



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

002348

23 NOV 1982

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Jay Ellenberger
Project Manager, No. 12
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D. *C.F. Chaisson* 11/23
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Methidathion/Alfalfa Risk Assessment, No. 7F1983

Action Requested:

- 1) Ciba-Geigy has submitted for review a risk assessment based on the 2-year mouse oncogenicity study conducted by IBT.
- 2) Ciba-Geigy has also submitted for review 12 mutagenicity studies on methidathion and its metabolites .
- 3) Ciba-Geigy has petitioned for an increase in the tolerance for methidathion on alfalfa from 6 ppm to 12 ppm.

Review and Discussion:

- 1) The risk assessment submitted by Ciba-Geigy is based on a mouse oncogenicity study conducted by IBT. This study has been declared invalid by the Canadian Government in their IBT Validation Program. Therefore, at this time the Toxicology Branch will not use this study as the basis for an oncogenicity risk assessment. If, in the future, a risk assessment using these data is performed, the risk assessment submitted by Ciba-Geigy will be evaluated at that time.
- 2) The 12 mutagenicity studies submitted by Ciba-Geigy in Appendix H of their petition are listed below:

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BibliographyAppendix II: Mutagenicity Reports

1. Salmonella/mammalian - microsome mutagenicity test with GS-13005, 4/17/80. Exp. #79/1556, MRID 00078329.
2. ibid, 10/29/80. Exp. #801488, MRID not assigned.
3. In vitro microbial assays for mutagenicity testing of DMTP (methidathion), 8/31/79. Exp. #NRI-79-2884, MRID 00078330.
4. Intravasanguine host - mediated assay with S. Typhimurium with GS-13005, 10/31/80 Exp. #801494, MRID 00078331.
5. Point mutation assay with mouse lymphoma cells with GS-13005, 10/21/80. Exp. #801495 MRID 00078332.
6. In vitro and in vivo microbiological assays of six Ciba-Geigy Chemicals, 3/77. Exp. # SRI Project LSC -- 5686, MRID 00060642.
7. Nucleus anomaly test in somatic interphase of Chinese Hamster (GS-13005), 7/2/80. Exp. #80-0437, MRID 00078334/6.
8. Sister chromatid exchange study, GS-13005, Chinese Hamster, 11/4/80. Exp. #801489, MRID 00078335.
9. Salmonella/mammalian-microsome mutagenicity test with GS-12956, 10/27/80, Exp. #801377. MRID not assigned.
10. Nucleus anomaly test in somatic interphase nuclei, GS-12956, Chinese hamster, 10/30/80, Exp. #801378. MRID not assigned.
11. Salmonella/mammalian - microsome mutagenicity test with GS-28369, 12/15/80. Exp. #801427. MRID not assigned.
12. Salmonella/mammalian - microsome test with GS-28370, 10/24/80. Exp. #801428. MRID not assigned.

The various studies on the mutagenicity of methidathion and three metabolites are summarized in Tables 1 and 2, respectively (Attachment A).

In addition, detailed reviews of each study are contained in Attachment B of this report.

When methidathion was tested in a mammalian cell point mutation test, using L5178Y mouse lymphoma cells in DBA/Bom/SFP mice at 15 mg/kg, no increase in the mutation frequency for resistance to methotrexate, cytosine arabinoside, or thymidine was reported (Strasser 1980, MRID 00078332, report #5).

Methidathion was also tested for mutagenicity in several point mutation systems employing microbial cells. A report by Simmon 1977 (MRID 00060642, report #6) showed marginally detectable activity with several strains of S. typhimurium. The responses were scattered, were not dose related and occurred only in the presence of metabolic activation. These results were not substantiated in studies reported by Arni 1980 (MRID 00078329, report #1; and report #2, MRID not assigned) or Satou 1979 (MRID 00078330, report #3) who tested methidathion against the same strains of S. typhimurium at comparable doses in the presence or absence of metabolic activation. Methidathion was not mutagenic in host-mediated assays, in mice, employing S. typhimurium strains TA 1535 and TA 1538 (Simon 1977, MRID 00060642, report #6) but induced a slight but less than the required 2-fold increase in the mutation rate of strain TA 100 (Arni 1980, MRID 00078331, report #4). However, there was no evidence of mutagenicity in the standard plate test with strain TA 100 when methidathion was tested in the presence or absence of metabolic activation provided by rat liver S9 (Arni 1980; MRID 00078329, report #1; and Satou 1979, MRID 00078330, report #3).

In addition, there was no evidence of mutagenicity when methidathion was tested in the rec assay employing B. subtilis, strains H17 and M45, or when tested against E. coli, strain B/r WP2 at 10-5000 ug/plate (Satou 1979, MRID 00078330, report #3).

Methidathion was tested for the capacity to induce sister chromatid exchanges in bone marrow cells of Chinese hamsters (Hool 1980, MRID 00078335, report #8). A marginal response was observed at only one of the three dosage levels tested. It was statistically significant at the 1% level but was considered not to be biologically relevant in the absence of a clear dose response.

No increase in the percentage of bone marrow cells with nuclear anomalies was reported following the treatment of Chinese hamsters with methidathion (Hool 1980, MRID 00078334/6, report #7).

Methidathion metabolite GS 12956 showed no evidence of mutagenicity when tested in the presence or absence of metabolic activation in a point mutation assay employing *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 (Arni 1980, Exp. # 801377, report #9), or in a nucleus anomaly test employing Chinese hamster bone marrow cells (Hool 1980, Exp. #801378, report #10). There was no evidence of mutagenicity when metabolite GS 28369 was tested against *S. typhimurium* strains TA 98, TA 100 and TA 1535 (Arni 1980, Exp. # 801427, report #11) or when metabolite GS 28370 was tested against the same strains, as well as TA 1537, in the presence or absence of metabolic activation (Arni 1980, Exp. # 801428, report #12).

Based upon the above information, neither methidathion, nor its metabolites GS 12956, GS 28369 and GS 28370, are considered to be mutagenic. Where marginal detectable activity was observed the biological relevance of the results was not clearly demonstrated. Further testing is not required at this time. However, the agency requests that Ciba-Geigy submit the original data on nucleus anomaly tests of methidathion (Hool 1980, MRID 00078334; report #7) and metabolite GS 12956 (Hool 1980, Exp. #801378, report #10) so that an in-depth analysis of these studies may be done. Pending the results of these evaluations, additional testing may be required.

3) Ciba-Geigy has requested an increase in the alfalfa tolerance for methidathion from 6 ppm to 12 ppm. Several issues are pertinent to this request:

- a) RCB has concluded that a tolerance of 12 ppm for methidathion on alfalfa will result in significant combined residues of methidathion, its oxygen analogue and methidathion sulfoxide and sulfone in meat (0.15 ppm), milk (0.10 ppm), poultry (0.15 ppm) and eggs (0.05 ppm) (R.B. Perfetti to J. Ellenberger, April 14, 1982; Attachment C). Therefore, the Toxicology Branch concurs with RCB in their conclusion that the alfalfa tolerance request be proposed in terms of the combined residues of the parent methidathion and metabolites in the commodities mentioned above.
- b) The level of the combined residues of methidathion and its metabolites cited in (a) above have been used to calculate the % ADI. These levels in meat, milk, poultry and eggs alone represent 126.93% of the current ADI. Therefore, the Toxicology Branch would not recommend a 12 ppm tolerance for alfalfa until the combined residues of methidathion and its metabolites are lowered substantially.

Conclusions

1) The Ciba-Geigy risk assessment based on an invalid IBT oncogenicity study will not be evaluated at this time nor used as a basis for a regulatory decision.

2) Based on the 12 mutagenicity studies submitted by Ciba-Geigy, neither methidathion, nor its metabolites GS-12956, GS-28369 and GS-28370 are considered mutagenic. However, the agency requests that Ciba-Geigy submit the original data on the nucleus anomaly tests of methidathion (Hool, 1980, MRID 00078324, Report #7) and metabolite GS-12956 (Hool, 1980, Report #10) so that an in-dept analysis of these studies may be done

In addition, the registrant is advised to submit any additional information or discussion which supports the rationale for selecting the test systems employed to test the capacity of methidathion and its metabolites to cause gene or chromosomal mutations. Guidance for this is available from the Toxicology Branch upon request.

3) Ciba-Geigy should submit a request for a tolerance for combined residues of methidathion, its oxygen analogue and methidathion sulfone and sulfoxide in meat, milk, poultry and eggs as per the RCB request (R.B.Perfetti to J. Ellenberger, April 14, 1982; Attachment C). The levels of these combined residues resulting from a 12 ppm tolerance of methidathion on alfalfa must be submitted to RCB for review before the Toxicology Branch can evaluate the requested increase in the current tolerance from 6 ppm to 12 ppm.



Chad E. Sandusky, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Attachments

Table 1

Mutagenicity Studies of Methidathion

Report No.	Test System	Test Organism	Dose	Results	Reference/MRID #
No. 1	Point mutation	<u>S. typhimurium</u> ; strains TA 1535, TA 1537, TA 98, and TA 100	25-2025 ug/ plate in pre- sence and ab- sence of rat liver S9	No evidence of mutagenicity	Arni, 1980; 00078329; Exp. #79/1556
No. 2	Point mutation	<u>S. typhimurium</u> ; strains TA 1535, TA 1537, TA 100, and TA 98	25-2025 ug/ plate in pre- sence and ab- sence of rat liver S9	No evidence of mutagenicity	Arni, 1980; Exp. # 801488; MRID Not Assigned
No. 3	Point mutation	<u>S. typhimurium</u> ; strains TA 1535, TA 1538, TA 98, TA 100, and 1538	50-5000 ug/ plate, in pre- sence and ab- sence of rat liver S9	No evidence of mutagenicity	Satou, 1979; C0078330; Exp. NRI-79-2884
No. 4	Point mutation; Host mediated	<u>S. typhimurium</u> ; strains TA 1537, TA 98, TA 100, in albino mice	5-20 mg/kg	Slight but less than 2-fold in- crease in muta- tion rate with TA 100 only	Arni, 1980; 00078331; Exp. #801494
No. 5	Point mutation; Resistance to toxicity of methotrexate, thymidine, or cytosine arbinoside	Mouse lymphoma cells (L5178Y) and DBA/2om/SPF mice	15 mg/kg only. This was 1/9 LD50.	No increase in mutation frequency for resistance to arabinoside, or thymidine	Strasser, 1980; 00078332; Exp. #801495

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Attachment A

Cont.

No. 6	Point mutation, Host-mediated	<u>S. typhimurium</u> ; strains TA 1535, TA 1538 in male Swiss Webster mice	1X at 10-20 mg/kg; 5X at 5-20 mg/kg	No evidence of mutagenicity	Simmon, 1977; 00060642; Exp. #SRI Project LSC-5686
No. 7	Nucleus Anomaly	Chinese hamster bone marrow cells	17-68 mg/kg	No increase in percentage of nucleus anomalies	Hool, 1980; 00078334/6; Exp. #80-0437.
No. 8	Sister chromatid exchange	Chinese hamster bone marrow cells	17-68 mg/kg	Marginal response at one dose only (34 mg/kg); statisti- cally significant at 1% level. Biological relevance questionable in absence of a dose response.	Hool, 1980; 00078335; Exp. #801489

Table 2

Mutagenicity Studies of Methidathion (Metabolites)

Report No.	Test System	Test Organism	Metabolite	Dose Range	Results	Reference
No. 9	Point mutation	<u>S. typhimurium</u> strains TA 98, TA 100, TA 1535, and TA 1537	GS-12956	10-810 ug/ 0.1 ml, with and without rat liver S9	No evidence of mutagenicity	Arni, 1980; Exp. #801377
No. 10	Nucleus anomaly	Chinese hamster bone marrow cells	GS-12956	121-484 mg/kg	No evidence of mutagenicity	Hool, 1980; Exp. #801378
No. 11	Point mutation	<u>S. typhimurium</u> strains TA 98, TA 100, TA 1535	GS-28369	15-960 ug/0.1 ml, with and without rat liver S9	No evidence of mutagenicity	Arni, 1980; Exp. #801427
No. 12	Point mutation	<u>S. typhimurium</u> strains TA 98, TA 100, TA 1535, TA 1537	GS-28370	25-2025 ug/0.1 ml, with and without rat liver S9	No evidence of mutagenicity	Arni, 1980; Exp. #801428

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ATTACHMENT B

12 Data Evaluation Records on Mutagenicity*

Ciba-Geigy, Petition 7F1983

Appendix H, Reports 1-12

*Note: Although these DER's are stamped DRAFT, they are officially FINAL and have all received secondary review and approval.

REPORT #1

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MRID: 00078329

1. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Active Ingredient, purity unspecified

3. Citation or Citations:

Arni, P. (1980) Salmonella/Mammalian-microsome Mutagenicity Test with GS 13005. (Test for Mutagenic Properties in Bacteria): No. of Experiment 79/1556. (Unpublished study received Aug 13, 1981 under 7F1983; prepared by Ciba-Geigy, Ltd., Switzerland, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:070213-D)

4. Reviewed by:

Roman J. Pienta
Systems Scientist
The MITRE Corporation
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Signature: *Roman J. Pienta*

Date:

5. Approved by:

Signature: *Charles J. Fenollosa*

Date: *November 18, 1982*

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 through 4.

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7. Conclusions:

Methidathion was tested for mutagenicity on histidine-auxotrophic mutants of Salmonella typhimurium strains TA 1537 and TA 98 which detect frame-shift mutations and strains TA 1535 and TA 100 which detect base-pair substitution mutations. The material was tested with and without microsomal metabolic activation at 25, 75, 225, 675, and 2025 µg/0.1 ml. While the controls showed clear dose-related mutagenic activity, no mutagenic activity was observed with methidathion at any dose tested (see results in Table 1).

CORE CLASSIFICATION: Not applicable. Guidelines for minimum requirements for mutagenicity tests are not available.

8. Materials and Methods:

The test material was designated as GS 13005, batch mg 32289/4239 and was not further characterized.

The bacteria on which the test was performed were the histidine-auxotrophic strains of Salmonella typhimurium which detect point mutations by reversion to histidine-prototrophic mutants. Strains TA 1535 and TA 100 detect base-pair substitution mutations and strains TA 1537 and TA 98 detect frame-shift mutations. Tests were carried out according to the procedure of Ames, et al., (Proc. Natl. Acad. Sci. USA 70:782-786, 1973; 70:2281-2285, 1975; and Mut. Res. 31:347-364, 1975).

The test was performed with and without microsomal activation (S9), at 25, 75, 225, 675, and 2025 µg methidathion per 0.1 ml. The test

TABLE 1
IN VITRO ASSAY OF METHIDATHION WITH SALMONELLA TYPHYMURIUM

Test Substance	Dose (µg/0.1 ml)	Histidine-Positive Revertants per Plate on Strain							
		TA 98		TA 100		TA 1535		TA 1537	
		-a	+	-	+	-	+	-	+
GS 13007	Control	16 ^b	27	168	157	11	12	5	4
	25	20	28	150	153	13	11	8	5
	75	20	27	168	142	14	10	4	5
	225	21	27	155	151	12	9	2	8
	625	14	24	168	170	12	12	3	6
	2025	17	23	158	171	3	5	1	4
<u>Positive Controls</u>									
Daunorubicin.HCl	Control	12							
	5	128							
	10	276							
4-Nitroquinoline-N-Oxide	Control			168					
	0.125			868					
	0.25			>1300					
N-Methyl-N'-nitro-N-nitroso-guanidine	Control					10			
	3					1433			
	5					>2150			
9(5)Aminoacridine.HCl	Control							6	
	50							177	
	100							>700	
Cyclophosphamide	Control						10		
	250						391		

^a - Indicates test was performed without microsomal activation; + indicates test was performed with microsomal activation.

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material was dissolved in DMSO. DMSO alone was used for negative controls. The metabolic activation mixture contained 0.3 ml S9 fraction of liver from rats induced with Aroclor 1254 and 0.7 ml of a solution of necessary co-factors.

Positive control experiments were run simultaneously with the following compounds: 1) for strain TA 98: daunorubicin-HCl; 2) for strain TA 100: 4-nitroquinoline-N-oxide; 3) for strain TA 1535: N-methyl-N'-nitro-N-nitrosoguanidine; and 4) for strain TA 1537: 9(5) aminoacridine hydrochloride monohydrate. The efficiency of the activation mixture was tested with strain TA 1535 and cyclophosphamide.

Three petri dishes were used per strain and per concentration of test material or control group. The plates were incubated for 48 hours at 37°C in darkness.

After counting the colonies, the arithmetic mean was calculated. A test substance was considered to be non-mutagenic if the count of revertant colonies was not doubled that of the negative control at any concentration.

In the absence of any two-fold increase in numbers of revertant colonies, statistical analyses were not done.

9. Results and Discussion:

In tests performed with and without metabolic activation by rat liver S9 microsomal enzyme preparations, methidathion did not cause an increase in the incidence of histidine-prototrophic mutants in comparison with the controls (Table 1).

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At the highest concentration tested (2025 $\mu\text{g}/0.1\text{ ml}$) methidathion precipitated in soft agar. At this dose level in the test without microsomal activation for strains TA 1535 and TA 1537, the number of colonies of histidine-prototrophic mutants was lower than that found in controls. This indicates an inhibitory effect of the compound on the growth of the bacteria.

10. Technical Review Time: 5.75 hours.

Report #2

1. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

GS-13005 Technical

3. Citation or Citations:

Arni, P. (1980) Salmonella/Mammalian-microsome Mutagenicity Test with GS 13005. (Test for Mutagenic Properties in Bacteria): No. of Experiment 801488. (Unpublished study received Aug. 13, 1981 under 7F1983; prepared by Ciba-Geigy, Ltd., Switzerland, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL: 070213-D)

4. Reviewed by:

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Toxicology Branch
Hazard Evaluation Division (TS-769)

Signature: *Chad B. Sandusky*

Date: 11/23/82

5. Approved by:

Christine F. Chaisson, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Signature: *C.F. Chaisson*

Date: 11/23/82

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 through 4.

7. Conclusions:

Methidathion was tested for mutagenicity on histidine-auxotrophic mutants of Salmonella typhimurium strains TA 1537 and TA 98, which detect frame-shift mutations, and strains TA 1535 and TA 100, which detect base-pair substitution mutations. The material was tested with and without microsomal metabolic activation at 25, 75, 225, 675, and 2025 ug/0.1 ml. While the positive controls showed clear dose-related mutagenic activity, no mutagenic activity was observed with methidathion at any dose tested (see results in Tables 1 and 2).

CORE CLASSIFICATION: Not applicable. Guidelines for minimum requirements for mutagenicity tests are not available.

8. Materials and Methods

The test material was designated as GS 13005, batch No. OP. 25-572 and was not further characterized.

The bacteria on which the test was performed were the histidine-auxotrophic strains of Salmonella typhimurium which detect point mutations by reversion to histidine-prototrophic mutants. Strains TA 1535 and TA 100 detect base-pair substitution mutations and strains TA 1537 and TA 98 detect frame-shift mutations. Tests were carried out according to the procedure of Ames, et. al., (Proc. Natl. Acad. Sci. USA 70:782-786, 1973; 70:2281-2285, 1973; and Mut. Res. 31:347-364, 1975).

The test was performed with and without microsomal activation (S9), at 25, 75, 225, 675, and 2025 ug methidathion per 0.1 ml. The test material was dissolved in DMSO. DMSO alone was used for negative controls. The metabolic activation mixture contained 0.3 ml S9 fraction of liver from rats induced with Aroclor 1254 and 0.7 ml of a solution of necessary co-factors.

Positive control experiments were run simultaneously with the following compounds: 1) for strain TA 98: daunorubicin-HCl; 2) for strain TA 100: 4-nitroquinoline-N-oxide; 3) for strain TA 1535: N-methyl-N-nitro-N-nitrosoguanidine; and 4) for strain TA 1537: 9 (5) aminoacridine hydrochloride monohydrate. The efficiency of activation mixture was tested with strain TA 1535 and cyclophosphamide.

Three petri dishes were used per strain and per concentration of test material or control group. The plates were incubated for 48 hours at 37°C in darkness.

After counting the colonies, the arithmetic mean was calculated. A test substance was considered to be non-mutagenic if the count of revertant colonies was not double that of the negative controls at any concentration.

In the absence of any two-fold increase in numbers of revertant colonies, statistical analyses were not done.

9. Results and Discussion:

In tests performed with (Table 2) and without (Table 1) metabolic activation by rat liver S9 microsomal enzyme preparations, methidathion did not cause an increase in the incidence of histidine-prototrophic mutants in comparison with the controls.

At the 2 highest dose levels in the test without microsomal activation for strains TA 98, the number of colonies of histidine-prototrophic mutants was lower than that found in controls. This indicates an inhibitory effect of the compound on the growth of the bacteria.

10. Technical Review Time 4.00 hours.

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Table 1

Salmonella/Mammalian-Microsome Mutagenicity Test
Experiments without microsomal activation
Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

		<u>Strain of S. typhimurium used</u>			
		TA 98	TA 100	TA 1535	TA 1537
		3.10.80	12.9.80	12.9.80	12.9.80
<u>Test substance</u>					
GS 13 005	Control	21	125	7	6
	25 µg/O.1 ml	22	120	10	8
	75 µg/O.1 ml	30	131	7	10
	225 µg/O.1 ml	24	127	11	8
	675 µg/O.1 ml	17	138	7	8
	2025 µg/O.1 ml	12	131	12	4
<u>Positive controls</u>					
Daunorubicin-HCl	Control	21			
	5 µg/O.1 ml	325			
	10 µg/O.1 ml	446			
4-Nitroquinoline-N-oxide					
	Control		157		
	0.125 µg/O.1 ml		710		
	0.25 µg/O.1 ml		~1210		
N-Methyl-N'-nitro-N-nitrosoguanidine					
	Control			11	
	3 µg/O.1 ml			~1380	
	5 µg/O.1 ml			~2050	
9(5)Aminoacridine hydrochloride					
	Control				8
	50 µg/O.1 ml				19
	100 µg/O.1 ml				53

Table 2

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Salmonella/Mammalian-Microsome Mutagenicity Test
Experiments with microsomal activation
Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

		<u>Strain of S. typhimurium used</u>			
		TA 98 3.10.80	TA 100 12.9.80	TA 1535 12.9.80	TA 1537 12.9.80
<u>Test substance</u>					
GS 13 005	Control	46	128	11	7
	25 µg/O.1 ml	45	131	10	6
	75 µg/O.1 ml	44	113	10	9
	225 µg/O.1 ml	34	99	10	10
	675 µg/O.1 ml	44	117	12	7
	2025 µg/O.1 ml	35	116	15	4

Positive control of the
microsomal activation

Cyclophosphamide

12. 9.80	Control	15
	250 µg/O.1 ml	374
3.10.80	Control	13
	250 µg/O.1 ml	393

REPORT #3

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MRID: 00078330

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1. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Active Ingredient, 99.95% pure

3. Citation or Citations:

Satou, S.; Kimura, Y.; Yamamoto, K.; et al. (1980?) In Vitro Microbial Assays for Mutagenicity Testing of DMTP (Methidathion): Project No. NRI-79-2884. Final rept. (Translation; unpublished study, including letter dated May 29, 1980 from G. Voss and K. Kikawa to G. Dupuis, received Aug 13, 1981 under 7F1983; prepared by Nomura Research Institute, Japan, submitted by Ciba-Giegy Corp., Greensboro, N.C.; CDL:070213-F)

4. Reviewed by:

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System Scientist
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Signature:

Date:

Roman J. Pienta
June 3, 1982

5. Approved by:

Signature:

Date:

Charles B. Jenclo
July 4, 1982

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 through 4.

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7. Conclusions:

Methidathion was not mutagenic when tested with several in vitro microbiological systems which included positive controls and metabolic activation. These included the rec-assay using Bacillus subtilis H17 and M45 (Table 1), and reverse mutation with both Escherichia coli B/r WP2 Try⁻ Hcr⁻ (Table 2) and Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 (Tables 3 and 4) in the presence and absence of a rat liver-homogenate metabolic activation enzyme system (S-9).

CORE CLASSIFICATION: Not applicable. Guidelines for minimum requirements for mutagenicity testing are not available.

8. Materials and Methods:

The test material, designated as DMTP in the study, was reported to be 99.95% pure, and was a colorless crystalline solid which was stable for at least 12 months at 0°C. The material was supplied by Ciba-Giegy (Japan) Ltd.

Several in vitro test systems were used, namely 1) rec-assay in Bacillus subtilis H17 and M45; 2) reverse mutation with Escherichia coli B/r WP2 Try⁻Hcr⁻ in the presence and absence of a rat liver-homogenate metabolic activation system (S-9); and 3) reverse mutation with strains TA 1535, TA 100, TA 1537, TA 1538, and TA 98 of Salmonella typhimurium in the presence and absence of S-9.

Rec-assay

The ability of methidathion to induce DNA damage was tested with two strains of B. subtilis; H17 which is capable of repairing DNA damage, and

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MRID: 00078330

TABLE 1

ASSAY OF METHIDATHION IN THE REC ASSAY WITH B. SUBTILIS H17 AND M45

Test Compound	Concentrations	µg/well	Inhibitory Zone (mm)		Difference (mm)	Muta-genicity
			H17	M45		
Methidathion						
	5 mg/ml	250	0	0	0	-
			0	0	0	-
		500	0	0	0	-
			0	0	0	-
25		1250	0	0	0	-
			0	0	0	-
		2500	0	0	0	-
			0	0	0	-
1000		5000	0	0	0	-
			0	0	0	-
		10,000	0	0	0	-
Negative Controls						
DMSO		100	1	0	0	-
				0	0	-
NaOH	7.5N	100	1	20	1	-
				23	1	-
HCl	2N	100	1	19	1	-
				21	0	-
Kanamycin	1 mg/ml	100	1	8	1	-
				9	0	-
Positive Control						
AF-2	0.1/µg/ml	10		9	11.5	+
				11	13	+

TABLE 2

REVERSE MUTATION ASSAY OF METHIDATHION WITH E. COLI WP2 TRY⁻HCR⁻

Test Compound	Concentration (mg/ml)	µg/plate	S-9 Mix	No. of Colonies per plate ^a	Ratio ^d	Muta-genicity
Methidathion	0	0	-	35		
	0.1	10	-	40	1.1	-
	0.5	50	-	45	1.3	-
	1	100	-	37	1.1	-
	5	500	-	40	1.1	-
	10	1000	-	41	1.2	-
	50	5000	-	41	1.2	-
	0	0	+	46	---	-
	0.1	10	+	43	0.9	-
	0.5	50	+	42	0.9	-
	1	100	+	52	1.1	-
	5	500	+	35	0.8	-
	10	1000	+	50	1.1	-
	50	5000	+	51	1.1	-
<u>Positive Controls</u>						
AF-2 ^b	0.0004	0.04	-	70	2.0	+
2AA ^c	0.4	40	-	20	0.6	-
		40	+	553	12.0	+

^aMean number from duplicate plates.^bAF-2 = 2-(2-furyl)-3(5-nitro-2-furyl) acrylamide.^c2AA = 2-aminoanthracene.^dColonies from treated plates divided by colonies from control plates.

TABLE 3

MUTAGENICITY ASSAY OF METHIDATHION WITH SALMONELLA TYPHIMURIUM

Test Compound	Dose $\mu\text{g}/\text{plate}$	S-9 Mix	CPP ^e	TA1535 R ^f	MG	TA100 CPP	R	M	TA1537 CFP	R	M
Methidathion	0	-	43			62			17		
	10	-	63	1.5	+	61	1.0	-	6	0.4	-
	50	-	34	0.8	-	46	0.7	-	12	0.7	-
	100	-	32	0.7	-	55	0.9	-	9	0.5	-
	500	-	36	0.8	-	67	1.1	-	7	0.4	-
	1000	-	30	0.7	-	47	0.8	-	12	0.7	-
	5000	-	11	0.3	-	20	0.3	-	0 ^h	0	-
	0	+	16			69			27		
	10	+	11	0.7	-	68	1.0	-	10	0.4	-
	50	+	17	1.0	-	67	1.0	-	18	0.7	-
	100	+	9	0.6	-	59	0.9	-	29	1.1	-
	500	+	11	0.7	-	68	1.0	-	27	1.0	-
	1000	+	13	0.8	-	62	0.9	-	21	0.8	-
	5000	+	3	0.2	-	41	0.6	-	20	0.7	-
<u>Positive Controls</u>											
-PL ^a	10	-	1962	45.6	+						
2AA ^b	1	-	30	0.7	-						
	1	+	34	2.1	+						
	0.5	-				52	0.8	-			
	0.5	+				245	3.6	+			
	5.0	-							16	0.9	-
	5.0	+							411	15.2	+
AF-2 ^c	0.5	-				387	6.2	+			
9AC ^d	50.0	-							60	3.5	+

^a β -PL = β -propiolactone. In the original document "ENNG" is listed and is obviously an error since β -PL is used as the positive control for TA1535 according to the text.

^b 2AA = 2-aminoanthracene;

^c AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide.

^d 9AC = 9-aminoacridine;

^e CPP = mean number of colonies per plate from duplicate plates;

^f R = ratio of treated over control number of colonies;

M = mutagenicity;

^h no lawn, test material inhibitory;

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MRID: 00078330

TABLE 4

MUTAGENICITY ASSAY OF METHIDATHION WITH SALMONELLA TYPHIMURIUM

Test Compound	Dose μ g/plate	S-9 Mix	CPP ^d	TA1538 R ^e	M ^f	CPP	TA98 R	M
Methidathion	0	-	28			22		
	10	-	7	0.3	-	27	1.2	-
	50	-	21	0.8	-	20	0.9	-
	100	-	20	0.7	-	27	1.2	-
	500	-	20	0.7	-	21	1.0	-
	1000	-	14	0.5	-	18	0.8	-
	5000	-	0	0g	-	0g	0	-
	0	+	29			32		
	10	+	9	0.7	-	26	0.8	-
	50	+	24	0.8	-	38	1.2	-
	100	+	23	0.8	-	40	1.3	-
	500	+	20	0.7	-	35	1.1	-
	1000	+	18	0.6	-	42	1.3	-
	5000	+	0	0h	-	0h	0	-
<u>Positive Controls</u>								
2NF ^a	5	-	443	15.8	+			
AF-2 ^b	0.2	-				52	2.4	+
2AA ^c	0.5	-	27	1.0	-	25	1.1	-
	0.5	+	557	19.2	+	528	18.4	+

^a2NF = 2-Nitrofluorene;

^bAF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide;

^c2AA = 2-Aminoanthracene;

^dCPP = mean numbers of colonies per plate from duplicate plates;

^eR = ratio of treated over control number of colonies;

^fM = mutagenicity;

gno lawn, compound inhibitory;

hweak lawn, compound inhibitory.

M45 which lacks the repair mechanism. A well 1 cm in diameter, was made in the center of each nutrient agar test plate. Overnight cultures of H17 and M45 were streaked radially from the edge of the well. Methidathion dissolved in dimethylsulfoxide (DMSO) was tested at 250 to 10,000 µg/well by applying 50 µl of solution to the well. After overnight incubation at 37°C the length of the inhibitory zone was measured. Hydrochloric acid (2N), sodium hydroxide (7.5N) and Kanamycin were used as negative controls. The compound 2-(2-furyl)-3(5-nitro-2-furyl)acrylamide (AF-2) served as a positive control. A two-fold increase over the controls was considered a positive response. Duplicate plates were used throughout.

Reverse Mutation - Agar Overlay Plate Method Using E. coli B/r WP2
Try⁻Hcr⁻

An overnight culture of the test bacteria was adjusted to contain 3.3×10^9 cells/ml. A mixture of 2.5 ml of molten top agar, 0.1 ml of the bacterial culture, and 0.1 ml of the test compound dissolved in DMSO were mixed in a test tube and poured onto each agar plate of Vogel-Bonner E medium. After incubation for 3 days at 37°C, the number of colonies was counted. The mean number of colonies was determined from duplicate plates for each dose level. Methidathion was tested at 10 to 5000 µg per plate in the presence and absence of rat liver S-9 (see below for preparation and use of S-9 mix). DMSO was used as the negative control. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 2-aminoanthracene (2AA) were used as positive controls in the absence and presence of S-9 mix, respectively.

In vitro Reverse Mutation Assay Using *S. typhimurium*

S. typhimurium tester strains TA 1535, TA 100, TA 1537, TA 1538, and TA 98, which are auxotrophic for histidine and biotin were used. The cultures were grown overnight in nutrient broth. Two and one-half ml of molten top agar containing appropriate minimal amounts of histidine and biotin were mixed with 0.1 ml of bacterial culture and 0.1 ml of test compound dissolved in DMSO. Methidathion was tested at 50 to 5000 µg/plate. The mixture was poured onto plates of Vogel-Bonner E medium. S-9 was incorporated as required. DMSO was used as a negative control; 2AA was used as positive control for all tests using S-9. Positive controls in the absence of S-9 were as follows: β-propiolactone (-pl) for TA 1535, AF-2 for TA 100 and TA 98, 9-aminoacridine (9AC) for TA 1537, and 2-nitrofluorene (2NF) for TA 1538. After incubation for 2 days at 37°C, the mean number of colonies per plate was obtained from duplicate plates.

Preparation of S-9 Mix

Rat liver S-9 homogenate fractions were prepared from Sprague-Dawley rats treated intraperitoneally with polychlorobiphenyl, Tetra (Wako Pure Chemical Industries, Ltd) according to standard procedures (Ames, et al., Proc. Natl. Aca. Sci. USA 70:2281, 1973; Ames et al., Mut. Res. 31:347, 1975). The homogenate was centrifuged at 9000 X G and the supernatant material was stored as S-9 at -80°C. S-9 mix was prepared just before assay experiments by adding NADPH, NADH, ATP, and G-6-P to S-9 according

to the procedure used in Sugimura's laboratory (see Nagao, et al., Mut. Res. 42:335, 1977). For use in the assay 0.5 ml of S-9 mix, 2.5 ml of top agar, 0.1 ml of bacterial culture, and 0.1 ml of test compound solution were mixed in a tube and poured onto each agar plate.

No statistical analyses were performed.

9. Results and Discussion:

Rec-assay - The data summarized in Table 1 show that no inhibitory zones were observed with the M45 or H17 strains of B. subtilis when treated with methidathion at doses from 250-10,000 µg per plate. This indicates that under the conditions of the test the compound does not induce DNA damage.

Reverse mutation with E. coli - The results summarized in Table 2 show that the number of revertant colonies obtained with cultures treated with methidathion at 10-5000 µg/plate in the presence or absence of a rat liver S-9 metabolic activation system were not significantly increased over the untreated controls. A two-fold increase is considered positive. An increase of 1.3-fold was obtained at 50 µg/plate. However, the maximum increase obtained with the highest dose tested (5000 µg/plate) was only 1.2 fold.

The positive control compounds, AF-2 and 2AA, elicited responses of 2.0- and 12.0-fold, respectively. The authors claimed to have used MNNG as another positive control, but the data were not given in the report.

In vitro reverse mutation assay with S. typhimurium - Methidathion was tested at doses of 10-5000 µg/plate with S. typhimurium strains TA

1535, and TA 100, which detect base-pair substitution mutants, and strains TA 1537, TA 1538 and TA 98, which detect frame-shift mutations. All tests were performed in the absence and presence of a rat liver S-9 metabolic activation system. Methidathion was not mutagenic since no 2-fold or more increase in the number of revertants was observed with any of the indicator strains whether tested in the presence or absence of S-9 as shown in Tables 3 and 4. Positive control chemicals gave typical positive responses indicating that the tests were functioning properly.

In Table 3 of the original report, a compound designated as "ENNG" was listed as a positive control although the text refers to β -propiolactone as the control compound for strain TA 1535. This may be a typographical error in translation from Japanese into English.

From the overall observations in this study it is concluded that methidathion is nonmutagenic.

10. Technical Review Time: 8.5 hours.

REPORT #4

Page 1 of 5
MRID: 000783311. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Active Ingredient, purity unspecified

3. Citation or Citations:

Arni, P. (1980A) Intravascular Host-mediated Assay with S. typhimurium with GS 13005: (Test for the Demonstration of Point Mutation in Bacteria in vivo): No. of Experiment 801494. (Unpublished study received Aug 13, 1981 under 7F1983; prepared by Ciba-Geigy, Ltd., Switzerland, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:070213-G)

4. Reviewed by:

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Signature: Roman J. Pienta
Date: June 14, 19825. Approved by:Signature: Charles Indusky
Date: June 14, 19826. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 through 4.

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MRID: 000783317. Conclusion:

Methidathion was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium in an intrasanguine host-mediated system with strains TA 98, TA 100, and TA 1537 in albino mice. When the compound was tested at 5, 10, and 20 mg/kg body weight, no significant increases were observed in the mutation rate or number of revertant mutant colonies with strains TA 98 and TA 1537. With strain TA 100, a slight increase in the mutation rate $8.8/10^7$ cells in controls vs 12.1 and $12.4/10^7$ cells, at 10 and 20 mg/kg), respectively, but not in the number of mutant colonies per plate was observed. However, these observations were not considered to be biologically relevant.

CORE CLASSIFICATION: Not applicable. Guidelines for minimum requirements for mutagenicity tests are not available.

8. Materials and Methods:

Methidathion was tested for mutagenicity on histidine-auxotrophic mutants of S. typhimurium in an intrasanguine host-mediated assay. Male albino mice, of unspecified strain, weighing 22-34 g, were used. They were fed a standard diet with water ad libitum.

The test material was designated as GS 13005, batch number Op. 25-572 and was not further characterized. It was tested at 5, 10, and 20 mg/kg body weight.

The assays were performed according to previously reported procedures (Ames, et al., Mut. Res. 31:347-364, 1975; Arni, et al., Mut. Res. 45:291-307, 1977).

An overnight culture of each indicator bacteria culture was concentrated by centrifugation to obtain a cell density of approximately 10^{11} bacteria/ml.

Groups of 6 mice each were used. After the mice had been fasted for 16 hours, the test compound was administered orally in 0.5% carboxymethylcellulose (CMC). Treatment was repeated at one hour and two hours after the first dosage. One group of animals served as a control and received CMC only. Immediately after the last dose of test substance, 0.3 ml of the bacterial suspension was injected intravenously into appropriate mice.

Sixty minutes after injection of the bacteria, the mice were killed. Their livers were removed and homogenized; the bacteria were recovered by centrifugation of the liver homogenate.

After determination of the total bacterial count and the number of revertant mutants, the mutation rate for each animal was calculated.

The authors verified the significance of any differences observed by the X^2 -test. The level of the significance was set at $p < 0.05$.

9. Results and Discussion:

When tested at 5, 10, or 20 mg/kg body weight, methidathion did not induce a significant increase in the mutation rate or in the number of mutant colonies per plate of S. typhimurium strains TA 98 and TA 1537 (Table 1). With strain TA 100, there was no significant increase in the number of revertant mutant colonies. However, there was a slight

TABLE 1

INTRASANGUINE HOST-MEDIATED ASSAY OF METHIDATHION WITH S. TYPHIMURIUM

Strain	Test Substance	Dose (mg/kg)	No. Mice	Total Bacterial Count Per Ml	Revertant Mutant Colonies Per Ml	Mutation Rate per 10 ⁷ Cells
TA 98	0.5% CMC ^a	Control	6	3.02 X 10 ⁷	3.35	1.11
	Methidathion ^b	5	6	6.55 X 10 ⁷	2.65	0.40
		10	6	4.28 X 10 ⁷	3.0	0.70
		20	3	1.23 X 10 ⁷	1.33	1.08
TA 100	0.5% CMC	Control	6	5.69 X 10 ⁷	50.0	8.79
	Methidathion	5	5	7.75 X 10 ⁷	64.0	8.26
		10	6	3.05 X 10 ⁷	37.0	12.13
		20	6	4.68 X 10 ⁷	62.5	13.35
TA 1537	0.5% CMC	Control	5	1.17 X 10 ⁷	4.5	3.85
	Methidathion	5	5	1.98 X 10 ⁷	1.8	0.91
		10	5	1.65 X 10 ⁷	2.2	1.33
		20	0 ^c	-	-	-

^aCMC = Carboxymethylcellulose.^bGS 13005 batch no. Op. 25-572.^cAll animals died.

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increase in the mutation rate at the doses of 10 and 20 mg/kg, but not at 5 mg/kg. The increases were less than two-fold that of the control values. Only mean values were reported in the table, thus variations in the total bacterial counts cannot be verified.

Therefore, in the absence of any significant responses with the other indicator strains and since the increases in mutation rates with TA 100 were small and not dose related, the responses are considered to have no biological relevance.

10. Technical Review Time: 5.25 hours.

1. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Active Ingredient of Unspecified Purity

3. Citation or Citations:

Strasser, F.F. (1980) Point Mutation Assay with Mouse Lymphoma Cells: Host-mediated Assay with GS 13005: (Test for Mutagenic Properties in Mammalian Cells): No. of Experiment 801495. (Unpublished study received Aug 13, 1981 under 7F1983; prepared by Ciba-Geigy Ltd., Switzerland, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL: 070213-H)

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Date:5. Approved by:Signature: Charles L. Lundy
Date: November 1, 19826. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC
MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement
163.84-1 through 4.

7. Conclusions:

Methidathion, designated as GS 13005, was tested, at 15 mg/kg, for the ability to induce point mutations in a host-mediated mutagenicity system employing mouse lymphoma cells (L5178Y) and DBA/Bom/SPF mice.

The mutation frequency in the cultures of treated cells as reported in this study was not increased. Hence, a mutation factor (mutation frequency of treated cells/mutation frequency of control cells) of 2.5, which was arbitrarily selected as the cut-off point for mutagenic activity, was not attained. The mutation factor for resistance to the control antimetabolites was 1.23 for thymidine, 1.02 for methotrexate, and 0 for cytosine arabinoside. Therefore, based on the limited information presented, methidathion is considered to be nonmutagenic in this test system. However, only summary data was presented (see Table 1) and proper evaluation of this study is not possible.

CORE CLASSIFICATION: Not applicable. Guidelines for minimum criteria for mutagenicity testing are not available.

8. Material and Methods:

The test material was identified as GS 13005, batch No. 25-572. The purity was unspecified.

The material was tested for mutagenic effects on mouse lymphoma cells (L5178Y) in a host-mediated assay system employing mice of the DBA/Bom/SPF strain. The test system permits the detection of forward mutations in mammalian cells. Mutagenic effects are manifested by the

TABLE 1

HOST-MEDIATED POINT MUTATION ASSAY OF METHIDATHION

Test Substance	System	Antimetabolites					
		Cytosine Arabinoside		Methotrexate		Thymidine	
		MF ^c	MFF ^d	MF	MFF	MF	MFF
Control (CMC) ^a	In vivo ^b	0		7.42		2.88	
GS 13005 (15 mg/kg)	In vivo	0.07	0	7.59	1.02	3.54	1.23

^aCarboxymethylcellulose. All doses were contained in 10 ml/kg.

^bL5178Y mouse lymphoma cells inoculated intraperitoneally into DBA/Bom/SPF mice.

^cMF = Mutant Frequency per 100,000 viable cells.

^dMFF = Mutation Factor.

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occurrence of mutants that are resistant to any of the three antimetabolites used (methotrexate, cytosine arabinoside or thymidine).

The L5178Y target cells were inoculated intraperitoneally (10^6 cells/animal) into two groups of four mice each. Three days after inoculation of the target cells the test material, suspended in 0.5% carboxymethylcellulose (CMC), was administered by intubation to one group of four animals at 15 mg/kg. This dosage is 1/9 of the acute LD_{50} as determined in a previous toxicity test. A second group of four animals, treated with CMC only, served as a control. All doses were contained in 10 ml CMC/kg. Three days after treatment, the cells were aseptically removed from the peritoneal cavity. After centrifugation and washing the cells were placed in culture tubes (4×10^5 cells/5 ml) in semisolid agar containing the appropriate antimetabolite (methotrexate, thymidine, or cytosine arabinoside) to detect mutagenic effects. A cell-viability control was carried out by seeding 100 cells per 5 ml agar without anti-metabolites. The incubation time was 10 days for the cell viability test and 14 days for the mutagenicity test.

The values obtained from the viability control served to normalize results from the mutagenicity test. Colonies were counted. The mutant frequency (MF) was determined by dividing the number of colonies by 100,000 viable cells. The mutation factor (MFF) was calculated by dividing the MF of the treated cells by the MF of the control cells. The test substance was arbitrarily considered to be mutagenic if the MFF was greater than 2.5.

No statistical analyses were performed.

9. Results and Discussion:

In a preliminary toxicity test, four groups of mice were treated with methidathion at ranges from 1.5 to 45 mg/kg. Three of six mice treated with methidathion at 45 mg/kg died, therefore, the mutagenicity test was performed with the next lower dose of 15 mg/kg. No actual data from individual animals were presented, instead calculated mutation frequencies per 100,000 viable cells (MF) and a mutation factor (MFF), or ratio of the MF treatment group/MF control group were given (Table 1). This under-reported data made it difficult to assess the soundness of the study. In spite of these shortcomings, the results presented show that the mutation frequency was not increased and the test material was not considered to be mutagenic since a maximum MFF of only 1.23 (with thymidine) was reached. This was below the MFF of 2.5 arbitrarily required for mutagenicity.

10. Technical Review Time: 5.50 hours.

REPORT #6

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Page 1 of 12
MRID: 000606421. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Active ingredient, purity unspecified

3. Citation or Citations:

Simmon, V.F., Poole, D. (1977) Final Report: In Vitro and In Vivo
Microbiological Assays of Six Ciba-Geigy Chemicals: SRI Project
LSC-5686. (Unpublished study received Dec 29, 1977 under 100-542;
prepared by Stanford Research Institute, submitted by Ciba-Geigy
Corp., Greensboro, N.C.; CDL: 232550-B.)

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Signature: Roman PientaDate: May 7, 19825. Approved by:Signature: Charles SanduskyDate: June 15, 19826. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC
MUTAGENICITY

This study relates to the Proposed Guidelines data requirement
163.84-1 through 4.

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Page 2 of 12
MRID: 000606427. Conclusions:

Methidathion was not mutagenic when tested at 10-5000 $\mu\text{g}/\text{plate}$ in vitro in the Salmonella typhimurium bioassay employing strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 in the presence of or absence of a rat liver homogenate metabolic activation system except with TA 1538 at 50 μg . In a repeat experiment minimally detectable activity was observed with strains TA 1535, TA 1537, and TA 1538 at 1, 2, or 3 doses, respectively, in the presence of metabolic activation including TA 1538 at 50 μg (see Tables 1 and 2). These results may not be biologically relevant however, since the test values were much lower than those observed with positive control compounds and no linear dose response was observed.

Methidathion was not mutagenic in the in vivo host-mediated assay employing S. typhimurium (strains TA 1535 and TA 1538) and male Swiss Webster mice. The material was tested via two dosing schedules. In the acute test, animals were treated once only with methidathion at the LD_{50} (40 mg/kg body wt.), $1/2 \text{ LD}_{50}$ (20 mg/kg) or $1/4 \text{ LD}_{50}$ (10 mg/kg). In the subacute test, animals were treated once daily for five days with $1/2 \text{ LD}_{50}$, $1/4 \text{ LD}_{50}$, or $1/8 \text{ LD}_{50}$ methidathion.

CORE CLASSIFICATION: Not applicable. Guidelines for minimum requirements are not available for mutagenicity tests.

8. Materials and Methods:

Methidathion was provided by Ciba-Geigy (Japan). The purity was not specified.

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MRID: 00060642

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TABLE 1

IN VITRO ASSAYS OF METHIDATHION WITH SALMONELLA TYPHIMURIUM

Compound	Dose µg/plate	Metabolic Activation	Histidine-Positive Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
<u>Experiment-1</u>							
Negative control		-	30	11	11	41	79
		+	48	20	15	53	90
Positive controls ^a							
BPL	50	-	111				
9-AA	100	-		410			
2-NF	50	-			1348		
AF2	0.5	-				61	165
2-AA	20	+	48	16	48	652	2740
Methidathion	10	-	26	5	7	27	85
	50	-	35	7	7	22	50
	100	-	36	8	4	22	51
	500	-	39	12	7	18	82
	1000	-	27	3	12	27	52
	5000	-	26	1	6	0	7
	10	+	28	8	13	26	80
	50	+	37	15	64 ^b	33	80
	100	+	35	4	14	37	59
	500	+	27	12	12	34	89
	1000	+	34	3	9	22	83
	5000	+	11	0	3	6	45

^aBPL = 3-Propiolactone; 9-AA = 9-Aminoacridine; 2-NF = 2-Nitrofluorene;
AF2 = 2-(2-furyl)-3(5-nitro-2-furyl)acrylamide; 2-AA = 2-Anthramine

^bMarginally detectable activity

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TABLE 2

IN VITRO ASSAYS OF METHIDATHION WITH SALMONELLA TYPHIMURIUM

Compound	Dose ug/plate	Metabolic Activation	Histidine-Positive Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
<u>Experiment-2</u>							
Negative control		-	16	7	13	11	137
		+	11	2	8	26	131
Positive controls ^a							
BPL	10	-	1085				
9-AA	100	-		2085			
2-NF	10	-			1815		
AF2	0.1	-				371	
2-AA	2	+	636	89	340	1868	
Methidathion	50	-	24	7	9	16	119
	100	-	24	9	8	18	107
	500	-	24	2	10	18	110
	1000	-	19	5	13	20	111
	2500	-	1	0	10	6	95
	5000	-	0	2	2	3	86
	50	+	15	<u>18</u>	<u>27</u>	36	98
	100	+	12	<u>13</u>	<u>16</u>	34	115
	500	+	20	8	22	28	113
	1000	+	<u>26</u>	8	14	44	108
	2500	+	4	1	12	23	18
	5000	+	1	0	1	8	96

^a BPL = β -Propiolactone; 9-AA = 9-Aminoacridine; 2-NF = 2-Nitrofluorene;
AF2 = 2-(2-furyl)-3(5-nitro-2-furyl)acrylamide; 2-AA = 2-Anthramine.

^b marginally detectable activity is underlined

Methidathion was tested in the in vitro Ames microbial mutagenesis assay employing S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 which are all histidine auxotrophs. The compound was also tested in vivo in the host-mediated mutagenicity assay employing strains S. typhimurium, TA 1535 and 1538, with male Swiss Webster mice. When these histidine-dependent cells are grown on a minimal medium containing a trace of histidine, only those cells that revert to histidine independence (his⁺) are able to form colonies. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar medium the mutation frequency is increased 2- to 100-fold.

The combination of the two assay procedures significantly enhances the probability of detecting potentially hazardous chemicals.

Ames Salmonella/Microsome Mutagenesis Assay

All indicator strains were checked to verify their genotypic characteristics and stock cultures were stored in 10% sterile glycerol at -80°C until needed.

Since some carcinogenic chemicals must be metabolized to active forms in order to be mutagenic in Salmonella, a metabolic activation system is incorporated into the assay. The activation system consists of the 9000 xg supernatant fraction (S9) prepared from the livers of rats treated with the polychlorinated biphenyl, Aroclor 1254, according to

standard procedures (Ames, et al., Mut. Res. 31:347-361, 1975). The S9 fraction is used with necessary cofactors.

In vitro Assay - To a sterile 13 X 100 mm test tube heated to 43°C are added 1) 2.00 ml of 0.6% agar, 2) 0.05 ml of indicator organisms, 3) 0.50 ml of metabolic activation mixture, i.e., S9 plus cofactors (this step optional), and 4) 0.05 ml of a solution of test chemical.

Negative controls consist of 1), 2), and 3) (optional) and 0.05 ml of dimethylsulfoxide (DMSO) which is used as the solvent for methidathion. Positive controls consisted of β -propiolactone, 2-(2-furyl)-3(5-nitro-2-furyl)acrylamide, 2-nitrofluorene, 9-aminoacridine, and 2-anthramine which are specific mutagens to revert particular strains of Salmonella.

The mixture is stirred gently and then poured onto petri plates containing solidified minimal agar. After the top agar has set, the plates are incubated at 37°C for 2 days. The number of his⁺ revertant colonies is counted and recorded.

In vivo Host-mediated Assay

Generally, in this assay the animal host receives simultaneously the test compound by oral intubation and the indicator microorganism by intraperitoneal (ip) injection. The microorganisms incubate in the peritoneal cavity while the animal presumably metabolizes the compound. After four hours the microorganisms are removed from the animal and assayed for mutations.

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Male Swiss Webster mice, weighing 28 to 30 g were injected ip with 2 ml of an overnight suspension (the concentration of which was not specified) of the appropriate strain of S. typhimurium (TA 1535, TA 1538). Mice received methidathion orally via intubation. The compound was dissolved in dimethylsulfoxide (DMSO) to a concentration requiring a 0.2 ml volume. Negative control animals received DMSO only. The compound was tested at three dose levels, with from 10-15 mice per dose. To enhance sensitivity of the assay, it was modified so that two dosage regimes were used. In the acute test, treated mice received a single dose of the compound. In the subacute test, treated mice received a daily dose for 5 consecutive days. On the fifth day the mice received the indicator microorganisms.

Preliminary toxicity experiments were conducted to determine the maximum tolerated dose of test material. In the acute test the dose was the LD₅₀. In the subacute test the dose was one-half the LD₅₀. The lower doses were one-half and one-fourth of the highest dose administered. The dosing schedule is summarized in Table 3.

TABLE 3

IN VIVO HOST-MEDIATED TEST OF METHIDATHION

Acute Test		Subacute Test	
Dose mg/kg	Treatment Schedule	Dose mg/kg	Treatment Schedule
40	single	20	1 X 5 days
20	single	10	1 X 5 days
10	single	5	1 X 5 days

Two strains of S. typhimurium were used; TA 1535 (which detects base-pair substitution mutations) and TA 1538 (which detects frame-shift mutations).

The positive control for TA 1535, dimethylnitrosamine (DMNA) was dissolved in DMSO and administered at a dose of 200 mg/kg in a 0.05 ml volume by intramuscular injection. The positive control for strain TA 1538, 2-anthramine, was dissolved in DMSO and administered by oral intubation at 1500 mg/kg. Negative control animals received 0.2 ml DMSO by oral intubation.

All mice were sacrificed four hours after the simultaneous inoculation of the indicator organism and administration of the test compound at times indicated for the acute and subacute tests. Two ml of sterile saline was injected into the peritoneal cavity. The peritoneal cavity was opened and the exudate from each mouse was treated individually.

Immediately, serial dilutions (10^0 to 10^{-6}) were made for each peritoneal exudate. To determine total viable bacteria, 0.2 ml of the 10^{-6} dilution sample was plated in triplicate onto tryptone-yeast extract (TYE) agar medium. To determine mutant bacterial cells, 0.2 ml of the 10^0 dilution was plated onto minimal medium in five replicate plates. All bacteria were incubated at 37°C (18 hours for TYE and 2 days for minimal medium).

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The number of bacteria recovered was determined by:

- 1) Bacteria/ml undiluted peritoneal exudate
= Number of colonies on a TYE plate X 5 X 1/dilution factor
- 2) Histidine-positive revertants/ml
= Number of colonies on a minimal medium plate X 5
- 3) Histidine-positive revertants/ 10^8 cells
= $\frac{\text{Histidine-positive revertants/ml}}{\text{Bacteria recovered/ml peritoneal exudate}}$

No statistical analyses were reported.

9. Results and Discussion:

In two separate experiments methidathion was tested (at 10-5000 $\mu\text{g}/\text{plate}$) for mutagenicity in the in vitro reverse mutation assay employing S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, or TA 100. An Aroclor-1254-stimulated, rat-liver-homogenate metabolic activation system (S9) was included in the assay procedures.

In the initial experiment (summarized in Table 1), except for the marginally detectable activity with TA 1538 treated with 50 $\mu\text{g}/\text{plate}$ in the presence of S9, (values underlined in the table), no mutagenic activity was observed with any of the strains either in the absence or presence of metabolic activation. In a repeat of the experiment (summarized in Table 2), marginally detectable activity (values underlined in the table) was observed only in the presence of S9 with strain TA 1535 at 1000 $\mu\text{g}/\text{plate}$, TA 1537 at 50 or 100 $\mu\text{g}/\text{plate}$, and TA 1538 at 50, 100 or 500 $\mu\text{g}/\text{plate}$. No activity was observed with any of the strains treated in the absence of S9. The minimal responses elicited

by methidathion have dubious biological relevance for the following reasons: 1) no dose responses were elicited; 2) the numbers of revertant mutants were significantly lower than those observed with positive control compounds, and 3) background levels appear to be lower than those for historical controls. Since only one petri dish per dose was employed, it is not possible to assess variability among responses.

Methidathion was also tested in vivo in the host-mediated mutagenicity assay employing Swiss Webster mice and S. typhimurium strains TA 1535 and TA 1538 which detect base-pair substitution or frameshift mutations, respectively. Two dosage regimens were employed. In the acute test, methidathion was administered only once to mice. In the subacute test, mice were treated with methidathion once daily for five days. Methidathion was negative in either case when tested at 10-40 mg/kg in the acute exposure test (Table 4) or at 5-20 mg/kg in the subacute exposure test (Table 5).

10 Technical Review Time: 14.50 hours.

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TABLE 4

HOST-MEDIATED ASSAY OF METHIDATHION
WITH SALMONELLA TYPHIMURIUM: ACUTE EXPOSURE^a

Treatment	Dose (mg/kg)	Number Mice	Bacteria Recovered per ml (X10 ⁸ CFU)	Revertants per ml	Revertants per 10 ⁸ Cells
<u>STRAIN TA1535</u>					
Negative Control (DMSO)		8	9.7 ± 3.7	85.9 ± 35.6	9.2 ± 3.2
Positive Control (DMNA) ^b	120	7	10.6 ± 6.1	149.6 ± 84.2	59.4 ± 119.6
Methidathion	40	6	10.9 ± 7.0	131.5 ± 105.2	11.6 ± 5.2
	20	7	9.6 ± 5.7	91.1 ± 29.1	12.6 ± 6.7
	10	7	16.1 ± 5.6	111.1 ± 50.2	7.3 ± 2.5
<u>STRAIN TA1538</u>					
Negative Control (DMSO)		9	1.6 ± 1.1	9.1 ± 3.8	11.9 ± 11.9
Positive Control (2-AA) ^c	1500	9	1.6 ± 1.2	372.6 ± 164.4	272.2 ± 126.7
Methidathion	40	8	3.5 ± 2.9	11.9 ± 11.8	5.6 ± 3.9
	20	9	1.7 ± 1.1	8.8 ± 3.6	7.9 ± 8.5
	10	9	1.8 ± 0.9	5.6 ± 2.5	3.9 ± 2.7

^a Mice given one oral dose of test material; treated simultaneously with bacteria intraperitoneally and then sacrificed four hours later.^b DMNA = Dimethylnitrosamine.^c 2-AA = 2-Anthramine.

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TABLE 5
HOST-MEDIATED ASSAY OF METHIDATHION
WITH SALMONELLA TYPHIMURIUM: SUBACUTE EXPOSURE^a

Treatment	Dose (mg/kg)	Number Mice	Bacteria Recovered per ml ($\times 10^8$ CFU)	Revertants per ml	Revertants per 10^8 Cells
<u>STRAIN TA1535</u>					
Negative Control (DMSO)		8	10.3 ± 3.2	19.1 ± 8.9	2.0 ± 1.2
Positive Control (DMNA) ^b	200	9	7.8 ± 2.3	363.7 ± 208.1	46.6 ± 25.6
Methidathion	20	10	8.4 ± 4.5	33.9 ± 15.2	5.2 ± 3.7
	10	7	5.4 ± 2.3	39.4 ± 12.3	5.6 ± 1.6
	5	9	8.2 ± 2.1	37.6 ± 10.2	4.8 ± 1.8
<u>STRAIN TA1538</u>					
Negative Control (DMSO)		6	0.22 ± 0.1	6.0 ± 3.0	27.6 ± 14.9
Positive Control (2-AA) ^c	1500	9	0.38 ± 0.4	499.1 ± 364.8	1864.8 ± 1500.1
Methidathion	20	10	0.70 ± 0.5	7.9 ± 3.9	13.3 ± 5.6
	10	7	0.48 ± 0.3	6.3 ± 5.6	11.6 ± 4.0
	5	7	0.42 ± 0.3	7.4 ± 7.4	20.9 ± 21.6

^aMice treated with test substance for 5 days. On the fifth day the mice received the test microorganism and were sacrificed four hours later.

^bDMNA = Dimethylnitrosamine.

^c2-AA = 2-Anthramine.

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MRID: 00078334;
000783361. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Technical, Active Ingredient, 96.9% pure

3. Citation or Citations:

Hool, G; Langauer, M.; Loos, H.; et al. (1980) Nucleus Anomaly Test
in Somatic Interphase Nuclei of Chinese Hamster: Test No. 80-0437.
(Unpublished study received Aug 13, 1981 under 7F'983, prepared by
Ciba-Geigy, Ltd., Switzerland, submitted by Ciba-Geigy Corp.,
Greensboro, N.C.; CDL: 070213-J).

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Signature: Roman J. Pienta
Date: June 9, 19825. Approved by:Signature: Charles B. Lencusky
Date: June 9, 19826. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC
MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement
163.84-1 through 4.

7. Conclusions:

There was no increase in the percentage of nuclear anomalies of bone marrow cells from Chinese hamsters treated orally on 2 consecutive days with methidathion at dosages of 17, 34, or 68 mg/kg body weight. Consequently, methidathion is considered to be nonmutagenic in this system which is designed to evaluate the mutagenic effects of chemicals on somatic interphase cells in vivo.

CORE CLASSIFICATION: Not applicable. Guidelines for minimum criteria for mutagenicity tests are not available.

8. Materials and Methods:

The test material was designated as GS 13005 technical, batch number Op. 91046, and was 96.9% pure.

Chinese hamsters (Cricetulus griseus) of both sexes were used. The females were 6-10 weeks old and weighed 22-30 g; the males were 4-9 weeks old and weighed 22-31 g. They were fed a standard diet (NAFAG No. 924) and tap water ad libitum. The animals were kept in an air-conditioned room which was illuminated for 12 hours daily and held at a temperature of 24°-25°C and a relative humidity of 50-60%. The animals were housed individually and identified by cage number.

In this system, mutagenic effects present themselves in the form of nuclear anomalies in bone marrow cells as a consequence of damage during mitosis.

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The test materials were administered orally by intubation to groups of 6 female and 6 male animals. Methidathion suspended in a 0.7% solution of Na-carboxymethylcellulose (CMC) was administered at 17, 34, and 68 mg/kg body weight so that each animal received 20 ml/kg. Cyclophosphamide, which served as a positive control, was administered at 128 mg/kg in 20 ml CMC. Negative control animals received 20 ml/kg of 0.7% CMC. Treatment consisted of one daily application on 2 consecutive days.

Twenty-four hours after the second application the animals were sacrificed by cervical dislocation.

Bone marrow was collected from the shafts of both femurs. Spread-slides were prepared and air-dried. On the next day, the slides were stained in undiluted May-Grunwald solution for 2 minutes then in May-Grunwald solution/water (1:1) for 2 minutes and finally in 40% Geimsa solution for 20 minutes. The slides were then rinsed in 55% methanol for 5-8 seconds and washed off twice in water. After rinsing with distilled water and air-drying, the slides were cleared in xylol and mounted in Eukitt.

Slides of 3 female and 3 male animals each from the negative control group, the positive control group, and the groups treated with methidathion were examined. For each animal, 1000 bone marrow cells were scored and the percentage of the following anomalies were reported: a) Single Jolly bodies, b) fragments of nuclei in erythrocytes,

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c) micronuclei in erythroblasts, d) micronuclei in leukopoietic cells, and e) polyploid cells.

The data were analyzed for increased incidence of nuclear anomalies by the χ^2 -test. The differences were considered significant at $p \leq 0.05$.

9. Results and Discussion:

In all dosage groups of animals treated with methidathion the percentage of cells displaying nuclear anomalies did not differ significantly from the negative control (Table 1).

A significant increase in the percentage of cells with anomalies was observed for cells from animals treated with the positive control, cyclophosphamide. The mean percentage of anomalies was 7.97 for cyclophosphamide compared with a percentage of 0.12 for the negative control animals treated with CMC alone. The difference was significant at $p \leq 0.05$.

Therefore, under the conditions of the test methidathion is considered nonmutagenic.

10. Technical Review Time: 5.50 hours.

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00078336

TABLE 1
PERCENTAGE OF BONE MARROW CELLS FROM THE CHINESE HAMSTER
DISPLAYING NUCLEAR ANOMOLIES FOLLOWING TREATMENT WITH METHIDATHION

Treatment ^a	Animal	Sex	Single Jolly Bodies	Fragmentation of Nuclei in Erythrocytes	Micronuclei in Erythroblasts	Micronuclei in Leukopoietic Cells	Polyploid Cells	Total Percentage	Mean Percentage
Control (CMC, 0.7%)	1	F	0.2	- ^d	-	0.1	-	0.3	0.12
	2	F	0.1	-	-	-	-	0.1	
	3	F	0.1	-	-	-	-	0.1	
	4	M	-	-	-	-	-	0.0	
	5	M	0.1	-	-	-	-	0.1	
	6	M	0.1	-	-	-	-	0.1	
Cyclophosphamide ^b (128 mg/kg)	1	F	3.3	0.5	1.7	1.2	0.1	6.8	7.97
	2	F	5.1	1.1	0.7	0.6	-	7.5	
	3	F	5.5	0.4	0.7	0.3	0.1	7.0	
	4	M	8.0	1.0	-	-	-	9.0	
	5	M	7.2	1.2	1.0	0.6	-	10.0	
	6	M	5.1	0.8	0.8	0.8	-	7.5	
Methidathion ^c (68 mg/kg)	1	F	0.3	-	-	-	-	0.3	0.13
	2	F	0.2	-	-	-	-	0.2	
	3	F	0.1	-	-	-	-	0.1	
	4	M	-	-	-	-	-	0.0	
	5	M	-	-	-	-	0.1	0.1	
	6	M	0.1	-	-	-	-	0.1	
Methidathion (34mg/kg)	1	F	-	-	-	-	-	0.0	0.08
	2	F	0.2	-	-	-	-	0.2	
	3	F	0.1	-	-	-	-	0.1	
	4	M	-	-	-	-	-	0.0	
	5	M	-	-	-	-	0.1	0.1	
	6	M	0.1	-	-	-	-	0.1	
Methidathion (17 mg/kg)	1	F	-	-	-	-	-	0.0	0.10
	2	F	0.1	-	-	0.1	-	0.2	
	3	F	0.1	0	0.1	-	-	0.2	
	4	M	0.1	-	-	-	-	0.1	
	5	M	0.1	-	-	-	-	0.1	
	6	M	-	-	-	-	-	0.0	

^a Daily oral application on 2 consecutive days. Animals sacrificed 24 hours after last application.

^b Positive control.

^c GS 13005, technical batch No. Op. 910046.

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MRID: 000783351. Chemical or Chemicals:

Methidathion

2. Type or Formulation:

Technical, Active Ingredient, purity unspecified

3. Citation or Citations:

Hool, G. (1980) Sister Chromatid Exchange - Study in Somatic Cells, Bone Marrow, GS 13005, Chinese Hamster: Dosage 17, 34, 68 mg/kg: No. of Experiment 801489. (Unpublished study received Aug 13, 1981 under 7F1983; prepared by Ciba-Geigy Ltd., Switzerland, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL: 070213-K)

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Date:5. Approved by:Signature: Charles B. Sandusky
Date: November 1, 19826. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 through 4.

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MRID: 000783357. Conclusions:

Methidathion was examined for possible mutagenic effects of the substance as manifested by the enhancement of the exchange of chromatin between two sister chromatids of a duplicated chromosome, i.e. sister chromatid exchange (SCE).

Methidathion, designated as GS 13005 was administered orally to Chinese hamsters. The animals were treated with a single dose of methidathion at levels of 17, 34, and 68 mg/kg. Bone marrow preparations were examined.

The number of SCEs in animals from the control group and in those treated with either the low or high dose of methidathion was not different. There was a slight but statistically significant increase ($t = 2.59$) in the SCE rate in animals treated with the intermediate dose of methidathion. However, the SCE rate in cells from this group (5.1 ± 2.41) was within the range observed in the negative control group (4.27 ± 2.16). A non-parametric test for a dose-dependent trend gave a negative result.

In view of the marginal response (less than 50% increase over the controls) at only one dose and the absence of a dose response, it is concluded that under conditions of this test methidathion is not clearly mutagenic.

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MRID: 00078335

CORE CLASSIFICATION: Not applicable. Guidelines for the performance of mutagenicity tests are not available.

8. Materials and Methods:

The test material was designated GS 13005, batch No. Op. 25-572. Purity was unspecified and the material was not described further.

The test system employed Chinese hamsters (Cricetulus griseus) of both sexes. Females weighed 20-27 g and males weighed 20-23 g when used. The animals were provided a standard diet and tap water ad libitum. They were housed in an air-conditioned room held at a temperature of 21-22°C and a relative humidity of 52-70%. The room was illuminated for 12 hours daily.

The treated groups and control groups consisted of 4 female and 4 male animals each. On the first day of the experiment, a 45-mg tablet of 5-bromodeoxyuridine (BUdR) was implanted subcutaneously in the neck of each animal. Two hours later the animals were treated by oral intubation with methidathion at dosages of 17, 34 and 68 mg/kg contained in 20 ml/kg of a 0.5% aqueous solution of sodium carboxymethylcellulose (CMC).

Animals in the positive control group were treated with 7,12-dimethylbenz(a)anthracene (DMBA) at 100 mg/kg in 20 ml/kg CMC. Negative controls received 20 ml/kg CMC. On the following day (22 hours post-treatment) each animal received an intraperitoneal injection of 10 mg colcemide/kg body weight. Two hours later the animals were sacrificed by cervical dislocation.

Bone marrow was collected from the shafts of both femurs of each animal. The cells were suspended in balanced salt solution, diluted to hypotonicity with distilled water while kept in a waterbath at 4-6°C, and then centrifuged for 10 minutes at 200xg. The pellets were then fixed in methanol/acetic acid (3:1) for 30 minutes, resuspended, centrifuged for 5 minutes at 150xg for 5 minutes and stored in fresh fixative overnight at 40°C. Finally the pellets were centrifuged at 150xg for 5 minutes and resuspended in approximately 0.5 ml fixative. Drop-preparations were made by pipetting the suspensions onto glass slides and allowing them to air-dry.

The air-dried slides then were treated with a solution of bisbenzimidazole for 15 minutes, rinsed in McIlvaine buffer, pH 8.0, and irradiated in this buffer at 50°C with UV light (350 nm). Following development of the fluorochrome-UV-light reaction in 60°C double-strength sodium citrate for 90 minutes, the slides were stained in 40% Giemsa for 20-40 minutes, rinsed well, cleared in Xylol and mounted in Eukitt.

The slides from 2 female and 2 male animals from each group were examined. For each animal 25 differentially stained metaphases of the second cell cycle with BUdR-substitution were analyzed for the number of SCEs following specific criteria for identification of SCEs so described by Marquardt and Bayer (Mut. Res. 56:169-176, 1978).

The significance of differences of the treatment groups compared to the negative control group was assessed by a t-test on the level of one

percent. The authors used the Jonckheere test as a non-parametric test for a dose-dependent trend on the number of SCEs per cell.

9. Results and Discussion

Following treatment of animals with either the low dose (17mg/kg) or high dose (68 mg/kg) of methidathion no statistically significant difference in the number of SCEs in bone marrow cells was found in comparison with the negative control group. The number of SCEs per cell in the negative control group was 4.27 ± 2.16 . The values were 4.58 ± 2.38 per cell for the low dose group and 4.28 ± 2.11 for the high dose group. In the intermediate dose group (34 mg/kg) the number of SCEs per cell was slightly (5.10 ± 2.42 vs 4.27 ± 2.16) but significantly ($p < 0.01$) increased. The authors reported that a non-parametric test (Jonckheere) for a dose-related trend gave a negative result. The positive control group treated with DNBA showed a marked increase in the number of SCEs per cell in comparison with the negative control group (8.8 ± 3.73 vs 4.27 ± 2.16 ; $p < 0.01$). It is difficult to verify some of the statistical analyses since only summarized data are presented.

In summary, a slight, but significant, difference in the rate of SCEs was shown for one dose. However, in the absence of a dose response the biological relevance of the findings is questionable.

10. Technical Review Time: 13.00 hours.

TABLE 1

THE EFFECT OF METHIDATHION ON BONE MARROW CELLS OF THE CHINESE HAMSTER

Group	Number	Sex	Sister Chromatid Exchanges (SCEs) per cell				Observed t Value
			Animal $\bar{X}$	(S)	Group $\bar{X}$	(S)	
Control (0.5% CMC) (Negative Control)	1	F	4.20	(2.00)	4.27	(2.16)	--
	2	F	4.28	(2.51)			
	3	M	4.52	(2.57)			
	4	M	4.08	(1.53)			
DMBA (100 mg/kg) (Positive Control)	1	F	9.60	(3.56)	8.81	(3.73)	10.56a
	2	F	7.16	(2.27)			
	3	M	8.92	(4.66)			
	4	M	9.56	(3.73)			
Methidathion (17 mg/kg)	1	F	4.32	(2.67)	4.58	(2.38)	0.97
	2	F	4.76	(1.85)			
	3	M	4.60	(2.61)			
	4	M	4.64	(2.41)			
Methidathion (34 mg/kg)	1	F	5.12	(2.44)	5.10	(2.41)	2.59a
	2	F	4.88	(2.19)			
	3	M	5.32	(2.59)			
	4	M	5.08	(2.53)			
Methidathion (68 mg/kg)	1	F	4.04	(1.65)	4.28	(2.11)	0.03
	2	F	4.32	(1.97)			
	3	M	4.32	(2.66)			
	4	M	4.44	(2.14)			

 $\bar{X}$ = mean value of SCEs per cell

s = standard deviation

a = statistically significant at 1% (critical t-value = 2.34, d.f. = 198)

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REPORT #9

Page 1 of 6
MRID: Not assigned

1. Chemical or Chemicals:

Methidathion Metabolite GS 12956

2. Type or Formulation:

N/A

3. Citation or Citations:

Arni, P. (Oct 27 1980B) Salmonella/Mammalian-Microsome
Mutagenicity test with GS 12956 (Test for mutagenic properties
in bacteria). No. of experiment: 801377; Ciba-Geigy Ltd.,
Basle, Switzerland

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Date: *June 8, 1982*

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Signature: *Charles B. Andrusky*
Date: *June 8, 1982*

6. Disciplin/Topic or Test Type:

This study has information pertinent to discipline toxicology,
TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement
163.84-1 thru 4.

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MRID: Not assigned

7. Conclusions:

Methidathion metabolite GS 12956 was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium, strains TA 98, TA 100, TA 1535, and TA 1537. The material was tested with and without metabolic activation at concentrations of 10, 30, 90, 270, and 810 µg/0.1 ml.

No evidence of the induction of point mutations by GS 12956 was detectable in these strains of S. typhimurium although the material was tested up to toxic concentrations.

CORE CLASSIFICATION: Not applicable. Guidelines for mutagenicity tests are not available.

8. Materials and Methods:

The test material was described only as GS 12956, batch No. RU-1932/51-59. It was tested in the Salmonella/microsome test according to the method described by Ames et al. The histidine-auxotrophic strains of Salmonella typhimurium employed were TA 98, TA 100, TA 1535, and TA 1537.

The material was tested at the following concentrations with and without microsomal activation: 10, 30, 90, 270, and 810 µg/0.1ml. The substance was dissolved in DMSO. DMSO alone was used for the negative controls. Vehicles for positive controls are indicated below.

Each petri dish contained: 1) approximately 20 ml of minimum agar, plus salts (Vogel-Bonner medium E) and glucose, 2) 0.1 ml of

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MRID: Not assigned

the solution of the test material or the vehicle and 0.1 ml of a bacterial culture (in nutrient broth) in 2.0 ml of soft agar. The soft agar was composed of: 100 ml of 0.6% agar solution with 0.6% NaCl and 10 ml of a solution of L-histidine (0.5 mM) and +biotin (0.5 mM).

In the experiments in which the test substance was metabolically activated, 0.5 ml of an activation mixture was added. One ml activation mixture contained 0.3 ml S9 fraction of liver from rats induced with Aroclor 1254 and 0.7 ml of a solution of co-factors.

Positive control experiments were carried out with substances appropriate for the specific bacterial strains, as indicated in Table 1.

In the experiments with and without the addition of microsomal activation mixture, three petri dishes were used per strain per group per dose. The dishes were incubated for about 48 hours at 37°C in darkness. When the revertant colonies had been counted, the arithmetic mean was calculated. The test substance was considered to be nonmutagenic if the colony count in relation to the negative control was not doubled at any concentration.

No statistical analyses were performed.

9. Results and Discussion:

Methidathion metabolite GS 12956 was tested in experiments performed without and with microsomal activation. Minimally

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MRID: Not assigned

TABLE 1

POSITIVE CONTROL CHEMICALS

Strain	Chemical	Doses Tested (μ g/0.1 ml)	Vehicle
TA 98	Daunorubicin-HCl	5, 10	phosphate buffer
TA 100	4-nitroquinoline-N-oxide	0.125, 0.25	phosphate buffer
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine	3, 5	phosphate buffer
TA 1537	Aminoacridine hydrochloride monohydrate	50, 100	DMSO
TA 1535	Cyclophosphamide ^a	250	phosphate buffer

^aPositive control for testing activation mixture.

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MRID: Not Assigned

detectable activity was observed only with strain TA 98 when GS 12956 was tested in the absence of metabolic activation. Although this occurred at the three lowest doses, the response rate was barely 2-fold that of the control and was not dose related. (The authors attributed the increase to fluctuations in the rate of spontaneously occurring back-mutants). Results were negative at all doses when the material was tested in the presence of metabolic activation. None of the tested concentrations of GS 12956 caused an increase in the incidence of histidine-prototrophic mutants of the other tester strains (TA 100, TA 1535, TA 1537) in comparison with controls. In contrast with the positive control, daunorubicin, the number of revertant colonies observed was increased 22 to 39 times that observed with the vehicle control. The trial material was tested at concentrations up to toxic levels. This was indicated by the observation that the number of revertant colonies was reduced when GS 12956 was tested at 810 $\mu\text{g}/0.1$ ml against strains TA 98 and TA 100, with metabolic activation. Similarly, inhibition was observed when GS 12956 was tested at 270 $\mu\text{g}/0.1$ ml against TA 100 without metabolic activation.

10. Technical Review Time: 4.5 hours.

TABLE 2

NUMBER^a OF HISTIDINE-PROTOTOPIIC REVERTANT COLONIES OBSERVED IN
THE SALMONELLA TYPHIMURIUM MICROsome MUTAGENICITY TEST OF GS 12956

Test Substance	Dose μg/0.1 ml	Strains of <u>S. typhimurium</u> Used							
		TA 98		TA 100		TA 1535		TA 1537	
		- ^b	+ ^b	-	+	-	+	-	+
GS 12956	Control	6	22	147	151	8	12	5	6
	10	13	20	151	126	6	3	5	3
	30	15	16	130	113	5	11	4	5
	90	14	19	140	109	5	8	3	7
	270	10	17	46	110	7	3	2	4
	810	3	9	16	46	1	2	3	4
<u>Positive Controls</u>									
Daunorubicin-HCl	Control	14							
	5	319							
	10	551							
4-NQO ^c	Control			152					
	0.125			504					
	0.25			744					
MNNG ^d	Control					5			
	3					>2000			
	5					>2500			
9-AA ^e	Control							5	
	50							191	
	100							>1500	
Cyclophosphamide	Control					9			
	250					239			

^aArithmetic mean from three dishes.^b- = without metabolic activation; + = with metabolic activation.^c4-nitroquinoline-N-oxide.^dN-methyl-N¹-nitro-N-nitrosoguanidine.^e9(5) Aminoacridine hydrochloride.

DRAFT

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REPORT #10

002348

Page 1 of 5
MRID: Not assigned

1. Chemical or Chemicals:

Methidathion metabolite GS 12956

2. Type or Formulation:

N/A

3. Citation or Citations:

Hool, G. (Oct 30, 1980) Nucleus Anomaly Test in Somatic Interphase Nuceli; GS 12956, Chinese Hamster; (Test for mutagenic effects on bone marrow cells); Experiment No. 801378

4. Reviewed by:

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Signature: *Roman J. Pienta*

Date: *June 9, 1982*

5. Approved by:

Signature: *Charles J. Emdustry*

Date: *June 9, 1982*

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 thru 4.

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MRID: Not assigned

7. Conclusions:

Methidathion metabolite GS 12956 was administered by gavage to Chinese hamsters at 121-484 mg/kg daily on two consecutive days. The animals were sacrificed 24 hours after the second application. Bone marrow cells were examined for evidence of mutagenic effects presenting themselves in interphase cells in the form of nucleus anomalies as a consequence of damage during the mitotic process. No evidence of mutagenicity was reported. The bone marrow smears from animals treated with the various doses of GS 12956 showed no significant differences from the control.

CORE CLASSIFICATION: Not applicable. Guidelines for mutagenicity testing are not available.

8. Materials and Methods:

The test material was described as GS 12956, batch No. RU-1932/51-59.

Animals: Chinese hamsters (Cricetulus griseus) of either sex, (females weighing 19-24 g, males weighing 21-26 g) were used. They were fed a standard diet (NAFAG No. 924) and tap water ad libitum. The animals were kept in an air-conditioned room held at 22-24°C and a relative humidity of 52-58%. The room was illuminated for 12 hours daily.

Treatment Schedule: GS 12956 was administered at 121, 242, and 484 mg/kg in 20 ml/kg polyethylene glycol 400 (PEG 400). [The oral

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LD₅₀ was previously found to be 1451 (550-3866 mg/kg) in Chinese hamsters of either sex according to Lab. Report GU2.1, dated Oct 2, 1980, and cited by the authors)].

Cyclophosphamide served as the positive control and was administered at 128 mg/kg in 20 ml/kg PEG 400. Negative control animals received 20 ml PEG 400/kg.

The preparations were administered orally by gavage to groups of 6 female and 6 male animals each. Treatment consisted of one daily application on 2 consecutive days. Animals were sacrificed 24 hours after the second application.

Preparation of test material: Bone marrow was harvested from the shafts of both femurs. The bone marrow was drawn up into a siliconized pipette filled with approximately 0.5 µl rat serum. In order to receive a homogenous suspension, the content of the pipette was aspirated gently about three times. Small drops of the mixture were transferred on the end of a slide, spread out by pulling it behind a polished cover glass and the preparations were air-dried. On the next day the slides were stained in undiluted May-Grunwald solution for two minutes, then in May-Grunwald solution diluted 1:1 with water for two minutes and then in 40% Giemsa stain for 20 minutes. After being rinsed in 55% methanol for 5-8 seconds and washed off twice in water, they were immersed in water for approximately 2 minutes. After rinsing with distilled water and air-drying the slides were cleared in xylol and mounted in Eukitt.

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MRID: Not assigned

Scoring of slides: The slides from three female and three male animals per group were examined. For each animal, 1000 bone marrow cells were scored and the following anomalies were recorded: a) Single Jolly bodies, b) fragments of nuclei in erythrocytes, c) micronuclei in erythroblasts, d) micronuclei in leukopoietic cells, and 3) polyploid cells.

The statistical significance of difference was assessed by χ^2 -test.

9. Results and Discussion:

In all dosage groups treated with GS 12956, the percentage of cells displaying anomalies of nuclei did not differ significantly from the negative control (Table 1). By contrast the positive control, cyclophosphamide induced a marked increase in the mean percentage of cells with anomalies (9.07% vs. 0.12%; $p < 0.05$). The study was difficult to evaluate further since only summarized data were presented.

10. Technical Review Time: 2.5 hours.

TABLE 1

PERCENT OF BONE MARROW CELLS WITH ANOMALIES OF NUCLEI
FOLLOWING TREATMENT OF CHINESE HAMSTERS WITH GS 12956

	Animal Number	Sex	Single Jolly Bodies	Fragments of Nuclei in Erythrocytes	Micronuclei in Erythroblasts	Micronuclei in Leukopoietic Cells	Polyploid Cells	TOTAL
Negative Control (PEG 400)	1	F	0.1			0.1		0.0
	2	F	0.2					0.2
	3	F	0.1					0.2
	4	M	0.1					0.1
	5	M	0.2					0.2
	6	M						0.0
Positive Control Cyclophosphamide (128 mg/kg)	1	F	7.9	1.0	1.2	0.2		10.3
	2	F	7.3	1.1	0.7	0.5	0.1	9.7
	3	F	7.0	0.5	0.5	0.1		8.5
	4	M	8.9	1.2		0.5		10.2
	5	M	7.1	1.1	1.7	0.5	0.1	10.5
	6	M	3.7	0.6	0.7	0.2		5.2
GS 12956 (121 mg/kg)	1	F	0.3			0.1		0.3
	2	F	0.1					0.1
	3	F	0.1					0.1
	4	M	0.1					0.1
	5	M	0.3					0.3
	6	M	0.1					0.1
GS 12956 (242 mg/kg)	1	F	0.2			0.1		0.2
	2	F	0.3					0.4
	3	F	0.4					0.4
	4	M	0.3					0.0
	5	M	0.3			0.1		0.4
	6	M	0.1					0.1
GS 12956 (484 mg/kg)	1	F	0.1					0.1
	2	F	0.2					0.0
	3	F	0.2					0.2
	4	M	0.2			0.1		0.3
	5	M	0.2					0.2
	6	M						0.2

REPORT #11

Page 1 of 4
MRID: Not assigned1. Chemical or Chemicals:

Methidathion metabolite GS 18369

2. Type or Formulation:3. Citation or Citations:

Arni, P. (Dec 15, 1980C) Salmonella/mammalian-microsome mutagenicity test with GS 28369. (Test for mutagenic properties in bacteria. Experiment No. 801427.) Ciba-Geigy Ltd., Basle, Switzerland

4. Reviewed by:

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Signature: Roman J. PientaDate: June 9, 19825. Approved by:Signature: Charles S. SmolenskyDate: June 9, 19826. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 thru 4.

Page 2 of 4
MRID: Not assigned

7. Conclusions:

There was no evidence of mutagenicity when methidathion metabolite GS 28369 was tested on histidine-auxotrophic mutants of Salmonella typhimurium. The substance was tested with strains TA98 and TA100 at 15, 30, 60, 120, 240, 480, and 960 µg/0.1 ml in the presence or absence of microsomal activation by rat liver microsomes. Comparison of the number of back-mutant colonies in the controls and the cultures treated with the various concentrations of GS 28369 revealed no marked increases.

CORE CLASSIFICATION: Not applicable. Guidelines for mutagenicity testing are not available.

8. Materials and Methods:

The test substance was identified as GS 28369 (>98% ex W. Mucke). The tests were performed on histidine-auxotrophic strains of S. typhimurium strains TA98 and TA100 according to the method described by Ames et al. Strain TA1538 was used to test the efficacy of the activation mixture.

The following concentrations of test substance were employed with and without microsomal activation: 15, 30, 60, 120, 240, 480, and 960 µg/0.1 ml. The substance was dissolved in DMSO which also served as the negative control. Positive control experiments were performed simultaneously with the following substances dissolved in phosphate buffer: 1) for strain 98: daunorubicin-HCl, at 5 and 10 µg/0.1 ml; 2) for strain TA100: 4-nitroquinoline-N-oxide, at 0.125

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MRID: Not assigned

and 0.25 $\mu\text{g}/0.1\text{ ml}$; 3) for strain TA1535: N-methyl-N'-nitro-N-nitrosoguanidine, at 3 and 5 $\mu\text{g}/0.1\text{ ml}$. The activation mixture was tested with strain TA 1535 and cyclophosphamide, at 250 $\mu\text{g}/0.1\text{ ml}$ phosphate buffer.

In all experiments two petri dishes were prepared per strain and per group. The dishes were incubated for about 48 hours at 37°C in darkness. The colonies were then counted and the arithmetic mean was calculated. The test substance was considered to be nonmutagenic if the colony count of revertant mutants in relation to the negative control was not doubled at any concentration.

No statistical analyses were done.

9. Results and Discussion:

The data summarized in Table 1 show that GS 28369 was not mutagenic to S. typhimurium strains TA98 and TA100 when tested at 15-960 $\mu\text{g}/0.1\text{ ml}$ with or without microsomal activation.

10. Technical Review Time: 3.0 hours.

TABLE 2

NUMBER^a OF REVERTANT COLONIES OBSERVED IN THE SALMONELLA
MAMMALIAN-MICROSOME MUTAGENICITY TEST OF METHIDATHION METABOLITEGS 12956

Test Substance	Dose µg/0.1 ml	Strains of <i>S. typhimurium</i> Used					
		TA98		TA100		TA1535	
		- ^b	+	-	+	-	+
GS 28369	Control	14	24	88	92		
	15	15	2	106	97		
	30	9	26	83	81		
	60	14	24	82	90		
	120	9	27	79	92		
	240	8	24	70	85		
	480	9	17	84	94		
	960	11	21	82	64		
<u>Positive Controls</u>							
Daunorubicin-HCl	Control	15					
	5	88					
	10	181					
4-NQO ^c	Control			93			
	0.125			494			
	0.25			647			
MNNG ^d	Control					10	
	3					1200	
	5					3150	
<u>Positive Control of Microsomal Action</u>							
Cyclophosphamide	Control 250					12 447	

^aArithmetic mean from two dishes.

^b- = without metabolic activation; + = with metabolic activation.

^c4-nitroquinoline-N-oxide.

^dN-methyl-N'-nitro-N-nitrosoguanidine.

REPORT #12

Page 1 of 4
MRID: Not assigned

002348

1. Chemical or Chemicals:

Methidathion Metabolite GS 28370

2. Type or Formulation

N/A

3. Citation or Citations:

Arni, P. (Oct. 24, 1980D). Salmonella/mammalian-microsome mutagenicity test with GS 28370. (Test for mutagenic properties in bacteria). Experiment No. 801428. Ciba-Geigy Ltd., Basle, Switzerland.

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Roman J. Pienta
June 9, 1982

5. Approved by:

Signature
Date:

Charles B. Landrusky
June 9, 1982

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC MUTAGENICITY.

This study relates to the Proposed Guidelines data requirement 163.84-1 through 4.

002348

7. Conclusion

There was no evidence of mutagenic activity when methidathion metabolite GS 28370 was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium, strains TA98, TA100, TA1535, and TA1537. No increase in revertant mutant colonies, compared with the controls, was observed when the substance was tested with and without microsomal activation at 25, 75, 225, 675 and 2025 µg/0.1 ml, indicating that GS 28370 is not mutagenic for these strains of S.typhimurium.

CORE CLASSIFICATION: Not applicable. Guidelines for mutagenicity tests are not available.

8. Materials and Methods

The test article was described only as GS 28370 (>98%; ex W. Mucke). It was tested for mutagenic effects on histidine-auxotrophic mutants of S.typhimurium, strains TA98, TA100, TA1535, and TA1537. The tests were reported to be carried out according to the standard methods described by Ames, et al. The following concentrations of trial substance were tested with and without metabolic activation: 25, 75, 225, 675 and 2025 µg/0.1 ml. The substance was dissolved in DMSO, which was used alone for the negative controls. Positive experiments were carried out simultaneously with the following substances: 1) for strain TA98 daunorubicin-HCl, 5 and 10 µg/0.1 ml; 2) for strain TA100: 4-nitroquinoline-N-oxide, 0.125 and 0.25 µg/0.1 ml; and 3) for strain TA1535: N-methyl-N'-nitro-N-nitrosoguanidine, 3 and 5 µg/1.0 ml.

For strain TA1537, 9(5)aminoacridine hydrochloride monohydrate was dissolved in DMSO and tested at 50 and 100 $\mu\text{g}/0.1 \text{ ml}$. The activation mixture was tested with strain TA1535 and cyclophosphamide at 250 $\mu\text{g}/0.1 \text{ ml}$ phosphate buffer. In all experiments three petri dishes were prepared per strain and per group. The dishes were incubated for about 48 hours at 37°C in darkness.

When the colonies had been counted, the arithmetic mean was calculated. The test substance was considered to be nonmutagenic if the colony count, in relation to the negative control was not doubled at any concentration. Statistical analyses of the data were not performed.

9. Results and Discussion

Methidathion metabolite GS 28370 was tested for mutagenic effects on histidine-auxotrophic mutants of S.typhimurium, strains TA98, TA100, TA1535, and TA1537. When the substance was tested at 25, 75, 225, 675 and 2025 $\mu\text{g}/0.1 \text{ ml}$ in the presence or absence of microsomal activation, provided by rat liver microsomes and cofactors, no increase in the number of back-mutant colonies was observed (see Table 1).

10. Technical Review Time: 4.0 hours

TABLE 1

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MRID: Not assigned

002348

NUMBER^a OF REVERTANT COLONIES OBSERVED IN THE
SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
OF METHIDATHION METABOLITE GS 28370

Test Substance Dose μg/0.1 ml		Strains of <i>S.typhimurium</i> Used							
		TA98		TA100		TA1535		TA1537	
		-b	+	-	+	-	+	-	+
GS 28370	Control	17	28	150	135	8	11	7	4
	25	14	26	153	127	9	10	3	5
	75	18	30	152	140	10	9	5	5
	225	20	29	147	137	8	11	6	4
	675	13	27	151	139	7	15	3	6
	2025	14	31	144	164	9	12	3	5
<u>Positive Controls</u>									
Daunorubicin-HCl	Control	16							
	5	606							
	10	754							
4-Nitroquinoline-N-oxide	Control			171					
	0.125			626					
	0.25			1008					
N-methyl-N'-nitro-N-nitrosoguanidine	Control					7			
	3					>1450			
	5					>2200			
9(5)Aminoacridine hydrochloride	Control							4	
	50							102	
	100							>850	
<u>Positive Control of the Microsomal Activation</u>									
Cyclophosphamide	Control						13		
	250						406		

^aArithmetic mean from 2 dishes

b- = without metabolic activation; + = with metabolic activation

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

002348

*Received via CBS
6/21/82*

APR 14 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

Subject: PP#7F1983. Methidathion on alfalfa.
Amendment of 8/13/81.

From: R. B. Perfetti, Ph.D., Chemist
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

Thru: Charles L. Trichilo, Chief
Residue Chemistry Branch
Hazard Evaluation Division

To: J. Ellenberger, Product Manager #12
Insecticide-Rodenticide Branch
Registration Division (TS-767)

and

Toxicology Branch
Hazard Evaluation Division (TS-769)

This amendment is in response to our memo of 12/3/80 in which we concluded that, as a result of the TOX concern over methidathion sulfoxide and sulfone, methidathion must be classed in category 2 of Section 180.6(a) with respect to meat, milk, poultry and eggs. In light of this conclusion we advised the petitioner that tolerance proposals, appropriate methodology including validation data and blank values; completion of a successful method trial and either an additional cattle feeding study at a higher level (at least 12 ppm in the diet) or reanalysis of meat and milk samples, if any, retained from the latest feeding study for methidathion sulfoxide and sulfone would be needed in order to determine appropriate levels of total residues in meat and milk. Also the tolerance regulation should be proposed in terms of total residues of parent and sulfoxide and sulfone metabolites. Finally, appropriate tolerance levels in poultry and eggs would be 0.15 ppm and 0.05 ppm respectively.

The petitioner has responded to the above by submitting a cow feeding study. This feeding study was submitted previously in conjunction with PP#8F2046. The samples were reanalyzed in order to determine residues of methidathion sulfoxide and sulfone in the milk and tissues of medicated cows.

Residues of methidathion sulfoxide and sulfone in milk plateaued within 3 to 5 days at the 72 ppm feeding level. Residues of the sulfoxide and sulfone in milk were all given as <0.01 ppm and <0.005 ppm respectively at the lowest feeding level. At the 72 ppm dosage level residues in milk ranged from <0.01 to 0.092 ppm for the sulfoxide and from <0.005 to 0.058 ppm for the sulfone.

Residues in all tissues were <0.025 ppm of either methidathion sulfoxide or sulfone at the lowest dosage level. At the 72 ppm feeding level, residues of the sulfoxide in muscle, liver, kidney blood and fat ranged from <0.025 to 0.058 (liver) ppm. The highest combined residue in tissue was 0.12 ppm in muscle.

The analyses for residues of methidathion in bovine tissues and milk were performed using methods AG-334 (milk) and AG-335 both of which were submitted in conjunction with PP#8F2046.

Briefly the milk method involved addition of acetone to milk after which precipitated milk solids are filtered off. The solids are reextracted with toluene and the acetone and toluene solutions are combined in a separatory funnel. The organic phase contains methidathion, its oxygen analog, methidathion sulfide, sulfone and most of the sulfoxide. The remaining sulfoxide is found in the aqueous phase and is removed by extraction with methylene chloride. The toluene fraction is evaporated to dryness and the residue redissolved in hexane.

The hexane solution is partitioned with acetonitrile and the acetonitrile layer containing the residues is taken to dryness, redissolved in toluene and analyzed via glc using a flame photometric detector operating in the sulfur mode. The dichloromethane fraction is taken to dryness and the residue redissolved in toluene and also analyzed via glc as above.

Validation data reflected fortification of milk with 0.01 ppm of each of the 4 compounds above (excluding the oxygen analog) and recoveries were 90 and 120%, 60 and 70%, 130 and 160% and 150 and 160% for methidathion, methidathion sulfide, sulfone and sulfoxide respectively. Check values were <0.01 ppm for parent, <0.005 ppm for sulfide, <0.01 ppm for sulfoxide and <0.005 to 0.006 ppm for sulfone.

The method for tissues was similar to that above. The sample is blended with acetone/water and toluene is added and the organic phase is separated. The aqueous phase is again extracted with dichloromethane and all organic phases are combined, taken to dryness and partitioned between hexane and acetonitrile. The acetonitrile layer is evaporated to dryness and the residue is dissolved in toluene and further cleaned-up on a silica gel column. The eluates are taken to dryness redissolved in acetone and analyzed via glc using a flame photometric detector in the sulfur mode as above.

No validation data for sulfoxide or sulfone in tissues was submitted.

With respect to the initial analyses of the tissues and milk for methidathion and its oxygen analog, all tissue samples at the 14.2 or 72 ppm feeding levels were <0.01 ppm for either compound. Several milk samples showed oxygen analog residues ranging from 0.01 to 0.04 ppm at the 72 ppm feeding level. All values for parent compound were <0.01 ppm.

Based on the information above we conclude that appropriate tolerance levels for methidathion and metabolites in meat and milk would be 0.15 ppm and 0.10 ppm respectively. The tolerance regulation should be proposed in terms of methidathion, its oxygen analog, and methidathion sulfoxide and sulfone. These proposals should be submitted in a revised Section F. The tolerance of 0.15 and 0.05 ppm for poultry and eggs respectively should also be proposed in the same terms as for meat and milk above. The petitioner should be so informed. The petitioner should also be informed that successful completion of a method trial will be needed before any tolerances could be established. This method trial will be initiated at such time as deficiencies in the petition are resolved.

regulation and levels for combined residue
its metabolites in these commodities are

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Conclusions

- 1) The metabolism of methidathion in animals is adequately understood. The terminal residue of concern will consist of methidathion, its oxygen analog and methidathion sulfoxide and sulfone.
- 2) Tolerances are needed for combined residues of methidathion and its metabolites in meat, milk, poultry and eggs. Appropriate levels would be as follows:

meat	0.15 ppm
milk	0.10 ppm
poultry	0.15 ppm
eggs	0.05 ppm

These proposals should be submitted in a revised Section F.

- 3) The tolerance regulation should be proposed in terms of combined residues of the parent and metabolites named in conclusion 1 above. The new tolerance proposals including the previously proposed 12 ppm level for alfalfa should be submitted in a revised Section F.
- 4) A method trial for meat and milk must be successfully completed before any proposed tolerances could be established. This method trial will be initiated at such time as the other deficiencies in the petition are resolved.

Recommendations

We recommend that the proposed tolerances not be established for the reasons given in conclusions 2 and 3 above. Requirements for resolution of these deficiencies are also discussed in the appropriate conclusion above. The petitioner should also be informed of the method trial requirement discussed in conclusion 4.

Note to the Product Manager: When and if the meat, milk, poultry and egg tolerances are established, 40 CFR 180.298 should be modified to include a paragraph (b) in which the tolerance regulation and levels for combined residues methidathion and its metabolites in these commodities are expressed.

TS-769:RCB:R.Perfetti:MCH:CM#2:RM810:X77484

cc: R.F., Circu., R. Perfetti, Thompson, TOX, EEB, EFB, FDA,
PP#7F1983

RDI: Quick, 4/7/82; Schmitt, 4/7/82

END