REVIEWER

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

005227

JUN 29 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#4G3047 - Pyridate Herbicide - Review of Chronic Toxicity and Carcinogenicity Studies in Rats in Support of Tolerances for Corn, Wheat, and Rice (EPA REG. NO. 42545-EUP-R)

Tox. Chem. No. 716A

FROM: Yiannakis M. Ioannou, Ph.D.
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

J M Ioannou 5-27-86

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D.
Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 6/12/86

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

6/29/86

Registrant: Chemie Linz Ag, Austria

Toxicology Branch has reviewed three studies concerning the chronic toxicity and carcinogenicity of Pyridate in rats, submitted by the registrant in support of tolerances of Pyridate for corn, wheat, and rice. The studies reviewed included:

1. One-Year Chronic Feeding Study
2. Two-Year Chronic Feeding Study
3. Lifetime Carcinogenicity (Feeding) Study

All studies were initiated by the sponsor at the same time carried out at the same laboratory, using the same strain of rats, and the same Pyridate dose levels (0, 80, 400, 2500 ppm). Thus, in the absence of more information from the sponsor, it is not clear whether we are dealing with three distinct studies or one study subdivided into three parts as the study progressed.

The following comments pertain to the individual studies as follows:

A. One-Year Chronic Feeding Study (Assay #170; Pyridate Technical, 90.3% purity): This study apparently represents an interim sacrifice (1-year) of the 2-year chronic toxicity study. Ten rats were used/sex/dose level. The study was classified as Core-Supplementary due mainly to the following deficiencies (see also DER):

1. The analytical Pyridate dietary concentrations varied considerably from the nominal dietary concentrations usually being significantly lower than the target concentrations for long-time intervals.

2. Pyridate appears to be highly unstable when mixed with the diet and exposed to room temperature (23 °C). Within 24 hours 18 to 36 percent of Pyridate is lost and within 72 hours 49 to 73 percent is lost (either through volatilization or degradation). The sponsor failed to provide an explanation for these losses and no steps were taken to maintain nominal diet concentrations either by minimizing losses or by increasing dose levels.

3. No urinalysis or hematology measurements were carried out.

4. Food consumption was not measured.

B. Two-Year Chronic Toxicity (Feeding) Study (Assay #171; Pyridate Technical, 90.3% purity): For this study 15 rats were used/sex/dose level. The study was classified as Core-Supplementary due mainly to the following deficiencies (see also DER):

1. The analytical Pyridate dietary concentrations varied considerably from the nominal concentrations, usually being significantly lower than the target concentrations for long-time intervals.

2. Pyridate appears to be highly unstable when mixed with the diet and exposed to room temperature (23 °C). Losses (either through volatilization or degradation) ranged from 15 to 27 percent within 24 hours and 44 to 60 percent within 72 hours. The sponsor failed to provide an explanation as to why the study was still carried out under these conditions or why no corrective measures were taken to adjust for these losses.

3. Food consumption was not measured.

4. The high dose tested (2500 ppm) does not appear to approximate the MTD especially in female animals.

C. Lifetime Feeding Carcinogencity Study (Assay #172; Pyridate Technical 90.3% purity): This study utilized 50 rats/sex/dose level. The study was classified as Core-Supplementary due to the following deficiencies:

1. The analytical Pyridate dietary concentrations varied considerably from the nominal concentrations usually being significantly lower than the target concentrations for long-time intervals.

2. Pyridate appears to be highly unstable when mixed with the diet and exposed to room temperature (23 °C). Losses (either through volatilization or degradation) ranged from 15 to 27 percent within 24 hours and 44 to 60 percent within 72 hours. The sponsor failed to provide an explanation as to why the study was still carried out under these conditions or why no corrective measures were taken to adjust for these losses.

3. Urinalysis measurements were not conducted.

In view of the aforementioned deficiencies, the following problems pertaining to all three studies need to be resolved by the sponsor before a final evaluation of these studies is considered:

1. The analytical data presented by the sponsor show that diet concentrations were on the average 13, 10, or 9 percent lower than the target concentrations of 80, 400, or 2500 ppm, respectively. Our examination

of the analytical data shows that individual values deviated from the nominal concentrations by unacceptable levels (up to -41, -45, or -34 percent for the low-, mid-, and high-dose levels, respectively), for considerable time intervals. We request that the sponsor provide the Agency with a written explanation as to why these low concentrations were used when they could have been adjusted to approximate the nominal concentrations.

2. Stability data obtained by the sponsor prior to the initiation of the above studies showed conclusively that Pyridate is very unstable at room temperature resulting in up to 36 percent loss in 24 hours and up to 73 percent loss in 72 hours, when mixed with the diet. We would like to know why the sponsor knowing the unstable nature of Pyridate, and the potential complexities which may be involved in the interpretation of the study results, still conducted the above major studies. The sponsor should have attempted prior to the initation of these studies to determine whether Pyridate losses were due to volatilization and/or metabolism. If losses were due to metabolism then metabolic products should have been identified and quantitated. If Pyridate and/or metabolites are shown to be volatile then obviously all personnel that conducted these studies were exposed to high levels of this chemical by the inhalation route.

   The sponsor needs to also address the quality control in this laboratory. It is possible that the analytical methodology used was not appropriate. Thus, we would like to know:

   a. Type of HPLC equipment used.

   b. Solvent system used; were these solvents capable of separating the parent compound from any metabolites?

   c. Were any reference standards (internal or external) used so that the HPLC system could be shown to be functioning at all times?

   d. How were the solutions for HPLC prepared (including extraction procedures), and how long were they exposed to room temperature before analyzed? (Knowing the unstable nature of Pyridate and/or metabolites the great variations in diet concentrations might

be partially explained by the procedures used in preparing samples for HPLC analysis.)

e. Were these analyses carried out by the same trained technicians thoughout the study?

3. Major disparities were reported between the three studies concerning body weight data. Thus, the HDT (2500 ppm) resulted in statistically significantly decreased body weight gains in males of the 2-year chronic feeding study (but not in males of the other two studies) and in females of the lifetime feeding study (but not in females of the other two studies). These inconsistencies raise questions on the actual conduct of the experiment with regard to animal husbandry. Therefore, the sponsor should supply the Agency additional information addressing such points as, for example:

   a. Were all the animals treated the same?

   b. Were all the animals kept in the same room, or moved from room to room, or cages rotated within the rack, or racks rotated within the room?

   c. Were room conditions identical (temperature, humidity, light, etc.)?

   d. Was there a turnover in personnel?

   e. Other considerations.

The Toxicology Branch is unable to come to any definitive conclusions concerning the reported results of all three studies (discussed above) including the establishment of NOEL and LEL. This is primarily due to the unresolved questions regarding the fate of Pyridate in the diet and the apparent lack of mutually supporting data between the three studies within the experiment. Additionally, depending on how the aforementioned questions are resolved by the registrant, the Toxicology Branch might request the statistical reanalysis of data in these studies.

An additional study entitled "Maximum Tolerated Dose of Pyridate in dogs"was also submitted by the registrant and reviewed by Toxicology Branch.This study was classified as Core-Supplementary mainly due to the failure of the sponsor to include a vehicle control group in this study (see also DER).Additionally, we like to point out that the registrant conducted this study after

the initiation or completion of other major long term studies (chronic toxicity and/or carcinogenicity studies in rats, mice or dogs) and thus any findings from this study could not be utilized (mainly in dose selection) for the long term studies.

We are also taking this opportunity to ask the following questions with regard to the 12-month dog study.

- How and why was the high dose so greatly increased above the target concentration when determined analytically and why was the dose level not decreased to the nominal concentration ?

- Additionally in light of the disparities seen in the doses (nominal vs. analytical)administered in either the 12-month dog or these rat studies (i.e., Assay # 170,171,and 172),we believe it prudent not to accept any results in the 12-month dog study beyond the 12th to 15th week.

We would also like to know why there is such a disparity between measurements for batch 11-02-82 as shown on page 001063 (34) [Study #171 table # 2] for parent compound after 3 days at 23 °C.

We would also like a study conducted where the parent compound is placed in feed and the residue of the parent conpound is measured (i.e., concentration remaining in feed, concentration in air of both, metabolites and parent).

We also find the wholesale loss of some tissues very disturbing and not reflective of good quality control or good laboratory practices.

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.

In all cases, high pressure liquid chromatography (HPLC) was used for analysis.

All animals were observed daily for signs of toxicity and/or mortality. Body weight measurements were taken at initiation of the study (day 0), at weekly intervals for the first 14 weeks and every 2 weeks thereafter. Food consumption was not measured in this study.

At termination (week 52) blood samples were taken from the aorta of all animals while under ether anesthesia, using heparin solution (30 mL solution of 5000 IU/mL) as the anticoagulant. The plasma, obtained by centrifuging blood at 2000 rpm for 15 minutes, was used to measure the following parameters:

Urea	Creatinine	
Albumin	Cholesterol	
Globulin	Electrolytes:	Na
Total Protein		K
Alkaline Phosphatase		Ca
Glutamic Oxaloacetic Transaminase		Cl
Glutamic Pyruvic Transaminase	Thyroid Function:	T_3 - uptake
		T_4 - content
Lactic Dehydrogenase		
Bilirubin	Glucose*	

[Although hematology parameters were not measured in this study, the sponsor reported the results from hematological investigations performed on blood samples obtained from 10 rats/sex/group on weeks 0, 4, 26, and 52, or 53 from a 2-year toxicity study where the same test article dose levels were used. Hematology parameters measured in the 2-year toxicity study (and reported here) included:

Red blood cells
White blood cells
Hemoglobin
Packed cell volume
Differential count
Reticulocyte count
Thrombocytes
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration

Urinalysis parameters were not measured in this study.]

*Glucose was determined from blood samples obtained from the tail of overnight fasted rats (week 52).

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:

	Percent of Pyridate Lost	
Target Level (mg/kg)	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

005227

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.

Subject: Pyridate: 2-Year Dietary Chronic Toxicity Study in Rats

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

Accession Number: 072342

Sponsor: Chemie Linz AG, Austria

Testing Facility: Netherlands Organization for Applied Scientific Research

Study Number: B80-0223 (Assay #171)

Testing Period: February 1980 - February 1982

Report Submitted to Sponsor - February 1983

Materials and Methods:

Pyridate technical (CL-11344), a brown viscous liquid used in this study, had a purity of 90.3 percent. All samples were stored at 5 °C until used.

Male and female weanling SPF rats (Cpb:WU; Wistar random), obtained from the Central Institute for the Breeding of Laboratory Animals, TNO, Zeist, the Netherlands, were used throughout this study. Upon arrival, all animals were checked for general health and acclimated to laboratory conditions for 7 days before being used. Healthy animals were divided into four groups (15 rats/sex/treatment level) and fed diets containing pyridate at 0, 80, 400, or 2,500 ppm (approximately 0, 4, 20, or 125 mg/kg/day). At the initiation of the study the rats were 4.5 weeks old and their mean body weight (per group) was approximately 80 g for males and 70 g for females. The animals were housed (5 per/cage) in suspended, stainless steel cages and identified (within each group) individually by an earmark. The temperature in the animal room was maintained at 23 ± 1 °C, the relative humidity between 40 to 70 percent, with a 12-hour light/dark cycle and 8 to 10 air changes/hour. Basal diets containing the test article were prepared weekly (in batches of 30 kg) and stored along with the control diets at 5 °C (41 °F) until use. Diets and tap water were available to all animals ad libitum.

Analysis of the diets for the level of test article concentration was conducted immediately after mixing on a weekly basis for the first 14 weeks and then every other month. Homogeneous distribution and stability of pyridate in the diet was determined once during the study.

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

Table 1 (Cont'd)

Urinalysis

pH
Protein
Glucose
Occult blood
Ketones
Density
Volume
Appearance
Sediment

At termination of the study (week 105) all surviving animals were killed and subsequently examined for gross pathological changes. A complete gross examination was also carried out on animals that died during the study or were sacrificed in moribund condition. Samples from tissues shown in table 2 were dissected from all animals and preserved in an aqueous, neutral, 4 percent phosphate-buffered formaldehyde solution.

The weight of the following tissues were recorded at sacrifice from all surviving animals:

heart	brain	pituitary
kidneys	lungs	thyroid and parathyroid
liver	testes	adrenals
spleen	ovaries	

The organ weight to body weight ratios (relative organ weight) were also calculated.

A detailed histopathological examination was carried out on all male and female rats of all groups. All tissues listed in table 2 were embedded in Paraplast®, sectioned at 5 µm, stained with hematoxylin-eosin and examined microscopically.

All nodules, tissue masses and other lesions suspected of being a tumor were preserved, along with samples of adjacent tissue where appropriate.

Statistical Analysis:

Data on body weights and organ weights were evaluated by one-way analysis of (co-)variance, followed by Dunnett's multiple comparison test.

Data on hematology, clinical chemistry and urinalysis were analyzed by the Mann-Whitney U-test. The gross and histopathological findings were examined by the chi-square test. Data on mortality were analyzed by the Fisher exact probability test.

Table 2

Samples of the Following Tissues were Dissected and Preserved from all Animals at the Time of Necropsy.

adrenals
aorta
axillary lymph nodes
bone (femur)
brain (brainstem, cerebrum and cerebellum)
caecum
cervix
coagulating glands
colon
duodenum
epididymides
esophagus
eyes
Harderian glands
head
heart
ileum
jejunum
kidneys
liver (at least two lobes)
lungs (all lobes with main stem bronchi)
mammary glands (females only)
mesenteric lymph nodes
ovaries
pancreas
parotid glands
pituitary
prostate
sciatic nerve
seminal vesicles
skeletal muscle
skin
spinal cord (at least two levels)
spleen
sternum with bone marrow
stomach (glandular and non-glandular)
submaxillary salivary glands
sublingual salivary glands
testes
thyroid with parathyroids
trachea
urinary bladder
uterus
thymus (if present)

The authors reported the following deviations from the protocol:

- A number of hematological and clinical chemistry determinations in individual animals was not carried out because of shortage of blood, loss of samples, clotting of sample or because of death of the animals.

- Some parameters were determined by other methods than those mentioned in the protocol, viz. glucose, urea and thrombocyte count. This was done because new and more reliable methods became available.

- By oversight a number of organs were not weighed.

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

  (Note: It is noted here that these deviations from the protocol did not appear to affect the outcome of the analysis or the interpretation of the findings.)

Results:

Diet analysis showed that the mean levels of pyridate concentration in the diets immediately after mixing as reported by the sponsor were 13, 10, or 9 percent lower than the target concentrations of 80, 400 or 2500 ppm, respectively. However, the individual values from which the mean is derived indicate a much greater variation than that represented by the mean above, and for intervals not reflected in the reporting of the mean alone. These deviations seriously challenge the quality (control) of the study and cast doubt on the findings of the study (see attached). Stability tests indicated that when pyridate (mixed with the diet) is stored at 23 °C, 27, 24, and 15 percent is lost in 24 hours and 60, 53, and 44 percent is lost in 72 hours in the low, mid- and high-dose levels, respectively. The test article appeared to be homogeneously distributed in the diet as indicated by the low coefficient of variation of 3.2, 3.8, and 2.3 for the low-, mid- and high-dose levels, respectively. (Note: [1] the same pyridate concentration levels, homogeneity and stability values were reported for the oncogenicity study with rats which was conducted concurrently with this study. [2] See Materials and Methods. All animals received one serving of feed to hold them over from Friday to Monday.)

All animals were palpated for masses during the study. Grossly visible masses were present in all groups of male and female animals. Statistically significantly higher number of masses was reported in the high-dose group of male rats compared to controls.

Clinical observations did not reveal any overt signs of pyridate toxicity to rats within the first 18 months of the study. After 18 months, a variety of aging symptoms were seen in all groups and the number or intensity of symptoms was similar between treated and control groups.

Table 3. Summary of Cumulative Mortality (%)[1]

Time (Days)	Males				Females			
	Dose (ppm)				Dose (ppm)			
	0	80	400	2500	0	80	400	2500
28	0	0	0	0	0	0	0	0
168	7	0	0	0	0	0	0	0
364	7	0	13	0	27	0	0	0
532	20	0	20	0	33	0	0	0
616	27	40	33	0	47	7	27	0
728[2]	33	53	60	0	80	13	73	13

[1] 15 animals were used per group.
[2] Day of terminal sacrifice.

Mortality figures (table 3) indicate that the rate of mortality was relatively low the first 18 months for all groups. Mortality increased gradually the last 6 months of study in all groups except the high-dose males (no mortality) and the low- and high-dose group females (13% mortality).

Mean body weights in male rats of the high dose group were statistically significantly lower than controls for the first 64 weeks of the study. Numerically lower, but not statistically significant mean body weights were also recorded in male rats of the high dose from week 64 to termination of the study. In females, statistically significantly higher mean body weights were observed in the low dose group beginning on week 72 until the termination of the study. Numerically higher body weights were seen also in the high dose females after week 70 of the study.

Organ weights were for the most part similar between treated and control groups in both sexes. The absolute lung weight, however, was statistically significantly lower in the low and high dose groups in males and females as compared to controls. The relative organ weight to body weight ratio of the adrenals, brain, and lungs in female rats was statistically significantly lower in the low and high dose groups as compared to controls. With the exception of the lung to body weight ratio in females at the high dose tested, the other organs showing a statistically significant decrease did not reveal a dose response. Additionally, for lungs, a comparison of the control group (N = 3; 15.20 $\pm$ 4.97 g/kg) against the mid-dose group (N = 4; 11.67 $\pm$ 2.85) indicated that the means were generally comparable numerically and not statistically significant. However, in the low dose tested (N = 13; 6.65 $\pm$ 0.89 g/kg) and the high dose tested (N = 13; 5.41 $\pm$ 0.57 g/kg) both groups were statistically significantly decreased from controls, but were comparable numerically. The statistical significance may therefore be attributed to sample size (N = 3 vs. N = 13). Additionally, histopathology was considered negative for the lung.

Hematology data indicated that a variety of parameters measured resulted in values which were statistically significantly different between treated and control groups in male and female rats. In male rats statistically significant decrease was observed in red blood cell counts, packed cell volume, reticulocytes and white blood cell counts while increases were observed in mean corpuscular volume, mean corpuscular hemoglobin concentration and packed cell volume in the various dose groups at different time intervals. In females, a statistically significant decrease in hemoglobin, thrombocytes, packed cell volume, reticulocytes, red blood cells, mean corpuscular hemoglobin counts and white blood cells was observed in various dose groups at different time intervals. However, neither in male nor in female rats was a dose-response relationship observed for any of the aforementioned parameters at any of the time points examined.

Clinical chemistry determinations indicated statistically significant differences between treated and control groups of male and female rats for a variety of parameters at different time intervals as shown in table 4. Although, most of the changes were seen with the high dose groups, the biological significance of these observations is not clear since no consistent dose-response relationship was established, and changes generally occurred in a direction which did not reflect a toxic response.

Urinalysis results have shown that urine volume was slightly lower while urine density was significantly higher in the high dose group of male rats. In females statistically significantly decreased urine volume was recorded in week 53 and urine density in weeks 13 and 53. None of the other parameters measured showed any changes between treated and control groups in either sex.

Table 4. Summary of Clinical Chemistry Determinations

Sex	Time Point (Weeks)	Parameter	Dose (ppm) 0	80	400	2500
Male	28	Urea	5.4[1]	6.4	5.8	6.1
	80	Urea	10.1	5.6	5.1	5.1
	28	Lactate Dehydrogenase	186.0	175.0	157.0	126.0*
	28	Calcium	2.6	2.6	2.6	2.4***
	105	Calcium	2.7	2.6	2.6	2.5*
	28	Chloride	100.0	100.0	101.0	102.0**
	28	Potassium	5.1	5.0	5.3	5.5*
	28	Sodium	192.0	171**	178.0	180.0
	80	Sodium	149.0	141***	142***	143.0***
	105	Total Globulin	37.0	33.0	34.0	32.0*
	105	Creatinine	70.0	78.0*	72.0	66.0
	105	Alkaline Phosphatase	62.0	56.0	54.0	47.0**
Female	28	Creatinine	67.0	68.0	75.0**	77.0***
	28	Alkaline Phosphatase	101.0	100.0	84.0*	91.0
	28	Lactate Dehydrogenase	162.0	202.0	79***	88.0***
	28	Chloride	101.0	103.0*	104.**	104.0***
	80	Chloride	98.0	97.0	98.0	101.0**

[1]Mean value from 10 animals.
* Statistical difference from control Mann/Whitney U-test.
* $P < 0.05$, **$P < 0.02$, ***$P < 0.002$.

Table 4. Summary of Clinical Chemistry Determinations (Cont'd)

Sex	Time Point (Weeks)	Parameter	Dose (ppm) 0	80	400	2500
Female	80	Albumin	47.0	50.0*	47.0	49.0
	105	Albumin	39.0	46.0*	45.0	45.0
	80	Total Globulin	34.0	31.0*	34.0	31.0*
	105	Total Globulin	41.0	32**	34.0	34.0*
	80	Urea	6.0	5.6	6.2	6.8*
	80	Potassium	3.9	3.9	4.0	4.3*
	105	Potassium	2.6	3.1	3.2	3.2**
	80	Sodium	142.0	136.**	136.**	135.0***
	105	Total Bilirubin	3.3	2.6	2.4	2.3**
	105	T_4	22.5	36.1	34.3	33.5*

* Statistical difference from control Mann/Whitney U-test.
* $P < 0.05$, **$P < 0.02$, ***$P < 0.002$.

Gross pathology performed on all animals that died spontaneously, sacrificed at a moribund state or necropsied at the end of the study did not reveal any statistically significant increase in the incidence of macroscopic lesions in pyridate treated animals as compared to controls. However, a variety of macroscopic lesions were seen in several tissues and the incidence of these lesions was higher in one or more treated groups as compared to controls. Some of the tissues with macroscopic lesions are listed in Table 5.

Table 5. Summary of Macroscopical Observations

Macroscopical Observations	Males				Females			
	Dose (ppm)				Dose (ppm)			
	0	80	400	2500	0	80	400	2500
Mammary glands: Tumor or suspected tumor	0/15[1]	1/15	0/15	0/15	1/15	3/15	2/15	2/15
- Evidence of secretory activity	0	0	0	0	3	9	2	8
Adrenals: Tumor or suspected tumor	1	0	0	1	0	3	0	0
- Spotted	0	1	0	0	1	5	2	5
Kidneys: Granular surface	2	4	2	0	0	1	0	2
Testes: Atrophy								
-Unilateral	1	3	2	1	-	-	-	-
-Bilateral	1	0	0	2	-	-	-	-
Ovaries: Cyst(s)	-	-	-	-	1	6	1	0
-Enlarged	-	-	-	-	0	1	2	0
Liver: Pronounced lobular pattern	1	2	0	1	0	3	1	3
Pituitary: Tumor or suspected tumor	1	0	0	1	1	3	1	1

[1]Number of rats with specified observation/total number of rats examined.

Histopathological examination revealed a variety of non-neoplastic and neoplastic lesions in several tissues of male and female rats. Although in most instances the incidence of histopathological lesions was approximately similar between the treated and control groups, statistical significance was observed with several non-neoplastic lesions. In lungs, the accumulation of alveolar macrophages was statistically significantly higher in female rats of the mid-dose group, while in male rats of the low dose group, the incidence of thickened alveolar septa was higher than controls (table 6). Myocardial degeneration (heart) was statistically significantly higher in female rats of the low-dose group, while hyperplasia (small intestines) was higher in male rats of the high-dose group. Focal proliferation of parafollicular cells was significantly higher in the thyroid of female rats of the low-dose group.

None of the neoplastic lesions were of statistically significantly higher incidence in treated male or female animals as compared to controls. Certain lesions that are of higher incidence (numerically) in treated than control animals are listed in table 6. However, the incidence of these lesions does not appear to be dose-dependent.

Table 6. Summary of Histopathological Observations

Histopathological Observation	Males Dose (ppm)				Females Dose (ppm)			
	0	80	400	2500	0	80	400	2500
Non-neoplastic Lesions								
Kidneys: Focal infiltration inflammatory cells - slight	6/15[1]	9/14	5/14	10/15	1/15	8/15	3/15	6/15
-Nephrosis - slight to moderate	10/15	10/14	8/14	10/15	2/15	5/15	2/15	4/15
Spleen: Extramedullary hematopoiesis - slight to moderate	5/15	5/14	1/14	4/15	2/14	4/15	0/15	5/15
							***	
Lungs: Accumulation of alveolar macrophages-slight to moderate	5/13	2/14	4/14	3/15	2/15	5/15	11/15	3/15
-Thickened alveolar septa with increased cellularity -slight to moderate	0/13	*4/14	3/14	3/15	3/15	2/15	4/15	0/15
						*		
Heart: Myocardial degeneration - slight to moderate	6/15	7/14	6/14	7/15	1/15	6/15	4/15	2/15
Small intestines: Hyperplasia of patches of Peyer - slight to moderate	1/15	1/13	2/12	*6/15	1/14	4/15	3/14	2/15
Thyroid with Parathyroid: Focal proliferation of parafollicular cells - slight to moderate	2/14	3/13	0/12	2/15	0/13	5/15	3/15	1/14
Adrenals: Foci or areas of degenerated cortical cells -one to a few	0/15	0/14	0/14	0/15	0/14	2/15	3/15	0/15

Table 6. Summary of Histopathological Observations (Cont'd)

Histopathological Observation	Males Dose (ppm)				Females Dose (ppm)			
	0	80	400	2500	0	80	400	2500
Non-neoplastic lesions (Continued)								
Ovaries: Cyst(s) small to medium sized - Atrophy	-	-	-	-	2/14	6/15	6/15	5/15
-slight to moderate	-	-	-	-	1/14	1/15	4/15	1/15
Thymus: Presence of involution	2/7	3/5	3/5	2/6	2/6	4/10	7/8	5/10
Pituitary: Focus of cellular alteration hypertrophic cells (pale to dark)	2/15	5/14	3/13	2/15	1/13	4/14	4/15	0/14
Neoplastic Lesions								
Uterus: Fibromatous polyp(s) -single	_	_	_	_	2/15	3/15	3/15	4/15
Adrenals: Pheochromocytomas-benign and malignant	2/15	3/14	0/14	2/15	0/15	0/15	0/15	0/15
Mammary glands: Fibroadenoma	-	-	-	-				
-Single	-	-	-	-	0/15	3/15	2/15	2/15
-Multiple	-	-	-	-	1/15	1/15	0/15	0/15
-Adenocarcinoma	-	-	-	-	0/15	1/15	0/15	0/15
TOTAL					1/15	5/15	2/15	2/15

Number of rats with specified observation/total number of tissues examined.
*Significant difference from control. Chi-square test: *P < 0.05, **P < 0.01, ***P < 0.001 .

Discussion:

The present study has investigated the chronic toxicity of Pyridate technical material in the rat. It is pointed out here that the sponsor in essence appears to have conducted one experimental study but subdivided and reported this study as three separate studies conducted in parallel. The studies were designated as follows:

Assay #170 = 10 animals per dose per sex per group - all animals sacrificed at 1 year. A chronic toxicity study.

Assay #171 = 15 animals per dose per sex per group - all animals sacrificed at 2 years. A chronic toxicity study.

Assay #172 = 50 animals per dose per sex per group. A lifetime feeding study. All animals remaining at termination were sacrificed. Each assay had its own control groups.

Assays #170, 171, and 172 were all started from the same pool of animals. Assay #170 and #171 are the chronic feeding study, with Assay #170 being the interim 1-year kill. Assay #171 was the continuation of the chronic feeding study to the terminal sacrifice of 2 years. Assay #172 was the lifetime oncogenicity portion of the study. It can therefore be said that the chronic feeding and the oncogenicity portions of each study appeared to have started out with the minimally acceptable number of animals.

Our examination of the analytical data with respect to the target concentration of the parent compound in the diet leads us to the conclusion that the deviations from the nominal concentration are much more serious than the reported means for the values and time period indicated. We are seriously concerned, at least with ~~with~~ the following:

- The loss of quality control for the analytical concentrations in the low-dose group (80 ppm) after May 19, 1980 (ca. 90 days, 13 weeks), in the mid-dose group (400 ppm) after October 6, 1980 (ca. 180 days, 26 weeks), and almost immediately in the high dose group (2500 ppm) from February 8 through May 12, 1980 (i.e., the first 90 days; 13 weeks).

- The apparent randomness with which the deviations occurred between dose groups for the periods of time indicated and yet the presence of apparent uniformity of decrement within dosing periods.

- The failure to take corrective measures to regain the nominal concentration as revealed by the analytical findings.

- The fact that the high dose tested (HDT) was at decreased levels of target concentration during the period of most rapid biological growth (i.e., first 90 days).

- The fact that the low- (80 ppm) and mid-dose (400 ppm) were severely decreased for the majority of the animal's life span (i.e., low-dose duration 90 weeks; mid-dose duration 78 weeks).

- That there has been no apparent effort to explain the deterioration or analyze for possible degradation products of the parent compound in the feed.

- That stability data for the period Monday through Friday was not reported.
- We are also concerned with the decrease of the parent chemical in the diet between Friday and Monday and its likely affect on the experimental results. It is not clear why this part of the protocol would be accepted by the sponsor, knowing the deterioration rate and potential complexities which may be involved in the interpretation of the study results.

We are, therefore, requesting that the sponsor provide the Agency with a written narrative explanation as to the conduct of this portion of the study.

We are also taking this opportunity to ask the following questions with regard to the 12-month dog study.

- How and why was the high dose so greatly increased above the target concentration when determined analytically and why was the dose level not decreased to the nominal concentration?

- Additionally, in light of the disparities seen in the doses (nominal vs. analytical) administered in either the 12-month dog or these rat studies (i.e., Assay #170, 171, and 172), we believe it prudent not to accept any results in the 12-month dog study beyond the 12th to 14th week.

We are also concerned with the results of the body weight data in this 2-year chronic feeding study (Assay #171) as well as the way they relate to the studies conducted in parallel, namely the 1-year study (Assay #170) and the lifetime feeding study (Assay #172) for males and females. The statistically significant decrease in male body weights during the first year for the HDT (2500 ppm) in the 2-year chronic feeding study was not corroborated in the lifetime feeding study where the sample size was 50 animals versus 15(25) in the 2-year study, nor was it corroborated in the 1-year study where the sample size was somewhat equivalent (N = 10). On the other hand in the HDT, in the 2-year chronic feeding study, females showed no statistically significant decreases in body weight as was also the case in the 1-year study. However, in the lifetime feeding study females in the HDT showed a statistically significant weight decrement for the entire length of the study.

We are therefore requesting from the sponsor a written narrative as to the actual conduct of the experiment with regard to the animal husbandry which should address such points as, for example,

- Were all the animals treated the same?

- Were they all in one room, or moved from room to room, or cages rotated within the rack as the experiment progressed?

- Were room conditions identical?

- Was there a turnover in personnel; were the personnel adequately trained, etc.

We would also like to know why there is such a disparity between measurements for batch 11-02-82 as shown on page 001063(34) [Study #171 table #2] for parent compound after 3 days at 23 °C.

We would also like a study conducted where the parent compound is placed in feed and the volatility of the parent compound is measured (i.e., concentration remaining in feed, concentration in air of both metabolites and parent).

Histopathological examinations revealed statistically significant differences between control and treated groups in both sexes in the formation of non-neoplastic lesions. The statistically significant increases in the accumulation of alveolar macrophages in females at the mid-dose, and the statistically significant increase of the alveolar septa in males at the low-dose, do not appear to be toxicologically significant as there was no dose response as evidenced in the accompanying table. However, the statistically significant increased hyperplasia of Peyer's patches in the small intestine of males at the high dose may be related to the administration of the parent compound. However, the evidence does not appear to be strong in that this response was not seen in females, nor was this effect observed in the oncogenicity study. It does not appear, therefore, to be a compound-related effect.

Neoplastic lesions of slightly numberically higher incidence were observed in one or more treated groups when compared to controls. However, the incidence of neoplastic lesions in the treated groups does not appear to be of toxicological concern since a dose-response was not established for any of these lesions and statistical significance was not in evidence.

Conclusion:

The Toxicology Branch is unable to come to any definitive conclusion with regard to the reported results of this 2-year chronic-feeding rat study, including the establishment of a NOEL. 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.

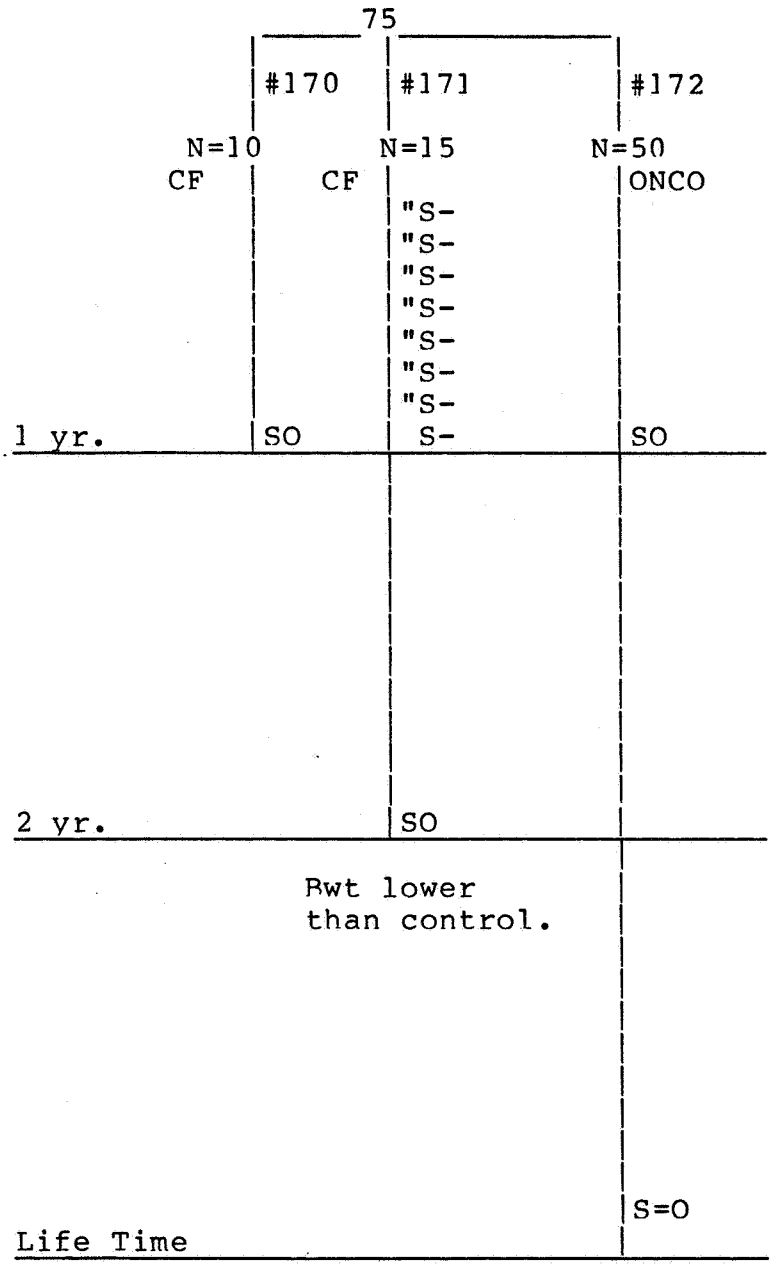
Classification:

Core-Supplementary for reasons presented in the review.

75 CONTROLS

Males

TREATED (HDT)



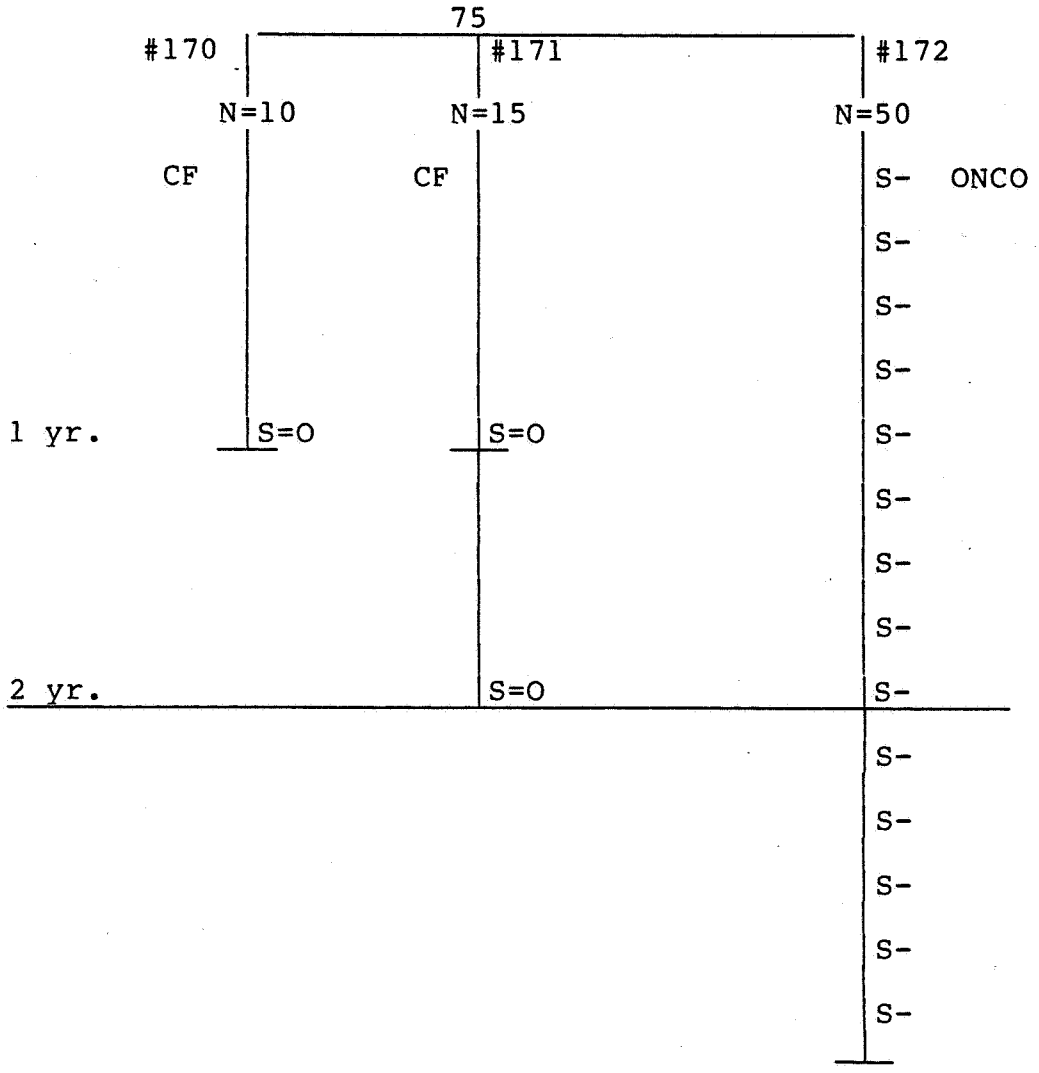
S=O Not Stat. Sig.
S- Stat. Sig. Decrease

"N" is equivalent for each group of controls.

CF CHRONIC FEEDING

75 CONTROLS

Females



"N" is equivalent for each group of controls.

Loss of Pyridate (Mean Percent Loss) (Assay #171)

Nominal Conc.	+ 24 Hours (23 °C)	+ 72 Hours (23 °C)
80 ppm	-27 (-35)*	-60 (-73)
400 ppm	-24 (-21)	-53 (-61)
2500 ppm	-15 (-13)	-44 (-49)

*Values in parenthesis indicate individual measurements where the percent loss has been higher than the mean.

Levels of Pyridate in Diet Immediately After Mixing

(Assay #171)

Target Date		80 ppm Percent (%) Change	400 ppm	2500 ppm
02/08/80		+12	+6	-8
02/14/80		-2	-5	-8
02/21/80		-5	-1	-18
02/29/80		-8	-5	-13
03/07/80		-15	-22	-27
03/14/80		-22	-3	-10
03/21/80		-8	+0.5	-14
03/30/80		+11	-8	-19
04/09/80*		+3	-1	-18
04/09/80*		-1	-9	-22
04/18/80		+8	+8	-9
05/01/80		0	-4	-13
05/12/80	90-day mark	+8	+1	-10
05/19/80	weekly thru	-22	-5	-3
05/28/80	(14th week)	-20	-2	-1
06/24/80	every other	-22	-4	+4
08/22/80	month (26	-20	0	+4
10/06/80	weeks 180	-28	-13	-8
12/11/80	days)	-31	-45	-33
02/17/81	(1-yr. mark)	-16	-14	-4
04/14/81		+6	-10	+6
06/17/81		-36	-23	-7
08/28/81		-15	-18	-1
01/07/81		-41	-32	+8
11/24/81		-27	-35	-7
02/11/81		-16	-16	-15
		$\bar{X}$-13%	$\bar{X}$-10%	$\bar{X}$-9%

Subject: Pyridate: Carcinogenicity Study in Rats. Lifetime Feeding Study

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

Accession Number: 072343

Sponsor: Chemie Linz AG, Austria

Testing Facility: Netherlands Organization for Applied Scientific Research

Study Number: B 80-0223 (Assay #172)

Testing Period: February 1980 to June 1982

Report Submitted to Sponsor: June 1983

Materials and Methods:

The purity of Pyridate technical (CL-11344), a brown viscous liquid used in the study, was 90.3 percent. All samples were stored at 5 °C until used.

SPF (Cpb:WU; Wistar random) male and female weanling rats, obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, the Netherlands, were used in this study. After an acclimation period of 7 days, male and female healthy animals were randomly divided into 4 groups (50/sex/treatment level) and fed diets containing 0, 80, 400, or 2500 ppm (approximately, 0, 4, 20, or 125 mg/kg/day) of Pyridate.

At the initiation of the study, the rats were approximately 5 weeks old and their mean body weight was approximately 80 g for the males and 70 g for the females. The animals were housed in suspended stainless steel cages (5/sex/cage) and identified individually by an earmark. The temperature in the animal room was kept at 23 ± 1 °C, the relative humidity between 40 to 70 percent, with a 12-hour light/dark cycle and 8 to 10 air changes/hour. Basal diets containing the test article were prepared weekly and stored, along with control diets, at 5 °C until use. Diets and tap water were available to control and treated rats ad libitum. The animals were given fresh portions of the diets every day from Monday through 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 or Sunday. The test article concentrations in the diet were determined immediately after mixing on a weekly basis for the first 14 weeks of the study and then every other month. The homogeneity and stability of the Pyridate in the diet were determined once during the study.

All animals were checked for clinical signs of toxicity or mortality once daily for the first 18 months on study and twice daily thereafter. Ophthalmoscopic examinations were carried out prior to the study (week 0) and on weeks 6, 13, 26, 60, 77, and 104 on all rats of the control and high dose (2500 ppm) groups.

Body weights were recorded at weekly intervals in the first 14 weeks and every 2 weeks thereafter. Food intake was measured at weekly intervals and water consumption was recorded daily for the control and high dose group animals in weeks 7, 13, and 25.

Hematology measurements were carried out in 10 rats/sex/dose group on day 839 for males and 840 for females from blood taken from the tip of the tail. For clinical chemistry measurements blood was taken from the abdominal aorta of 10 rats/sex/dose/group on days 834 and 845 (28 months) while the rats were under ether anesthesia (immediately prior to terminal sacrifice). Urinalysis was not performed. All hematology and clinical chemistry parameters measured are shown in Table 1.

All animals were killed at the termination of the study (week 121) and subjected to a complete gross examination. Animals that were sacrificed in a moribund condition or died during the study were also necropsied (if autolysis was not too advanced). A list of all organs and tissues dissected from all animals on the study is presented in Table 2. Samples of these organs and tissues were preserved in an aqueous, neutral, 4% phosphate-buffered formaldehyde solution. For histopathological examination, organs and tissues (listed in Table 2) were embedded in Paraplast®, sectioned at 5 µm and stained with hematoxylin-eosin. The pituitary, adrenals, thyroid, and ovaries were sectioned at three levels. All sections were examined microscopically for the presence of hyperplastic, preneoplastic, and neoplastic lesions.

Organ weights (absolute) were recorded at sacrifice from all surviving animals as follows:

heart	brain	pituitary
kidneys	lungs	thyroid and parathyroids
liver	testes	adrenals
spleen	ovaries	

The organ weight to body weight ratios (relative organ weight) were also calculated.

Statistical Analysis:

Data on body weights and organ weights were evaluated by one-way analysis of (co-) variance, followed by Dunnett's multiple comparison test. Data on food and water intake were evaluated by analysis of variance followed by the L.S.D. test. The gross and histopathological findings and mortality data were analyzed by the Fisher exact probability test.

Table 1

Hematology

red blood cells
white blood cells
hemoglobin
packed cell volume
differential count
recticulocyte count
thrombocytes
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration

urea
albumin
alkaline phosphatase activity
glutamic-oxaloacetic transaminase activity
glutamic-pyruvic transaminase activity
lactic dehydrogenase activity
total protein
bilibubin
globulin
creatinine
cholesterol
electrolytes: Na, K, Ca, Cl
thyroid function: T_3 uptake
T_4
glucose

Table 2

Samples of the Following Tissues, were Dissected and Preserved from All Animals at the Time of Necropsy.

aorta
adrenals
axillary lymph nodes
bone (femur)
brain (brainstem, cerebrum and cerebellum)
caecum
cervix
coagulating glands
colon
duodenum
epididymides
eyes
Harderian glands
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 glands
pituitary
prostate
sciatic nerve
seminal vesicles
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 (if present)

All nodules, tissue masses, and other lesions suspected of being a tumor were preserved, along with samples of adjacent tissue where appropriate.

The authors reported the following deviations from the protocol:

- By oversight and tumorigenic enlargement a number of organs were not weighed.
- A few organs could not be examined microscopically because they were not collected for fixation or were lost during processing.
- The ophthalmoscopic examination - scheduled for week 52 - had to be postponed till week 60, because of an outbreak of sialodacryoadenitis around week 52.

Results:

Pyridate analytical concentrations in the diet immediately after mixing, as reported by the sponsor were on the average 13, 10, and 9 percent lower than the target concentrations of 80, 400, and 2500 ppm, respectively. However, the individual values from which the mean is derived indicate a much greater variation than that represented by the mean alone and for intervals not reflected in the reporting of the mean alone. These deviations seriously challenge the quality (control) of the study and cast doubt on the findings of the study (see attached). Stability tests indicated that when Pyridate is stored (mixed with diet) at 23 °C for 24 hours, approximately 27, 24, and 15 percent is lost from the low-, mid- and high-dose levels, respectively. Storage (23 °C) for 72 hours resulted in 60, 53, and 44 percent loss of Pyridate from the low-, mid- and high-dose levels, respectively. (Note: see Materials and Methods. All animals were given one serving of feed to hold them over from Friday through Monday.) The test article appeared to be homogeneously distributed in the diet with a coefficient of variation of 3.2, 3.8, and 2.3 for the low-, mid-, and high-dose levels, respectively.

All animals were palpated for masses throughout the study. Grossly visible masses were observed in all groups (control and treated) in male and female animals. No significant differences in total number of animals with masses were seen between control and treated groups in both sexes with the exception of a statistically significantly lower incidence of masses seen in the high dose group of female rats as compared to control.

Clinical observations did not reveal any overt signs of Pyridate toxicity during the first 18 months of the study and animal mortality (Table 3) was very low during this period. A variety of symptoms were observed in all animals after 18 months on study. No significant differences in the number or intensity

of symptoms were seen between control and treated groups in both sexes. Increasing mortality was also recorded in all groups after 18 months so that at termination up to 72 percent of animals of both sexes died (Table 3). The observed symptoms and high mortality towards the last stages of the study can be attributed mainly to animal aging rather than Pyridate toxicity. No dose-response relationship was observed at any time point with respect to clinical symptoms or mortality.

Ophthalmoscopic examinations revealed that some lesions observed in week 60, 77, and 104 were of higher incidence in the high dose group of males and females compared to controls as shown in Table 4. Statistical analysis was not performed for the above data and the biological significance of these findings is not clear.

Mean body weights of male rats of the high dose group were statistically significantly lower than controls in the first 3 weeks and on weeks 94, 96, 98, and 106. In general, throughout the study, lower mean body weights were observed in male rats with the high-dose group as compared to controls. Mean body weights of female rats of the high-dose group were statistically significantly lower than controls throughout the first 2 years of the study. No significant mean body weight differences were seen between the control and the low- and mid-dose groups of male and female rats.

Mean food consumption in male rats was statistically significantly lower in the high-dose group as compared to controls on weeks 1, 2, 3, and at a few other random time points during the study. In females, however, statistically significantly lower food consumption was recorded in the high-dose group from week 1 through week 62. Occasional significant differences from the control were also observed after week 62 for the high dose group and throughout the study for the low- and mid-dose groups in female rats. Food efficiency (weight gain/food consumed) calculated for weeks 1 to 4 (period of rapid growth) was found to be comparable in all groups. Mean water consumption was also comparable between the control and high-dose groups.

The absolute as well as the relative organ weights were comparable in all groups of male and female animals. The statistically significant decrease in absolute weight in testes with the mid-dose group as compared to control, does not appear to be of any biological significance since no dose-response relationship was seen.

Hematology data indicated that in male rats most parameters measured were comparable between control and treated groups. Statistically significant differences were seen only in lower mean corpuscular volume with the high-dose group and in higher percent eosinophils with the mid- and high-dose groups. In females statistically significant differences from control values were

Table 3

Summary of Cumulative Mortality (%)[1]

Time (Days)	MALES Dose (ppm)				FEMALES Dose (ppm)			
	0	80	400	2500	0	80	400	2500
28	0	0	0	0	0	0	0	0
168	0	0	2	0	0	0	0	0
364	0	0	2	0	0	0	0	0
532	0	6	6	4	4	0	2	2
616	18	18	12	14	8	8	4	14
728	34	36	36	38	34	30	18	38
845[2]	60	70	66	72	72	46	52	72
% Survivors	40	30	34	28	28	54	48	28

[1] 50 animals were used per group.
[2] Day of terminal sacrifice (845 days = 28 months).

Table 4

Time[1] (weeks)	Ophthalmoscopic Observation	MALE Dose (ppm)		FEMALE Dose (ppm)	
		0	2500	0	2500
60	Vascular infiltration of cornea	6/50	13/49	8/50	5/10
77	do.	2/48	4/48	1/50	1/47
104	do.	10/33	11/31	9/34	13/31
60	Focal opacity of the cornea	1/50	0/49	2/50	8/50
77	do.	3/48	6/48	8/50	5/47
77	Cataract - total	1/48	3/48	1/50	1/47
104	Cataract - focal	18/33	19/31	11/34	16/31

[1] No adverse effects were seen at week 0, 6, 13, or 26.

seen in the following parameters: hemoglobin, lower in low-dose group; packed cell volume, lower in all groups; red blood cells, lower in low- and high-dose groups; white blood cells, lower in low- and high-dose groups; and mean corpuscular hemoglobin concentration, higher in mid-dose group.

Clinical chemistry determinations have shown that statistically significant differences from the control values were seen in higher values of lactate dehydrogenase with the high-dose group and potassium with the mid-dose group in male rats and lower total bilirubin with the high-dose group and creatinine with the low- and high-dose groups, in female rats.

Gross pathology performed on all female rats that died spontaneously, moribund sacrificed or necropsied at the end of the study did not reveal any significant treatment-related effects. However, for males, a slight downward trend appeared to be evident with an increasing dose for enlarged heart and enlarged unilateral adrenals.

Histopathological examination revealed a variety of neoplastic and preneoplastic lesions in several tissues of male or female rats. Although, in most instances these lesions were of comparable incidence in control and treated groups, statistically significant differences between control and treated groups were observed in several tissues and are listed in Table 6 (neoplastic lesions) and Table 7 (preneoplastic lesions). In adrenals, the incidence of total benign pheochromocytomas was statistically significantly higher in male rats of the low-dose group than the control (3/50 and 11/48 in control and low-dose, respectively). A decreasing trend with an increasing dose appeared evident. Additionally, the number of pheochromocytomas classified as "medium" were statistically significantly higher in the low dose and appeared to show a decreasing trend with an increased dose. The incidence of malignant pheochromocytomas was slightly higher in the mid-dose group of male rats compared to control. The combined incidence of benign and malignant pheochromocytomas in male rats was again statistically significantly higher in the low-dose group and numerically slightly higher in the mid- and high-dose groups as compared to control (5/50, 13/48, 10/49, and 6/47 in control, low-, mid-, and high-dose groups, respectively). A downward trend with increasing dose also appeared to be evident. Females showed no essential differences between treated and control groups.

In female mammary glands, statistically significantly higher incidence of multiple fibroadenomas was observed in the low- and mid-dose groups. A high incidence of fibroadenomas (single) was observed in all groups (control and treated) of female rats. The combined incidence of single and multiple fibroadenomas in female rats did not show statistically significant differences between control and treated groups. In male rats no fibroadenomas were seen in any group.

Table 5

Summary of Macroscopical Observations

Macroscopical Observations	Males				Females			
	Dose (ppm)				Dose (ppm)			
	0	80	400	2500	0	80	400	2500
SKIN: Tumor or suspected tumor	3/50[1]	2/49	2/49	7/47	2/50	0/50	4/50	1/50
ABDOMINAL CAVITY: Ascites	1	3	3	3	0	0	0	1
SPLEEN: Splenomegaly	0	1	3	0	2	0	1	0
ADRENALS: Tumor or suspected tumor	1	3	5	0	1	2	2	2
Enlarged-unilateral	1	4	3	1	6	3	4	3
KIDNEYS: Surface lesions (cysts, spots)	3	5	6	4	0	1	0	0
-Enlarged	1	7	0	3	0	0	0	1
MESENTERIC LYMPH NODES: Enlarged/swollen	0	2	4	2	2	1	1	0
-Hemorrhagic	0	3	2	1	0	0	1	0
TESTES: Atrophy-unilateral	4	2	13	8				
OVARIES: Tumor or suspected tumor					1	0	0	3
cyst					3	4	7	5
HEART: Enlarged	5	10	4		6	1	0	3

[1] Number of rats in specified observation/total number of tissues examined.

Table 6

Summary of Histopathological Observations (Tumors)

Histopathological Observation (Tumors)		Males				Females			
		Dose (ppm)				Dose (ppm)			
		0	80	400	2500	0	80	400	2500
ADRENALS:	Pheochromocytoma-Small	1/50[1]	3/48	2/49	3/47	1/49	2/49	0/50	1/49
	-Medium	1	8*	3	0	0	0	0	0
	-Large	1	0	1	1	0	1	0	0
	Total (Benign)	3	11*	6	4	1	3	0	1
	Pheochromocytoma-Malignant	2	2	4	2	0	0	1	0
	Total: Benign & Malignant	5	13*	10	6	1	3	1	1
MAMMARY GLANDS:									
	Fibroadenoma-Single	0/30	0/26	0/17	0/18	9/48	8/48	10/49	5/48
	-Multiple	0	0	0	0	0	5*	7*	2
	Total Single & Multiple	0	0	0	0	9	13	17	7
	Fibroadenoma with focal malignant area	0	0	0	0	1	1	1	0
	Adenocarcinoma	0	0	0	0	0	1	3	0
	Adenoma	0	0	0	1	0	1	1	0
THYROID:	Light cell solid carcinoma	0/47	2/47	0/43	0/44	0/46	0/47	0/50	0/48
	Light cell solid adenoma	2	7	9*	3	8	7	5	5
	Follicular adenoma	1	1	0	0	0	2	1	1
	Papillary cystadenoma	1	0	0	0	1	0	0	0

[1] Number of rats with specified observation/total number of tissues examined.

* Significant difference between treated and control incidence (Fisher's exact test), $P < 0.05$.

Table 7

Summary of Histopathological Observations (Preneoplastic Lesions)

Histopathological Observation (Tumors)	Males Dose (ppm) 0	80	400	2500	Females Dose (ppm) 0	80	400	2500
ADRENALS:								
Focal medullary hyperplasia-Small	4/50[1]	3/45	4/47	5/43	4/49	4/48	4/50	1/49
Medium	0	6**	8**	1	1	0	2	1
Large	1	2	2	3	2	0	0	0
Total	5	11	14	9	7	4	6	2
Light-cell focus in zonaglomerulosa		2	1*	3	13	15	13	12
HEART: Focal cartilagenous metaplasia	0/50	5/49	4/49	4*/45	4/49	2/49	3/50	1/49
LIVER:								
Foci of cellular alteration-Basophilic	0/48	1/48	0/44	0/42	4/48	4/49	2/49	3/49
-Mixed	1	5	2	0	0	2	0	1
-Clear	2	4	3	6	1	0	0	1
Total	3	10	5	6	5	6	2	5
MAMMARY GLAND: Lobular hyperplasia	1/30	0/25	0/17	0/18	18/48	27/48	28/48	11/48
PITUITARY:								
Foci of cellular alteration-Acidophilic	3/47	0/42	7/44	7/40	4/46	1/47	1/48	1/49
-Clear	1	0	2	0	4	2	4	2
-Basophilic	3	1	3	2	0	1	3	4
Total	7	1	12	9	8	4	8	7
THYROID: Parafollicular cell proliferation	7/47	9/44	11/40	13/41	9/46	7/45	7/48	10/47
URINARY BLADDER: Epithelial hyperplasia	0/50	1/48	3/45	1/42	0/47	0/47	0/48	0/47
UTERUS: Papillary endometrial hyperplasia					1/47	3/48	0/50	0/49
PARATHYROID: Diffuse epithelial hyperplasia	3/33	6/36	4/25	5/32	0/35	1/43	1/43	0/44

[1] Number of rats with specified observation/total number of tissues examined.
* Significant difference between treated and control incidence (Fisher's exact test), $P < 0.05$.
**Significant differences between treated and control incidence(Fisher's exact test), $P < 0.01$.

Light-cell solid adenomas of the thyroid were statistically significantly higher in the mid-dose group of male rats while the incidence in the low-dose group was close to significance compared to controls. Additionally, two light cell solid carcinomas were noted in the low dose group but none were found in the other dose groups. In female rats, the incidence of these adenomas was comparable in control and treated groups.

Preneoplastic and/or hyperplastic lesions were present in several tissues of male and female animals (Table 7). In adrenals, focal medullary hyperplasia (small-, medium-, or large-sized) was of statistically significantly higher incidence in the low- and mid-dose group of male rats as compared to controls. The total was statistically significantly higher in the mid-dose. In female rats, the incidence of hyperplasia was higher in control rather than treated groups.

Focal cartilagenous metaplasia of the heart was significantly higher in all treated than control groups in male rats. Females showed a slightly higher incidence in controls. The total incidence of combined foci of cellular alteration in liver of male rats was statistically significantly higher in the low-dose group than control and numerically slightly higer in mid- and high-dose groups. The frequency of this lesion in female rats was comparable in all groups. In the mammary gland of female rats, lobular hyperplasia was statistically significantly higher in the mid-dose group and close to significance in the low-dose group as compared to controls.

In the pituitary of male rats, the combined incidence of foci of cellular alteration (acidophilic, clear, or basophilic cell type) was numerically higher in the mid- and high-dose groups than the control group. In the thyroid of male animals, parafollicular cell proliferation was increasingly higher with the higher dose level although statistical significance between control and treated groups was not established (Table 7 14%; 20%; 27%; 31% respectively). Epithelial hyperplasia of the urinary bladder in male rats was of slightly higher incidence in the mid-dose group, while papillary endometrial hyperplasia of the uterus was slightly higher in the low-dose group. Slight but nonsignificant numerical increase in the incidence of diffuse epithelial hyperplasia of the parathyroid was seen with all treated groups.

Discussion:

The present study has investigated the oncogenic potential of Pyridate in male and female rats.

Examination of the analytical data with respect to the target concentration of the parent compound in the diet leads us to the conclusion that the deviations from the nominal concentration are

much more serious than the reported means for the values and time period indicated. We are seriously concerned, at least with the following:

- The loss of quality control for the analytical concentrations in the low-dose group (80 ppm) after May 19, 1980 (ca. 90 days, 13 weeks), in the mid-dose group (400 ppm) after October 6, 1980 (ca. 180 days, 26 weeks) and almost immediately in the high-dose group (2500 ppm) from February 8 through May 12, 1980 (i.e., the first 90 days, 13 weeks).

- The apparent randomness with which the deviations occurred between dose groups for the periods of time indicated and yet the presence of apparent uniformity of decrement within dosing periods.

- The failure to take corrective measures to regain the nominal concentration as revealed by the analytical findings.

- The fact that the high dose tested (HDT) was at decreased levels of target concentration during the period of most rapid biological growth (i.e., the first 90 days).

- The fact that the low- (80 ppm) and mid-dose (400 ppm) was severely decreased for the majority of the animals' life-span (i.e., low-dose duration 90 weeks; mid-dose duration 78 weeks).

- That there has been no apparent effort to explain the deterioration or analyze for possible degradation products of the parent compound in the feed.

- That the stability data for the period Monday through Friday was not reported.

- We are also concerned with the decreased presence of the parent chemical in the diet between Friday and Monday and its likely effect on the experimental results. It is not clear why this part of the protocol would be accepted by the sponsor knowing the deterioration rate and potential complexities which may be involved in the interpretation of the study results.

We are, therefore, requesting the sponsor to provide the Agency with a written narrative explanation as to the conduct of this portion of the study.

We are also taking this opportunity to ask the following questions with regard to the 12-month dog study.

- How and why was the high dose so greatly increased above the target concentration when determined analytically and

why was the dose level not decreased to the nominal concentration?

- Additionally, in light of the disparities seen in the doses (nominal vs. analytical) administered in either the 12-month dog study or these rat studies (i.e., Assay #170, #171, #172), we believe it prudent not to accept any results in the 12-month dog study beyond the 12th to 14th week.

We are also concerned with the results of the body weight data in this oncogenicity study (Assay #172) as well as the way it relates to the studies conducted in parallel, namely, the 1-year study (Assay #170) and the 2-year chronic feeding study (Assay #171) for both males and females. The statistically significant decrease in male body weights during the first year for the HDT (2500 ppm) in the 2-year chronic feeding study was not corroborated in the lifetime feeding study where the sample size was 50 animals versus 15 (25) in the 2-year study, nor was it corroborated in the 1-year study where the sample size was somewhat equivalent (N = 10). On the other hand, in the HDT in the 2-year chronic feeding study females showed no statistically significant decreases in body weight as was also the case in the 1-year study. However, in this lifetime feeding study females in the HDT showed a statistically significant weight decrease for the entire length of the study.

We are therefore requesting from the sponsor a written narrative as to the actual conduct of the experiment with regard to the animal husbandry, which should address such points as for example,

- Were all the animals treated the same?
- Were they all in one room or moved from room to room, or cages rotated within the rack as the experiment progressed?
- Were room conditions identical?
- Was there a turnover in personnel; were the personnel adequately trained?

We would also like a study conducted where the parent compound is placed into the feed and the volatility of the parent compound is measured (i.e., concentration remaining in feed, concentration in air of both parent and metabolites).

Mean food consumption in male rats was statistically significantly lower in the high dose group when compared to control during the first 3 weeks, and at a few random time points during the study. Body weight decreases in male rats for these 3 weeks paralleled decreased food consumption, and it seems reasonable

to assume that the decreased body weights for this time interval could be attributed to palatability. Body weight decreases and decreased food consumption values generally paralleled one another for the remainder of the experiment. During the first 18 months, body weight decreases and food consumption values generally ranged between 1 and 3 percent below control values. After 18 months, values for decreased food consumption and decreased body weight generally ranged between 5 and 10 percent for both parameters. These values for males taken together with the absence of any evidence of toxicity or tumors which could be related to the HDT question the selection of the HDT for this experiment in males.

Mean food consumption for female rats was consistently and statistically significantly decreased for the test animals in the HDT for the first 14 months of the experiment (434 days; 62 weeks), and generally comparable to control values thereafter. Body weights for females were consistently decreased from controls from the beginning of the experiment (with the exception of possibly days 7-35) through days 720 (24 months). The body weight decrements from 14 through 24 months in the absence of a statistically significant decreased food intake, followed by body weight decreases of 10 percent unaccompanied by decreased food intake from 24 through 28 months suggest that for females the high dose tested appeared to be adequate for this study, even though no overt toxicity (or tumors) was observed at the HDT.

Other parameters examined, i.e., water intake, absolute and relative organ weights, and hematology did not reveal any apparent treatment related effects of biological significance. However, the gross pathology indicated a variety of lesions and suggested that some may have been treatment related.

Statistically significant responses were observed in liver, adrenals, heart, and thyroid with a slight downward trend with increasing dose for adrenals, heart, and possibly thyroid. [It is also suggested here that if the pheochromocytomas are a primary cause then there appears to be a secondary relationship (toxicologically) between the other organs mentioned here as well as the increased LDH values noted in clinical chemistry.]

Additionally, differences in tumor incidence cannot be explained by differences in mortality rates since the death rate was comparable in all groups. However, until the question of the dietary stability is resolved, these findings cannot be considered conclusive.

Conclusions

The Toxicology Branch is unable to come to any definitive conclusion with regard to the reported results of this lifetime feeding study. 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:

Core-Supplementary for the reasons presented in the review. However, the study may be upgraded depending upon the resolution of issues raised in the review.

Subject: Maximum Tolerated Dose (MTD) of Pyridate in Dogs (Oral Gavage-Capsule)
(Note: This study was conducted after a 90-day oral gavage study dated 12/78[Accession # 072340] and after the 1-year chronic feeding study[4/81-4/82; Accession # 072350])

Test Material: Pyridate Technical (CL-11344) 90% ai

Accession Number: 072340

Sponsor: Chemie Linz Ag, Austria

Testing Facility: OEFZS

Study Number: OEFZS Ber. No. A0403

Testing Period: May 1982

Report Submitted to Sponsor: March 1983

Materials and Methods:

Male and female beagle dogs (supplied by Velaz/CSSR) approximately 8 months of age and weighing between 11.2 and 15.2 kg, were used in this study. The animals were divided into three groups, one male and one female dog/group, placed in individual metabolism cages and kept in an air-conditioned room with a temperature of 20 to 22 °C, 8 air changes/hour and a 12-hour light/dark cycle. Pelleted diet (Altronim No. 4024) and water were available to all dogs ad libitum. Dogs were identified by cage number and by markings inside the left auricle. The animals were acclimated to laboratory conditions for at least 7 days, fasted for 12 to 18 hours and then each group was administered orally technical Pyridate (oily liquid, 90% pure) using a gelatine capsule (Lilly size 00). The capsules were administered to each dog over a period of 10 minutes using about 50 mL of water to facilitate swallowing. Animals were allowed to resume feeding 3 hours after administration. Pyridate was administered as a single oral dose at 1000, 3000, or 5000 mg/kg. No control group was used in this study.

All animals were observed for 14 days after administration for signs of toxicity and/or mortality and palpated for tumors.

Specifically, clinical examinations were carried out 1 day prior to treatment, 3 hours posttreatment and on days 2, 8, and 15. Body weight measurements were recorded on days 0, 1, 2, 3, 8, and 15 on study. Ophthalmological examinations were conducted on all animals on day 1 and on day 15 on study.

The animals from all groups were sacrificed (exsanguinated under anesthesia, 50 mg thiopental/0.5 mL A dest/kg body weight I.V.), on the 15th day on study and examined for gross pathological lesions. Histopathology examination was performed on hematoxylin-eosin stained paraplast sections of liver, kidneys, lung and other organs appearing to be remarkably altered by the treatment.

The authors have also reported that they investigated the in vitro disintegration of the gelatine capsules by submerging them in 0.5% hydrochloric acid solution at 37 °C.

Results

It was reported that gelatine capsules (used in administering Pyridate to dogs) under in vitro conditions (presumably approximating the in vivo conditions) open up within 2 minutes and they totally disintegrate within 20 minutes.

No mortality was observed during the observation period. Body weights remained constant throughout the observation period. However in the absence of concurrent control body weights, the effects of treatment on body weight gain cannot be fully assessed. Clinical signs of toxicity were reported by the sponsor in all treated animals. Major toxicity signs are listed below for each group.

Group 1 - (1000 mg/kg): Low grade trembling of the body (male), increased peristalsis (male and female), increased tonus of abdominal muscles (female), mercaptaneous smell (male & female).

Group 2 - (3000 mg/kg): Mercaptaneous smell (male and female), increased peristalsis (male), mucous-liquid diarrhea (male), vomiting (female), uncoordinated movement and disturbed balance (female), indistinct pale pink conjuctivae (female), massive salivations (female), increased tonus of abdominal muscle (female).

Group 3 - (5000 mg/kg): Vomiting (male and female); mercaptaneous smell (male and female), lung percussion sound too loud (male and female), loss of hair (male and female)

Eye Examination did not reveal any significant toxic effect of Pyridate to the eyes of treated animals.

Gross pathology did not reveal any treatment-related effects in the low-dose group (group 1, 1000 mg/kg). However, the authors reported several compound related effects in animals of the mid- and high-dose groups as follows:

Group 2 (3000 mg/kg): Liver surface was yellow-brown color (male and female); low grade bilateral auxocardia (enlargement of the heart),male and female.

Group 3 (5000 mg/kg): Liver surface was yellowish with marked borders of lobuli (male and female); low to medium grade dilatation of the right ventricle (male); bilateral medium grade auxocardia (female).

Histopathological examination of certain organs/tissues did not reveal, according to the authors, any compound-related effects in the animals of the low-dose group (1000 mg/kg). In the mid-dose group (3000 mg/kg) the only histopathology lesion attributed by the authors to the test article was a low grade cloudy swelling of the proximal tubules (female). In the high dose group (5000 mg/kg) the main lesions reported were: Low grade periportal mononuclear infiltrations of the liver (male & female); medium grade epidermic proliferations on the root of the tail (female).

Discussion

In the present study an attempt was made by the sponsor to establish the MTD for Pyridate in male and female beagle dogs. Initially, two dogs (1 male and 1 female) were administered orally Pyridate at 1000 mg/kg. Data collected from these dogs (clinical observations, gross pathology and histopathology) did not reveal any major adverse effects that could be attributed to the test article. Hence, a higher dose, 5000 mg/kg was administered to a second group of dogs (1 male and 1 female) and data were collected as previously. The high dose resulted in vomiting by both dogs 2 to 3 hours postadministration. The authors reported that no Pyridate was present in the emeses but they could not say with certainty whether or not most of the test article administered was absorbed from the gastrointestinal tract or not. It appears, however, that indeed most of the test article was absorbed resulting in higher systemic toxicity, gross pathology, and histopathology lesions than observed with the lower dose level (1000 mg/kg).

A third group of dogs (1 male and 1 female) received an intermediary dose of Pyridate at 3000 mg/kg. Data collected from this group concerning toxic signs, gross and histopathological lesions were evaluated and compared to the data obtained

from the other two groups. Vomiting also occurred with one animal (female) of this group at approximately 7 hours postadministration. Presumably most of the test article was absorbed by this time (in 7 hours) and no loss in the emeses was expected.

In the absence of concurrent or historical control data it cannot be established with certainty which of the effects observed could be attributed to the compound tested. The authors suggested that numerous findings observed in treated dogs were due to "poor hygienic status" of the animals. However, other findings were seen only in dogs administered the higher dose levels (3000 or 5000 mg/kg), but not with the lower dose level (1000 mg/kg) suggesting that they could be compound related effects. These effects included loss of balance, diarrhea, vomiting, increased peristalsis and slight renal damage in animals of the mid-dose group (3000 mg/kg) and vomiting, respiratory difficulties, auxocardia and some degeneration of liver cells in animals of the high-dose group (5000 mg/kg).

The mercaptaneous smell reported in all treated dogs was caused by a Pyridate metabolite and not by the parent compound itself according to the authors. This postulate might explain the persistent mercaptaneous smell until termination of the study.

Body weight measurements did not provide any evidence as to the effect of the test chemical on these animals. Generally, body weight gain or loss was minimal in all animals. However, in the absence of vehicle control animals, the significance of these values cannot be established. The authors did not provide any data pertaining to absolute or relative organ weights or organ weight to body weight ratios. Thus again, the effects of the test chemical cannot be fully evaluated.

Conclusions

Data provided have indicated that Pyridate (90% ai) induces a variety of adverse systemic effects when administered orally to male and female beagle dogs at dose levels of 3000 or 5000 mg/kg. Thus it appears (from the available data) that the dose of 3000 mg/kg is the MTD in both sexes of dogs. However, additional data (vehicle control, organ weights, etc.) are needed in order to say with certainty that the dose of 1000 mg/kg is not the MTD.

Classification: Core-Supplementary (for deficiencies noted above).

005227

87416:Ioannou:KENCO:C.DISK:3/31/86:DAA:VO