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Subject: Multigeneration (Reproduction) Study with Pyridate in Rats.

Test Material: Pyridate technical (CL-11344)

Accession Number: 072347

Sponsor: Chemie Linz A.G., Austria

Testing Facility: Netherlands Organization for Applied Scientific Research

Study Number: B80-0696 Assay No. 194

Testing Period: June 1980 to February 1982

Report Submitted to Sponsor: August 1982

Materials and Methods:

Test Material: The purity of pyridate technical (CL-11344). A brown viscous liquid used in this study was approximately 90.3 percent. The impurities (9.7%) were specified in the report and are attached (Annex 1). Samples of the test material were stored at 5 °C until use.

Animals: Ninety male and 90 female weanling SPF-bred rats (Cpb:WU; Wistar random) purchased from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, were used. The animals were about 3.5 weeks old with a body weight range of 35 to 50 g. All rats were checked for health status and acclimated to laboratory conditions for 7 days. The healthy animals were divided into 4 groups of 20 males and 20 females each by computer randomization, and individual rats within each group were identified by earmark. These rats formed the parent rats of the F_0 generation.

The rats were housed under conventional conditions, in a well-ventilated room (8 to 10 air changes per hour) with a temperature of 22 ± 1 °C, relative humidity of at least 40 percent, and a 12-hour light/dark cycle. Animals in the four groups were given fresh portions of the test diets every day from Monday to Friday. The amount given was somewhat more than the need for 1 day. On Friday the rats received a larger portion for over the weekend. Drinking water was supplied in glass bottles ad libitum.

During the premating period the rats were kept, five to a cage, separated by sex, in suspended stainless steel cages. During the mating period of 3 weeks the rats were housed two to a cage (one male and one female of the same group), in

suspended stainless-steel cages. At the end of the mating period the males were returned to their original cages and the females were placed individually in wire mesh breeding cages for the birth and rearing of their young.

The parent rats of the F_1 and F_2 generation were treated as the F_0 generation rats, except that they were also exposed to the test material *in utero* and during the lactation period.

Diets: For the test groups, pyridate was added to stock diet at levels of 80, 400, or 2500 ppm to provide an intake of about 4, 20, or 125 mg pyridate/kg body weight/day. Batches of 30 kg were prepared weekly, packed in closed metal containers and, together with the control diet, stored at about 5 °C until use. Test diets were analyzed bimonthly for test material concentration. Homogeneity and stability of the test material were established once during the study.

F_0 generation: After receiving the test diet for 12 weeks, the F_0 generation rats were mated within their diet group, one male to one female, for a period of 3 weeks. The males were rotated every week. The day that mating was established (vaginal plug) was considered day 0 of gestation. After the mating period, the males were returned to the original cages and the females were transferred to individual cages for the birth and rearing of their litters. Litters containing more than 10 siblings were reduced to contain 10 young. The offspring of the first mating--the F_1a litters--were discarded when 3 weeks old. Nine weeks after the first mating in week 22, the rats were mated again, for producing the F_1b litters. After weaning of the F_1b litters, the parent rats were killed by decapitation, the males in week 31 and the females in week 33, and subsequently examined grossly for pathological changes.

F_1 generation: When the F_1b litters had reached weaning age, 10 males and 10 females for one subgroup, and 20 males and 20 females for another subgroup were selected from each diet group, from as many litters as possible and maintained on the same test diets as their parents. The remaining young of the F_1b litters were discarded. The 10 rats/sex/group of the first set of subgroups were killed for interim gross pathological examination when about 6 weeks old. The rats of the second set of subgroups were mated in weeks 42 and 51 (weeks 13 and 22 after weaning). The same procedure as the one used for the F_0 generation was followed. The rats of the F_2a litters were discarded at weaning age. After weaning of the F_2b litters the parent rats of the F_1 generation were killed, the males in week 66 (week 37 after weaning) and the females in week 67 (week 38 after weaning) and subjected to gross pathological examination.

F_2 generation: At weaning age of the F_2b litters, 20 males and 20 females were selected from each group and continued on the respective diets to rear the next generation. In addition, 10 rats/sex were selected from each diet group and killed at 10 weeks of age for interim gross pathological examination. The rats of the F_2b generation (20 males and 20 females of each diet group) were mated in weeks 71 and 80 (weeks 13 and 22 after weaning). The rats of the F_3a litters were discarded at weaning age. After weaning of the second litter (F_3b) the parent rats were killed, the males in week 89 (week 31 after weaning) and the females in week 90 (week 32 after weaning) and examined grossly for pathological lesions.

F_3b generation: When the F_3b litters had reached weaning age, 10 males and 10 females were selected from each diet group and continued on the respective diets for a period of 4 weeks. They were killed in week 91 by decapitation for gross and detailed microscopic examination.

Parental observations: Each animal (F_0, F_1, F_2 generation) was observed for clinical signs of toxicity or mortality. All surviving parent rats of the F_1 and F_2 generations were examined by indirect ophthalmoscopy in weeks 63 and 88, respectively. Body weight for all male and female animals was recorded weekly during the premating period of 12 weeks. For female rats, body weight was also recorded weekly during pregnancy. Food consumption was recorded weekly for all male and female animals during the premating period of 12 weeks, and food efficiency (g weight gain/g food consumed) was calculated for each generation. The gestation period, fertility index, and gestation index were also calculated for all generations.

Hematological investigations were performed on 10 rats/sex/dose group for F_1 generation on week 61. Blood samples were taken from the tail and analyzed for white blood cell count and differential white blood cell count. Blood samples were also taken at autopsy from the aorta of 10 rats/sex/dose group from F_1 (weeks 66 and 67) and F_2 (weeks 89 and 90) generation rats used for the clinical chemistry measurement of plasma chloride and potassium (F_1 generation) and lactic dehydrogenase activity (F_1 and F_2 generations).

At sacrifice (F_0, weeks 31 and 33; F_1, weeks 66 and 67; F_2, weeks 89 and 90 for male and female, respectively) all animals were examined for gross pathological changes. All tissues and organs listed in Table 1 were removed from all parent animals of all three generations and samples were preserved in 4 percent aqueous, phosphate-buffered formaldehyde solution. Organ weights were recorded for kidneys, liver, pituitary, and thyroid from all F_1 and F_2 generation parent animals. The pituitary glands from all male and female rats of the F_1 parent generation were embedded in paraffin wax, sectioned at 5 um, stained with hematoxylin-eosin, and examined for histopathological lesions.

TABLE 1

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

Litter Data: At parturition the birth date was recorded and considered to be day 0 of the lactation period. On day 1, the total number of pups (dead and alive), the number of male and female pups, the number of congenital defects in the litter, and the total litter weight were recorded. Litters were weighed again on days 4, 14, and 21 of lactation and the number of live pups was also recorded on these days. From these observations the following parameters were calculated: mean litter size at birth (MLSB); mean pup weight (on days 1, 4, 14, and 21); and pup survival indices (on days 1, 4, and 21).

Young rats (10 rats/sex/dose group) from the F_1b and F_2b litters were killed (by decapitation) when they were 6 and 10 weeks old, respectively, and subjected to detailed gross examination. Tissue samples from pituitary, thyroid, kidneys, and liver of all male and female animals were preserved in a 4 percent aqueous, neutral, phosphate-buffered formaldehyde solution.

The pituitary of 10 rats/sex/group of the F_2b litter was embedded in paraffin wax, sectioned at 5 um, stained with hematoxylin-eosin, and examined for histopathological lesions. For the F_3b generation rats, the body weight and food consumption were recorded weekly and the food efficiency calculated.

All rats of the F_3b generation belonging to the 4-week study were killed by decapitation on day 28 (males) or 29 (females) after weaning and examined for gross pathological changes. Tissue samples from pituitary, thyroid, kidneys, and liver from all animals were preserved in 4 percent aqueous, neutral, phosphate-buffered, formaldehyde solution.

Organ weights from F_3 rats were recorded for:

liver	heart	thymus	testes
kidneys	brain	thyroid	ovaries
spleen	lungs	adrenals	pituitary

For microscopic examination tissues were embedded in paraffin wax, sectioned at 5 um, and stained with hematoxylin-eosin. Microscopic examination was performed on all tissues (as listed in Table 1) of all rats of the high-dose and control groups of the F_3b generation. In addition, the kidneys were also examined in all animals of the mid- and low-dose groups. Special attention was paid to the reproductive organs.

Statistical Analysis: Body weights of the parent rats were subjected to analysis of covariance, with the initial body weights as covariables, followed by Dunnett's Multiple Comparison Test. Food intake and food efficiency figures were

subjected to analysis of variance followed by the L.S.D. test or Dunnett's Test. Relative organ weights and clinical chemistry data were subjected to analyses of variance followed by Dunnett's Multiple Comparison Test. Total and differential white blood cell counts were evaluated by the Mann/Whitney U-test.

Mean litter size at birth and mean pup weight on days 1, 4, 14, and 21 were analyzed by the student t-test. The chi-square test was applied to the viability index determined on days 1 and 4, to the lactation index, and to the histopathological lesions.

Deviations from the protocol:

a. By oversight a total of five thyroids were not weighed as follows:

   F_1 generation: one female mid-dose group
   F_2 generation: one female control
   F_2 generation: two males mid-dose group
   one female mid-dose group.

b. A total of 33 organs of the F_{3b} generation rats could not be examined microscopically because they were, by oversight, not collected or were lost during fixation or processing.

c. Although not mentioned in the protocol the extraorbital lachrymal glands and preputial/clitoral glands have been examined microscopically in the F_{3b} generation rats.

d. Because of holidays and workload the autopsy of the F_1 parent rats and of the F_{2b} young was postponed 4 to 5 weeks.

Results:

Analytical concentrations of pyridate in the diet (determined immediately after mixing) showed considerable variation between batches, especially with the low- and mid-dose levels as shown on Tables 1 and 2 (APPENDIX A, abstracted from the original report). When adjustments for recovery are made (recovery was reported by the authors to be 80 to 85 percent; however, the authors did not describe how this value was determined), the following deviations from the target concentrations were obtained:

Low dose (80 ppm) -29 to +28 percent, Mean -4 percent;
Mid dose (400 ppm) -22 to +20 percent, Mean +11 percent;
High dose (2500 ppm) +3 to +31 percent, Mean +17 percent.

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Although the distribution of pyridate in the diet appeared to be homogeneous (coefficient of variation < 5 %), pyridate appeared to be relatively unstable at room temperature (23 °C) resulting in losses from the diet as follows:

	24 hours	48 hours	72 hours
Low dose	18 percent	46 percent	63 percent
Mid dose	10 percent	45 percent	49 percent
High dose	4 percent	36 percent	39 percent

Parent Animal Data: Although no mortality of male or female animals was observed with the F_0 generation, two female rats (one from the control and one from the mid-dose group) of the F_1 generation and four female rats from the F_2 generation (one from the control, one from the low-, and two from the high-dose groups) died during the course of the study.

A variety of clinical signs were observed in some male and female animals of F_0, F_1, and F_2 generations. Monocclusion of incisors was the most common clinical sign and was observed mainly in female animals of the F_0 generation (3/20, 4/20, 8/20, and 10/20 animals of the control, low-, mid-, and high-dose groups, respectively). In F_1 generation, focal alopecia was observed in 0/20, 1/20, 1/20, and 3/20 male animals in control, low-, mid-, and high-dose groups, respectively).

Ophthalmoscopic examination performed on all surviving rats of the F_1 and F_2 generations did not reveal, according to the authors, any ocular lesions that were treatment-related. However the authors did not report the results of the ophthalmoscopic examinations of the F_1 generation rats either as individual animal data or in summary tables as was done for the F_2 generation. Vascular infiltration of the cornea was observed in male (2/20 with mid-dose and 2/20 with high-dose groups) and female (1/20, 1/20, and 2/20 with low-, mid-, and high-dose groups, respectively) F_2 generation rats.

The mean body weight for the high-dose group animals was numerically consistently lower throughout the study as compared to the corresponding control groups, for F_0 and F_1 generation animals. For F_0 generation animals, statistically significantly lower mean body weight as compared to controls, was reported for males (days 7, 14, and 21) and females (days 42, 93, 100, 156, 163, and 170). Food consumption by males of the F_0 and F_1 generations and by females of the F_1 generation was consistently lower mainly in the high-dose groups as compared to the corresponding control groups. Statistically significantly lower food intake was reported at different time intervals throughout the study for F_0, F_1, and F_2 generation rats, mainly with the high-dose groups as compared to controls but these differences were not consistent. Food efficiency

(g weight gain/g food consumed) was not significantly different between treated and control groups.

The reproductive parameters, gestation period, gestation index, and fertility index, calculated for F_0, F_1, and F_2 generation parent rats were found to be comparable between treated and control groups for both breedings for each generation.

The limited hematological parameters measured did not reveal any differences of toxicological significance between treated and control groups of the F_1 generation. Clinical chemistry measurements (F_1 and F_2 generation rats) revealed that males of the F_1 generation had statistically significantly lower lactic dehydrogenase activity in all treated groups as compared to the control group wth some evidence of a dose-related trend. All other clinical chemistry parameters measured were comparable between treated and control groups.

Absolute and relative organ weights found to be statistically significantly different from the control values are presented in Table 3. The relative weight of the kidneys was statistically significantly increased in the high-dose groups of male and female animals of the F_1 and F_2 generations, and in females of the mid-dose group of the F_2 generation. Absolute and relative liver weights in the high-dose group of F_2 generation female animals were statistically significantly increased, whereas relative thyroid weight was statistically significantly decreased in males of the mid- and high-dose groups. Absolute thyroid weight was also numerically lower in the males of mid- and high-dose groups of the F_2 generation.

Gross pathological (macroscopic) examinations performed on all parent animals of F_0, F_1, and F_2 generations, according to the authors, did not reveal any lesions that could be attributable to treatment. However, a summary table for gross pathology findings was not included in the report and thus no final evaluation could be made.

Histopathological examination of all pituitaries in male and female F_1 parent rats did not reveal, according to the authors, any treatment-related lesions (no individual animal data or summary tabulations were reported by the authors).

Litter Data: A number of reproductive parameters were recorded or calculated for the first and second breedings in each generation (F_0, F_1, and F_2). Results from all three generations, with statistically significant differences between treated and controls, are shown in Tables 4, 5, and 6. The mean litter size at birth was comparable between control and treated groups for all generations. The viability indices in the F_0 and F_1 generation, for both breedings at day 1 and 4,

ranged between 94 and 100 percent with no apparent treatment-related effects. In the F_2 generation, the viability index at 4 days was statistically significantly lower in all treated groups as compared to the control in the first breeding and lower in the low- and mid-dose groups in the second breeding. However, a dose-response was not evident when effects were compared between doses and the effect was not considered biologically significant. The lactation index (indicator of pup survival) was consistently high (in most cases higher than controls) in treated groups of F_0 and F_1 generations (both breedings) but statistically significantly lower than controls with the mid- and high-dose groups of the first breeding of F_2 generation. Mean pup body weight was comparable in all groups at day 1 and 4 for both breedings of all three generations. A statistically significant decrease in the mean pup body weight for days 14 and 21 was observed with the high-dose group of the first breeding of F_0, F_1, and F_2 generations, and on day 21 for the low-dose group for F_1 and F_2 generations (first breeding). A dose-response was not evident and the mid-dose was not statistically significantly different from control. No malformations at birth were seen in any of the pups of all three generations.

TABLE 3

Organ	Sex		F_1 Generation Dose (ppm)				F_2 Generation Dose (ppm)			
			0	80	400	2500	0	80	400	2500
Kidneys	M	R[1]	5.5[3]	5.6	5.6	6.0**	5.5	5.8	5.8	6.3**
Thyroid	M	A[2]					0.028	0.026	0.022	0.022
Thyroid	M	R					0.068	0.065	0.056**	0.053**
Kidneys	F	R	6.4	6.4	6.6	6.9**	6.5	6.7	7.1*	7.3**
Liver	F	A					7.8	8.0	7.7	8.7**
Liver	F	R					31.9	32.5	32.6	35.6**

[1] Relative organ weight (g/kg)
[2] Absolute organ weight (g)
[3] Mean absolute or relative organ weight
N = 20 for males F_1, and males and females F_2.
N = 19, 19, 20, and 18 for control, low-, mid-, and high-dose groups, respectively of females of F_1 generations.
* Significantly different from control values
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by student t-test or chi-square test.

TABLE 4

F_0 Generation: Reproductive Performance

Parameter	First Breeding Dose (ppm)				Second Breeding Dose (ppm)			
	0	80	400	2500	0	80	400	2500
Number of matings	20	20	20	20	20	20	20	20
Total pups born alive	194	216	206	206	209	185	194	192
Total pups born dead	1	1	5	0	0	12***	3	1
Mean litter size at birth	9.8	10.9	10.6	10.3	10.4	10.4	10.9	9.6
Sex ratio at birth (M/F)	0.96	0.98	1.06	1.17	1.05	0.71	0.85	1.02
Fertility index (%)	100	100	95	100	100	95	100	100
Gestation index (%)	100	100	100	100	100	95	90	100
Viability index (%)								
- Day 1	99	100	98	100	100	94***	98	99
- Day 4	100	100	99	99	96	99*	99*	99*
Lactation index (%)	92	98**	98*	95	81	94***	92*	91*
Mean pup weight (g): Day 1	5.9	5.9	5.6	5.8	5.9	6.0	5.6	5.9
Day 4	9.1	9.3	8.9	8.9	8.5	9.2	8.7	9.1
Day 14	27.8	27.6	27.4	24.6**	28.5	29.2	27.4	27.6
Day 21	40.9	40.9	41.2	36.2**	43.8	42.9	40.7	40.1

Values marked with asterisk(s) differ significantly from the control values.

* $p < 0.05$ by student t-test or by chi-square test.
** $p < 0.01$ by student t-test or by chi-square test.
*** $p < 0.001$ by student t-test or by chi-square test.

TABLE 5

F_1 Generation: Reproductive Performance

Parameter	First Breeding				Second Breeding			
	Dose (ppm)				Dose (ppm)			
	0	80	400	2500	0	80	400	2500
Number of matings	20	20	20	20	19	20	19	20
Total pups born alive	169	212	201	202	204	207	187	199
Total pups born dead	19	0***	2***	2***	0	1	8**	0
Mean litter size at birth	9.9	10.6	10.7	10.7	11.3	10.4	10.3	10.5
Sex ratio at birth (M/F)	1.25	1.06	1.28	0.98	1.19	0.90	1.17	0.95
Fertility index (%)	95	100	100	95	100	100	100	95
Gestation index (%)	95	100	95	100	95	100	95	100
Viability index (%)								
- Day 1	90	100***	99***	99***	100	100	96**	100
- Day 4	97	99	96	100	98	96	99	99
Lactation index (%)	91	97*	97*	91	95	97	100**	99**
Mean pup weight (g): Day 1	5.8	6.0	6.0	6.0	5.9	6.0	6.1	6.0
Day 4	9.4	9.4	9.4	9.1	8.8	9.0	9.5	9.2
Day 14	27.7	25.9	26.4	24.2**	24.6	25.9	26.9*	25.7
Day 21	40.2	36.5*	38.6	34.5**	38.3	38.8	39.7	38.1

Values marked with asterisk(s) differ significantly from the control values.

* $p < 0.05$ by student t-test or by chi-square test.
** $p < 0.01$ by student t-test or by chi-square test.
*** $p < 0.001$ by student t-test or by chi-square test.

TABLE 6

F_2 Generation: Reproductive Performance

Parameter	First Breeding Dose (ppm)				Second Breeding Dose (ppm)			
	0	80	400	2500	0	80	400	2500
Number of matings	19	20	20	20	19	19	20	18
Total pups born alive	194	195	206	210	186	191	203	170
Total pups born dead	3	5	3	0	10	6	2*	1*
Mean litter size at birth	10.4	10.5	10.4	10.5	10.3	10.4	10.3	10.1
Sex ratio at birth (M/F)	1.17	1.24	1.02	1.02	0.86	0.91	0.95	1.07
Fertility index (%)	100	95	100	100	100	100	100	94
Gestation index (%)	100	100	100	100	100	100	100	100
Viability index (%)								
- Day 1	98	98	99	100	95	97	99*	99*
- Day 4	97	91*	88***	92*	96	83***	91*	92
Lactation index (%)	90	85	71***	76***	81	76	85	90*
Mean pup weight								
(g): Day 1	5.7	5.7	5.6	5.5	5.8	5.9	6.0	6.0
Day 4	7.7	7.6	6.8	7.3	8.0	7.5	8.3	8.5
Day 14	20.4	18.6	19.1	17.1***	25.5	24.2	24.5	24.7
Day 21	30.9	27.7*	28.4	27.1*	36.0	36.5	34.4	34.6

Values marked with asterisk(s) differ significantly from the control values.

* $p < 0.05$ by student t-test or by chi-square test.
** $p < 0.01$ by student t-test or by chi-square test.
*** $p < 0.001$ by student t-test or by chi-square test.

Macroscopic examination of all F_1b and F_2b generation litters (killed when 6 and 10 weeks old, respectively) did not reveal any gross abnormalities (summary tables not provided in the report) which could be attributed to the ingestion of pyridate. Microscopic examination of the pituitaries of the F_2b generation litter did not reveal, according to the authors, any histopathological lesions. (Note: Neither individual animal data nor summary tables for histopathological lesions were provided.)

F_3b Generation Rats: None of these animals (10 rats/sex/dose level) showed any clinical signs of toxicity and no mortalities were reported during the 4-week observation period after weaning. (Note: No individual animal data or summary tables were provided for these observations.) There were no significant differences in mean body weights between treated and control groups, although food consumption was statistically significantly lower on days 7, 14, and 21 for low- and mid-dose groups, and on day 7 for the high-dose group in male rats. In female rats higher food consumptions were reported for all treated groups on day 7. Food efficiency was comparable in all groups for male and female rats. Mean absolute organ weights were not of significant difference between treated and control groups in either sex. Mean relative organ weights were, however, statistically significantly higher for the kidneys of male and female rats of the mid- and high-dose groups with an apparent dose-response trend. In male rats the mean relative liver weight of the high-dose group was also statistically significantly higher than controls.

Macroscopic examinations of all tissues from male and female rats from control and treated groups did not reveal any abnormalities of biological significance (tabulated data not reported). Microscopic examination of all tissues (mainly from the control and high-dose groups) revealed a variety of non-neoplastic pathological lesions which were, in most cases, of the same incidence in the treated as in the control group.

Discussion:

The present study has investigated the effects of pyridate on the reproduction of three consecutive generations of rats. Although the target concentrations for pyridate were specified as 80, 400, and 2500 ppm (for the low-, mid-, and high-dose levels, respectively) due to the unstable nature of pyridate, actual diet concentrations were in general lower, especially with the low- and mid-dose levels. Since all animals were given sufficient diet on Friday to last until Monday and a considerable portion of pyridate was lost during the 72-hour period (see "Results" section) the actual intake of pyridate by all groups was lower than reported. Since losses at

the high-dose group were not excessive we do not believe that the outcome of this experiment was affected.

A number of changes have been observed in all parental generations (F_0, F_1, and F_2) as well as in the litters. However, in most cases no dose-response relationships were established and thus, the biological significance of these changes is not fully understood. For parent rats, mortality was generally comparable between dose groups and between generations. Body weights were consistently significantly lower in male and female rats of F_0 and F_1 generations of the high-dose groups. The adverse effect of pyridate on body weight appears to be diminishing with successive generations (F0 > F1 > F2) possibly suggesting adaptation of rats to longer pyridate exposure. Relative weight of the kidneys was consistently significantly higher in male and female rats of the high-dose group for both F_1 and F_2 generations. Relative liver weight was also higher in the female rats of the high-dose group of F_2 generation, while relative thyroid weight was significantly lower in male rats of the mid- and high-dose groups of F_2 generation. Although these relative organ weight changes are suggestive of pyridate toxicity these changes were not accompanied by any gross lesions in the corresponding organs. The authors also state in their report that no "treatment-related histopathological changes" were seen in these tissues, although histopathology was performed only with pituitaries and not with kidneys, liver, or thyroids.

From the litter data, the main indication for adverse pyridate effects was the statistically significantly lower pup body weight in the high-dose group of the first breeding of F_0, F_1, and F_2 generation on days 14 and 21.

Additional data obtained from F_3b generation rats also revealed some pyridate toxicity as follows: Mean relative kidney weight was statistically significantly higher for male and female rats of the mid- and high-dose groups and mean relative liver weight was higher in male rats of the high-dose group.

The evaluation of the present study cannot be completed until additional information is provided by the registrant as follows:

1. Summary tables of the results of the ophthalmoscopic examinations of the F_1 generation male and female rats.

2. Summary tables of gross pathology data from all generations.

3. Histopathology summary tables and individual animal

incidence of lesions for liver, kidneys, pituitaries, and thyroids for all parent generations.

4. Summary tables and individual animal data for congenital defects in all litters.

5. Translate into English the attached analytical data on pyridate.

6. Describe the procedure used in establishing the percent recovery of pyridate from the diet.

7. The authors reported that "because of holidays and workload the autopsy of the F_1 parent rats and of the F_2b young was postponed 4 to 5 weeks." We request that the authors provide us with information as to how those animals were handled prior to autopsy.

Conclusions

Based on the available data the maternal NOEL (depressed body weight) was found to be 400 ppm and the LEL 2500 ppm. The NOEL (tentative) for fetotoxic effects (depressed pup body weight) was established at 400 ppm and the LEL at 2500 ppm (the highest dose level tested).

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

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