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DATA EVALUATION RECORD

Study Type: Subchronic Toxicity ( Rat ) Tox Chem. No.: 716A

MRID No.: 40157401

Test Material: Pyridate Technical

Synonyms: CL-11344; Lentagran

Project No.: TRL #043-005

Sponsor: Gilmore, Inc.
Memphis, TN

Testing Facility: Toxicity Research Laboratories, Ltd.
Muskegon, MI

Title of Report: 90-Day Rat Oral Subchronic Toxicity Study with a 28-Day Recovery Period of Pyridate Technical

Report Issued: April 6, 1987

Conclusions:

The LEL for general toxicity (hypoactivity, salivation) was found to be 177 mg/kg/day (the MDT) and the NOEL 62.5 mg/kg/day (the LDT) in both sexes of albino rats. The high dose tested, 500 mg/kg/day, exceeded the MTD for both sexes.

Classification: Core-Guideline.

Materials and Methods:

The test material, Pyridate Technical, a brown oily liquid (Lot No. EOA-Knr:2429966 CEO-Knr:2556520) with a reported chemical purity of 86.2 to 92.9 percent (list of contaminant(s) were not reported by the sponsor) was supplied by Chemie Linz AG, Austria, and used throughout this study. Samples of undiluted Pyridate Technical were analyzed for purity on weeks 8 and 13 on study while dosing preparations (diluted Pyridate) prepared on week 10 were analyzed on weeks 11 and 12 on study for stability. Analyses of the dosing solutions for Pyridate concentrations were done on weeks 8, 10, 12, and 13 on study.

Male and female albino rats (supplied by Charles River Breeding Laboratories, Portage, MI) were acclimated to the laboratory conditions for 14 days and observed for general health and behavior. At initiation of the study, the rats were approximately 35 days old and weighed approximately 183 g (males) or 151 g (females).

a. Study Design - Animals were assigned to the following test groups based on body weights:

| | Test Group | Dose (mg/kg/day) | Number of Rats/Group Male | Female |
|---|---|---|---|---|
| I | Control | 0 | 45 | 45 |
| II | Pyridate (LDT) | 62.5 | 45 | 45 |
| III | Pyridate (MDT) | 177.0 | 45 | 45 |
| IV | Pyridate (HDT) | 500.0 | 45 | 45 |
| V | Pyridate | 500/600 | 10 | 10 |
| VI | Control (Pretreatment) | 0 | 20 | 20 |

Rats were housed individually in suspended metal wire-bottom cages and kept in a room with a mean temperature of 74 ± 2 °F, mean humidity of 40 ± 10%, filtered room air and a 12-hour light/dark cycle. Purina Chow No. 5002, and water were available ad libitum.

The dosing solutions were prepared on a weekly basis by diluting the test material in corn oil. Each animal received the proper dose via oral gavage at a volume of 5 mL/kg body weight. Control animals received corn oil (at 5 mL/kg body weight). Ten rats from each group (groups I to IV) were dosed for 28 to 29 days and then sacrificed for interim necropsy. Twenty rats from each group (groups I to IV) were dosed for 91 to 93 days and then sacrificed while 15 rats from each group (groups I to IV) were dosed for 91 days and sacrificed after a

28-day recovery period. Rats (10 male and female) in group V were dosed for 7 days at 500 mg/kg/day and then dosed with 600 mg/kg/day for the remainder of the study (day 91). Group VI (20 males and females) were sacrificed 5 days prior to the initiation of the experiment.

Clinical observations were made daily at 1 and 3 hours after dosing for physical appearance and mortality. Body weights and food consumption were recorded weekly. Ophthalmoscopic examinations (using an indirect ophthalmoscope) were performed on all rats during the pretreatment period and on week 13 on study.

For hematology and clinical chemistry measurements, blood was collected by cardiac puncture from all rats (fasted overnight) scheduled for the pretreatment sampling, for the interim necropsy and from the nonperfused rats at the final and recovery necropsies. The checked (X) parameters were examined:

b. Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT)* | | Total plasma protein (TP) |
| X | Hemoglobin (HGB)* | X | Leukocyte differential count |
| X | Leukocyte count (WBC)* | X | Mean corpuscular HGB (MCH) |
| X | Erythrocyte count (RBC)* | X | Mean corpuscular HGB conc. (MCHC) |
| X | Platelet count* | X | Mean corpuscular volume (MCV) |
| X | Prothrombin time (PT) | X | Activated Partial Thromboblastin Time (APTT) |

c. Clinical Chemistry

| | | | |
|---|---|---|---|
| | Electrolytes: | | Other: |
| X | Calcium* | X | Albumin* |
| X | Chloride* | X | Blood creatinine* |
| | Magnesium* | X | Blood urea nitrogen* |
| X | Phosphorous* | | Cholesterol* |
| X | Potassium* | X | Globulins |
| X | Sodium* | X | Glucose* |
| | Enzymes | X | Total Bilirubin* |
| X | Alkaline phosphatase | X | Total Protein* |
| X | Cholinesterase | | Triglycerides |
| | Creatinine phosphokinase* | X | Thyroxine ($T_4$) |
| | Lactic acid dehydrogenase | X | Triiodothyronine ($T_3$) |
| X | Serum alanine aminotransferase (also SGPT)* | X | A/G Ratio |
| X | Serum aspartate aminotransferase (also SGOT)* | X | Total Carbon dioxide ($TCO_2$) |

*Recommended by Subdivision F (October 1982) Guidelines for Chronic Studies.

For hematology evaluations, only 10 rats/sex were used from the pretreatment group (group VI). The other 10 rats/sex were used for clinical chemistry evaluations. No prothrombin time (PT) or activated partial thromboblastin time (APTT) were measured (in group VI) because of limited blood supply. For the analysis of cholinesterase and free and total thyroxine ($T_4$) and triiodothyronine ($T_3$) blood was collected from five males and females from group I; five males and four females from group II; four males from group III; two males from group IV; and all surviving rats from group V.

For urinalysis, urine samples were collected on week 13 on study from 10 rats/sex from groups I to III and 10 males and 9 females from group IV. Prior to collection of urine, the rats were orally dosed with 5 to 7 mL of tap water. The checked (X) parameters were examined:

| X | | X | |
|---|---|---|---|
| X | Appearance* | X | Glucose* |
| | Volume* | X | Ketones* |
| X | Specific gravity* | X | Bilirubin* |
| X | pH | X | Blood* |
| X | Sediment (microscopic)* | X | Nitrate |
| X | Protein* | X | Urobilinogen |

d. Sacrifice and Pathology - All animals that died or were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

| X | | X | | X | |
|---|---|---|---|---|---|
| | **Digestive system** | | **Cardiovasc./Hemat.** | | **Neurologic** |
| | Tongue | | Aorta* | XX | Brain* |
| X | Salivary glands* | XX | Heart* | | Periph. nerve* |
| X | Esophagus* | | Bone marrow* | X | Spinal cord (3 level) |
| X | Stomach* | X | Lymph nodes* | XX | Pituitary* |
| X | Duodenum* | XX | Spleen* | | Eyes (optic n.)* |
| X | Jejunum* | XX | Thymus* | | **Glandular** |
| X | Ileum* | | **Urogenital** | XX | Adrenals* |
| X | Cecum* | XX | Kidneys* | X | Lacrimal gland |
| X | Colon* | X | Urinary bladder* | X | Mammary gland* |
| X | Rectum* | XX | Testes* | X | Parathyroids* |
| XX | Liver* | X | Epididymides | XX | Thyroids* |
| | Gallbladder* | | Prostate | | **Other** |
| X | Pancreas* | X | Seminal vesicle | | Bone* |
| | **Respiratory** | XX | Ovaries | X | Skeletal muscle* |
| X | Trachea* | | Uterus* | X | Skin |
| X | Lung* | | | | All gross lesions and masses |

*Recommended by Subdivision F (October 1982) Guidelines for Chronic Studies.

For histopathology, tissues were processed on a Fisher Scientific Histomatic or an AO TP/8000 and then embedded in paraffin using a Tissue Tek embedding system. Tissues were then sectioned at 5 to 6 microns, mounted on slides and stained with hematoxylin-eosin. Mesenteric lymph nodes were examined for mineralized cellular debris in the germinal follicles. Spleens were stained with Prussian blue stain for iron (hemosiderin). Mammary glands (females only) were examined for hyperplasia.

e. Statistics (Excerpted from the original report) - The body weight, food consumption, organ weight, (organ to body weight and organ to brain weight) clinical pathology, and ranked histopathology data were tested for homogeneity of variance by Bartlett's method. If the data were found to be homogeneous, differences between control and treatment means were tested for statistical significance by the method of Dunnett. If the data were found not to be homogeneous, the method of Gill (modified Dunnett's) was employed.

Results:

The chemical purity of Pyridate Technical, used throughout this study, was reported to range from 86.2 to 92.9 percent (average purity of 89.6%). The authors, however, failed to report the identity and quantities of impurities present in Pyridate Technical which ranged from 7 to 14 percent.

a. Mortality - As shown in Table 1 (excerpted from the original report) the incidence of mortality was considerably high in both sexes (especially females) of

Table 1: INCIDENCE OF MORTALITY

| Treatment Group | Dose (mg/kg/day) | Mortality[a] Male | Female |
|---|---|---|---|
| I | Vehicle Control | 0/45 (0%) | 0/45 (0%) |
| II | 62.5 | 0/45 (0%) | 1/45 (2.2%) |
| III | 177 | 1/45 (2.2%) | 1/45 (2.2%) |
| IV | 500 | 4/45 (8.9%) | 10/45 (22.2%) |
| V | 500/600[b] | 1/10 (10%) | 4/10 (40%) |

[a]Data presented as number dead/total number in the treatment group.
[b]Ascending dose group.

groups IV and V. Three rats (group II female; group III male and group IV female) died as a result of gavage or cage accidents. The death of the rest of the animals (19 animals in different groups) was due to Pyridate treatment, although, according to the authors, the exact cause of death could not be readily established from gross and histopathologic examination. Females of groups IV and V appeared to be more sensitive to Pyridate toxicity (higher mortality).

b. Clinical Observations - Although hypoactivity and salivation were the most prevalent clinical signs of toxicity, other clinical signs such as ataxia, alopecia, lacrimation, rapid respiration, scabbing, and diarrhea were also reported throughout the study. Hypoactivity was seen mainly in rats of the group IV and V (500 and 500/600 mg/kg/dose; respectively), immediately (1 hour) after dosing, reaching a peak on weeks 3, 4, and 5 on study. Up to 62 percent of males and 72 percent of females in group IV and all rats in group V were mildly hypoactive during this time. By weeks 7 to 10 on study, the incidence of hypoactivity decreased with the higher dose indicating, according to the authors, increased tolerance of these animals to Pyridate. Some animals of group III also showed hypoactivity throughout the study. The incidence of salivation reached a peak on weeks 4 and 5 on study. Up to 38 percent males and 18 percent females of group III, 75 percent males and 64 percent females of group IV and 80 percent of males and females of group V had this clinical sign at the 1-hour observations. Female rats of group V had a decreased incidence of salivation after week 5 (until termination of the study) indicating possibly the development of tolerance to Pyridate by these animals. Salivation was seen sporadically in some animals of group II throughout the study. None of the control animals (group I) showed any hypoactivity, salivation, or any other clinical signs.

c. Ophthalmological examinations did not reveal any ocular lesions that could be treatment-related.

d. Mean body weights were comparable between the control and the low (group II, 62.5 mg/kg/day) and mid (group III, 177 mg/kg/day) dose groups.

The mean body weight of the male and female animals in group IV (high dose group, 500 mg/kg/day) was statistically significantly lower than controls throughout the study. At termination of dosing (week 13) male rats in group IV weighed 28 percent less than the corresponding controls, and females of group IV weighed 15 percent less than

controls. The mean body weight gains were statistically significantly lower than controls in male rats of group IV throughout the study and in female rats of the same group at weeks 1, 5, 7, and 12 on study. Statistically significantly lower body weights than control were observed in male and female rats of group V (ascending group) throughout the study while body weight gains were statistically significantly lower than controls at weeks 1 through 10 in male rats and weeks 1, 2, and 10 in female rats.

During the first 3 weeks of the recovery period (weeks 14, 15, and 16) male and female rats of group IV had statistically significantly lower mean body weights than controls and body weight gains were statistically lower than controls during the first 2 weeks of recovery (weeks 14 and 15). By the end of the recovery period (week 18) animals of group IV (male and female) had comparable body weights and body weight gains as the controls.

e. Food consumption was found to be statistically significantly lower than control in male rats of groups IV and V throughout the study. In females, statistically significantly lower food consumption was observed for group IV at weeks 1, 2, 4, 10, 12, and 13, and for group V at weeks 1, 2, 7, 10, 12, and 13 on study. The lower food consumption correlated with the lower body weights and body weight gains in the same groups (groups IV and V) of rats throughout the study. In groups II and III, food consumption was only slightly lower than the controls.

Several hematology parameters were found to be statistically significantly different between the treated and control groups as shown in Table 2. In female rats, at the 14-week measurement, the erythrocyte count (RBC) and hemoglobin in the high-dose group (group IV, 500 mg/kg/day) were statistically significantly lower than control while the packed cell volume (PCV) was only numerically lower than the controls. The mean corpuscular volume (MCV) was statistically significantly higher and the mean corpuscular hemoglobin concentration (MCHC) statistically significantly lower than controls in female rats at the 14-week measurement. In male rats, statistically significantly lower leukocyte counts (WBC) were seen with the high-dose group (week 14) and statistically significantly higher mean corpuscular volume (MCV) in the mid- and high-dose groups with a dose-related trend (Table 2). At the 5-week time point, the prothrombin time (PT) and activated partial thromboblastin time (APTT) were statistically significantly

Table 2

Summary of Hematology Findings

| Parameter | Week of Test | Dose (mg/kg/day) Males 0 | Males 62.5 | Males 177 | Males 500 | Females 0 | Females 62.5 | Females 177 | Females 500 |
|---|---|---|---|---|---|---|---|---|---|
| PT (sec) | 5 | 14.0 | 13.9 | 14.3 | 15.7* | | | | |
| APTT (sec) | 5 | 21.6 | 22.8 | 22.9 | 31.6** | 21.6 | 21.8 | 17.9** | 21.1 |
| WBC (x $10^3$/ul) | 5 | | | | | 11.4 | 7.8* | 9.4 | 8.9 |
| | 14 | 15.9 | 15.2 | 16.3 | 11.8* | | | | |
| Abs. Lymph (x $10^3$/ul) | 5 | | | | | 10.0 | 6.2** | 8.0 | 7.5* |
| PCV (%) | 14 | 48.6 | 49.7 | 51.5* | 50.4 | 48.4 | 48.2 | 49.5 | 47.1 |
| RBC (x $10^6$/ul) | 14 | 8.64 | 8.65 | 8.83 | 8.43 | 8.46 | 8.38 | 8.65 | 7.75** |
| HGB (g/dl) | 14 | 15.7 | 15.8 | 16.1 | 16.3 | 16.3 | 15.9 | 16.4 | 15.4* |
| MCV (fl) | 14 | 56.5 | 57.6 | 58.7* | 60.1** | 57.4 | 57.8 | 57.6 | 61.0** |
| MCHC (g/dl) | 14 | 32.4 | 31.8 | 31.4* | 32.3 | 33.7 | 33.1 | 33.1 | 32.7* |
| Abs. Neut. (x $10_3$/ul) | 14 | 2.5 | 2.0 | 2.1 | 1.5* | | | | |
| | 18 | 2.7 | 1.9 | 2.2 | 1.7 | | | | |
| MCH (pg) | 18 | 19.4 | 19.6 | 20.2 | 20.4* | | | | |
| Lymph (%) | 18 | | | | | 82.3 | 86.3 | 89.8 | 69.4** |

*Statistically significantly different from control $P \leq 0.05$.
**Statistically significantly different from control $P \leq 0.01$.

higher in the high-dose group male rats than controls. These changes however, were not observed at later time points, i.e., weeks 14 or 18 on study.

A number of clinical chemistry parameters in male and female rats were found to be statistically significantly different between treated and control groups at different time intervals (Table 3). Total bilirubin was statistically significantly higher than controls in male and female rats of the high-dose group at the week 5 evaluation and in male rats at the week 14 evaluation, with a dose related trend. Alkaline phosphatase activity in female rats increased in a dose-related manner reaching significance at the high-dose level tested (week 14). Similar increase was observed with alanine aminotransferase (SGPT) activity in male rats at the week 14 evaluation. Aspartate aminotransferase (SGOT) was statistically significantly lower than controls in the high-dose group of female rats at week 5 of evaluations. Blood urea nitrogen (BUN), potassium and chlorine were statistically significantly decreased in female rats of the high-dose group on week 14. Other parameters that were affected, especially at the high dose level were: Albumin, globulin, A/G ratios, creatinine, total protein, and glucose.

Measurement of the thyroid hormones at the final necropsy revealed that total thyroxine ($T_4$) and free $T_4$ were statistically significantly decreased in male rats of groups IV and V. Total $T_4$ was also statistically significantly decreased in female rats of group V. Total and free triiodothyronine ($T_3$) were not affected compared to controls.

Cholinesterase activity was measured for brain, plasma, and erythrocytes in male and female animals of all groups. The results indicate that brain cholinesterase activity was not affected by Pyridate in either sex. Plasma cholinesterase was inhibited by approximately 22 percent in male rats of group V and by 60 percent and 57 percent in female animals of groups III and V, respectively. Erythrocyte cholinesterase activity was increased by approximately 63 percent in female rats of group V.

Most of the urinalysis parameters measured were found to be similar between the treated and control groups in both sexes with the exception of pH which was found to be statistically significantly lower in group IV of female rats.

Table 3

Summary of Clinical Chemistry Findings

| Parameter | Week of Test | Dose (mg/kg/day) Males 0 | Males 62.5 | Males 177 | Males 500 | Females 0 | Females 62.5 | Females 177 | Females 500 |
|---|---|---|---|---|---|---|---|---|---|
| Creat. (mg/dl) | 5 | | | | | 0.6 | 0.6 | 0.6 | 0.7* |
| | 18 | | | | | 0.7 | 0.7 | 0.7 | 0.6 |
| TP (g/dl) | 5 | 6.27 | 6.33 | 6.20 | 6.59* | | | | |
| ALB (g/dl) | 5 | 3.58 | 3.60 | 3.56 | 3.79* | | | | |
| | 14 | 3.67 | 3.73 | 3.77 | 3.99** | | | | |
| SGOT (u/l) | 5 | | | | | 57.0 | 59.3 | 57.6 | 48.8* |
| BUN (mg/dl) | 14 | | | | | 15.2 | 13.5 | 13.7 | 11.6* |
| Tot. Bili. (mg/dl) | 5 | 0.13 | 0.15 | 0.16 | 0.18** | 0.16 | 0.16 | 0.22** | 0.21 |
| | 14 | 0.19 | 0.20 | 0.21 | 0.27** | 0.22 | 0.21 | 0.22 | 0.25 |
| Alk Phos. (u/l) | 14 | 68.6 | 73.7 | 68.7 | 97.4 | 33.9 | 44.6 | 46.5 | 75.6* |
| SGPT (u/l) | 14 | 29.9 | 31.4 | 37.9 | 46.0* | 27.6 | 23.8 | 26.8 | 33.6 |
| K (meq/l) | 14 | | | | | 8.46 | 7.74 | 8.09 | 6.74 |
| Cl (Mmol/l) | 14 | | | | | 104.8 | 104.5 | 102.8* | 101.3 |
| GLOB (g/dl) | 14 | 3.52 | 3.30 | 3.35 | 3.08** | 3.43 | 3.52 | 3.43 | 3.06 |
| A/G | 14 | 1.05 | 1.13 | 1.13 | 1.31** | 1.20 | 1.14 | 1.21 | 1.29 |
| GLUC (mg/dl) | 18 | 250.9 | 261.3 | 235.0 | 207.0* | | | | |
| Total $T_4$ (ng/ml) | 14 | 29.46 | 27.82 | 29.65 | 12.65** | 22.86 | 24.08 | 22.82 | 16.07 |
| Free $T_4$ (ng/ml) | 14 | 28.16 | 26.32 | 26.38 | 10.95** | 14.18 | 15.25 | 15.54 | 12.12 |

*Statistically significantly different from control $P \leq 0.05$.
**Statistically significantly different from control $P \leq 0.01$.

f. Organ Weights (Nonperfused Rats)

1. Absolute Organ Weights - Absolute organ weights were found to be statistically significantly different between treated groups and controls mostly in male and to some extent in female rats. The weight of heart, liver, spleen, kidneys, and pituitary in male rats of group IV and V were statistically significantly lower than the controls. The absolute weight of thymus in male rats was statistically significantly lower than the control in groups III, IV, and V with an obvious dose-response relationship. The weight of the thyroids was statistically significantly lower than controls only in group V male rats, while the weight of the adrenals was higher than controls in groups IV and V. In female rats, the weight of thymus and pituitary was statistically significantly lower than controls in groups IV and V (also group II for pituitary). The weight of kidneys was higher than controls in groups IV and V. For thyroids, lower weight was seen with groups II, III, and IV. These differences are reported in Table 4 (excerpted from the original report - Table 15).

2. Relative Organ Weights - In male rats of groups IV and V, the relative weight of heart, brain, gonads, and adrenals, and the relative liver weight of group V was statistically significantly higher than controls. In kidneys, the relative weight was statistically significantly higher than controls in all dose groups (groups II-V) with an obvious dose-related trend. The relative weight of thyroids and pituitary was statistically significantly higher than controls only in group IV. In female rats, the relative weight of heart, brain, and adrenals (groups IV and V) and liver and kidneys (groups III, IV, and V), was statistically significantly higher than controls while the relative weight of thymus (groups IV and V) and thyroids (groups II and III) were statistically significantly lower than controls. These differences in relative organ weights are shown in Table 5 (excerpted from the original report - Table 16).

3. Organ to Brain Weight Ratios: In male rats, the organ to brain weight ratios for heart, liver, spleen (groups IV and V), thymus (groups III, IV, and V), and kidneys and thyroids (group V) were statistically significantly lower than the corresponding controls. The adrenal weight to brain weight ratio was statistically significantly higher for groups IV and V. In female rats, statistically significantly

Table 4

90 Day Rat Oral Subchronic Toxicity Study with a 28 Day Recovery Period of Pyridate Technical
Group Mean Absolute Organ Weights (grams)

Final Necropsy - Non-Perfused

TRL Study #043-005

| Dose Level (mg/kg/day) | sex | terminal body wt.(g)[a] | heart | liver | spleen | thymus | kidneys | brain | gonads | thyroids | pituitary | adrenals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | M | 540 | 1.64 | 18.0 | 0.794 | 0.376 | 3.87 | 2.07 | 3.61 | 0.026 | 0.016 | 0.056 |
| 62.5 | M | 502 | 1.64 | 17.7 | 0.795 | 0.333 | 3.99 | 2.09 | 3.44 | 0.022 | 0.015 | 0.056 |
| 177 | M | 499 | 1.64 | 16.6 | 0.755 | 0.284** | 4.11 | 2.07 | 3.51 | 0.023 | 0.014 | 0.054 |
| 500 | M | 349** | 1.33** | 12.1** | 0.480** | 0.168** | 3.24** | 1.94** | 3.21** | 0.021 | 0.012** | 0.075** |
| A[b] | M | 332** | 1.35** | 12.6** | 0.500** | 0.150** | 3.24** | 1.98 | 3.34 | 0.018* | 0.011** | 0.074** |
| 0 | F | 268 | 0.97 | 7.90 | 0.415 | 0.240 | 1.97 | 1.87 | 0.074 | 0.020 | 0.016 | 0.070 |
| 62.5 | F | 259 | 0.97 | 7.87 | 0.473 | 0.260 | 2.02 | 1.88 | 0.073 | 0.014** | 0.014* | 0.065 |
| 177 | F | 255 | 0.95 | 8.41 | 0.446 | 0.221 | 2.12 | 1.88 | 0.077 | 0.014** | 0.016 | 0.062* |
| 500 | F | 225** | 1.07 | 8.62 | 0.368 | 0.113** | 2.23* | 1.82 | 0.081 | 0.016* | 0.014* | 0.097** |
| A[b] | F | 214** | 1.03 | 8.95 | 0.374 | 0.113** | 2.22* | 1.82 | 0.094 | 0.016 | 0.013** | 0.091 |

[a] Terminal body weight taken on the day of necropsy.

[b] Ascending dose group.

* Significant at $p \geq 0.05$

** Significant at $p \geq 0.01$

Table 5

90 Day Rat Oral Subchronic Toxicity Study with a 28 Day Recovery Period of Pyridate Technical
Group Mean Relative Organ Weights (% of body weight)

Final Necropsy - Non-Perfused

TRL Study #043-005

| Dose Level (mg/kg/day) | sex | terminal body wt.(g)[a] | heart | liver | spleen | thymus | kidneys | brain | gonads | thyroids[b] | pituitary[b] | adrenals[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | M | 540 | 0.305 | 3.33 | 0.147 | 0.070 | 0.717 | 0.387 | 0.673 | 4.78 | 2.90 | 10.4 |
| 62.5 | M | 502 | 0.327 | 3.52 | 0.158 | 0.066 | 0.797** | 0.418 | 0.688 | 4.45 | 3.08 | 11.3 |
| 177 | M | 499 | 0.329 | 3.32 | 0.153 | 0.057 | 0.826** | 0.421 | 0.709 | 4.52 | 2.81 | 10.8 |
| 500 | M | 349** | 0.383** | 3.44 | 0.137 | 0.047** | 0.934** | 0.563** | 0.934** | 5.95* | 3.54* | 21.9** |
| A[c] | M | 332** | 0.407** | 3.79** | 0.151 | 0.045** | 0.974** | 0.597** | 1.008** | 5.40 | 3.42 | 22.4** |
| 0 | F | 268 | 0.364 | 2.95 | 0.156 | 0.089 | 0.738 | 0.705 | 0.028 | 7.57 | 6.17 | 26.5 |
| 62.5 | F | 259 | 0.374 | 3.05 | 0.183* | 0.101 | 0.783 | 0.731 | 0.028 | 5.46** | 5.43 | 25.2 |
| 177 | F | 255 | 0.374 | 3.29** | 0.176 | 0.086 | 0.833** | 0.738 | 0.030 | 5.42** | 6.20 | 24.2 |
| 500 | F | 225** | 0.476** | 3.83** | 0.163 | 0.050** | 0.944** | 0.810** | 0.036** | 7.09 | 6.15 | 43.4** |
| A[c] | F | 214** | 0.483** | 4.20** | 0.174 | 0.053* | 1.036** | 0.854** | 0.043 | 7.70 | 6.01 | 42.7* |

[a] Terminal body weight taken on the day of necropsy.

[b] Percent of body weight multiplied by 1000

[c] Ascending dose group.

* Significant at $p \geq 0.05$

** Significant at $p \geq 0.01$

higher organ weight to brain weight ratios were seen with liver and kidneys (groups IV and V), heart (group IV) and adrenals (groups III and IV). Statistically significantly lower organ weight to brain weight ratios were observed with thymus (groups IV and V), thyroids (groups II and III) and pituitary (groups II and V). Table 6 (excerpted from the original report - Table 17) shows these differences in organ weight to brain weight ratios in both sexes of rats.

The authors also presented data on organ weights from perfused animals at the final necropsy (week 13) and from nonperfused animals at the recovery necropsy (week 18). For perfused animals, very few differences in absolute and relative organ weights and organ weight to brain weight ratios were seen between the treated and control groups. The most significant changes were as follows: Absolute weight - Thymus, significantly lower and adrenals significantly higher in males of group IV than controls; adrenals significantly higher in females of group IV. Relative weight - Brain and gonads of males of group IV significantly higher than controls; heart, kidneys, and adrenals of females of group IV significantly higher than controls. Organ weight to brain weight ratio - Thymus of males, group IV, significantly lower and adrenals of males and females of group IV significantly higher than controls. for the recovery necropsy (week 18, nonperfused) the most significant changes in absolute and relative organ weights were: Absolute weight - Thymus of female rats, group IV, significantly increased. Relative weight - Brain of female rats, group IV, significantly increased; liver, thymus, kidneys, gonads, and adrenals of female rats, group IV, significantly greater than controls.

g. Gross Pathology was performed on all animals that died during the study or sacrificed on week 4 (interim necropsy), week 13 (final necropsy), or week 18 (recovery necropsy). In general, very few gross lesions were reported in these animals. The most prevalent gross lesions included smooth mucosa, red mucosa, dark spots, and smooth tan raised mucosa in the forestomach and fundic stomach (of animals that died during the study); small white raised areas in the forestomach (interim necropsy); raised white areas or spots, dark spots, or thick and spongy appearance of the forestomach.

Table 6

90 Day Rat Oral Subchronic Toxicity Study with a 28 Day Recovery Period of Pyridate Technical

Group Mean Organ to Brain Weight Ratios
Final Necropsy - Non-Perfused

TRL Study #043-005

| Dose Level (mg/kg/day) | sex | brain wt. (grams) | heart | liver | spleen | thymus | kidneys | gonads | thyroids | pituitary | adrenals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | M | 2.073 | 79.251 | 868.67 | 38.317 | 18.227 | 186.386 | 174.020 | 1.248 | 0.751 | 2.710 |
| 62.5 | M | 2.086 | 78.580 | 849.12 | 38.133 | 16.006 | 191.914 | 165.215 | 1.061 | 0.740 | 2.717 |
| 177 | M | 2.074 | 79.264 | 803.65 | 36.495 | 13.715* | 198.820 | 169.387 | 1.088 | 0.672 | 2.594 |
| 500 | M | 1.942** | 68.475** | 620.97** | 24.691** | 8.681** | 167.186 | 165.578 | 1.066 | 0.631** | 3.856** |
| A[a] | M | 1.977 | 68.148** | 636.53** | 25.220** | 7.588** | 163.693* | 168.941 | 0.905* | 0.573** | 3.753** |
| 0 | F | 1.872 | 51.861 | 421.36 | 22.201 | 12.797 | 105.024 | 3.954 | 1.068 | 0.875 | 3.755 |
| 62.5 | F | 1.881 | 51.303 | 419.05 | 25.119 | 13.889 | 107.429 | 3.878 | 0.753** | 0.744** | 3.466 |
| 177 | F | 1.875 | 50.774 | 448.13 | 23.853 | 11.775 | 113.257 | 4.108 | 0.735** | 0.841 | 3.283* |
| 500 | F | 1.818 | 58.810* | 474.88* | 20.229 | 6.208** | 122.940** | 4.489 | 0.880 | 0.763 | 5.363** |
| A[a] | F | 1.816 | 56.619 | 493.00* | 20.497 | 6.217** | 121.724** | 5.118 | 0.899 | 0.707** | 4.988 |

[a] Ascending dose group

* Significant at $p \geq 0.05$

** Significant at $p \geq 0.01$

h. Histopathological examinations revealed several lesions which were of higher incidence in the high-dose groups. The most prevalent lesions seen were:

1. Mesenteric lymph nodes - Mineralized cellular debris in the germinal follicles of the mesenteric lymph nodes. The incidence of this lesion in male and female rats of the final necropsy was as follows:

| Group | Dose (mg/kg/day) | Number of Rats with Lesions | |
|---|---|---|---|
| | | Males | Females |
| I | 0 | 0/20 | 0/20 |
| II | 62.5 | 0/20 | 0/19 |
| III | 177 | 2/19 | 1/20 |
| IV | 500 | 8/17 | 6/14 |
| V | 500/600 | 4/9 | 2/6 |

These data show that control animals and animals in group II were not affected. Approximately 11 percent of males and 5 percent of females in group III had this lesion. For the high dose tested (group IV) 47 percent of the males and 43 percent of the females had the lesion. These data show that the incidence of this lesion is directly related to Pyridate administration.

2. Spleen - Presence of hemosiderin in the spleen. The incidence of this lesion in male and female rats at the final necropsy (week 13) was as follows:

| Group | Dose (mg/kg/day) | Animals with Lesions | |
|---|---|---|---|
| | | Males | Females |
| I | 0 | 41.4[1] | 32.7 |
| II | 62.5 | 20.4* | 52.5 |
| III | 177.0 | 61.5* | 52.7 |
| IV | 500.0 | 90.3** | 70.9** |
| V | 500/600 | 87.8* | 90.0** |

[1]Ranking number.
*Statistically significantly different from control; $P \leq 0.05$.
**Statistically significantly different from control; $P \leq 0.01$.

These results indicate that at high dose levels, the content of hemosiderin (iron) in the spleens is significantly higher than controls in both sexes.

3. Mammary glands - Hyperplasia of mammary glands in female rats. The incidence of this lesion in female rats of the final necropsy was:

| Group | Dose (mg/kg/day) | Incidence of Hyperplasia Females |
|---|---|---|
| I | 0 | 46.1[1] |
| II | 62.5 | 38.8 |
| III | 177.0 | 46.7 |
| IV | 500.0 | 74.7* |
| V | 500/600 | 88.3* |

[1]Ranking number.
*Statistically significantly different from control; $P \leq 0.05$.

These results indicate that at high dose levels (groups IV and V) Pyridate induces the formation of hyperplastic lesions in the mammary gland of female rats. There was no significant difference in the incidence of hyperplasia between groups II and III and the controls. Male animals were not affected.

Discussion:

The present study has investigated the toxicity of Pyridate to male and female rats after repeated exposure for 13 weeks. Although the purity of the test article was reported by the authors to be between 86.2 and 92.9 percent, the authors failed to identify and quantify the impurities present. Thus, we request that the sponsor provide the Agency with the appropriate data on Pyridate contaminants.

Mortality data presented here indicate that unusually high mortality was observed in the high-dose groups (groups IV and V; 500 and 500/600 mg/kg/day, respectively) especially in female rats. This high incidence of mortality indicates that the MTD has been clearly exceeded in this study in both sexes of the high doses tested (groups IV and V).

A dose-related incidence and severity of several clinical signs of toxicity were observed in this study. Hypoactivity and salivation were prevalent throughout the study with the highest incidence in the high-dose groups at the first few weeks on study. According to the authors, the incidence of hypoactivity tended to be lower than peak values after week 5 on study indicating possible development of some tolerance in both sexes. The incidence of salivation reached peak values in the high-dose group on week 5 on study and although, in male rats, this high

incidence continued throughout the study, in female rats salivation diminished rapidly thereafter indicating possibly sex-related development in tolerance. During the recovery period (weeks 14 - 18) hypoactivity and salivation were not present in the high-dose groups suggesting that these toxicity signs were readily reversible. The incidence of hypoactivity and salivation was comparable between controls and the low-dose group (group II). Rats of the mid-dose group (group III) showed a mild incidence of hypoactivity and salivation in both sexes suggesting that this group (177 mg/kg/day) is the LEL for this study.

Mean body weights for male and female rats of groups IV and V were statistically significantly lower than controls throughout the exposure period (week 1 through 13). Mean food consumption with some exceptions, was also statistically significantly lower in these high-dose groups compared to controls. Thus, it appears that the lower body weight gains in these groups might be the result of lower food consumption due to the unpalatability of the test article. Once dosing was terminated (week 13), animals of group IV (males and females) had a higher food consumption during the 4-week recovery period, resulting in higher mean body weights which were comparable to (and in some instances higher than) controls. No significant differences between the controls and the low- and mid-dose groups were reported.

It appears from the hematology data that treatment of female rats with the test article at 500 mg/kg/day produces anemia in these animals as indicated by the statistically significant decrease in RBC and HGB and the numerical decrease in PCV (at the 14-week time point). The anemia was apparently the result of hemolysis as indicated by the statistically significant increase in bilirubin and the accumulation of iron (hemosiderin) in the spleen. Although other hematology parameters were found to be statistically significantly different between the treated and control groups in both sexes, these changes do not appear to be of major biological significance since most of them occurred randomly, they were not persistent throughout the study and did not appear to be dose-dependent.

From the clinical chemistry parameters measured, a great number of values were found to be statistically significantly different between control and treated groups. However, it appears that only very few of these changes were of some biological significance. Total bilirubin appeared to increase in a dose-related fashion especially in male rats at the 5 and 14 week evaluation. This increase is directly related (as discussed earlier) to the increased destruction of RBC resulting in hemolytic anemia. Alanine aminotransferase activity (SGPT) was increased, in male rats at the 14-week evaluation, in a dose-dependent manner, reaching statistical significance in the HDT. Although higher SGPT activity is usually indicative of liver dysfunction no pathological lesions were seen in the livers of these rats. A

dose-response relationship was also seen in alkaline phosphatase activity in female rats at the 14-week evaluation. However, these findings could not be correlated with apparent liver disease. Total thyroxine ($T_4$) production was found to be statistically significantly lower in male and female animals of the high-dose group while free $T_4$ was lower in the high-dose group males compared to controls. These results indicate that Pyridate interferes with thyroid function in the synthesis and secretion of thyroxine.

Mean organ weight from the treated groups (mainly groups III, IV, and V) were found in many cases to be statistically significantly different from the control values. Organ weight changes included: Decreased absolute weight in liver, kidney, heart, thymus, spleen, thyroids, pituitary, brain and gonads, and increased adrenal weight in male rats of groups IV and V mainly. In female rats, the absolute weight of kidneys and adrenals was increased while the weight of thymus, thyroids, and pituitary was decreased. The relative weight of heart, liver, kidneys, brain, gonads, and adrenals was increased significantly in male and female rats of groups IV and V mainly, while the relative weight of thymus was decreased in both sexes of groups IV and V. Although most of these changes in organ weights are considered treatment-related, none of these organs with significant weight changes showed any pathological lesions (macroscopic and/or microscopic) which could explain these changes.

Although none of the gross macroscopic lesions observed are considered of biological significance, some histopathological (microscopic) lesions appeared to be treatment-related with some biological significance. Hyperplasia of the mammary gland in female rats of groups IV and V is considered the most important Pyridate-induced lesion. This lesion was reversible, however, as no significant differences in the incidence of this lesion were seen between the control and treated groups at the end of the recovery period. Accumulation of hemosiderin (iron) in the spleen of male (groups III, IV, and V) and female (groups IV and V) rats is considered to be a secondary effect resulting from the accelerated destruction of erythrocytes (senescent) in the spleen. Thus, the primary toxicological effect of Pyridate is the induction of hemolytic anemia (slight) with the secondary effect of hemosiderin accumulation in the spleen.

The incidence of mineralized cellular debris in the germinal follicles of the mesenteric lymph nodes appeared to be dose-related in both sexes. The highest occurrence of this lesion was in group IV male and female rats (47 and 44 percent of male and female rats, respectively). Although this is a rare lesion (according to the authors), the cause of this lesion and its toxicological significance cannot be assessed at present.

Conclusions:

Administration of Pyridate Technical (via oral gavage) to male and female albino rats for 13 weeks resulted in high toxicity and mortality at the dose level of 500 mg/kg/day (the high dose tested). Moderate toxicity (and some mortality) was observed with the dose level of 177 mg/kg/day (the mid dose tested) while no toxicity was observed with the low dose of 62.5 mg/kg/day. Thus, the LEL for general toxicity (hypoactivity, salivation) is considered to be the dose level of 177 mg/kg/day (MDT) and the NOEL 62.5 mg/kg/day (LDT) in both sexes. The high dose tested (500 mg/kg/day) was apparently higher than the MTD.

Classification: Core-Guideline.

91507:I:Ioannou:C.Disk:KENCO:5/14/87:NeeCee:V0:NeeCee