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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: METIRAM - Review of Mutagenicity Studies Submitted (August 21, 1985) Under Accession No. 259123, in Response to Notice of Intent to Suspend (July 24, 1985).

NRDC Reassessment
Caswell No. 41A
TB Project: 7-0100

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C) 11/24/86

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THRU: Jane E. Harris, Ph.D., Head
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Hazard Evaluation Division (TS-769C) J.W. Hauswirth for JEH 11/6/86

and

Judy Hauswirth, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C) J.W. Hauswirth 11/6/86 11/21/86

Registrant: FMC Corporation
Philadelphia, PA

Action Requested (870):

Review and evaluate the following mutagenicity studies submitted under cover letter (FMC to Werdig, August 21, 1985) in response to Notice of Intent to Suspend (July 24, 1985),

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in order to comply with the Data Call-In (DCI) Notice of January 17, 1983:

1. Evaluation of Metiram in the C3H-10T 1/2 cell system for transformation and promotion activities, BASF RZ-Report No. 85/209. (Performed by Arthur D. Little, Inc., ADL Reference 54045 (1-5527), Final Report dated June 18, 1985.)

2. Mouse host-mediated assay of Metiram Tech. K38/33A, BASF RZ-Report No. 85/210. (Performed by Litton Bionetics, Inc., LBI Project No. 20988, Final Report dated June 1985.)

3. Mutagenicity evaluation of Metiram technical K 38/33A in an in vitro sister chromatid exchange assay in Chinese hamster ovary (CHO) cells, BASF RZ-Report No. 85/076. (Performed by Litton Bionetics, Inc., LBI Project No. 20990, Final Report dated March 1985.)

4. Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance Metiram (tech. purity) (Substance No. 84/28), BASF RZ-Report No. 85/238. (Conducted at BASF, Final Report dated July 31, 1985.)

Background:

The DCI Notice of January 17, 1983, specified that the following types of mutagenicity data for metiram were required (as identified in the Decision Document for the EBDC's Special Review, dated October 14, 1982, concluding the RPAR - see attached memorandum: Mauer to Werdig, October 19, 1983):

1. Assays for bacterial gene mutation in vitro as well as under host-mediated conditions (a, b in the attached), and for gene mutation in mammalian cells (c).

2. Cytogenetic assays for both gross chromosome aberrations, and for sister chromatid exchange (d).

3. An assay for DNA repair, specifically for mammalian unscheduled DNA synthesis (UDS) in primary hepatocyte cultures (e).

4. An assay for in vitro mammalian cell transformation as well as enhancement (promotion).

Adequate testing (ACCEPTABLE ASSAYS) to satisfy data requirements for bacterial mutation and for DNA damage/repair is on file (Reference #2 in the attached, which reported

negative results for reversion in bacteria and for recombination in yeast, but positive inhibition of UDS in mammalian cells). Except for gross chromosome aberrations (clastogenesis), the data in the current submission (Accession No. 259123) satisfies the remaining requirements specified in the DCI.

TB Conclusions/Recommendations:

The following summarizes our review and evaluation of the data submitted under Accession No. 259123 (see attached TB DATA REVIEWS):

	Study Type	Reported Results	TB Evaluation
1.	Transformation/ Promotion	Negative/ Weak Positive	Acceptable/ Inconclusive*
2.	Host-mediated	Negative	Acceptable
3.	*In vitro* SCE in CHO cells	Positive without S9 and with mouse S9 (negative with rat S9)	Acceptable
4.	Gene mutation (HGPRT) in CHO cells	Positive without S9, marginal positive with rat S9	Inconclusive*

*Repeat assays recommended to confirm presumptive positive results.

TB recommends further:

1. Since the SCE *in vitro* assay is a more sensitive test for chromosomal effects and the study submitted indicates metiram is definitively positive, submission of data for gross chromosomal aberrations (clastogenesis) will not be required. Metiram will be considered to be positive for chromosomal damage (Category II of FIFRA requirements). The registrant may wish to conduct a chromosome test in an appropriate animal species to assay the potential for metiram to induce chromosomal aberrations (and/or SCE) *in vivo*.

2. As indicated above, the marginal positive reported for mutation at the HGPRT locus in CHO cells should be confirmed (or refuted) in a repeat assay using a mammalian metabolic activation system derived from B6C3F1 mice (mouse S9, as indicated in the attached DER), i.e., under the same conditions of activation that provided a definitive positive SCE assay.

3. Also as indicated above and in the attached DER, metiram probably has no capacity to transform mammalian cells directly (i.e., is not an "initiating" agent). On the other hand, the assay submitted for epigenetic potential (i.e., as a promoter), although presumptively positive, is considered inadequate based on procedural and reporting deficiencies, and should be repeated.

INFORMATION ON PRODUCT IMPURITIES NOT INCLUDED

TOXICOLOGY BRANCH DATA REVIEW

Chemical: Metiram (Polyram®)

Caswell No. 41A
EPA Chem. No. 014601

Study Type: Transformation in vitro

Citation: Evaluation of Metiram in the C3H-10T 1/2 Cell System for Transformation and Promotion Activities

Accession No.: 259123

MRID No.: (TB:7-0100)

Sponsor: FMC, Philadelphia, PA (BASF, Ludwigshafen, West Germany)

Testing Lab.: A.D. Little, Inc., Cambridge, MA

Study No.: ADL #54045 (1-5527)/BASF #RZ No. 85/209

Date: June 18, 1985

Test Material: Metiram technical (Lot #CH-K-38/33A), a white powder (% purity not stated, but [redacted], suspended in water for testing.

Procedures:

A. Transformation Assay

Following preliminary cytotoxicity assays to select dosages (determined by reduction in cloning efficiency), cultures of C3H-10T1/2 cells (Clone 8, originally obtained from Dr. Charles Heidelberger, UCAL C.C.C.) were exposed for 24 hours to five concentrations of test material (0.10, 0.25, 0.50, 0.75, and 1.0 ug/mL), then incubated in fresh medium for approximately 6 weeks, at which time they were prepared for scoring transformed foci (Types II and III, according to standardized published criteria). Untreated solvent (water) and positive (MCA, 3-methylcholanthrene) controls were run concurrently.

B. Promotion Assay

Cultures were first exposed for 24 hours to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, an initiating agent), washed and incubated in fresh medium for 5 days, then treated continuously for 5 weeks with the highest nontoxic concentrations of test material derived from the cell transformation assay (A), or of the known promoter, 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Other cultures were treated with MCA alone.

Results:

A. Transformation

Metiram was cytotoxic to C3H-10T1/2 cells at concentrations of 1 ug/mL and above (little to no clonal growth). At no test level, however, was there an increase above control (0.05 foci/plate) in transformation to either Type II or III (or combined) (range, 0.05 to 0.10 foci/plate), in contrast to the expected response to 3-MCA (0.29 to 0.38 foci/plate).

B. Promotion

Based upon the dosage selection screens for the transformation assay as well as a concurrent cytotoxicity assay, 0.3 ug/mL metiram was selected as the single test dose for the promotion assay. This level of metiram enhanced the transformation to Type II (0.23 foci/plate) but not Type III (0.0) over that induced by the initiator (MNNG) alone (0.06). The combination of MNNG plus TPA caused the expected strong enhanced response (0.78 foci/plate). MCA alone caused both Type II (0.50 foci/plate) and Type III (0.50) transformation.

Study Conclusions:

The authors conclude that "Metiram did not induce a direct transformation response in (the) C3H-10T1/2 cells under assay conditions which produced a dose-dependent cytotoxic effect," but exhibited "weak promotion activity" (increase in Type II foci only) in cells initiated with MNNG.

TB Evaluation/Core:

The transformation assay is ACCEPTABLE in demonstrating metiram appears to have no capacity to transform C3H-10T1/2 directly.* The promotion assay, on the other hand, is considered inconclusive (although presumptively positive) since:

1. Only single doses of the agents were employed.

2. Response to the initiator alone (MNNG) was unsatisfactorily minimal (0.06 foci/plate), not different from metiram alone in the transformation assay (0.05 to 0.10 foci/plate); perhaps MCA should have been used as the initiating agent.

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*Adequate mammalian metabolic activation systems have not yet been developed for this assay; further, these cells appear to be capable of activating indirect mutagens and carcinogens, viz. MCA.

We recommend repeating the promotion assay, employing several concentrations of both MNNG and MCA as initiators (from no effect on transformation when used alone to highest effective dose) as well as of the test agent (into the toxic range), in order to provide a comprehensive test.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Date: 10-24-86

TOXICOLOGY BRANCH DATA REVIEW

Chemical: Metiram (Polyram®) Caswell No. 41A
EPA Chem. No. 014601

Study Type: Mutagenicity - Gene Mutation in Bacterial Host-mediated Assay (Mouse/S. typhimurium TA 1530)

Citation: Mouse Host-mediated Assay of Metiram Tech K38/33A

Accession No.: 259123

MRID No.: (TB:7-0100)

Sponsor: FMC, Philadelphia, PA (BASF, Ludwigshafen, West Germany)

Testing Lab.: Litton Bionetics, Kensington, MD

Study No.: LBI #20988 (Genetics Assay No. 7419)/BASF #RZ-Report 85/210

Date: June 1985

Test Material: Metiram technical (Lot #CH-K-38/33A), a yellow powder (% purity not stated, but [redacted], suspended in CMC for oral administration.

Procedures:

Groups of 10 male CD-1 mice were orally gavaged once with test material at doses of 0 (vehicle control), 500, 1670, and 5000 mg/kg, and 30 minutes later injected i.p. with 0.5 mL Salmonella typhimurium strain TA-1530 (ca 2 x 10^9 cells). Three hours later, the animals were killed, the peritoneal cavity drained by syringe, and the recovered bacterial cells incubated for 2 days on minimal medium to score revertents, and on nutrient medium for enumerating survivors. A positive control (dimethylnitrosamine, injected i.m. at 0.05 mL per mouse) was run concurrently.

Results:

No clinical toxicity was apparent at any test dose, and all test revertent frequencies (per 10^8 survivors) were comparable to the vehicle (CMC) control value (41.39), in contrast to the positive control which exhibited a 3.8-fold increase (82.57) over its vehicle (saline) control (21.68).

INFORMATION ON PRODUCT IMPURITY IS NOT INCLUDED

Study Conclusions:

Metiram technical was not mutagenic (to indicator TA 1530 cells) under host-mediated conditions using mice treated acutely up to 5000 mg/kg (limit dose).

TB Evaluation/Core:

ACCEPTABLE and negative.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Date: 10-24-86

INFORMATION ON PRODUCT IMPURITY NOT INCLUDED

TOXICOLOGY BRANCH DATA REVIEW

Chemical: Metiram (Polyram®) Caswell No. 41A
EPA Chem No. 014601

Study Type: Mutagenicity--Sister Chromatid Exchange (CA) In Vitro (CHO/SCE)

Citation: Mutagenicity Evaluation of Metiram Technical K38/33A (84/28) In an in vitro Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells

Accession No.: 259123

MRID No.: TB:7-0100

Sponsor: FMC, Philadelphia, PA (BASF Ludwigshafen, West Germany)

Testing Lab.: Litton Bionetics, Kensington, MD

Study No.: LBI Project No. 20990 (Genetics Assay No. 7419)/BASF No. RZ-Report 85/076

Date: March 1985

Test Material: Metiram technical (Lot #CH-K-38/33A), a yellow powder (% purity not stated, but [redacted] suspended in aqueous culture medium.

Procedures:

Following a thorough series of toxicity testing to select doses, exponential cultures of Chinese hamster ovary cells (CHO, clone WB1) were exposed for 2 hours to a range of test article concentrations up to cytotoxic levels (100 ug/mL, which was also the limit of solubility) in the absence of metabolic activation, or in the presence of a metabolic activation system (S-9) derived from Arochlor 1254-induced Fischer 344 rat as well as B6C3F1 mouse livers. Concurrent positive controls were run with every experiment, using mitomycin C (MMC) for nonactivated assays, and cyclophosphamide (CP) in S-9 supplemented tests.

Results:

Metiram caused significant toxicity at 100 ug/mL and above in nonactivated tests and at 200 ug/mL and above in the presence of S-9. Significant dose-related increases in SCE over controls were found in both nonactivated cultures treated at 40, 60, 80, and 100 ug/mL (11%, 19%, 35%, and 42%, respectively), as well as in mouse S-9 activated cultures exposed to levels of 125, 150, 175, and 200 ug/mL (21%, 33%, 26%, and 44%, respectively), but only

marginal (nonsignificant) increases (and a flat dose response) in rat S-9 supplemented cultures (5 to 15% increases).

Study Conclusions:

The authors conclude that metiram is positive ("active") for the induction of ". . . small but significant increases in sister chromatid exchanges in Chinese hamster ovary cells under the treatment conditions of nonactivation and metabolic activation by B6C3F1 mouse S9 . . . [but] . . . no significant increases in sister chromatid exchange were induced under the treatment conditions which included metabolic activation by Fis[c]her 344 rat S9."

TB Evaluation/Core:

ACCEPTABLE and positive for SCE induction both in the absence and presence of mouse (but not rat) activation.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Date: 10-24-86

TOXICOLOGY BRANCH DATA REVIEW

Chemical: Metiram

Caswell No. 41A
EPA Chem. No. 014601

Study Type: Mutagenicity - Gene Mutation in Mammalian Cells *in vitro* (CHO/HGPRT).

Citation: Report on a Point Mutation Test Carried Out on CHO Cells (HGPRT Locus) with the Test Substance Metiram (tech. purity) (Substance No. 84/28)

Accession No.: 259123

MRID No.: TB:7-0100

Sponsor: FMC, Philadelphia, PA

Testing Lab.: BASF Aktiengesellenschaft Department of Toxicology, Ludwigshafen, West Germany

Study No.: (BASF) RZ-Report #85/238

Date: July 31, 1985

Test Material: Metiram Technical (Lot #CH-K-38/33A) (% purity not stated, but [redacted] a light yellow powder, suspended in culture medium for testing.

INFO. ON PRODUCT IMPURITY IS NOT INCLUDED

Procedures:

Following preliminary range-finding toxicity testing, cultures of Chinese hamster ovary cells (K-1 clone, from Flow) were exposed for 4 hours, both in the absence and presence of a metabolic activation system from Arochlor 1254 stimulated Sprague-Dawley rat livers, S-9 mix, to test material (at concentrations of 0.681 to 100 and 0.681 to 10 ug/mL, respectively), rinsed and subcultured for an additional 11 days (expression period), at which time mutants were identified by selection in 6-thioguanine medium. Two experiments were conducted, each concurrent with the positive control substances ethylmethane sulfonate and bromdesoxyuridine (for nonactivated conditions) and 3-methylcholanthrene (for activation assays).

Results:

Both experiments demonstrate dose-related increases in mutant colonies in nonactivated test cultures treated with metiram in the range of 0.0681 to 4.64 ug/mL (at which level cytotoxicity approaches 50%), acknowledged as significant at the two highest dose levels. In the presence of rat S-9, only sporadic, non-dose-related increased mutation rates were found (i.e., values at

least twice the background/concurrent control rates), which the investigators ascribe to solubility problems and/or cytotoxicity not clearly related to these mutant values

Study Conclusions:

The authors concluded that ". . . Metiram may lead to a marginal increase of mutants in the culture . . . [an effect] . . . of borderline significance."

TB Evaluation/Core:

The study is judged inconclusive, since the presumptive positive results bear confirmation in a repeat study. TB recommends that this repeat study be conducted in the presence of mouse S9, under which conditions metiram has demonstrated mutagenic activity (SCE assay in Accession No. 259123).

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Date: 10-24-86

4. RESULTS

4.1. Mutagenicity and cytotoxicity (cloning efficiency)
First study: 04.03.a5

Substance No.: 84/28

Concentration µg/ml	with metabolizing system (S-9 mix)									without metabolizing system (S-9 mix)								
	Mutagenicity: Colonies per fl. (80 cm²) in selection medium					MR**	Cytotoxicity: CE*			Mutagenicity: Colonies per fl. in selection medium					MR**	Cytotoxicity: CE*		
0	0	0	0	0	0	0	180	178	89.5	0	1	0	0	0	0.7	198	190	97.0
0.0681										3	5	1	3	2	9.7	150	154	76.0
0.1										2	2	1	3	1	6.0	154	147	75.3
0.464										0	1	0	0	0	0.7	161	157	79.5
0.681	3	1	2	1	0	4.7	178	170	87.0	6	5	7	6	4	18.7	159	170	82.3
1.0	0	0	0	0	0	0	138	144	70.5	4	4	8	9	11	24.0	162	169	82.8
4.64	0	0	0	0	0	0	160	132	73.0									
6.81	3	4	2	7	4	13.3	162	140	75.5									
10.0	0	1	0	0	0	0.7	143	141	70.8									
Positive contr.: MCA 0.01	61	48	50	56	67	188.0	124	99	55.8									
Positive contr.: EMS 0.3										63	65	70	74	55	218.0	162	153	78.8

* = Cloning efficiency: no. of colonies and % relation to no. of seeded cells (approx. 200)
** = MR = mutation rate: number of mutants per 10^6 cells, not corrected for cytotoxicity

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Second study: 15.05.85

Substance No.: 84/25

Concentration µg/ml	with metabolizing system (S-9 mix)									without metabolizing system (S-9 mix)								
	Mutagenicity: Colonies per fl. (80 cm²) in selection medium					MR**	Cytotoxicity: CE*			Mutagenicity: Colonies per fl. in selection medium					MR**	Cytotoxicity: CE*		
0	0	3	0	1	0	2.7	141	151	73.0	0	0	0	0	0	0.0	169	178	86.75
0.681										0	0	2	1	0	2.0	136	163	74.75
1.0										1	0	2	1	0	2.7	170	139	77.25
4.64										4	5	3	3	5	13.3	98	99	49.25
6.81										1	3	2	1	1	6.0	103	91	48.5
10.0	0	0	0	0	0	0.0	99	14[illegible]	59.75									
46.4	0	0	0	0	0	0.0	90	89	44.75									
68.1	0	0	0	0	0	0.0	84	58	35.5									
100	2	1	2	0	3	5.3	47	39	21.5									
Positive contr.: MCA 0.01	211	188	225	201	191	677.3	113	105	54.5									
Positive contr.: BvdV 0.05										19	21	17	21	26	69.3	63	59	30.5

* = Cloning efficiency: no. of colonies and % relation to no. of seeded cells (approx. 200)

** = MR = mutation rate: number of mutants per 10^6 cells, not corrected for cytotoxicity

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