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MEMORANDUM NOV 3 1982

To: Robert Taylor, PM #25
Registration Division, (TS-767)

Subject: EPA Reg. No. 241-243. Review of pathology report submitted as an addendum to the rat 2-year chronic toxicity/oncogenesis study with pendimethalin.

TOX CHEM NO. 454BB

Background:

The rat chronic feeding/oncogenesis study with the herbicide AC 92,553 (pendimethalin) was previously rereviewed and it was determined to be INVALID (see J. Doherty memo dated Nov. 27, 1981). The registrant (American Cyanamid Co.) has provided additional data in the form of an addendum to the pathology report in order that this study may be upgraded. This submission is reviewed herein.

On June 16, 1982 at 2:00 p.m., a meeting was held between an American Cyanamid representative (Mr. F. Ray Barron) and EPA staff (E. Budd, L. Kasza, J. Doherty and R. J. Taylor) to discuss several problems related to this 2-year rat chronic-feeding/oncogenesis study with pendimethalin. Mr. Barron was informed of the following problems which will require clarification prior to Toxicology Branch's acceptance of this study to meet the requirement for a rat chronic-feeding/oncogenesis study.

Note: On July 9, 1982, Mr. Barron and the American Cyanamid toxicologist, Dr. Mel Johnson, and two other company representatives met with EPA staff (Doherty, Badd, Kasza and Taylor) to discuss in more detail the problems listed below. The American Cyanamid Company is expected to prepare a detailed reply to these problems.

1. The registrant must provide for EPA a statement from the pathologist (Dr. R. F. McConnell) that the lesions listed as "nodular hyperplasia (neoplastic nodule)" are or are not neoplastic and do or do not represent adenomas or hepatomas or other neoplastic changes. These lesions should be more fully described so as to justify their inclusion in Table 1 which lists nonneoplastic changes.

2. The bladders of males were found to have three incidences of transitional cell carcinoma in the high dose group vs. only two incidences in all other groups combined. Because bladder carcinomas are rare in rats, the bladder is being considered as a suspect target organ for a possible oncogenic effect of pendimethalin.

3. The high dose group females had 9 incidences (18.4%) of endometrial adenocarcinoma whereas only 4.5% of the control rats had this type of tumor (which is considered to be relatively rare in rats).

   The registrant was informed that the data related to urinary bladder carcinomas and uterine adenocarcinomas was referred to Toxicology Branch's statistician for analysis.

   The registrant was informed that a part of the reason that Toxicology Branch was concerned with bladder and endometrial tumors was because these types of tumors are known to be associated with nitroso compounds and pendimethalin contains nitrosamines.

4. The chronic feeding aspects of this study do not show a NOEL for lesions which develop in the liver. For example, the low dose group had many more incidences of rats with "periportal hepatocyte hypertrophy," "ground glass cytoplasmic changes" and "fatty changes." The mid dose group (females) and high dose group (males and females) also had "cytoplasmic laminated bodies."

   The registrant was invited to provide comments on the above mentioned problems. In particular, the registrant should describe more fully the lesions described as "fatty changes".

5. CORE classification of this study is reserved pending response from the registrant.

Review of Study Addendum

Twenty-four month oral toxicity and carcinogenicity study in rats - AC 92,553 - Pathology report

This pathology report was prepared by Robert F. McConnell, D.V.M., and is dated June 15, 1981. The report is in EPA Accession Nos. 246347, 246348 and 246349. The original study was conducted at Bio/Dynamics, Inc., East Millstone, New Jersey. The original report is dated August 21, 1974, and is study number 72R-749. The original report is in EPA Accession No. 112849.

Five groups of 120 (60 male and 60 female) Long-Evans rats were grouped as control group 1, control group 2, low dose test group (100 ppm), mid dose test group (500 ppm), and high dose test group (5,000 ppm), and dosed with herbicide AC 92,553 (pendimethalin) in the diet for 24 months. The high dose group was dosed with 2500 ppm for the first five weeks before the feeding level was raised to 5000 ppm.

For the first 5-6 months of this study, a high purity grade of AC 92,553 was used. In the succeeding months, a commercial grade of AC 92,553 was used. The commercial grade, but not the high purity grade, contained a nitrosamine contaminant [redacted]. (See H.L. Avallone memo dated May 12, 1980, concerning the audit of this study.)

After three months on the study, 5 males and 5 females from each of the control groups and 10 males and 10 females from each of the test groups were sacrificed and evaluated for toxicity effects. The remainder were kept on their respective diets until their deaths or were sacrificed after 24 months.

RESULTS

1. Survival: The following table demonstrates that there was no compound related increase in deaths in this study. The number of surviving animals was always greater than 25.

24 Month Survival

	Males	Females
Control Group 1	37/67%*	39/71%
Control Group 2	31/56%	35/64%
100 ppm	32/64%	35/70%
500 ppm	26/52%	32/64%
5000 ppm	29/58%	40/80%

* Number of survivors /% of possible survivors (55 for controls or 50 for test groups).

2. Body weight: The following table demonstrates that only the high dose test groups (male and female) had terminal body weights significantly different (lower) than the controls.

Mean Body Weight at 103 Weeks

	Males	Females
Control Group 1	587.3	352.8
Control Group 2	591.2 } 589.2+	335.4 } 343.7+
100 ppm	574.7 (97.5%)	365.7 (106%)
500 ppm	587.0 (99.6%)	342.4 (99.5%)
5000 ppm	506.7 (86.0%)**	282.9 (82.2%)**

( ) percent of combined control weight. + combined control average
**statistically significant $p<.01$

3. Histopathology was reported for the tissues:

Brain	preputial gland
Pituitary	seminal vesicles
Adrenal glands	ovaries
Lymph nodes	cervix
Thyroid	uterus
Parathyroid	stomach
Lung	colon

Spleen	duodenum
Heart	ileum
Aorta	skeletal muscle
Kidneys	skin
Pancreas	mammary gland
Urinary bladder	eye
Liver	Bone marrow
Testis	salivary gland
Prostate	thymus
mesentery	

Many of the tissues were held in storage (in formalin) between the years 1974 and 1981 before they were processed for microscopic evaluation. Except for the mammary gland and certain other smaller tissues, samples of tissues from all available rats were examined histologically and approximately equal samples from each group were evaluated.

The gross necropsy observations were somewhat limited but were followed up by histopathological observations. There were many more lesions noted by microscopy than there were gross observations.

4. The following tables present information related to the overall development of tumors for this study.

   a. Rats with one or more neoplasms.

Group	Males	Females
Control Group 1	37/54 (68.5)*	37/55 (67.3)
Control Group 2	34/53 (64.2)	32/55 (58.2)
Low Dose Group (100 ppm)	33/47 (70.2)	32/50 (64.0)
Mid Dose Group (500 ppm)	28/48 (58.3)	25/50 (50.0)
High Dose Group (5000 ppm)	32/49 (65.3)	27/50 (54.0)

   * Rats with a tumor/number of rats examined, ( ) as percent.
   No compound related or dose related increase in number of rats with neoplasms was noted.

   b. Total number of tumors.

	Males		Females	
	Total	Malignant	Total	Malignant
Control Group 1	58/54 (1.07)*	20/54(.37)	57/55 (1.04)	12/55(.22)
Control Group 2	43/53 (.81)	10/53(.19)	54/55 (.98)	12/55(.22)
Low Dose Group (100 ppm)	51/47 (1.09)	13/47(.28)	47/50 (.94)	11/50(.26)
Mid Dose Group (500 ppm)	38/48 (.79)	13/48(.27)	39/50 (.78)	6/50(.12)
High Dose Group (5000 ppm)	48/49 (.98)	18/49(.37)	38/50 (.76)	13/50(.26)
Total neoplasms	238		235	

   * Number of tumors/number of rats examined (as neoplasms/rat).

No obvious compound related or dose related increase in total number of tumors or animals with malignant tumors was noted.

Among the males, the pituitary (71 neoplasms or 29.8%), adrenal (49 neoplasms or 20.6%) and thyroid (34 neoplasms or 14.3%) accounted for most of the tumors. There was no indication that these common types of tumors which occurred in these tissues were related to the presence of the test chemical. They were uniformly distributed among the different groups and did not show evidence of earlier development.

Among the females, the pituitary (93 neoplasms or 39.6%) and the thyroid (24 neoplasms or 10%) accounted for most of the tumors. The uterus had 19 endometrial adenocarcinomas and is considered separately below.

The remaining 35% (or 69 incidences) of the neoplasms in males and 42% (or 99 incidences) in females occurred in assorted other tissues without an indication of the test groups showing higher frequencies than the control groups or earlier development.

Various nitrosamines have been reported to cause neoplasms in the lungs, liver, kidneys, esophagus, bladder, ductal pancreas, islet cells of the pancreas and nasal sinuses (see Casarett and Doull's Toxicology, Second Edition, p. 98). There were no suspicious patterns of development of oncogenic effects reported in the lungs, liver, kidneys, or pancreas in this study. The urinary bladders are considered separately below.

5. The following organs are given special consideration as follows:

Uterus. There were increased incidences of an uncommon neoplasm described as endometrial adenocarcinoma in this organ as indicated in the following table:

Group	Endometrial adenocarcinoma Rats Affected	Fibrovascular polyp Rats Affected
Control - 1	3/55 (5.5%)*	3/55
Control - 2	2/55 (3.6%)	4/55
Low - (100 ppm)	4/50 (8.0%)	3/50
Mid - (500 ppm)	1/49 (2.0%)	2/49
High - (5000 ppm)	9/49 (18.4%)	2/49

*Number of rats affected with adenocarcinomas/number of rats observed, () as percent.

NOTE: All of the rats with uterine adenocarcinoma except one (75 weeks to death) were survivors.

These data reach statistical significance, according to Mr. B. Litt, Toxicology Branch statistician (Fisher's exact test), when the control, low, and mid-dose groups are compared with the high-dose group ($p < .01$) and when the high-dose group is compared with the mid-dose group ($p < .01$). The conclusion of the pathologist who analyzed this study was that lifetime data analysis adjusted for mortality indicated no significant treatment-related or dose-related effect at the 5% level of significance for the development of uterine endometrial adenocarcinomas. (See Supplement IV of Acc. No. 246349)

These data, however, allow the conclusion that ingestion of pendimethalin at 5000 ppm may be associated with the development of neoplasms in the uterus. The more common type of uterine neoplasm (fibrovascular polyp) did not show indication of being affected by the test chemical. Endometrial adenocarcinomas have been reported to be associated with dosing of N-nitroso compounds (Baba and Von Haam, 1976).

Urinary Bladder - The urinary bladder was shown to be associated with development of a rare and unusual type of neoplasm in the high dose male test group.

	Male rats with transitional cell carcinoma
Control - 1	1/50
Control - 2	0/52 (0.98%)*
Low	1/46 (2.2%)
Mid	0/44 (0%)
High	3/46 (6.5%)

* Rats with tumor/rats observed, () as percent.

Note: None of the females was affected.

The rats in this study were also affected with chronic renal disease and certain nonneoplastic lesions described as hyperplasia of pelvic epithelium (kidney) and epithelial hyperplasia of the urinary bladder. The hyperplasias were more frequent in the treated groups. It was the conclusion of the pathologist that the test chemical was associated with a low level irritative effect in the pelvic and urinary bladder epithelium.

The development of transitional cell carcinoma in the urinary bladder was not statistically analyzed by the testing laboratory because of the few incidences involved. Urinary bladder carcinomas are rare in rats (Hicks et. al. 1976).

Liver

a. Neoplastic changes.

Among rats of both sexes, there were a total of six liver tumors (hepatocellular adenoma or adenocarcinoma), four in the controls (out of 216 rats) and two in the high dose test group (out of 97 rats). The incidences of "nodular hyperplasia (neoplastic nodule)" are shown in the following table:

"Nodular Hyperplasia (neoplastic ~~model~~ nodule)"

Group	Males	Females
Control (Combined)	2/107 (1.9%)*	8/110 (7.3%)
Low	3/47 (6.4%)	2/50 (4%)
Mid	1/48 (2.1%)	7/50 (14%)
High	5/49 (10.2%)	6/50 (12%)

* Rats affected/rats observed, () as percent.

The nature of this lesion is unclear because the pathologist listed the occurrences under nonneoplastic lesions and defined them as "neoplastic nodules." The proper classification of these lesions will have to be verified. The frequency of occurrence of this lesion in the high dose group is much higher than in the controls for the male groups.

b. Nonneoplastic effects.

There were apparent test-chemical-related incidences of four types of nonneoplastic lesions noted in the liver. These were "fatty change," "periportal hepatocyte hypertrophy," "ground glass cytoplasmic change" and "cytoplasmic laminated bodies." The following table shows the relationship between the test chemical in the diet and the development of these four lesions, and the sum of these lesions and their intensity.

	Males				Females			
	0	Low	Mid	High	0	Low	Mid	High
Total rats examined	107	47	48	49	110	50	50	50
Total incidences of nonneoplastic lesions (selected)	26	53	42	66	39	79	85	57
As lesion/rat	.24	1.13	.88	1.35	.36	1.58	1.70	1.14
Fatty change (incidences)	23	20	18	18	28	29	26	24
As percent	21.5	42.6	37.5	36.7	25.5	58	52	48
Severity*	1.78	1.79	2.18	2.44	1.71	1.73	1.92	1.42
Periportal hepatocyte hypertrophy	2	16	12	17	9	30	22	10
As percent	1.8%	34.0	25.0	34.7	8.2%	60%	44%	20%
Severity*	1.5	1.62	1.64	2.38	ND	ND	ND	ND

	Males				Females			
	0	Low	Mid	High	0	Low	Mid	High
Ground glass cytoplasmic changes	1	16	12	17	2	20	26	16
As percent	.9	34	25	35	1.8	40	52	32
Severity*	ND	1.63	1.67	2.47	ND	1.50	1.77	1.50
Cytoplasmic laminated bodies	0	1	0	14	0	0	11	7
As percent	0	2	0	29	0	0	22	14

* Severity was determined by grading the lesion as 1 = slight, 2 = minimal, 3 = moderate and 4 = marked. The severity index number given is the average score for the group. ND = not determined.

Of these four lesions, "fatty change," "periportal hepatocyte hypertrophy" and "ground glass cytoplasmic change" show effects at the low-dose level (100 ppm). Each of these lesion types are considered separately as follows:

i. Ground glass cytoplasmic change - This lesion was considered by the pathologist to be due to submicroscopic proliferation of the smooth endoplasmic reticulum. The available data were tabulated and examined for increases in intensity (i.e., 1 = slight, 2 = minimal, 3 = moderate, 4 = marked). The average scores were 1.59, 1.77 and 1.50 for the low-, mid- and high-dose groups for females and 1.63, 1.67 and 2.47 for the males. Thus, only in the high-dose male group was there a dose-related increase in intensity. (Note: the controls were not included because only one male and two females were affected.) This lesion was found mostly in the aged or surviving animals.

ii. Periportal hepatocyte hypertrophy - was a regionalized enlargement of hepatocytes around the portal triads. For females there was a very poor relationship between dose level and the number of animals affected (see preceding table). For males, all treated groups showed many more animals affected but there was no clear dose response. The intensity of this lesion was more pronounced in the high-dose male test group than in the lower male groups or the control. Except for four rats in the high-dose male group, the affected animals were aged or survivors.

iii. Fatty change - Both male and female dosed groups, at all dosage levels, showed approximately twice as many affected animals by percent than the controls. A dose related increase in intensity was noted for the males only with the progression being 1.78, 1.79, 2.18 and 2.44 for the controls; low-, mid- and high-dose groups.

iv. There were increases in the incidence of cytoplasmic laminated bodies in the mid-dose group females and high-dose male and female test groups to further indicate an effect of this chemical in the liver.

Liver weight of the survivors was also affected (increased) by the test chemical as follows:

Dose Level ppm		Males Terminal Body Weight[a] gm	Males Liver Weight[a] gm	Males Liver Ratio %	Females Terminal Body Weight[a] gm	Females Liver Weight[a] gm	Females Liver Ratio %
0	Mean	546.5	12.6753	2.3584	314.0	6.9423	2.2208
(Combined)	S.D.	76.9	1.9790	0.4950	47.9	1.1725	0.2807
	Mean	568.3	12.2248	2.1991	328.3	8.1575	2.4646
100	S.D.	65.9	1.5086	0.2289	43.2	2.2900	0.4560
	Mean	580.2	13.1283	2.2802	337.0	8.0244	2.3984
500	S.D.	79.8	1.4158	0.2386	59.5	1.3371	0.2507
	Mean	502.9	15.4772*	3.1186**	268.1	8.6984	3.2804**
5000	S.D.	100.6	3.5078	0.6702	51.7	1.6413	0.5317

[a] All mean values based on 10/sex/level.
* Statistically significantly different from combined control $p < .05$.
** Statistically significantly different from combined control $p < 0.01$.

The low-dose groups (male or female) were not statistically significantly different from the controls although the female group was 17.5% higher in absolute weight and 11% higher in relative weight.

A limited amount of clinical chemistry analysis did not give indication of advanced liver injury at any dose level. For example, BUN, alkaline phosphatase (some depression at mid- and high-dose levels but liver injury is associated with increases of this activity), and serum glutamic pyruvic transaminase did not show injury related deviations from the control. Assays were determined at months 3 and 24.

The increased incidences of "ground glass cytoplasmic change," "periportal hepatocyte hypertrophy" and "fatty change" which occur in the low dose groups (both male and female) cannot be dismissed as incidental. This experiment uses 110 control rats of each sex and only 55 of each sex for each dose level. The control groups show a very low frequency for these lesions. The test groups show much higher frequencies. A dose response for intensity for some of these lesions was demonstrated, but no true dose response in the number of animals affected was evident.

Thyroid:

a. Neoplastic - There were five types of neoplasms reported (medullary C-cell adenocarcinoma and adenoma, squamous papilloma, follicular adenocarcinoma and adenoma) and these five types were in all groups as 10/52,

6/53, 5/46, 6/48 and 7/47 for the males; and 7/52, 5/54, 3/48, 4/50, and 5/50 for the females, for the two controls, low-, mid- and high dose test groups, respectively. No neoplastic effect related to the test chemical based on these data is evident.

b. Non-neoplastic - The high dose (5000 ppm) test groups (both male and female) were associated with increased incidences of "increased secretory granules and/or globules in follicular epithelial cells." Five animals in the mid dose male test group were also affected. The following table shows the overall development of this lesion.

	Rats with thyroid lesion	
	Males	Females
Control 1	0/52*	0/52
Control 2	0/53	0/54
Low (100 ppm)	0/46	0/48
Mid (500 ppm)	5/46 (10.8%)	0/50
High (5000 ppm)	33/47 (67.4%)	47/50 (94%)

*incidences/rats observed (as percent) of available animals observed.

A NOEL for this lesion is set at 100 ppm. (Note: thyroid weights were not statistically significantly different from controls and there were no frank signs of physiological dysfunction indicative of thyroid injury presented in the report).

Conclusions:

CORE assignment for this study is reserved. This study has four areas of unresolved issues. These are:

1. Assignment of a NOEL for nonneoplastic effects (liver lesions in all test dose groups).

2. Full description of and proper classification of "nodular hyperplasia (neoplastic nodules)" in the liver.

3. Carcinomas in the bladder of males.

4. Endometrial adenocarcinomas of the uterus.

5. Full description of the fatty changes in the liver.

The registrant is invited to provide their comments on these issues.

John D. Doherty, Ph.D. 11/1/82
Toxicology Branch

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