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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Pesticide Petition Nos. 4G3047/5G3217 - Pyridate Herbicide - Evaluation of the Response by Gilmore, Inc. to EPA Comments Pertaining to Rat Oncogenicity Study (EPA Experimental Use Permit Nos. 42545-EUP-1/42545-EUP-2)

TOX Chem No.: 716A

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Registrant: Gilmore, Inc., Memphis, TN

Toxicology Branch (TB) has evaluated additional data and comments submitted by Gilmore, Inc., in an effort to satisfy EPA requirements for upgrading the rat combined chronic toxicity and oncogenicity study with Pyridate, which was earlier given a

Core-Supplementary classification. The sponsor has satisfactorily responded to all major comments raised earlier by TB and supplied additional data to supplement earlier inadequacies in the aforementioned study. For the record, the major issues raised by TB and resolution of these issues by the sponsor are discussed below.

TB has reviewed three studies submitted by Gilmore, Inc., entitled:

1. One-Year Chronic Feeding Study in Rats (Assay No. 170);

2. Two-Year Chronic Feeding Study in Rats (Assay No. 171); and

3. Lifetime Carcinogenicity (Feeding) Study in Rats (Assay No. 172).

All three studies were classified as Core-Supplementary due mainly to the fact that the test chemical, Pyridate, based on the analytical data supplied by the sponsor, was not stable

and up to 36 percent could not be accounted for in the diet (room temperature) within 24 hours, and up to 73 percent was lost within 72 hours. Thus, the dietary concentrations received by the test animals were consistently significantly lower than the target concentrations.

In their response, Gilmore, Inc. have clarified the fact that the aforementioned three studies were indeed a single chronic toxicity and carcinogenicity study which was subdivided into three separate parallel assays to allow for better handling. An identical protocol was used for all three assays. Thus, Assay No. 170 (1-year chronic study) represented the satellite group for the 1-year interim sacrifice (10 animals/sex/dose). Assay No. 171 (2-year chronic feeding study) represented a satellite group for the interim sacrifice after 2 years (15 rats/sex/dose). Assay No. 172 (lifetime carcinogenicity study) represented the main study (50 rats/sex/dose) and surviving animals were sacrificed at approximately 28 months after initiation of the study.

Gilmore, Inc. has also carried out supplementary studies and supplied the Agency with additional data concerning the stability of Pyridate and the concentrations of Pyridate in the diet at different time intervals at room temperature. The sponsor has conclusively shown that Pyridate is partially hydrolyzed to give CL-9673, and that the rate of Pyridate hydrolysis in the diet is proportional to the exposure of Pyridate to room

temperature. Thus, within 24 hours up to 15 percent of Pyridate is recovered from the diet as CL-9673 and in 72 hours 34 percent is recovered as CL-9673. The percent of Pyridate recovered from the diet was found to be 77 and 54 percent for 24 and 72 hours of exposure, respectively. Total recovery from the diet of Pyridate plus the hydrolysis product (CL-9673) was approximately 92 percent in 24 hours and 88 percent in 72 hours. The sponsor has also stated in his response that CL-9673 is only partially extractable from the diet and thus, it is possible that the unaccounted material (8-12%) is in the form of CL-9673. Thus, a total of approximately 33 and 46 percent of Pyridate is hydrolyzed to CL-9673 in 24 and 72 hours, respectively.

TB is satisfied that the earlier losses in Pyridate reported by the sponsor were indeed in error and that these losses were in reality the hydrolysis product of Pyridate, CL-9673. This metabolite was also shown to be the main Pyridate metabolite in plants.

Based on all aforementioned considerations, TB has decided to accept this study with the stipulation that the dose levels tested 80, 400, and 2500 ppm be lowered by 46 percent (the percent of hydrolyzed Pyridate) to represent the actual levels of Pyridate received by the test animals, i.e., 43, 216, and 1350 ppm.

Upon evaluation of additional data supplied by the sponsor on a 28-day range-finding study and the reanalysis of the data on body weights (from all three parts of this study), we accept the high dose tested (2500 ppm - or 1350 ppm analytical dose) to be the MTD for this study.

The LEL for chronic toxicity (depression of body weights) is considered to be:

Nominal Concentration- 400 ppm

Analytical Concentration- 216 ppm

The NOEL is considered to be:

Nominal Concentration- 2500 ppm

Analytical* Concentration- 1350 ppm

Pyridate was not oncogenic in male or female rats at the HDT, 2500 ppm (nominal concentration) or 1350 ppm (analytical concentration).

All other points noted by TB are considered to be resolved with the present response by the sponsor.

This study is thus upgraded to a <u>Core-Minimum</u> classification.

* The NOEL for the RfD will be based on the analytical concentration of 216 ppm

Subject: One-Year Chronic Feeding Study with Pyridate in Rats

Test Material: Pyridate Technical (CL-11344), 90.3% ai

Accession Number: 072350

Sponsor: Chemie Linz, AG, Austria

Testing Facility: Netherlands Organization for Applied Scientific Research

Project Number: B80-0223 (Assay #170; See also Assay #171)

Testing Period: February 7, 1980 to February 6, 1981

Report Submitted to Sponsor: April 1982

Materials and Methods:

Male and female SPF rats (Cpb:WU:Wistar random), obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, Netherlands, approximately 3 1/2 weeks old and weighing 35 to 50 g were used in this study. The animals were divided into four groups, 10 males and 10 females per group, identified individually by an earmark, placed in suspended stainless steel cages (five animals of the same sex/cage) and kept in a room where the temperature was maintained at 23 ± 1 °C, the relative humidity was at least 40 percent, 8 to 10 air changes per hour and a 12-hour light/dark cycle. All animals received food (powdered stock diet) and water ad libitum.

Following a 7-day acclimation period, each group was fed diets containing 0, 80, 400 or 2500 ppm of Pyridate, for a period of 52 weeks. Fresh diet portions were supplied to all groups every day except for the weekend (larger portions of diet were supplied on Fridays to last through the weekend).

Diets with the test article were prepared by initially preparing a premix of 4 kg of stock diet with Pyridate (using a mechanical blender) and then through serial dilution with stock diet the desired concentrations were obtained. For each dose level, homogeneity was achieved by mixing for 2 minutes in a mechanical blender. Diets containing Pyridate were prepared weekly in batches of 30 kg and stored refrigerated (along with control diets) at 5 °C until use. Test article concentrations in the diet were analyzed weekly for the first 14 weeks and every 2 months thereafter.

Test article concentrations in the diet were analyzed weekly for the first 14 weeks and every 2 months thereafter. Test article stability and homogeneity in the diet were determined once.

The animals were given fresh portions of the diets every day from Monday thru Friday. However, on Friday an extra-large portion of feed was given to cover the feeding requirements for Saturday and Sunday. A daily fresh portion was not given on Saturday and Sunday.

All animals were checked for clinical signs of toxicity or mortality once daily during the study. Body weights were recorded at the initiation of the study, on weekly intervals in the first 14 weeks and once every 2 weeks thereafter. Food and water intake were not measured in this study, but were measured in the oncogenicity study.

For hematological determinations blood samples were taken from the tip of the tail of 10 male rats/group on days 26, 180, 365, 551, and 718 and of 10 females rats/group and on days 27, 186, 368, (460)*, 552, and 719. For clinical chemistry measurements blood samples were taken by orbital puncture from 10 rats/sex/group on days 193/194 (week 28) and 557/558 (week 80). At autopsy, week 105, blood was taken from the aorta while the animals were under slight ether anesthesia. For urinalysis, individual urine samples were collected from 10 rats/sex/group on days 85, 188, 370, 554, and 722 following a 24-hour period of deprivation of water and a 16-hour period of deprivation of food. All hematology, clinical chemistry and urinalysis parameters measured are shown in table 1.

Table 1

Hematology	Clinical Chemistry
Red blood cells	Urea
*White blood cells (only)	Albumin
Hemoglobin	Alkaline phosphatase activity
Packed cell volume	Glutamic-oxaloacetic transaminase activity
Differential count	Glutamic-pyruvic transaminase activity
Reticulocyte count	Lactic dehydrogenase activity
Thrombocytes	Total protein
Mean corpuscular volume	Bilirubin
Mean corpuscular hemoglobin	Globulin
Mean corpuscular hemoglobin concentration	Creatinine
	Cholesterol
	Electrolytes: Na, K, Ca, Cl
	Thyroid function: T_3 uptake
	T_4
	Glucose

Necropsies were performed on all animals in this study. The animals were exsanguinated by cannulating the aorta under ether anesthesia and the organs and tissues listed in Table 1, were immediately dissected, embedded in Paraplast®, sectioned at 5 µm, stained with hematoxylin and eosin and examined for microscopic lesions. Only organ/tissues from the control group (male and female) and the high dose group (male and female) were used in histopathological observations. However, thyroids and pituitaries from the low- and mid-dose group males were also examined microscopically. Pituitary sections from all male animals were stained with Brookes' stain for differentiation of acidophilic cells and with Adams and Swettenham's performic acid-Alcian Blue-PAS-Orange G Stain (PAPO) for differentiation of basophilic cells.

Organ weights were recorded for all organs underlined in Table 1 and the organ weight to body weight ratios were determined using the terminal body weight.

TABLE 1

aorta

adrenals*

axillary lymph nodes

bone (femur)

brain (brainstem, cerebrum and cerebellum)

caecum

cervix

colon

duodenum

epididymides

eyes

harderian gland

head

heart

ileum

jejunum

kidneys

liver (at least two lobes)

lungs (all lobes with main stem bronchi)

mammary glands

mesenteric lymph nodes

oesophagus

ovaries

pancreas

parotid salivary glands

pituitary

prostate

sciatic nerve

skeletal muscle

skin

spinal cord (at least two levels)

spleen

sternum with bone marrow

stomach (glandular and nonglandular)

submaxillary salivary glands

sublingual salivary glands

testes

thyroid with parathyroids

trachea

urinary bladder

uterus

thymus (when present)

All nodules, tissue masses, and otherwise macroscopically abnormal tissues were preserved, along with samples of adjacent tissue when appropriate.

*Underlined tissues were weighed.

Statistical Analysis:

Body weight and organ weight data were subjected to one-way analysis of variance followed by Dunnett's multiple comparison test. Clinical chemistry values were analyzed by the Mann-Whitney U-test and hematological findings by the Wilcoxon test or the Mann-Whitney U-test. The incidence of histopathological changes was examined by the Fisher exact test and the percentage scores for Brookes or PAPO stained pituitaries were evaluated with the Mann-Whitney U-test.

The following deviations from the protocol were reported by the sponsor (abstracted from the original report):

a. The sodium content of the plasma (215 mmol/L) of one male control rat was omitted from the table because of an error in the determination.

b. Clinical chemistry, except for the determination of glucose, was not conducted in two males and one female of the 80 ppm group because of a mistake made during the sampling of the blood. In one male of the 400 ppm group the clinical chemistry could be carried out only partly because of shortage of plasma.

c. The creatinine concentration and the activities of LDH, GOT, and GPT were not determined in one female rat of the 80 ppm group because the plasma sample was too hemolytic.

d. By oversight, the thyroids of two males in the 400 ppm group, the testes of one rat in the 80 ppm group, and the adrenals of one male rat in the 400 and 2500 ppm groups were not weighed.

e. A few organs could not be examined microscopically because they were, by oversight, not collected for fixation or were lost during processing.

f. In addition to the H & E staining of the pituitaries of the male animals, two conventional special staining methods were also employed.

g. Some parameters were determined by other methods than stated in the protocol, namely glucose, urea, and thrombocyte count. This was done because new, more reliable methods were applied as indicated.

(Note: These deviations were judged by the Toxicology Branch to be not sufficiently great to influence the interpretation of the study results.)

Results:

Diet analysis throughout the study indicated that the actual levels of Pyridate concentrations in the diet (immediately after mixing) were on the average 9, 6, and 12 percent lower than the target concentrations of 80, 400, or 2500 mg/kg, respectively. More important however, was the fact that analytical concentrations varied considerably from target concentrations for substantial periods of time. Thus, for the low dose (80 ppm), concentrations varied (at different time points) between -31 percent and +14 percent, for the mid-dose (400 ppm) between -45 percent and +9 percent, and for the high-dose (2500 ppm) between -34 percent and +5 percent. The sponsor reported that the test article was homogeneously distributed in the diet as evidenced by the low coefficient of variation of 3.2, 3.8, and 2.3 percent for the low-, mid-, and high-dose levels, respectively. The test for homogeneity was conducted only once (approximately 5 weeks after the initiation of the study). Pyridate stability in the diet at 23 °C was reported to vary considerably with the dose level and the length of exposure to room temperature (23 °C). The following data were obtained:

Target Level (mg/kg)	Percent of Pyridate Lost	
	24 hours	72 hours
80	(35, 36) 36*	73
400	(21, 33) 27	61
2500	(13, 22) 18	49

Clinical signs: The sponsor reported that a number of clinical signs of toxicity were reported in some animals but it did not appear that these signs were due to the administration of Pyridate. The sponsors also reported that during the last months of the study (exact date not reported) there was an outbreak of infectious sialodacryoadenitis infecting most of the male animals in control and treated groups and some female animals in treated groups.

Mortality: No mortality was reported in male or female animals in control or treated groups.

Body weight gains were approximately the same between the controls and animals of the low-dose groups in males and females and high dose group males; slightly higher (4 to 7%) than controls in the mid-dose groups in males and females and lower (11%) than controls in the high-dose group females barely achieving

*Mean of two values shown in parentheses.

statistical significance (1 observation) towards the last 2 months of the study. Thus, the terminal body weights reported as percent change from control were as follows:

Dose Level (mg/kg/day)	Males	Females
80	+1.7	-5.1
400	+6.8	+4.0
2500	No Change	-10.8

A variety of clinical chemistry parameters (measured at terminal sacrifice) from the treated groups were found to be statistically significantly different from control groups. As shown in Table 2, in male animals statistically significant changes that may possibly be attributed to the test article administration were seen with alkaline phosphatase (high-dose group), glutamic pyruvic transaminase (high-dose group), calcium (high-dose group), and potassium (low-, mid-, and high-dose groups). In females, statistically significant decreases from the control were seen in the high-dose groups with alkaline phosphatase, lactate dehydrogenase, glutamic pyruvic transaminase, glutamic oxalacetic transaminase, and calcium.

[Although hematological measurements were not performed in this study, the sponsor supplied summary tables with data obtained from hematological determinations performed in a 2-year feeding study in rats using identical dose levels as in this study. Since these data were reviewed and discussed separately with the 2-year feeding study no review is presented here.]

Gross pathology did not reveal any compound-related effects.

Histopathological examinations (carried out for the most part in the control and high-dose groups) revealed a variety of lesions occurring randomly in both control and treated groups. In the pituitary (stained with hematoxylin-eosin), a statistically significant increase in the ratio in acidophilic and basophilic cells was observed in all treated groups of male rats, compared to controls. However, it was reported that staining of the pituitary with other special stains (designed to distinguish between the different endocrine-active cells in the pituitary) did not confirm the findings obtained from hematoxylin-eosin stained pituitaries.

In the small intestine of female rats, the incidence of slight hyperplasia of patches of Peyer was numerically (but not statistically) higher in the high-dose group compared to control (1/10 versus 4/10 for the control and high-dose groups, respectively).

Organ weights (absolute and/or relative) were found for some organs to be statistically significantly different between

TABLE 2

Effect of Pyridate on Clinical Chemistry Parameters

Parameter	Dose (mg/kg/day)							
	0		80		400		2500	
	M	F	M	F	M	F	M	F
Creatinine (umol/L)	72.8 ± 1.4†	76.3 ± 2.1	73.0 ± 3.2	72.5 ± 1.6	66.0 ± 1.2**	67.5 ± 1.7**	68.6 ± 1.4	74.9 ± 1.9
Chloride (umol/L)	107.4 ± 0.4		105.0 ± 0.5**		106.6 ± 0.6		106.7 ± 0.5	
Alkaline Phosphatase (u/L)	82.4 ± 4.4	66.5 ± 3.2	76.3 ± 3.6	67.0 ± 5.3	77.8 ± 4.1	60.8 ± 5.3	66.4 ± 3.5**	49.9 ± 5.2**
Glut. Pyr. Transaminase (u/L	49.9 ± 3.6	71.7 ± 5.8	58.9 ± 4.3	59.8 ± 6	60.4 ± 5.5	66.6 ± 9.3	42.3 ± 1.6	42.3 ± 2.8**
Glut. Oxal. Transaminase (u/L)	54.1 ± 1.9	65.8 ± 5.0	63.5 ± 4.6	57.0 ± 4.7	59.4 ± 3.4	64.8 ± 5.6	49.4 ± 1.5*	48.5 ± 2.6**
Potassium (mmol/L)	3.2 ± 0.02		3.5 ± 0.05***		3.7 ± 0.07***		3.7 ± 0.17***	
Calcium (mmol/L)	2.3 ± 0.02	2.5 ± 0.02	2.4 ± 0.02	2.5 ± 0.02	2.4 ± 0.03	2.4 ± 0.03	2.5 ± 0.03***	2.4 ± 0.02**
Lactate Dehydrogenase (u/L)		128.9 ± 12.5		83.6 ± 11.3				72.6 ± 6.0**

M = Males

F = Females

† Mean ± SD, N = 10

*Significantly different from control at P < 0.05.

**Significantly different from control at P < 0.02.

***Significantly different from control at P < 0.002.

Mann/Whitney U-Test, Two-sided.

Pyridate-treated and control groups, in both sexes. As shown in Table 3, absolute and relative thyroid weights in male rats were statistically significantly lower in all treated groups compared to controls. Absolute kidney weights were also higher than controls in the midand high-dose groups of male rats while the relative kidney weight was higher than controls in the high-dose group. In female rats statistically significantly higher relative weights were observed in pituitary and brain of the high-dose groups, while liver absolute weight in the high-dose group was significantly lower than controls.

Discussion:

Review of the analytical data presented in this study indicates that throughout the study test article concentrations in the diet varied considerably with all dose levels tested. In general, concentrations in the diet were significantly lower than the target concentrations for extended periods of time while in some cases nominal concentrations were moderately exceeded (up to 14 percent). If these results are correct (i.e., they do not represent errors in the analysis) then all animals received consistently fluctuating test article concentrations which were usually much lower than the dose levels specified. Additional data reported on the stability of Pyridate in the diet indicate that Pyridate is very unstable at room temperature and within 24 hours a high percentage (up to 36 percent) is lost either by degradation or volatilization and in 72 hours up to 73 percent is lost. Higher losses were consistently reported with the lower dose levels. The above results (i.e., lower diet concentrations coupled with high losses of Pyridate) strongly suggest that all animals were exposed to much lower concentrations of Pyridate than those reported by the sponsor, which makes the interpretation of the results more complicated.

The sponsor concluded that no compound-related signs of toxicity were seen in these animals.

Body weight gains in male rats were slightly higher than controls in the low- and mid-dose groups (1.7 and 6.8 percent higher in low- and mid-dose groups, respectively) while no change was observed with the high-dose group. In female rats, slightly lower (5.1 percent) body weights were observed with the low-dose group, slightly higher (4 percent) with the mid-dose group, and much lower (10.8 percent) with the high-dose group as compared to controls. (It is noted here however, that in the lifetime feeding study N = 50 food consumption was statistically significantly decreased the first 62 weeks.)

Evaluation of the clinical chemistry data revealed statistically significant changes in some parameters between treated and control groups. Although these changes may suggest some impairment of liver and/or kidney function, no correlation

TABLE 3

Effect of Pyridate on Organ Weights

Sex	Organ	A[1] or R[2]	Absolute or Relative Organ Weight Dose (ppm)			
			0	80	400	2500
Male	Thyroid	A	0.037 ± 0.002[3]	0.030 ± 0.002*	0.026 ± 0.002**	0.025 ± 0.002
		R	0.080 ± 0.004	0.063 ± 0.004*	0.053 ± 0.005**	0.054 ± 0.005
Male	Kidneys	A	2.484 ± 0.051	2.619 ± 0.065	2.875 ± 0.132**	2.780 ± 0.064
		R	5.3 ± 0.1	5.6 ± 0.2	5.8 ± 0.1	6.0 ± 0.1*
Female	Pituitary	A	0.016 ± 0.001	0.014 ± 0.001	0.016 ± 0.001	0.018 ± 0.001
		R	0.055 ± 0.003	0.050 ± 0.004	0.054 ± 0.003	0.071 ± 0.007
Female	Liver	A	9.00 ± 0.37	8.53 ± 0.32	8.84 ± 0.36	7.70 ± 0.81*
Female	Brain	R	6.3 ± 0.1	6.6 ± 0.2	6.3 ± 0.2	7.1 ± 0.2*

[1] Absolute tissue weight (g).
[2] Relative tissue weight (g/kg).
[3] Mean ± SD, N = 10 (N = 9 for adrenals).
*P < 0.05
**P < 0.01
Anova and Dunnett's Tests, Two-sided.

was observed between these changes and any histopathological changes in the same tissues or other tissues. Additionally, most of the changes seen did not show a clear dose-response relationship. Thus, the importance of the clinical chemistry changes cannot be fully assessed; however, it does not appear that the changes were compound-related.

Absolute and/or relative organ weights were found to be, in some cases, different between treated and control animals. In males, thyroid weights were statistically significantly lower in all treated groups as compared to controls. The decrease in thyroid weight appeared to be dose-related. Additional tests on thyroid function (T_3 uptake and T_4 content) did not reveal any differences between treated and control groups. Similarly, no histopathological changes were observed in the thyroid of male rats that could be ascribed to Pyridate administration. Decrease in liver weight in female rats of the high-dose group appear to correlate with the lower values seen in the HDT for alkaline phosphatase, lactate dehydrogenase, glutamic pyruvic transaminase, and glutamic oxalacetic transaminase activity in serum. However, the absence of any histopathological lesions in these livers and the lack of a dose response relationship, make it difficult to establish liver or any other tissue as a target tissue. Increase in kidney and brain weights were not associated with histopathological changes and are not considered to be biologically significant. Likewise, the higher relative pituitary weight observed in the high-dose group females was not associated with any obvious histopathological changes in the pituitary. The reported increase in the ratio of acidophilic/basophilic cells in pituitary slides stained with hematoxylin-eosin (male rats) could not be confirmed by other acceptable staining methods, according to the sponsor, and thus this finding is of doubtful significance.

Conclusions:

The Toxicology Branch is unable to come to any definite conclusion with regard to the reported results of this 1-year chronic-feeding rat study, including the establishment of a NOEL and LEL. This is primarily due to the unresolved questions regarding the fate of the test material in the diet and the apparent lack of mutually supporting data between the three studies within the experiment.

Classification:

The present study is classified as Core-Supplementary mainly for the following reasons:

1. Pyridate concentrations in the diet for all dose levels tested varied considerably from the target concentrations, usually being significantly lower than intended for extended periods of time.

2. Pyridate was found to be very unstable in the diet at room temperature with losses ranging from 18 to 36 percent within 24 hours and 49 to 73 percent at 72 hours.

3. No urinalysis was carried out.

4. Food consumptions were not measured.

5. The attached analytical data for Pyridate (technical) needs to be translated into English so that the identity of each impurity will be known.

6. Hematology was not measured in this experiment

It appears that this study may be upgraded depending upon the resolution of the issues raised in the review.

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R:87423:Ioannou:C.Disk:KENCO:5/19/86:DAA:VO

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