the guidelines. From the gross observations, we assess that this material may be oncogenic in the forestomach and the skin.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol and Protocol Amendments, CBI pp. 620-658.