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

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OFFICE OF
PESTICIDES AND TOXIC SUBS

MEMORANDUM

SUBJECT: FMC 54800 (Biphenthrin) Mutagenicity Studies

TO: Mr. Tim Gardner, PM 17
Registration Division (TS-767)

FROM: Byron T. Backus Byron T. Backus 11/29/85
Toxicologist
Toxicology Branch

THROUGH: Clint Skinner, Ph.D.
Head, Section III
and
Theodore Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

W Thomas Edwards 12-2-85
12/3/85

Chemical no. 463F

Project No. 793

Action Requested:

The Registration Division has requested a review of 3 mutagenicity studies on the technical material.

Comments and Conclusions:

1. The rat hepatocyte unscheduled DNA synthesis study under FMC no. A83-985 has been classified as acceptable in demonstrating UDS at 2.0 ul/ml (HDT), but as not acceptable in demonstrating lack of UDS at lower exposure levels, due to the high standard deviations associated with all mean net nuclear grain counts (including those of controls).

2. The rat hepatocyte unscheduled DNA synthesis study under FMC no. A83-1043 has been classified as not acceptable. The major problem is that such criteria as number of nuclei/exposure level with 6 or more net nuclear grains, as well as number of nuclei/exposure level showing 20 or more net nuclear grains, were not utilized in the evaluation of the study results, and the values for these parameters cannot be determined from the information presented.

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3. The in vivo chromosomal aberrations in rat bone marrow cells study has been classified as acceptable in demonstrating no increase in severity or incidence of chromosomal aberrations occurred following oral administration of FMC 54800 to male Sprague-Dawley rats at 3, 10 or 30 mg/kg/day over a 5-day period.

4. Copies of the individual data evaluation reports should be provided to the registrant.

Data Evaluation Reports (attached):

I. Thilagar, A. Unscheduled DNA Synthesis in Rat Primary Hepatocytes. FMC Study no. A83-985. Study conducted by Microbiological Associates, under study no. T2007.380. Report dated 9/26/83.

II. Thilagar, A. Unscheduled DNA Synthesis in Rat Primary Hepatocytes. FMC Study no. A83-1043. Study conducted by Microbiological Associates, under study no. T2007.380. Report dated 11/1/83.

III. Putnam, D. L. Subchronic in vivo cytogenetics assay in male rats. FMC Study no. A83-979. Study conducted by Microbiological Associates, under study no. T2007.102. Report dated 10/11/83.

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Data Evaluation Report (I)

Compound:

FMC 54800 (technical)

Study type:

Mutagenicity - Rat Hepatocyte Unscheduled DNA Synthesis

Citation:

Thilagar, A. Unscheduled DNA Synthesis in Rat Primary Hepatocytes. FMC Study no. A83-985. Study conducted by Microbiological Associates, under study no. T2007.380. Report dated 9/26/83. Study received at EPA 10/01/85; in Acc. 259434.

Reviewed by:

Byron T. Backus Byron T. Backus 11-29-85
Toxicologist
Toxicology Branch

Approved by:

Clint Skinner, Ph.D. W Thomas Edwards 12-2-85
Section Head
Review Section III
Toxicology Branch

Classification: Acceptable in demonstrating UDS at 2.0 ul/ml (HDT) FMC 54800
Not acceptable in demonstrating lack of UDS at lower exposure levels.

Comments and Conclusions:

1. The standard deviations associated with average mean net nuclear grain counts are so high that it has not been demonstrated, within 95% confidence limits, that the differences between values for the different exposure levels of FMC 54800 and their controls were not greater than 5. Therefore, the study is not acceptable in demonstrating lack of UDS at exposure levels of 0.01 to 1.0 ul/ml FMC 54800 in this assay.

2. Such criteria as number of nuclei/exposure level showing 6 or more net nuclear grains, and/or number of nuclei/exposure level showing 20 or more net nuclear grains, were not utilized in the evaluation of the study results, although these criteria were available from the information, as presented.

3. At 2.0 ul/ml FMC 54800 there was, in addition to a difference greater than 5 in average net grains/nucleus as compared with the solvent control, 4/75 (5.3%) cells showing 20 or more net nuclear grains, as compared to no more than 1/75 in any of the solvent control groups. The interpretation is then that UDS was induced as a result of exposure to 2.0 ul/ml FMC 54800.

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Materials:

Test material: FMC 54800 (Technical), Lot No. E2392-105, stored at room temperature. No indication as to purity. The test material was dissolved/diluted in acetone.

Positive control: 2-Acetoaminofluorene, dissolved in ethanol.

Procedure:

Rat hepatocytes were obtained by basically the method of Williams, et al. (1977), using adult male Sprague-Dawley rats from Charles River Laboratories. Rats were sacrificed by metofane inhalation, then were dissected and perfused with 0.5 mM ethylene-glycol-bis-(B-aminoethyl ether)-N-N'--tetraacetic acid (EGTA) followed by a collagenase solution. The liver was removed and cells were dissociated, counted, and seeded into 35 ml dishes (5×10^5 viable cells/dish) containing converslips. The cells were seeded in Williams Medium E (WME) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 ug/ml streptomycin. Cultures were incubated at 37° C in a humidified 5% CO_2 incubator for 2 hrs, washed and refed with serum free medium and used in the test.

Preliminary cytotoxicity was performed using 10 doses (0.005 ul/ml to 100 ul/ml) of FMC 54800, applied to replicate cultures of rat hepatocytes 2 hrs after seeding. 18 hrs later cells were washed with Ca^{++} and Mg^{++} free phosphate-buffered saline, trypsinized, stained (trypan blue) and counted in a hemacytometer. Relative survival indices (RSI) were calculated by comparing treated to control groups:

$$RSI = \frac{\text{Average \# of viable cells in test cultures}}{\text{Average \# of viable cells in solvent control}} \times 100$$

In the DNA repair assay three replicate plates seeded with 5×10^5 (viable?) hepatocytes/plate were exposed for 18 hrs to 0.01, 0.05, 0.1, 0.5, 1.0 or 2.0 ul/ml FMC 54800. 2-AAF, at 20 and 2 ug/ml was the positive control. Acetone (used for FMC 54800) and ethanol (used for 2-AAF) were solvent controls. Although not mentioned in the protocol, there must have been concomitant exposure to tritiated thymidine. In parallel with the test plates, 3 cultures/dilution were treated with the same compound for a parallel [illegible]icity test; relative survival indices were calculated as in the preli[illegible] cytotoxicity assay.

After 18 hrs of exposure, cells in the DNA repair assay plates were washed in serum-free WME, swollen in 1% sodium citrate, and were fixed in ethanol-acetic acid. Coverslips were air-dried, mounted cell surface up on glass slides (presumably; glass slide:mounting medium:coverslip:cells) and were allowed to dry. Slides were coated with Kodak NTB emulsion, then were stored 8 days at 4° C in light-proof boxes. Autoradiographs were developed with D19, photographically fixed, and preparations were then stained in hematoxylin-sodium acetate-eosin.

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Counts were made of the number of black silver grains for each of 75 randomly-selected nuclei/dose level (25/coverslip) using an ARTEK colony counter with microscope-mounted auxiliary T.V. camera. Net nuclear counts were computed by counting 3 nucleus-sized areas adjacent to each nucleus and subtracting the average from the count for this particular nucleus. Blackened nuclei (considered engaged in replicative synthesis), nuclei which were darkly stained, with disrupted membranes or irregular shape, or having a projected image of less than 4 mm^2, were not counted. All counts were converted to net nuclear grain counts using a programmable calculator with tape. For each treatment level an average net nuclear count with S.D. was determined.

"The results will be considered significant if the mean net nuclear count at a dose level is increased by at least five counts over the control. A test article will be judged positive if it induces a dose-related response and at least two successive doses exhibit a significant increase in the average net nuclear grains when compared to that of the negative control. In the absence of a dose response, the test article should show a significant increase in the mean net nuclear grain counts in at least 3 dose levels. If a test article showed a significant increase in the mean net nuclear grain count at one of two dose levels without any dose response, the test article will be considered to have a marginal positive activity. The test article will be considered negative if it did not cause a significant increase in the mean net nuclear grain counts at any dose level."

Results:

Preliminary cytotoxicity assay (as reported from table 1, p. 9):

Treatment & Dose	% Viable Cells	Ave. Viable Cells/Dish ($x10^4$)	Survival Index	Relative Survival	Relative Toxicity
FMC 54800 100 ul/ml*	49.30	3.5	7.0	25.55	74.45
FMC 54800 50 ul/ml*	59.21	4.5	9.0	32.85	67.15
FMC 54800 10 ul/ml*	59.26	4.8	9.6	35.04	64.96
FMC 54800 5.0 ul/ml	24.14	0.7	1.4	5.43	94.57
FMC 54800 1.0 ul/ml	47.78	4.3	8.6	33.33	66.67
FMC 54800 0.50 ul/ml	71.00	7.1	14.2	55.04	44.96
FMC 54800 0.10 ul/ml	78.51	9.5	19.0	73.64	23.36
FMC 54800 0.050 ul/ml	76.98	9.7	19.4	75.19	24.81
FMC 54800 0.010 ul/ml	74.84	11.6	23.2	89.92	10.08
FMC 54800 0.005 ul/ml	79.75	13.0	26.0	100.78	-
Acetone (solvent control)	86.00	12.9	25.8	100.00	-
WME (untreated control)	81.07	13.7	27.4	100.00	-

5×10^5 cells were plated per dish

*Test article was added directly and the data were compared to WME control

$$\text{Survival Index} = \frac{\text{Average Viable Cells per Dish}}{\text{Cells plated per Dish}} \times 100$$

$$\text{Relative Survival} = \frac{\text{Survival Index}}{\text{Survival Index of Control}} \times 100$$

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Parallel cytotoxicity assay (as reported from table 2, p. 10):

Treatment & Dose		% Viable Cells	Ave. Viable Cells/Dish ($x10^4$)	Survival Index	Relative Survival	Relative Toxicity
FMC 54800	2.0 ul/ml	40.42	2.13	4.26	14.72	85.28
FMC 54800	1.0 ul/ml	59.34	4.67	9.34	32.27	67.73
FMC 54800	0.50 ul/ml	60.95	5.93	11.86	40.98	59.02
FMC 54800	0.10 ul/ml	62.26	6.60	13.2	45.61	54.39
FMC 54800	0.050 ul/ml	80.04	11.80	23.6	81.55	18.45
FMC 54800	0.010 ul/ml	81.68	12.80	23.2	88.46	11.54
Acetone (solvent control)		84.47	14.47	28.94	100.00	-
2AAF	2.0 ug/ml	76.80	9.47	18.94	65.45	34.55
2AAF	20.0 ug/ml	61.11	6.60	13.20	45.61	54.39
Ethanol		81.61	14.47	28.94	100.00	-
WME (untreated control)		85.30	14.27	28.54	98.62	1.38

5×10^5 cells were plated per dish

Unscheduled DNA Synthesis Summary Results (as reported in table 3, p. 11):

Treatment & Dose		Relative Survival	Average Net Grains per Nucleus ± S.D.	
FMC 54800	2.0 ul/ml	14.72	9.3 ± 6.6	
FMC 54800	1.0 ul/ml	32.27	5.5 ± 5.2	
FMC 54800	0.50 ul/ml	40.98	4.9 ± 5.4	
FMC 54800	0.10 ul/ml	45.61	7.9 ± 6.4	All grain counts
FMC 54800	0.050 ul/ml	81.55	4.0 ± 4.2	were for 75
FMC 54800	0.010 ul/ml	88.46	6.0 ± 5.1	nuclei/dosage level
Acetone (solvent control)		100.00	3.8 ± 4.4	
2AAF	2.0 ug/ml	65.45	26.0 ± 7.6	
2AAF	20.0 ug/ml	45.61	27.0 ± 15.4	
Ethanol		100.00	2.5 ± 6.9	
WME (untreated control)		98.[illegible]	3.6 ± 4.1	

5×10^5 cells were plated [illegible] dish

Grain counts for individual nuclei in the UDS assay are given on p. 24-29 (FMC 54800 at 0.10 ul/ml); 30-34 (FMC 54800 at 0.50 ul/ml); 35-39 (WME3); 41-45 (FMC 54800 at 2.0 ul/ml); 46-50 (FMC at 0.050 ul/ml); 51-56 (ethanol); 57-61 (FMC 54800 at 1.0 ul/ml); 62-67 (? p. 67 is illegible; probably 2AAF at 2 ug/ml); 68-73 (acetone); 74-79 (FMC 54800 at 0.010 ul/ml); and 80-85 (2AAF at 20 ug/ml).

There was no confirmatory assay (although there was a subsequent study under FMC #A83-1043).

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Discussion:

The report states (p. 12) that FMC 54800 caused a significant increase in UDS (as manifested by a higher grain count) at the highest dose level of 2 ul/ml, but not at the remaining 5 doses.

Average net nuclear grain counts of five or greater above control average are usually assumed to constitute a positive response in this type of assay, since five is normally more than twice the standard deviation associated with the control count. In this study, however, the average grain count with S.D. for the solvent (acetone) control was 3.8 $\pm$ 4.4, essentially the same as for the WME (3.6 $\pm$ 4.1). The average grain count of 9.3 $\pm$ 6.6 for the 2.0 ul/ml dose level may not have been significantly different from control values then. However, since S.D.'s associated with average nuclear grain counts were usually above 5, 95% confidence limits for differences between net grain counts for cells exposed to FMC 54800 and their respective controls usually included values of five or more. The study has therefore not demonstrated within 95% confidence limits that UDS does not occur as a result of exposure to dosage levels of 0.01 to 1.0 ul/ml FMC 54800.

No additional criteria for UDS were utilized (number of nuclei/exposure level showing 6 or more net nuclear grains; number of nuclei/exposure level showing 20 or more net nuclear grains). These are indicated in Brusick, D. Principles of Genetic Toxicology. Plenum Press, 1980. pp. 224-228: This reference states (p. 227):

> The test article should be considered active in the UDS assay at applied concentrations that cause (1) An increase in the mean nuclear grain count to at least six grains per nucleus in excess of the concurrent negative control value; and/or (2) the percentage of nuclei with six or more grains to increase above 10% of the examined population, in excess of the concurrent negative control; and/or (3) the percentage of nuclei with 20 or more grains to reach or exceed 2% of the examined population.

This reference continues:

> Generally, if the first condition is satisfied, the second and often the third condition will also be met. However, satisfaction of only the second or third condition can also indicate UDS activity...all three of the above conditions should be considered in an evaluation.

It is also stated that the net nuclear grain count should be determined for 50 randomly selected cells on each coverslip (instead of the 25 in this study). However, this is a moot point if the statistical resolution of the data is such that definite conclusions can be drawn.

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The following table is constructed from the data in Appendix 2 (p. 24-85):

Treatment & Dose	Number of nuclei with 6 or more net grains: total	Number of nuclei with 6 or more net grains: less solvent control	Number of nuclei with 20 or more net grains	Percentage of nuclei with 20 or more net gra
FMC 54800 2.0 ul/ml	50	27	4	5.3
FMC 54800 1.0 ul/ml	33	10	0	0.0
FMC 54800 0.50 ul/ml	29	6	1	1.3
FMC 54800 0.10 ul/ml	48	25	1	1.3
FMC 54800 0.050 ul/ml	20	-	0	0.0
FMC 54800 0.010 ul/ml	38	15	1	1.3
Acetone (solvent control)	23	-	0	0.0
2AAF 20.0 ug/ml	72	62	49	68.1
Ethanol	10	-	1	1.3
WME (untreated control)	19	-	0	0.0

If percentage of nuclei with 6 or more grains in 10% of the population in excess of the concurrent negative control is evidence of UDS, this tentatively suggests activity at 2.0, 1.0, 0.1 and 0.01 ul/ml (but not at 0.5 or 0.05 ul/ml) FMC 54800, as well as the positive control.

Only the 2.0 ul/ml dose level FMC 54800 (as well as the positive control) had more than 2% of the examined nuclei with 20 or more grains.

The conclusion of this reviewer is that UDS was probably induced at an exposure level of 2.0 ul/ml FMC 54800. Conversely, because of the high standard deviations associated with average net nuclear grain counts for both control and exposed hepatocytes, it cannot be stated that increased UDS did not occur at lower exposure levels of FMC 54800.

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Data Evaluation Report (II)

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Compound:

FMC 54800 (Technical)

Study type:

Mutagenicity - Rat Hepatocyte Unscheduled DNA Synthesis

Citation:

Thilagar, A. Unscheduled DNA Synthesis in Rat Primary Hepatocytes. FMC Study no. A83-1043. Study conducted by Microbiological Associates, under study no. T2007.380. Report dated 11/1/83. Study received at EPA 10/01/85; in Acc. 259434.

Reviewed by:

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Toxicologist
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Approved by:

Clint Skinner, Ph.D. W. Thomas Edwards 12-2-85
Section Head
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Toxicology Branch

Classification: Not acceptable

Comments and Conclusions:

Using an increase in average net nuclear grains/nucleus as the only evaluation criterion, there was no evidence of UDS induction. In contrast to a previous UDS study performed on FMC 54800 at the same laboratory the mean net grains per nucleus were below 2.0 for the negative (WME) and solvent (acetone and ethanol) controls, as well as at all exposure levels (up to 2.5 ul/ml) FMC 54800. However, the study is currently classified as not acceptable because such criteria as number of nuclei/exposure level showing 6 or more net nuclear grains, as well as number of nuclei/exposure level showing 20 or more net nuclear grains (or criteria similar to these) were not utilized in the evaluation of the study results, and the values for these parameters cannot be determined from the information presented.

Materials:

Test material: FMC 54800 (Technical), Lot No. E2392-105, stored at room temperature. No indication as to purity. The test material was dissolved/diluted in acetone.

Positive control: 2-Acetoaminofluorene, dissolved in ethanol.

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Procedure:

Rat hepatocytes were obtained by basically the method of Williams, et al. (1977), using adult male Sprague-Dawley rats from Charles River Laboratories. Rats were sacrificed by metofane inhalation, then were dissected and perfused with 0.5 mM ethylene-glycol-bis-(B-aminoethyl ether)-N-N'--tetraacetic acid (EGTA) followed by a collagenase solution. The liver was removed and cells were dissociated, counted, and seeded into 35 ml dishes (5×10^5 viable cells/dish) containing converslips. The cells were seeded in Williams Medium E (WME) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 ug/ml streptomycin. Cultures were incubated at 37° C in a humidified 5% CO_2 incubator for 2 hrs, washed and refed with serum free medium and used in the test.

The preliminary cytotoxicity study which served as a basis for dose levels tested was that which had been previously performed in the study reported 9/26/83.

In the DNA repair assay three replicate plates seeded with 5×10^5 (viable?) hepatocytes/plate were exposed for 18 hrs to 0.5, 1.0, 1.5, 1.75, 2.0, 2.25 or 2.5 ul/ml FMC 54800. 2-AAF, at 20 and 2 ug/ml was the positive control. Acetone (used for FMC 54800) and ethanol (used for 2-AAF) were solvent controls. Although not mentioned in the protocol, there must have been concomitant exposure to tritiated thymidine. In parallel with the test plates, 3 cultures/dilution were treated with the same compound for a parallel toxicity test; relative survival indices were calculated by:

$$RSI = \frac{\text{Average \# of viable cells in test cultures}}{\text{Average \# of viable cells in solvent control}} \times 100$$

After 18 hrs of exposure, cells in the DNA repair assay plates were washed in serum-free WME, swollen in 1% sodium citrate, and were fixed in ethanol-acetic acid. Coverslips were air-dried, mounted cell surface up on glass slides (presumably; glass slide:mounting medium:coverslip:cells) and were allowed to dry. Slides were coated with Kodak NTB emulsion, then were stored 8 days at 4° C in light-proof boxes. Autoradiographs were developed with D19, photographically fixed, and preparations were then stained in hematoxylin-sodium acetate-eosin.

Counts were made of the number of black silver grains for each of 75 randomly-selected nuclei/dose level (25/coverslip) using an ARTEK colony counter with microscope-mounted auxiliary T.V. camera. Net nuclear counts were computed by counting 3 nucleus-sized areas adjacent to each nucleus and subtracting the average from the count for this particular nucleus. Blackened nuclei (considered engaged in replicative synthesis), nuclei which were darkly stained, with disrupted membranes or irregular shape, or having a projected image of less than 4 mm^2, were not counted. All counts were converted to net nuclear grain counts using a programmable calculator with tape. For each treatment level an average net nuclear count with S.D. was determined.

"The results will be considered significant if the mean net nuclear count at a dose level is increased by at least five counts over the control. A test article will be judged positive if it induces a dose-related response and at least two successive doses exhibit a significant increase in the average net nuclear grains when compared to that of the negative control. In the absence of a dose response, the test article should show a significant increase in the mean net nuclear grain counts in at least 3 dose levels. If a test article showed a significant increase in the mean net nuclear grain count at one of two dose levels without any dose response, the test article will be considered to have a marginal positive activity. The test article will be considered negative if it did not cause a significant increase in the mean net nuclear grain counts at any dose level."

Results:

Unscheduled DNA Synthesis Summary Results (as reported in table 2, p. 10):

Treatment & Dose		Relative Survival	Average Net Grains per Nucleus ± S.D.	
FMC 54800	2.50 ul/ml	3.9	0.4 ± 1.4	
FMC 54800	2.25 ul/ml	12.6	0.7 ± 1.2	
FMC 54800	2.00 ul/ml	26.8	0.1 ± 1.2	
FMC 54800	1.75 ul/ml	27.6	0.4 ± 1.4	All grain counts were for 75 nuclei/dosage level
FMC 54800	1.50 ul/ml	32.3	1.0 ± 1.7	
FMC 54800	1.00 ul/ml	52.0	1.1 ± 1.3	
Acetone (solvent control)		100.00	0.5 ± 1.1	
2AAF	2.0 ug/ml	45.2	22.8 ± 7.6	
2AAF	20.0 ug/ml	65.9	22.7 ± 15.4	
Ethanol		100.00	0.9 ± 1.6	
WME (untreated control)		97.6	0 ± 0.9	

5×10^5 cells were plated per dish

Parallel cytotoxicity assay (as reported from table 1, p. 9):

Treatment & Dose	% Viable Cells	Ave. Viable Cells/Dish ($x10^4$)	Survival Index	Relative Survival	Relative Toxicity
FMC 54800 2.50 ul/ml	11.63	0.5	1.0	3.9	96.1
FMC 54800 2.25 ul/ml	37.21	1.6	3.2	12.6	87.4
FMC 54800 2.00 ul/ml	45.95	3.4	6.8	26.8	73.2
FMC 54800 1.75 ul/ml	51.47	3.5	7.0	27.6	72.4
FMC 54800 1.50 ul/ml	57.75	4.1	8.2	32.3	67.7
FMC 54800 1.00 ul/ml	67.35	6.6	13.2	52.0	48.0
FMC 54800 0.50 ul/ml	74.17	11.2	22.4	88.2	11.8
Acetone (solvent control)	74.71	12.7	25.4	100.00	-
2AAF 20.0 ug/ml	76.80	9.47	18.94	65.45	34.55
2AAF 2.0 ug/ml	61.11	6.60	13.20	45.61	54.39
Ethanol	81.61	14.47	28.94	100.00	-
WME (untreated control)	85.30	14.27	28.54	98.62	1.38

5×10^5 cells were plated per dish

$$\text{Survival Index} = \frac{\text{Average Viable Cells per Dish}}{\text{Cells plated per Dish}} \times 100$$

$$\text{Relative Survival} = \frac{\text{Survival Index}}{\text{Survival Index of Control}} \times 100$$

Discussion:

Using an increase in average net nuclear grains as the only criterion for evaluation, there was no evidence of induction of UDS. In contrast to a previous UDS study performed on FMC 54800 at the same laboratory the average net grains per nucleus were below 2.0 for the negative (WME) and solvent (acetone and ethanol) controls.

However, such evaluation criteria as number of nuclei/exposure level with 6 or more net nuclear grains, and number of nuclei/exposure level with 20 or more net nuclear grains, were not utilized in the evaluation of the study results, and the values for these parameters cannot be determined from the information presented. Without this information, the study cannot be classified as acceptable.

Data Evaluation Report (III) 004820

Compound:

FMC 54800 (Technical)

Study type:

Mutagenicity - Chromosomal aberrations in vivo in rat bone marrow cells.

Citation:

Putnam, D. L. Subchronic in vivo cytogenetics assay in male rats. FMC Study no. A83-979. Study conducted by Microbiological Associates, under study no. T2007.102. Report dated 10/11/83. Study received at EPA 10/01/85; in Acc. 259434.

Reviewed by:

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Toxicologist
Toxicology Branch

Approved by:

Clint Skinner, Ph.D. W Thomas Edwards 12-2-85
Section Head
Review Section III
Toxicology Branch

Classification: Acceptable

Comments and Conclusions:

No increase in severity or incidence of chromosomal aberrations was observed following oral administration of FMC 54800 to male Sprague-Dawley rats at 3, 10 or 30 mg/kg/day over a 5-day period. The highest dose tested was sufficiently adequate in terms of acute toxicity as 2/7 males died; a previously accepted study defined the male rat oral LD_{50} as approximately 70 mg/kg/day.

Materials:

Test material: FMC 54800 (Technical), Lot No. E2392-105, stored at room temperature. No indication as to purity. The test material was administered in a corn oil suspension.

Positive control: Triethylenemelamine, dissolved in distilled water.

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Procedure:

Groups of 5 male Sprague-Dawley rats received, by gavage, 0 (vehicle control), 3 or 10 mg/kg/day FMC 54800. An additional group of 7 males received 30 mg/kg/day of FMC 54800. The test material was suspended in corn oil and administered as a 5 ml/kg suspension on 5 consecutive days. A positive control group of 5 males received a single 0.5 mg/kg intraperitoneal injection on day 4.

2-4 hours after receiving the last gavage, rats were injected IP with colchicine at 1 mg/kg, with sacrifice 2-4 hrs later by CO_2 asphyxiation. Immediately after sacrifice bone marrow was aspirated from the femur into a syringe containing Hank's balanced salt solution (HBSS). The cell suspension was transferred to a capped centrifuge tube containing 5 ml cold HBSS, and maintained in an ice bath until samples had been collected from all rats. After centrifugation, then resuspension in 0.075 M KCl at 37° C. and incubation at 37° C for 10 minutes, cells were centrifuged, then were resuspended and fixed in 3:1 v/v methanol:acetic acid. Cells were recentrifuged twice, and resuspended twice in fresh fixative. At least 3 slides were prepared from each rat using the final suspensions. Slides were allowed to air-dry, stained with 4% Giemsa, and permanently mounted.

Stained slides were coded and scored blind. Where possible, a minimum of 50 metaphases (no information as to number of slides) from each rat was examined and scored for chromatid and chromosome breaks, fragments, structural rearrangements and ploidy. The proportion of mitotic cells in a sample of 500 was determined for each rat.

Results:

At 30 mg/kg/day of FMC 54800 there was a decrease in mean body weight, all rats exhibited tremors, hyperactivity and irritability, and 2/7 died. No symptoms were observed in lower dose animals.

There was no noticeable change in ploidy, nor was there an effect on mitotic indices as a result of treatment. There was no indication of an increase in number of aberrations or evidence of any dose-related trend. A summarization of the data in tables 2 (p. 9) and 3 (p. 10) is given below:

	GROUP TOTALS							
				BREAKS				
	# Rats	cells scored	Gaps	Chromatid	Chromosome	Fragments	Exchange Rearrangements	Cells with 10+ aberrations
Corn Oil (5 ml/kg/day)	5	250	0	1	0	0	0	0
FMC 54800 (3 mg/kg/day)	5	250	0	0	0	1	0	0
FMC 54800 (10 mg/kg/day)	5	250	0	0	0	2	0	0
FMC 54800 (30 mg/kg/day)	5	250	0	0	0	1	0	0
TEM (0.5 mg/kg)	5	250	3	21	1	28	5	6

Approximately 11% of all cells in the TEM-treated group contained one or more aberrations.

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Discussion:

The study gives no indication of an increase in chromosomal rearrangements or other cytogenetic abnormalities as a result of exposure of male rats to FMC 54800 technical at levels up to and including 30 mg/kg/day over a 5-day period.

While it would have been somewhat more reassuring to have data from females as well as males, other studies have shown that the female oral LD_{50} is reasonably close to that of males (53.8 to 70.1 mg/kg), and the symptoms (primarily tremors) are identical in both sexes, strongly suggesting that effects and/or metabolism of FMC 54800 are essentially the same in males and females.

Additionally, while no evidence is presented that the target cells were actually exposed to FMC 54800 (since all members of the high dose group exhibited symptoms it is known that some unknown proportion of FMC 54800 was absorbed from the GI tract in at least the rats of this group, but this does not mean it was transported to the bone marrow of the femur), the study meets the criteria for acceptance under current OECD Guidelines.