004208

EPA: 68-01-6561
TASK: 65
November 7, 1984

Caswell # 535

DATA EVALUATION RECORD

CYTHION

Chronic Toxicity/Oncogenicity-Rats

CITATION: Rucci, G., Becci, P.J., Parent, R.A. The evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley Rats for 24 consecutive months. An unpublished study (No. 5436) prepared by Food and Drug Research Laboratories, Inc. for Agricultural Division, American Cyanamid Co. Princeton, N.J. Dated May 13, 1980.

REVIEWED BY:

Paul Wennerberg, D.V.M., M.S.
Project Scientist
Dynamac Corporation

Signature: [signature]
Date: 11-7-84

William L. McLellan, Ph.D.
Senior Scientist
Dynamac Corporation

Signature: [signature]
Date: 7 November, 1984

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

Signature: [signature]
Date: 11-7-84

APPROVED BY:

Keto Engler, Ph.D.
EPA Scientist
John Quest, Ph.D.
EPA Scientist

Signature: _____
Signature: [signature]
Date: 1/10/85

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity/Oncogenicity-Rats.

CITATION: Rucci, G., Becci, P.J., Parent, R.A. The evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley Rats for 24 consecutive months. An unpublished study (No. 5436) prepared by Food and Drug Research Laboratories, Inc. for Agricultural Division, American Cyanamid Co. Princeton, N.J. Dated May 13, 1980.

ACCESSION NUMBER: 248179.

LABORATORY: Food and Drug Research Laboratories, Inc.

QUALITY ASSURANCE STATEMENT: Present, signed, and dated May 13, 1982.

TEST MATERIAL: Technical Cythion (malathion), Lot No. W70225-1, 92.1 percent purity, inert ingredients 7.9 percent, supplied by American Cyanamid Co.

METHODS:

1. Sprague-Dawley rats (source: Blue Spruce Farms, Altamont NY.) acclimated to laboratory conditions for 10 days, were used in the study. At study initiation, the mean weights of males and females were 76 and 71 g, respectively. Cythion was administered at levels of 0, 100, 1000, or 5000 ppm in the diet to groups of 50 male and 50 female rats. The rats were individually housed in wire mesh bottom cages. They were maintained in an environmentally controlled room at a temperature of 70 ± 3° F and a 12 hour light-dark cycle. Feed and fresh tapwater were provided ad libitum.

2. A premix of test compound in basal feed (Charles River RHM3200 meal) was prepared fresh weekly by grinding test compound with powdered feed in a mortar. Diets containing the required concentration of the test material were prepared by mixing appropriate amounts of the premix and basal feed in a Hobart mixer. Samples of the prepared diets were collected weekly for analysis by the sponsor, and samples were taken quarterly for analysis of stability and homogeneity of the diets.

3. Although it was stated in the report that clinical observations for signs of toxicity were made daily and palpations of all animals were performed weekly throughout the study, there were no individual animal data presented in the report to support this statement. Body weights and food consumption were measured at week 1 through 13 and at the end of weeks 24, 53, 79, and 103.

4. Hematology, urinalysis, and cholinesterase determinations were conducted on 5 males and 5 females in each group at 3, 6, 12, and 24 months. The hematologic parameters measured were: hemoglobin, hematocrit, erythrocyte and platelet counts, and total and differential leukocyte counts. Cholinesterase activity of red cells and plasma were determined. Urinalyses included observation of appearance, color, and microscopic sediment and measurement of specific gravity, pH, glucose, and semiquantitative determination of albumin. Blood urea nitrogen, glucose, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined at 104 weeks only.

5. Postmortem examinations were conducted on all animals that died, or were sacrificed moribund and on all animals that survived to study termination. Organ weights were determined at study termination (24 months) for the following organs: liver, adrenal, spleen, thyroid, kidney, pituitary, heart, testes/ovaries, and brain.

6. Hematoxylin-eosin stained slides were prepared and the following tissues were examined histologically:

lungs	large intestine	mammary glands
spleen	urinary bladder	sternum
pancreas	salivary glands	eyes
thymus	lymph nodes (M & C)	trachea
testes	thyroid & parathyroid	esophagus
prostate	adrenals	ears
seminal vesicles	liver	tongue
heart	kidney	ovaries
aorta	brain (2 levels)	uterus
muscle & nerve	spinal cord	vagina
stomach (gl. & sq.)	pituitary	and all gross lesions
small intestines (3)	skin	

7. Body weight data, food consumption data, hematological, biochemical and urinalysis parameters, organ weights and relative organ weight data were analyzed by the investigator, using one-way completely randomized design analysis of variance. Differences between treatment groups were determined using Tukey's LSD test assuming a two tailed critical region. Survival data and incidence of tissue masses were analyzed using a chi-square test with Yates correction for 2 x 2 contingency tables. Incidence of gross lesions, tumors, and non-neoplastic microscopic lesions were analyzed by this reviewer using the Fisher exact test.

RESULTS:

Observations and Mortality: It was reported that "daily observations revealed no overt test effects of Cythion at any level", however, clinical observations for individual animals were not presented in the report. It 3

was reported that there was no differences between treated or control animals in the incidence of palpable masses; however, only summary group incidence data were presented for each 13-week interval in the study and no individual animal data on tissue masses were reported. Table 1 presents the investigator's summary of incidences of palpable masses at weeks 78, 91, and 103.

TABLE 1. Percent Incidence of Palpable Tissue Masses at Selected Study Intervals[a]

Group/ppm	Week 78	Week 91	Week 103
Males			
0	2	6	6
100	2	2	3
1000	4	5	7
5000	4	5	5
Females			
0	5	12	15
100	9	18	24
1000	6	11	19
5000	7	8	10

[a]It was not possible for the reviewer to validate incidences or to determine the locaton of masses.

The administration of test compound caused no statistically significant increase in mortality in dosed animals when compared to controls; in fact, survival in high dose females was remarkably higher than in control or mid-dose females. Mortality and percent survival at 18 and 24 months are summarized in Table 2. Survival in all groups ranged from 90 to 98 percent at 18 months; at 24 months, survival ranged from 48 to 88 percent (Table 2).

TABLE 2. Number of Mortalities and Percent Survival in Rats Fed Cythion[a]

	Males Dose level (ppm)				Females Dose level (ppm)			
	0	100	1000	5000	0	100	1000	5000
78 Weeks								
No. of deaths	2	2	5	5	1	2	2	2
Percent survival	96	96	90	90	98	96	96	96
104 Weeks								
No. of deaths	21	21	25	26	13	18	19	6
Percent survival	58	58	50	48	74	59	62	88

[a]Individual animal data presented were validated by this reviewer. Fifty animals per group were initiated in this study.

Body Weight and Food Consumption: Table 3 presents mean body weight data for male and female rats at selected intervals during the study. As

TABLE 3. Mean Body Weights of Rats Fed Cythion at Selected Intervals

Group/dose (ppm)	Mean body weight (grams)				
	Week 0	13	53	79	103
Males					
0	78	429	539	567	534
100	75	436	548	531	513
1000	75	412*	524	529*	482*
5000	76	405*	520	506*	482*
Females					
0	74	254	313	367	365
100	70*	253	316	354	365
1000	71*	251	309	341*	345*
5000	70*	237*	294*	333*	345*

*Statistically different from controls at a p value < 0.05.

stated in the report, there was throughout most of the study a slight but statistically significant decrease in mean body weights of both males and females in the 5000 ppm group when compared to controls and also a decrease in males at 1000 ppm. However, body weights of males at the high dose were only 6 to 11 percent lower than controls and in high dose females 4 to 9 percent lower than controls.

There were no effects of test compound on food consumption. There were sporadic statistically significant increases in females and decreases in males but they were not consistent with time or dose.

Hematology and Clinical Chemistry: There were no test compound-related effects on hematology parameters. Although there was a statistically significant decrease in hemoglobin in 5000 ppm females at 3 months when compared to controls (13.3 vs 14.9 g/100 ml), the decreased value was within the normal range, and values at other time periods were not different from controls. Mean values for SGOT, SGPT, BUN, and glucose were similar in control and dosed groups. Mean data for hematology and clinical chemistry were supported by individual data. However, data were not present for the 18 month test interval for hematology and were present only for the 24 month interval for blood chemistry; the number of animals tested was limited to 5/group/sex.

Cholinesterase Activity: Red cell cholinesterase activity was statistically significantly lower in 5000 ppm males than in controls at 3, 6, and 12 months but not at 24 months when some recovery of activity was apparent (Table 4). There was a 42% or less depression of activity in 1000 ppm males. In females at 5000 ppm there was a statistically significant maximum depression of activity of 71% at 6 months, but by 24 months some recovery was apparent as indicated by a depression of activity of only 36% in comparison to controls; in 1000 ppm females there was a 36% or less depression of activity. There were no compound-related changes in levels of plasma cholinesterase when dosed animals were compared to controls. Analysis of brain cholinesterase activity was not reported.

TABLE 4. Red Cell Cholinesterase Activity as Percent of Control at Various Study Intervals[a]

Group/dose (ppm)	Months			
	3	6	12	24
Males				
1000	75	73	58*	72
5000	34*	18*	25*	55
Females				
1000	80	64	55*	64*
5000	51*	29*	34*	64*

[a]Mean percent values of 5 animals/group/sex.

*Statistically different from control, $p < 0.05$.

6

Urinalysis: There were no compound-related effects on the urinalysis parameters reported (pH, specific gravity, albumin and glucose). The results were based on five animals per group per sex.

Organ Weights: There was a statistically significant increase in the means of both liver weights and percent liver weights relative to body weight in 5000 ppm males when compared to controls; 15.6 g (3.1%) in controls and 17.8 (3.9%) in the 5000 ppm group. There were slight increases (statistically significant) in kidney weights relative to body weights but not in the absolute kidney weights of the 1000 and 5000 ppm males and 5000 ppm females as compared to controls. Mean brain weights in males and females at 1000 and 5000 ppm were slightly lower than controls, but there were no changes in relative brain weights. It was noted that in calculating mean values for pituitary weights, several individual data points were excluded (see Discussion).

Gross pathology: Several statistically significant gross findings were reported and are included in Table 5. These include color alterations in males of the cecum at 5000 ppm and kidneys at 1000 ppm.

There were significantly increased incidence of size alterations in mesenteric lymph nodes of the 100 and 1000 ppm males; thyroid of the 100 ppm males; and ovaries of the 100 ppm group. No other statistically significant gross lesions were noted. There were other gross lesions observed (Table 5) for which the incidence suggested a positive trend; nowever, these findings were not statistically significant and are not considered by this reviewer to be compound-related.

Histopathology: In the females the incidence of mammary fibroepithelial tumors (adenomas, fibromas, fibroadenomas, and papillary cystadenomas) in the 1000 ppm group and uterine polyps in the 100 and 5000 ppm groups were significantly higher than in controls. An increased incidence of accumulation of pus within the uterus (pyometra) was also noted in the low and mid dose groups. No other statistically significant increase in tumors was detected. Data on neoplasms are summarized in Table 6.

Significant incidences of non-tumor pathology are presented in Table 7. Tissues of dosed animals showing significantly increased incidence of lesions as compared to controls are: heart in males at 5000 ppm (chronic inflammation); kidneys in males at 5000 ppm (both glomerulesclerosis and casts); liver in males at 5000 ppm (sinusoidal dilation); lung in females at 100 ppm (bronchopneumonia with bronchiectasis); lymph nodes in males at 5000 ppm (reticuloendothelial hyperplasia); spleen in females at 5000 ppm (extrahepatic hematopoiesis); and the uterus at 100 ppm (pyometra). Table 7 also presents the incidence of other lesions suggestive of a positive trend; however, these findings were not statistically significant and are not considered by this reviewer to be compound related.

Table 8 was compiled by this reviewer to assess the major lesions associated with illness and premature death. There was no statistically significant compound related lesion in these animals.

TABLE 5. Incidence of Selected Gross Findings in Rats Fed Cythion[a]

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
I. Color alterations:								
brain	6	10	11	14	1	4	1	3
cecum	1	7	3	9*	3	0	4	2
small intestine	4	6	4	9	3	2	1	1
kidneys	5	8	14*	10	6	6	4	2
liver	21	29	23	28	18	22	14	18
II. Size alterations								
liver	5	8	13	17	2	1	0	4
kidney	1	2	3	6	0	0	0	0
mes. lymph nodes	6	16*	16*	11	12	10	18	19
mammary gland	3	0	0	0	0	5	1	1
ovaries					0	6*	3	1
thyroid	2	5	7*	6	2	1	1	0

[a] This table presents only findings that were significant by the Fisher Exact Test or nonsignificant findings for data in either sex suggestive of a positive trend. The table was prepared by this reviewer based on reported information for 50 animals/ sex/group.

* Significant by Fisher Exact Test, $p < 0.05$; analysis by this reviewer.

8

TABLE 6. Tumor Incidence in Rats Fed Cythion[a]

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
Adrenals	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
adenoma	1	0	0	?	1	0	1	1
pheochromocytoma	0	2	1		0	0	1	1
Mammary gland	(49)	(48)	(49)	(47)	(50)	(50)	(50)	(50)
fibroadenoma[d]	0	0	1	3	9	13	15*	6
adenocarcinoma	0	0	0	1	1	0	3	1
Pancreas	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
adenoma	3	3	4	2	2	1	0	2
Pituitary	(49)	(48)	(45)	(49)	(49)	(46)	(48)	(49)
chromophobe adenoma	18	11[c]	11[c]	10[c]	29	27	30[c]	25
Skin	(50)	(5)	(50)	(50)	(50)	(50)	(50)	(50)
papilloma	0	1	1	1	0	0	0	0
histocytoma	0	0	0	1	0	0	1	1
Thyroid	(50)	(49)	(50)	(50)	(50)	(48)	(50)	(50)
C-Cell carcinoma	1	1	0	1	2	5	2	0
Uterus					(50)	(50)	(50)	(50)
polyps					3	10*	9	10*
other benign tumors					1	1	2	0

[a] This table was compiled by this reviewer and does not include sites where tumor incidence in treated groups are equal to or less than the control group.

[b] Number of tissues examined.

[c] "One animal has a tumor which histologically is a carcinoma" (as stated by applicant).

[d] Combined adenomas, fibromas, fibroadenomas, and papillary cystadenomas.

* Significant by Fisher Exact Test, $p < 0.05$; analysis by this reviewer.

9

TABLE 7. Non-Neoplastic Lesions in Rats Fed Cythion[a]

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
	(no. examined = 50 unless noted)							
Bone marrow hyperplasia	2	5	4	5	1	6	2	2
Heart chronic inflamation	4	11	9	13[c]	5	3	5	5
Large intestine inflamation	1	1	(49) 4	4	3	1	(49) 1	2
Kidneys	(49)							
glomerulesclerosis	0	3	6	7[c]	3	0	2	3
hydropelvis	1	1	3	4	6	3	5	3
acute inflammation	7	4	1	5	0	0	2	3
tubular casts	9	19[c]	13	15	3	7	7	10[c]
tubular dilation	6	9	9	13	4	2	5	8
Liver	(49)							
cytoplasmic vacuolization	10	6	7	7	6	9	10	10
sinusoidal dilation	1	3	1	10[b]	3	0	1	1
Lung								
acute bronchitis with bronchiectasis	5	10	11	0	0	(49) 4	1	4
bronchopneumonia with bronchiectasis	5	9	12	13	6	15[c]	10	1
Lymph nodes							(49)	
inflamation, chronic	24	31	29	29	28	29	30	32
lymphoid hyperplasia	4	17	17	9	16	16	15	22
reticuloendothelia hyperplasia	7	10	12	16[c]	11	17	11	11
Ovaries cysts					17	25	19	18
Pancreas duct dilation	0	3	1	4	0	1	1	4
Pituitary	(49)	(48)	(45)	(49)	(49)	(46)	(48)	(49)
cyst	1	2	3	5[c]	3	0	1	2

TABLE 7. Non-Neoplastic Lesions in Rats Fed Cythion[a] (Continued)

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
Prostate calcification	8	10	(48) 12	16[c]				
Spleen extramedullary hematopoiesis	6	7	7	8	13	(49) 17	18	26[b]
Thymus	(39)	(46)	(49)	(48)	(48)	(42)	(46)	(48)
cysts	2	3	1	3	1	5	3	6
Uterus pyometra					0	7[c]	4	0

[a] This table was compiled by this reivewer and presents only histologic lesions which had a statistically increased incidence in a dosed group or the data appeared suggestive of a positive trend.

[b] Significant by Fisher Exact Test, $p < 0.05$; analysis by applicant.

[c] Significant by Fisher Exact Test, $p < 0.05$; analysis by reviewer.

TABLE 8. Percent Incidence of Lesions Associated with Animals that Died or were Moribund Sacrificed[a]

	Dose Level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
Ear	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
inflamation of middle or inner ear	4	14	12	11	14	13	11	0
Lungs	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
bronchiectasis, abcesses	9	5	20	15	14	13	21	17
bronchitis, acute	9	0	4	4	0	0	5	17
bronchopneumonia, acute	22	48	32	48	14	26	21	17
Mammary gland	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
fibroadenoma	0	0	0	0	0	4	11	0
Pituitary	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
adenoma	2	5	4	11	21	26	47	33

[a] The numbers in parenthesis are the number of animals whose tissues were examined. The results are presented as the percent of animals with the lesions in the specified group. A few of the animals that died or were moribund sacrificed were not examined (see No. of deaths, Table 2).

DISCUSSION:

1. The results of the present study suggest that the 5,000 ppm dose of Cythion approximated a maximum tolerated dose. That is:

   a. There were significant decreases in body weight in males and females in the mid and high-doses; the final body weights at the high-dose were 6 and 11 percent lower than controls in females and males, respectively (Table 2).

   b. There was marked inhibition of erythrocyte cholinesterase in the high dose animals particularly during the first year of the study. Cholinergic toxic signs were not reported and there was no inhibition of plasma cholinesterase noted. Adaptation and recovery from inhibition in erythrocyte cholinesterase activity was evident, particularly in high-dose animals (Table 4). Data on the levels of brain cholinesterase could have facilitated interpretation of the importance of these effects; however, brain analyses data were not available.

2. The authors of the report concluded that "there were no biologically or toxicologically important test material related histopathologic effects or tumorigenic effects." In this study, there were statistically significant ($p < 0.05$) increases in fibroadenomas of the mammary gland (9/50 in controls versus 15/50 in the 1000 ppm females). This was dismissed in the report on the basis that there were comparable incidences of mammary tumors in "control and high-dose females similar to what would be expected in rats of this strain and age." Similarly, benign tumors of the uterus were statistically significant ($p \leq 0.05$) when the 100 and 5000 ppm groups were compared to control using the Fisher exact test. These were not considered meaningful in the report since they "occurred at the expected incidence typical of rats of this age and strain." While it is generally recognized that both of these types of tumors are characteristic of aging control rats, historical tumor incidence for this strain and laboratory is essential and needs to be provided for proper evaluation of the data.

3. The following ambiguities in the classification of tumors were noted:

   a. "All focal proliferative lesions of chromaffin cells were diagnosed as pheochromocytomas."

   b. "C-cell tumors of the thyroid appearing cytologically benign were considered malignant because of the tendency of progressive growth characteristic of thyroid tumors."

   c. Some pituitary tumors with areas appearing malignant microscopically were classified as adenomas "because of the tendency of pituitary tumors to remain localized."

   Based on such ambiguities, it is considered that the histopathology of endocrine tissues needs further clarification and the slides of these tissues should be re-examined.

4. Although the report noted that extramedullary hemopoiesis of the spleen in high dose females was statistically different from control, this was "not considered due to compound administration since it is a common finding in aging rats." Similarly, other non-neoplastic lesions were noted but were not considered important in the investigator's conclusions. Many of these lesions are generally present in aged rats, and a relationship to test chemical cannot be definitely established. However, many of them were found on our analyses to be statistically significant in the high dose animals when compared to controls and are indicated in Table 7.

   It is considered that the dismissal of statistically significant effects on the basis of a common finding in aging animals must be supported by specific laboratory historical control data.

5. The study has several deficiencies that limit its usefulness for evaluating chronic toxicity:

   a. Blood chemistry determinations, hematology, and urinalyses were not made at the required number of test intervals, only five animals/sex/group were examined, and their estimating of the platelet number alone was not a sufficient examination of the clotting potential of the blood.

   b. Cholinesterase determinations were performed on insufficient numbers of animals to give adequate values for analyses; the lack of an early effect on plasma cholinesterase followed by recovery may have been missed since the earliest interval for analysis was at 3 months; no analyses were reported at 18 months.

   c. Although there was a concluding statement in the report that there were "no overt test effects of Cythion," there were no clinical observations or tissue mass palpation data reported. Clinical observation would have been useful to assess possible cholinergic toxic signs.

6. There were minor errors in recording data. These were noted in comparing tables of pathology incidence and tables correlating gross and histologic findings. In calculating organ weight means, several values were not reported: For example, for mean pituitary weights for females of the 5000 ppm group, weights of 22 pituitaries were omitted; 20 of these were diagnosed as having adenomas. Inclusion of these values did not reveal any differences in mean weights in dosed animals when compared to controls due to the large variance of the population.

CONCLUSIONS:

As noted above in items 2-6 of the Discussion section, several deficiencies involving histopathology diagnoses and interpretation, clinical observations, and blood chemistry limit the usefulness of these data for the assessment of chronic toxicity and oncogenic potential. Despite such

deficiencies, however, statistically increased incidences in female rats of uterine polyps and mammary gland fibroadenomas were observed. These tumorigenic responses were all benign in nature, exhibed no dose-response relationships, and displayed no decrease in latency compared to control animals. Thus, a definite relationship between test material and the increased incidences of tumors cannot be conclusively established. The historical control data is necessary; it should be from the same laboratory and from as many experiments as possible, but not less than from five consecutively performed studies.

Administration of Cythion in the diet to rats for two years caused a slight decrease in weight gain in males and females at 1,000 and 5,000 ppm, a transient decrease (inhibition) of cholinesterase at 1,000 and 5,000 ppm in males and at 5000 ppm in females, and an increase in liver weights in males at 5,000 ppm which was accompanied by an increased incidence of sinusoidal dilation. No adverse effects appeared to be associated with administration of the chemical at a dose of 100 ppm. However, due to the absence of data on clinical observations for toxic signs, the limited blood chemistry analyses in terms of time frequency of sampling and the number of animals and parameters tested, and the limited hematology, urinalyses, and cholinesterase determinations performed, a definitive NOEL or LEL cannot be established.

CORE CLASSIFICATION: Supplementary.