UNDATED

006964

(5)

Reviewed By: Y.M. Ioannou
Section III, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: M. Copley
Section III, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Mutagenicity (84-2) TOX Chem. No.: 716A

Test Material: Pyridate Technical MRID No.: 408570-01

Synonyms: CL-11344, Lentagran

Study Number: T8186.381

Sponsor: Gilmore, Inc., Memphis, TN

Testing Facility: Microbiological Associates, Inc., Bethesda, MD

Title of Report: In Vivo-In Vitro Rat Hepatocyte Unscheduled DNA Synthesis Assay.

Author: Roger D. Curren

Report Issued: August 29, 1988

Conclusions

Pyridate Technical at single oral dose levels of 40, 160, and 800 mg/kg did not induce unscheduled DNA synthesis in male Fischer 344 rat primary hepatocytes established in culture after either 3 to 4 hours or 12 to 18 hours post-dose.

Classification : Unacceptable

Materials and Methods:

Test Material - Pyridate Technical, a brown oily liquid, with a reported purity of 91.5 percent, was used for this study. The test material was diluted in corn oil for preparing the dose solutions for this study. Methylmethanesulfonate (MMS), the positive control compound used for the 2- to 4-hour exposure, was diluted in water; 2-acetylaminofluorene (2-AAF), the positive control compound for the 12- to 18-hour exposure, was diluted in corn oil.

Test Animals - Male Fischer 344 rats (obtained from Harlan Sprague-Dawley, Inc., Frederick, MD) approximately 10 weeks old and weighing between 241 and 269 g at the time of dosing, were quarantined for at least 10 days and kept in animal rooms with a temperature of 68 to 75 °F, 30 to 90 percent relative humidity, and a 12-hour light/dark cycle. The animals were housed 3/cage and received Purina certified rodent chow and water ad libitum.

The animals were divided into 10 groups (5 rats/group) based on body weight and administered a single dose of the material (by oral gavage) as shown below:

Treatment Group	Treatment	Dose Level (mg/kg)	Exposure Time (H)	Number of Males
1	Corn Oil	10 mL/kg	2-4	5
2	Pyridate Technical	800	2-4	5
3	Pyridate Technical	160	2-4	5
4	Pyridate Technical	40	2-4	5
5	MMS	100	2-4	5
6	Corn Oil	10 mL/kg	12-18	5
7	Pyridate Technical	800	12-18	5
8	Pyridate Technical	160	12-18	5
9	Pyridate Technical	40	12-18	5
10	2AAF	100	12-18	5

Indicator Cells - Three rats from each group were sacrificed (by inhalation of metofane) either 2 to 4 or 12 to 18 hours after dosing, and their livers perfused with an 0.5 mM EGTA solution followed by a collagenase solution; the liver was then removed from each animal, the cells dissociated and counted, and seeded into 35 mm dishes (5×10^5 viable cells/dish). The cells were seeded in Williams' Medium E supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 ug/mL gentamicin (complete WME). Six cultures per rat, containing coverslips, were incubated at 37 ± 1 °C in a humidified 5 ± 1 percent CO_2 incubator.

Processing of the Hepatocyte Cultures (Abstracted from the Original Report):

"Approximately two hours after the cultures were seeded, the plates were washed with complete WME and refed with serum-free WME containing 10 uCi/ml ^{3}H-thymidine. Approximately four hours later, the cultures were washed three times and refed with serum-free WME containing 0.25 mM thymidine.

"Seventeen to twenty hours after the ^{3}H-thymidine-containing medium was removed, the cells were washed in serum-free WME, swelled in 1% sodium citrate and fixed in ethanol-glacial acetic acid fixative. The coverslips were air-dried, mounted (cell side up) on glass slides, and allowed to dry. The slides were coated with Kodak NTB2 emulsion and stored for three days at 4°C in light tight boxes with desiccant. The slides were then developed in Kodak D-19 developer, fixed in Kodak fixer and stained with hematoxylin-sodium acetate-eosin.

"The slides were read 'blind' on an automated colony counter. Nuclear grains were counted in 50 cells in random areas of each of three coverslips per animal where possible. The net nuclear counts were determined by counting one nuclear-sized area in the cytoplasm adjacent to each nucleus and subtracting that cytoplasmic count from the nuclear count. Replicative synthesis was identified by nuclei completely blackened with grains and such cells were not counted. Nuclei exhibiting toxic effects of treatment, such as dark or uneven staining, disrupted membranes or irregular shape, were not counted.

"Criteria for Evaluation of Test Results

"The results of this study were evaluated according to the following criteria. If the mean net nuclear count was increased by at least five counts over the control, the results for a particular dose level were considered significant. A test article was judged positive if it induced a dose-related response and at least one dose produced a significant increase in the average net nuclear grains when compared to that of the control. In the absence of the dose response, a test article which showed a significant increase in the mean net nuclear grain count in at least two successive doses was considered positive. If a test article showed a significant increase in the net nuclear grain counts at one dose level without any dose response, the test article was considered to have a marginal positive activity. The test article was considered negative if no significant increase in the net nuclear grain counts at any dose level was observed.

"Presentation of Data

"For each treatment slide, the net nuclear counts were averaged and the standard deviation (S.D.) determined and recorded on a summary form. Also reported are the mean, S.D. and the percent of cells in repair (cells with $\geq$ 5 net nuclear grains) for each animal and the mean and S.D. per treatment group.

"Criteria for Determination of a Valid Test

"The test was considered valid if the positive control compound induced a significant increase in the net nuclear grains and the number of cells in repair in the vehicle control was less than 20%."

Results and Discussion:

The authors reported that one rat exposed to the high dose (800 mg/kg) of Pyridate Technical, at the 2- to 4-hour exposure, died during this study, and thus was not used in the assay. At the 12- to 18-hour exposure, one animal of the high-dose group exhibited clinical signs of diarrhea; hepatocytes from this animal were, however, used in the assay.

Unscheduled DNA Synthesis - Results presented in Table 1 (abstracted from the original report) at the 2- to 4-hour exposure indicate that (a) the average net grain counts/nucleus were similar between hepatocytes from the three Pyridate-treated dose groups (40, 160, and 800 mg/kg) and the vehicle control group i.e., there was no evidence of unscheduled DNA synthesis (UDS) in the treated or control groups; (b) the positive control group (MMS at 100 mg/kg) had a statistically significant increase (greater than 5 net nuclear grain counts over the vehicle control) in the average net grain count/nucleus (2.2 vs. -4.0 mean net nuclear grain counts for the positive control and vehicle control, respectively).

Results from the 12- to 18-hour exposure (Table 2, abstracted from the original report) also show that (a) hepatocytes from Pyridate-treated groups and the vehicle control group had comparable mean net grain counts/nucleus and thus, no evidence of UDS; (b) hepatocytes obtained from animals treated with 2-AAF at 100 mg/kg (positive control group) had a statistically significant increase in the mean net nuclear grain count (3.3) as compared to hepatocytes from the vehicle control group (-3.0).

Results presented here from both exposure groups (i.e., 2- to 4-hour exposure and 12- to 18-hour exposure) have shown conclusively that the test system used had the ability to detect UDS as shown by the findings with both of the positive control groups (MMS and 2-AAF, Tables 1 and 2). Furthermore, Pyridate Technical at the dose level tested does not appear to induce UDS.

Conclusions:

The present study has investigated the ability of Pyridate Technical to induce UDS in rat hepatocytes when the test article was administered to rats by oral gavage at dose levels of 40, 160, and 800 mg/kg. The results show conclusively that Pyridate Technical does not induce UDS in rat hepatocytes at any of the dose levels tested.

Results obtained from the positive control groups indicate that all criteria for a valid test were met in this study.

Classification: The present study is classified as Unacceptable based on the following deficiencies:

1. The authors failed to provide information concerning the total concentration of the test article absorbed by the gastrointestinal tract and thus cabable of causing toxicity at the target cells.

2. The authors did not justify why only one sex of rats was used for this study and not both.

3. The authors failed to provide documentation as to whether storage of slides coated with Kodak NTB2 emulsion for three days was sufficient time to develop enough grains from a weakly positive chemical.

006964

TABLE 1

SUMMARY OF UDS ASSAY WITH PYRIDATE TECHNICAL (2-4 HOURS)

TREATMENT	ANIMAL NUMBER	SLIDE DESIGNATION	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS	S.D.		MEAN PER ANIMAL	S.D.a	CELLS IN REPAIR	MEAN PER GROUP	S.D.#
Pyridate Technical											
800 mg/kg	31	41A	50	-4.8	+/-	2.5	-4.5	0.3	0%		
		41C	50	-4.2	+/-	2.0					
		41F	50	-4.6	+/-	2.8					
	34	43A	50	-4.4	+/-	2.9	-4.4	0.1	0%	-4.3	0.3
		43C	50	-4.4	+/-	2.9					
		43F	50	-4.3	+/-	2.7					
	35	32A	50	-3.9	+/-	2.0	-4.0	0.3	0%		
		32B	50	-3.8	+/-	2.1					
		32F	50	-4.3	+/-	2.4					
160 mg/kg	37	37A	50	-3.7	+/-	2.3	-3.7	0.3	0%		
		37C	50	-3.4	+/-	2.1					
		37D	50	-4.0	+/-	1.8					
	38	44A	50	-4.8	+/-	3.1	-4.2	0.8	0%	-4.0	0.3
		44B	50	-4.5	+/-	2.2					
		44F	50	-3.3	+/-	2.1					
	40	34A	50	-4.8	+/-	2.7	-4.1	1.4	0%		
		34C	50	-2.5	+/-	1.9					
		34D	50	-5.0	+/-	2.3					
40 mg/kg	41	39C	50	-4.6	+/-	1.8	-4.3	0.3	0%		
		39D	50	-4.2	+/-	2.6					
		34F	50	-4.1	+/-	2.1					
	42	42A	50	-5.3	+/-	2.8	-5.1	0.3	0%	-4.4	0.6
		42C	50	-5.1	+/-	2.8					
		42F	50	-4.8	+/-	2.8					
	45	33B	50	-4.7	+/-	3.0	-4.0	0.7	0%		
		33D	50	-3.4	+/-	2.2					
		33F	50	-3.8	+/-	2.4					
MMS (Positive Control)											
100 mg/kg	46	40A	50	4.0	+/-	4.5	4.4	0.5	42%		
		40C	50	4.9	+/-	4.4					
		40E	50	4.4	+/-	4.6					
	47	38A	50	0.6	+/-	4.6	0.0	0.5	7%	2.2 *	2.2
		38B	50	-0.5	+/-	3.1					
		38C	50	0.1	+/-	2.8					
	50	46A	50	2.7	+/-	3.8	2.2	0.5	23%		
		46D	50	2.2	+/-	3.8					
		46E	50	1.8	+/-	3.5					
CORN OIL (Vehicle Control for Test Article)											
10 ml/kg	26	31C	50	-4.7	+/-	2.3	-4.2	1.4	0%		
		31D	50	-2.7	+/-	1.8					
		31E	50	-5.3	+/-	2.4					
	27	45A	50	-3.5	+/-	2.5	-3.5	0.1	0%	-4.0	0.5
		45B	50	-3.4	+/-	2.2					
		45E	50	-3.5	+/-	1.9					
	28	36A	50	-4.0	+/-	2.2	-4.3	0.4	0%		
		36E	50	-4.2	+/-	2.5					
		36F	50	-4.7	+/-	2.3					

* Significant (See Protocol: Section 8.0, Evaluation of Test Results)
S.D.a Standard Deviation Reflecting Slide to Slide Variation
S.D.# Standard Deviation Reflecting Animal to Animal Variation

006964

TABLE 2

SUMMARY OF UDS ASSAY WITH PYRIDATE TECHNICAL (12-18 HOURS)

TREATMENT	ANIMAL NUMBER	SLIDE DESIGNATION	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS	S.D.	MEAN PER ANIMAL	S.D.@	CELLS IN REPAIR	MEAN PER GROUP	S.D.#
Pyridate Technical										
800 mg/kg	6	24A	50	-3.2	2.0	-2.8	0.4	0%		
		24B	50	-2.6	2.2					
		24F	50	-2.5	1.7					
	7	16A	50	-3.0	1.9	-2.4	0.7	0%	-2.4	0.4
		16B	50	-1.7	1.5					
		16D	50	-2.4	1.5					
	8	12A	50	-2.1	1.5	-2.0	0.1	0%		
		12B	50	-2.1	1.7					
		12C	50	-1.9	1.2					
160 mg/kg	11	20A	50	-3.3	2.0	-2.7	0.5	0%		
		20C	50	-2.5	1.5					
		20F	50	-2.4	1.4					
	12	18C	50	-2.8	1.7	-2.9	0.3	0%	-2.9	0.2
		18D	50	-2.7	1.5					
		18F	50	-3.2	1.6					
	13	25A	50	-2.9	1.6	-3.2	1.0	0%		
		25C&25F	50	-4.3	2.1					
		25D	50	-2.4	1.9					
40 mg/kg	16	13D	50	-1.8	1.3	-1.9	0.6	0%		
		13E	50	-1.6	1.5					
		13F	50	-2.3	1.4					
	17	15B	50	-1.8	1.7	-2.0	0.3	0%	-2.4	0.8
		15C	50	-2.3	1.6					
		15D	50	-1.8	1.4					
	20	22B	50	-3.5	2.0	-3.3	0.2	0%		
		22C	50	-3.2	1.9					
		22E	50	-3.1	1.7					
2AAF (Positive Control)										
100 mg/kg	21	23A	50	3.5	2.9	3.7	1.1	37%		
		23D	50	2.7	3.0					
		23F	50	4.8	4.6					
	24	19B	50	5.4	4.8	4.3	1.4	45%	3.3 *	1.3
		19E	50	4.8	4.4					
		19F	50	2.8	4.0					
	25	17C	50	1.7	3.1	1.9	0.5	21%		
		17E	50	2.5	3.6					
		17F	50	1.5	3.2					
CORN OIL (Vehicle Control for Both Test Article and 2AAF)										
10 ml/kg	1	11A	50	-2.1	1.8	-2.9	0.7	0%		
		11B	50	-3.5	2.2					
		11D	50	-3.0	2.6					
	2	14A	50	-3.1	2.0	-3.0	0.1	0%	-3.0	0.1
		14B	50	-3.0	1.8					
		14E	50	-2.9	2.0					
	3	21B	50	-2.7	1.8	-3.0	0.5	0%		
		21E	50	-2.8	1.7					
		21F	50	-3.6	1.7					

* Significant (See Protocol: Section 8.0, Evaluation of Test Results)
S.D.@ Standard Deviation Reflecting Slide to Slide Variation
S.D.# Standard Deviation Reflecting Animal to Animal Variation