RB-3547
TXR-1198
12-28-83

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Terbufos; EPA Reg. #241-241. Review of an Addendum to the 24-Month Oral Toxicity and Oncogenicity Rat Study (Bio/dynamics Inc., Project #71R-725, 7/31/74); A Histopathological Re-evaluation, Bio/dynamics #72R-725 RFMc 4/83, 4/22/83; Accession Nos.: 251232, -3, -4 and -5. CASWELL#131A

TO: William Miller, PM#16
Registration Division (TS-769)

FROM: Amal Mahfouz, Toxicologist
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Amal Mahfouz 12/22/83
for IDC

THRU: William L. Burnam, Chief
Toxicology Branch
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WLB 12.28.83

Registrant: American Cyanamid Company
Agricultural Research Division
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Background:

A two year chronic feeding/oncogenicity study in rats (#71R-725, 7/31/74, Bio/dynamics) was classified as Core Supplementary because the histopathological data were considered incomplete by this reviewer, (see our review dated 2/2/82 for PP#1F2433).

On 9/8/83, the registrant submitted a histopathological re-evaluation report (72R-725, RFMc 4/83, 4/22/83, Bio/dynamics) for all the rats in this study. The newly submitted histopathology data are the subject of our present review.

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Recommendations:

The histopathological re-evaluation report reflects the following findings:

Neoplastic data. Terbufos is not oncogenic in rats. This portion of the 2-year chronic/oncogenic feeding study in rats is classified as Core-Minimum. The Registration Standard for Terbufos should be amended to indicate that this data gap has been filled.

Non-Neoplastic data. The histopathological data in this newly submitted report reflect the following findings:

- Increased incidence of histopathological lesions in the high dose group: lung granuloma and bronchiopneumonia in both males and females; stomach ulceration and erosion of the non-glandular and glandular mucosa in both sexes; lymphocytic myocarditis in males and epicarditis in both sexes; bone marrow hyperplasia in both sexes (hyperplasia in males was much higher than in females) in the rats that were sacrificed at termination; and bone marrow hypoplasia in the animals that died during the study.

- Increased incidence of lymphoid depletion in the spleen and in the mesenteric lymph nodes in both sexes in the three dosage groups for rats that died or were sacrificed moribund during the study.

- Increased incidence of spleen hemosiderosis in the 3 treated female groups and in the high dose male groups.

Although the newly submitted histopathological data successfully answers our concerns relative to the inadequacy of the number of animals and tissues microscopically examined in the previously submitted final report of the two year chronic feeding/oncogenic study in rats(see our review of 2/2/82), the chronic toxicity section of this study still remains classified as Core Supplementary.

A NOEL for chronic toxicity cannot yet be determined from this study due to a dose-related increase in both male mortality and male and female CHE inhibition at all dose levels. Hence, the chronic toxicity study in rats will remain as a data gap, and lower dosages of Terbufos should be tested.

Review:

The initial number of rats in this study was 60 animals per sex in each of the two control groups (Control I and Control II) and in each of the three treatment groups, 0.25, 1.00 and 2.0 ppm. We note that the dosage in the high dose group, 2.0 ppm, was first elevated to 4.0 ppm and then to 8.0 ppm at the beginning of the 6th and 12th weeks respectively. Due to severe toxicity in the high-dose female group, the 8.0 ppm dosage was decreased back to 4.0 ppm during the 16th week of treatment; however, the high dose male group continued to receive the 8.0 ppm dosage until the end of the study period.

At 3 months, 5 animals per sex from the control groups and 10 animals per sex from the treated groups were sacrificed as part of a three month study. The histopathological examination of these animals was not performed, however all the remaining animals, with few exceptions (due to autolysis or missing tissues), were histologically examined in this newly submitted histopathological re-evaluation report (#72R-725,4/22/83) for animals that died during the study or that were killed at the end of the two year study period , see the table below:

	GROUP									
	CONTROL				AC 92,100					
	I		II		III		IV		V	
	M	F	M	F	M	F	M	F	M	F
RATS STARTED ON TEST	60	60	60	60	60	60	60*	60	60	60
3 MONTH INTERIM STUDY RATS KILLED	5	5	5	5	10	10	10	10	10	10
TOTAL ON 2 YEAR SEGMENT	55	55	55	55	50	50	50	51	50	50
TOTAL RATS WHOSE TISSUES WERE EVALUATED AS PART OF THIS PATHOLOGY REPORT	54	55	53	55	50	50	44	50	47	49

*Group IV males should have been 59 due to an addition of a female in the male group at the initiation of the test. Animal counts in this group totaled 60. The discrepancy was not resolved

Apparently, all tissue masses described at necropsy were available for microscopic examination with the exception of the subcutaneous masses from three animals (one Control #I female, one Control #II female, and one low dose male). However, it was also stated in the newly submitted report that specimens of several pituitaries, which were described as enlarged in the path sheet, were not available for examination. The author explained that "often pituitary tumors become extremely friable especially after sitting in fixative for a long period of time. It was deemed probable that the tumors probably crumbled into small pieces during handling and were subsequently lost. In animals where the pituitary was described as being enlarged but no specimen was available, the lesion was considered to have been a pituitary tumor and was diagnosed as an adenoma".

In the opinion of the Toxicology Branch, all tissues (including the pituitary) when adequately fixed should remain in good condition for examination for a longer time than the period which elapsed between fixation of tissues and histopathological re-evaluation of these tissues in this study.

Results:

A. Neoplastic Lesions

Discussions

The incidence of tumors in the control group and in the Terbufos treated groups did not reflect a compound-related oncogenic effect in this study, see the next page for a copy of table #1 (Tumor Incidence in Rats of Different Treatment Groups) as submitted by the registrant in Volume IV, Accession #251235. However, note in this table that the low-dose was erronously recorded as 0.5 ppm instead of 0.25 ppm; that the recorded high dose, 2.0 ppm was actually increased to 8 ppm for males a d 4 ppm for females during the first four months of the study and remained at these levels until termination; and that data for Control #I and Control #II were combined.

In addition to the tissues listed in the above mentioned table #1, we also noted a few incidences of brain tumors (the incidences of brain tumors were not listed in table #1). These tumors were observed in the treated male groups and in the control female groups i.e., astrocytoma in 1/48 low dose males and 2/44 mid dose males, granular cell tumor in 1/44 mid dose males, and malignant meningioma in 1/44 high dose males; in the female control groups, malignant meningioma in 1/55 females and ependymoma in another female of Control #1, and astrocytoma in 2/55 females of Control #2 . No brain tumors were reported in the male control groups or in the three dosage groups in females.

It appears that the incidence of brain tumors increased in the treated males as compared to the male control groups. The mid dose males reflected the highest incidence of brain tumors. However, these incidences of brain tumors in treated males were similar to the reported incidences in the female control groups.

Hence, it appears that the above noted incidences of brain tumors in this study are not significant.

In Conclusion this study reflects a negative oncogenic potential for Terbufos in rats.

Classification: Core-Minimum for the oncogenicity section of this study.

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Table I

Tumor Incidence in Rats of Different Treatment Groups

(A) Male Rats

Tumor Type	Treatment Group (ppm)	No. At Risk			Adenoma			Carcinoma			Adenoma/Carcino		
		DS*	TK+	Total	DS	TK	Total	DS	TK	Total	DS	TK	Tot
Pituitary	0	32	64	96	9	21	30	0	0	0	9	21	3
	0.5	20	25	45	4	11	15	0	0	0	4	11	1
	1.0	20	21	41	9	9	18	1	1	2	10	10	2
	2.0	20	18	38	4	4	8	0	0	0	4	4	
Thyroid	0	39	66	105	2	13	15	0	1	1	2	14	16
	0.5	20	26	46	0	3	3	1	0	1	1	3	4
	1.0	21	18	39	0	1	1	0	0	0	0	1	1
	2.0	24	19	43	0	2	2	0	0	0	0	2	2
Lung	0	39	68	107	1	1	2	0	2	2	1	2	3
	0.5	24	26	50	0	0	0	0	0	0	0	0	0
	1.0	23	21	44	0	0	0	0	0	0	0	0	0
	2.0	28	19	47	0	0	0	0	0	0	0	0	0
Adrenal Medulla	0	75	124	199	1	4	5	4	9	13	5	13	18
	0.5	38	50	88	0	0	0	2	3	5	2	3	5
	1.0	43	42	85	0	2	2	2	2	4	2	4	6
	2.0	52	38	90	0	1	1	0	0	0	0	1	1
Liver	0	38	68	106	0	1	1	0	1	1	0	2	2
	0.5	24	26	50	0	0	0	0	1	1	0	1	1
	1.0	23	21	44	0	0	0	0	0	0	0	0	0
	2.0	26	19	45	0	0	0	0	0	0	0	0	0
Pancreas	0	32	68	100	2	4	6	0	3	3	2	7	6 9
	0.5	21	26	47	0	4	4	1	0	1	1	4	5
	1.0	20	21	41	2	1	3	0	0	0	2	1	3
	2.0	23	18	41	0	0	0	0	0	0	0	0	0

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Table I (Continued)

Tumor Type	Treatment Group (ppm)	No. At Risk			Adenoma			Carcinoma			Adenoma/Carcino		
		DS*	TK+	Total	DS	TK	Total	DS	TK	Total	DS	TK	Tot
Kidney	0	39	68	107	1	0	1	0	0	0	1	0	
	0.5	24	26	50	0	0	0	0	0	0	0	0	
	1.0	23	21	44	1	1	2	0	0	0	1	1	
	2.0	27	19	46	0	0	0	0	0	0	0	0	
Testes	0	38	68	106	0	2	2	0	0	0	0	2	2
	0.5	24	26	50	0	1	1	0	1	1	0	2	2
	1.0	23	21	44	0	0	0	0	0	0	0	0	0
	2.0	26	19	45	0	1	1	0	0	0	0	1	1
(B) Female Rats													
Pituitary	0	32	71	103	13	30	43	1	0	1	14	30	44
	0.5	13	37	50	6	13	19	0	0	0	6	13	19
	1.0	11	34	45	4	18	22	0	0	0	4	18	22
	2.0	18	18	36	3	3	6	0	0	0	3	3	6
Thyroid	0	34	72	106	0	6	6	2	4	6	2	10	12
	0.5	13	37	50	0	4	4	0	0	0	0	4	4
	1.0	13	33	46	0	1	1	0	2	2	0	3	3
	2.0	29	18	47	0	2	2	0	1	1	0	3	3
Lung	0	36	74	110	1	1	2	0	0	0	1	1	2
	0.5	13	37	50	0	0	0	0	0	0	0	0	0
	1.0	16	34	50	0	0	0	1	0	1	1	0	1
	2.0	28	20	48	0	0	0	0	0	0	0	0	0
Adrenal Medulla	0	68	145	213	0	1	1	0	0	0	0	1	1
	0.5	23	73	96	0	0	0	0	0	0	0	0	0
	1.0	32	66	98	0	0	0	0	1	1	0	1	1
	2.0	56	40	106	0	0	0	1	0	1	1	0	1

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Table I (Continued)

Tumor Type	Treatment Group (ppm)	No. At Risk			Adenoma			Carcinoma			Adenoma/Carcino		
		DS*	TK+	Total	DS	TK	Total	DS	TK	Total	DS	TK	Tot
Liver	0	36	74	110	0	2	2	0	0	0	0	2	
	0.5	13	37	50	0	0	0	0	0	0	0	0	
	1.0	16	34	50	0	1	1	0	0	0	0	1	
	2.0	28	20	48	0	0	0	0	0	0	0	0	
Pancreas	0	32	74	106	2	5	7	0	0	0	2	5	
	0.5	12	37	49	0	1	1	0	0	0	0	1	
	1.0	15	33	48	0	0	0	0	0	0	0	0	
	2.0	27	20	47	1	0	1	0	0	0	1	0	
Kidney	0	36	74	110	0	0	0	1	1	2	1	1	2
	0.5	13	37	50	0	0	0	0	0	0	0	0	0
	1.0	16	34	50	0	0	0	0	0	0	0	0	0
	2.0	29	20	49	0	0	0	0	0	0	0	0	0
Ovary	0	34	74	108	1	1	2	1	0	1	2	1	3
	0.5	13	36	49	1	0	1	0	0	0	1	0	1
	1.0	16	34	50	1	0	1	0	0	0	1	0	1
	2.0	28	19	47	0	0	0	0	1	1	0	1	1
Uterus	0	36	74	110	2	6	8	0	5	5	2	11	13
	0.5	13	37	50	0	1	1	0	2	2	0	3	3
	1.0	16	34	50	0	1	1	1	2	3	1	3	4
	2.0	28	20	48	0	0	0	0	0	0	0	0	0

*DS:Died/Sacrificed

+TK:Terminal Kill

B. Non-Neoplastic Lesions: Review of the recently submitted histopatholgical re-evaluation report of all animals in this study reflected the following findings.

1. Evaluation of lesions which were previously reported in our 2/2/83 review.

°Liver: The previously noted increase of bile duct hyperplasia in males and females of the 3 dosage groups which was reported in our 2/2/82 review appears to be an artifact. The incidences of this lesion appear to be not compound-related in the new report after examination of all animals . However, a slight increase was reported in the incidence of leucocytic infiltration in the intralobular and portal triads of the high dose animals as compared to the control groups.

°Heart: No significant increase was noted in this new report in the incidence of myofibril degeneration (necrosis) or lymphocytic myocarditis in the 3 dosage groups as compared to the control groups. However, 2/49 males and 1/5 females in the low dose group, 2/50 females in the mid dose, and 5/47 males and 1/48 females in the high dose group had lymphocitic myocarditis as compared to zero incidence in the remaining male groups and zero in the female control group. Also, the incidence of epicarditis appeared to slightly increase in both sexes in the high dose group as compared to the control group.

°Kidney: A slight increase in the incidence of chronic interstitial nephritis was observed in the 3 male dosage groups; no effect in females. A slight increase was noted in the incidence of mineralized concretions in both sexes in the dosage groups; however, the increase was not dose dependent and appeared to be higher at the low dose in both males and females than in the mid and high dose groups as compared to the control groups.

°Lungs: Lung granulomas (apparently due to food particles inhalation) and bronchiopneumonia significantly increased in the high dose group in both sexes as compared to the control group; no significant effect was noted at the lower dosage groups. These lesions were previously reported in our review of 2/2/82.

°Bone marrow: Bone marrow hyperplasia appeared to increase significantly at the high dose level in both sexes which were killed at termination. This effect was higher in males of this group than in females. No significant effect was noted in the lower dosage groups or in the high dose animals that died during the study. These findings were also noted in our review of 2/2/82. In this new report, a significant increase was also noted in the incidence of bone marrow hypoplasia in males and females of the high dose group that died during this study; no data were available for the animals that were killed at termination.

°Thyroid: The new histopathology report does not reflect an increase in the incidence of medullary cell hyperplasia in the high dose male group as was previouly noted in our 2/2/82 review. Upon evaluation of data from all animals in this study, it appears that the incidence of this lesion is higher in Control #1 than in any treatment group.

In summary, from all the previously reported lesions in our 2/2/83 review, only the following lesions appear to have significantly increased with Terbufos administration:

a. Lung granulomas and bronchiopneumonia at the high dose level in both males and females.

b. Lymphocytic myocarditis in high dose males, and epicarditis in both high dose males and females.

c. Bone marrow hyperplasia in males and females of the high dose group that were killed at termination, and hypoplasia in both sexes of this group for the rats that died during the study.

2. Evaluation of additional findings in this new report:

°Stomach: The following lesions were noted to significantly increase in both sexes at the high dose level: ulcer of the nonglandular mucosa, erosion of the glandular mucosa, submucosal erosion and epithelial hyperplasia. No effect was noted at the mid-dose level. Incidences of submucosal edema appeared to significantly increase in the low-dose females and the incidences of epithelial hyperplasia appeared to increase in the low dose males. However, the effects noted in the low-dose appear to be an artifact because they were not observed in the females or in the mid dose.

°Hematopoitic system: It was already mentioned under section B-1 above that hyperplasia and hypoplasia were noted to increase in the high dose animals. In addition to this finding, lymphoid depletion was noted to increase in both sexes of the three 3 dosage groups in the spleen and in the mesenteric lymph nodes of the animals that died during the study.

In summary, the following additional effects appear to have significantly increased in this study:

a. Stomach ulceration and erosion of the non-glandular and glandular mucosa in both males and females of the high dose groups.

b. Increased lymphoid depletion in the spleen and mesenteric lymph nodes in both sexes at the three dosage levels in the animals that died during the study.

Conclusions:

1. Neoplastic Data. Terbufos is not oncogenic in rats. This oncogenic section of the study is classified as Core-Minimum. The Terbufos Registration Standard should be amended in order to reflect the filling of this data gap.

2. Non-Neoplastic Data. The histopathological re-evaluation reflected the following findings:

- Increased incidence of histopathological lesions in the high dose group, i.e. lung granuloma and bronchiopneumonia in both males and females; stomach ulceration and erosion of the non-glandular and glandular mucosa in both sexes; lymphocitic myocarditis in males and epicarditis in both sexes; bone marrow hyperplasia in both sexes in the animals that were sacrificed at termination (the incidence of hyperplasia was much higher in males than females); and hypoplasia in the animals that died during the study.

- Increased incidence of lymphoid depletion in the spleen and mesenteric lymph nodes in both sexes of the 3 dosage groups in the animals that died during the study.

However, the chronic toxicity section of this two year feeding study in rats remains classified as Core-Supplementary due to the biologically significant, dose-related increase in male mortality i.e., 48%, 57.1% ($p < 0.05$) and 62.0% ($p < 0.01$) in the low, mid and high dose levels respectively as compared to 38.2% in the combined control group.

No NOEL can be determined from this study for mortality or ChE inhibition, consequently lower dosages should be tested to determine a NOEL for Terbufos chronic toxicity in rats.