DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §84-2; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 2-01-35 I; formerly 1-01-35 I (MRID 45710213)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** (*Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 081901 **DP BARCODE:** 301496
TXR#: 0052493 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Chlorothalonil technical (98.74% a.i., Batch # 46/87)

SYNONYMS: 2,4,5,6 Tetrachloro-1,3-benzenedicarbonitrile

CITATION: Forster, R. (1988) Reverse mutation in *Salmonella typhimurium*. Life Science Research, Rome Toxicology Centre S.p.A., Pomezia (Rome), Italy. Laboratory Report No.: LSR-RTC 128006-M-10587, May 6, 1988. MRID 45710213. Unpublished.

SPONSOR: Vischim S.R.L., Via Friuli, 55, Cesano Maderno (Milan), Italy

EXECUTIVE SUMMARY - In two independent reverse gene mutation assays in bacteria (MRID 45710213), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to Chlorothalonil technical (98.74% a.i., Batch # 46/87) in dimethylsulfoxide at concentrations of 0, 3.13, 6.25, 12.5, 25, or 50 µg/plate in the presence of mammalian metabolic activation (+S9) and 0, 1.56, 3.13, 6.25, 12.5, or 25 µg/plate in the absence of metabolic activation (-S9). The standard plate incorporation method was performed (+/-S9). Standard strain-specific mutagens served as positive controls.

Chlorothalonil technical was tested up to cytotoxic concentrations. In the presence of mammalian metabolic activation, cytotoxicity (as indicated by reduction in number of revertants or thinning of the background lawn) was observed at >=25 µg/plate (TA1537) and at 50 µg/plate (TA98, TA100, TA1535, and TA1538). In the absence of mammalian metabolic activation, cytotoxicity was observed at >=12.5 µg/plate (TA100, TA1535 and TA1537) and at 25 µg/plate (TA98 and TA1538). No marked increases in the number of revertants were observed at any concentration in any strain in either trial. The positive controls induced marked increases in

revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE - Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlorothalonil technical

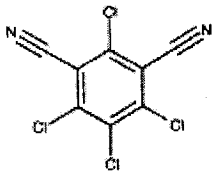
Description: White microcrystalline powder

Batch #: 46/87

Purity (w/w): 98.74% a.i.

CAS # of TGAI: 1897-45-6

Structure:



Solvent used: Dimethylsulfoxide (DMSO)

2. Control materials

Negative - The solvent alone and untreated cultures served as negative controls.
Solvent - DMSO (0.1 mL/plate)
Positive

Non-activation

Sodium azide (in distilled water)	1 µg/plate	TA100, TA1535
2-Nitrofluorene (in DMSO)	2 µg/plate	TA98, TA1538
9-Aminoacridine (in DMSO)	50 µg/plate	TA1537

Activation

2-Aminoanthracene (in DMSO)	1 µg/plate	TA98, TA100, TA1535, TA1537, TA1538

3. Activation - The S9 fraction was derived from young male Sprague-Dawley rats (Charles River, Como, Italy) weighing approximately 200-250 g:

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the study laboratory and stored at -80°C prior to use. Each batch was checked for protein content; aminopyrene demethylase activity, sterility, and efficacy. The S9 fraction was mixed with the following cofactors to make the S9 mix: glucose-6-phosphate (100 mM), NADP (100 mM), $MgCl_2$ (100 mM), KCl (330 mM), phosphate buffer (200 mM) at pH 7.4, and distilled water. The final S9 culture concentration was approximately 1.9%.

4. Test organisms - *S. typhimurium* and *E. coli* strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537	X	TA1538		WP2 *uvrA*		WP2 (pKM101)

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	X	Yes		No

5. Test compound concentrations used

Preliminary cytotoxicity assay - All strains

Non-activated conditions: 25, 79, 250, 790, or 2500 µg/plate (Trial 1); 0.250, 0.791, 2.5, 7.9, or 25 µg/plate (Trial 2)

Activated conditions: 25, 79, 250, 790, or 2500 µg/plate (Trial 1); 0.250, 0.791, 2.5, 7.9, or 25 µg/plate (Trial 2)

Mutagenicity assay - All strains

Non-activated conditions: 0, 1.56, 3.13, 6.25, 12.5, or 25 µg/plate (Trials 1 and 2)

Activated conditions: 0, 3.13, 6.25, 12.5, 25, or 50 µg/plate (Trials 1 and 2)

All concentrations of the test article and positive controls were plated in triplicate, both in the presence and absence of S9-activation, for each tester strain (*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538). Solvent and untreated controls were plated in triplicate both in the presence and absence of S9.

B. TEST PERFORMANCE

1. Type of assay

x standard plate test
_ pre-incubation (_ minutes)
_ "Prival" modification *(i.e. azo-reduction method)*
_ spot test
_ other

2. Protocol - Two independent mutagenicity trials, each using the standard plate incorporation method were conducted both in the presence and absence of S9. Prior to plating, inocula of the tester strains were cultured in nutrient broth overnight at 37°C. Bacteria (0.1 mL); test compound, solvent, or positive control (0.1 mL); and 0.5 mL of S9 mix (for tests requiring metabolic activation) or phosphate buffer were added to 2 mL of melted top agar supplemented with histidine-biotin solution. The top agar components were thoroughly mixed and poured into triplicate plates containing solidified minimal agar. After solidification, the plates were inverted and incubated for approximately 72 hours at 37°C. After incubation, the plates were scored (or held at 4°C prior to scoring) for number of revertant colonies using an automatic counting system (Artek Model 890).

3. Statistical analysis - A regression analysis of the data was performed.

4. Evaluation criteria

Assay validity - The assay validity criteria were not provided; however, typically the following criteria indicate a valid experiment:

- The negative control plates showed regular background growth.
- The spontaneous reversion rates in the negative controls were within the historical control range.

- The positive controls induced clear increases in revertant colonies compared to solvent controls.

Positive result - The test article was considered to be mutagenic if the following criteria were met:

- The mean number of revertants was more than 2x the solvent control rate in any strain at two consecutive concentrations or at the last non-toxic concentration.
- A concentration-related increase in the number of revertants was observed.
- The results were reproducible.

II. REPORTED RESULTS

The test substance was soluble in DMSO at 25 mg/mL. Therefore, 2500 µg/plate was selected as the maximum concentration for the preliminary toxicity test. Dose formulations were not analyzed for actual concentrations.

A. PRELIMINARY CYTOTOXICITY ASSAY - Precipitation was observed at ≥790 µg/plate (±S9). In two trials (-S9), toxicity was observed at concentrations ≥25 µg/plate in all strains and ≥2.5 µg/plate in TA1537. In two trials (+S9), toxicity was observed at concentrations ≥79 µg/plate in strains TA98, TA100, and TA1535, ≥25 µg/plate in TA1537 (one trial), and ≥250 µg/plate in TA1538. Therefore, 25 µg/plate (-S9) and 50 µg/plate (+S9) were selected as the highest concentrations for the mutagenicity assays.

B. MUTAGENICITY ASSAY - The results of the mutagenicity trials were tabulated for each strain, Tables 3-12 on Study Report pages 18-27. The results of these assays were negative; therefore, copies of Table 4 (Trial 1) and Table 9 (Trial 2) for strain TA1537 are included as representative examples in the Attachment to this DER. In both trials, tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to the test article at concentrations of 0, 1.56, 3.13, 6.25, 12.5, or 25 µg/plate (-S9) and 0, 3.13, 6.25, 12.5, 25, or 50 µg/plate (+S9). Cytotoxicity (as indicated by reduction in number of revertants or thinning of the background lawn) was observed (-S9) at ≥12.5 µg/plate (TA100, TA1535 and TA1537) and at 25 µg/plate (TA98 and TA1538). Cytotoxicity was observed (+S9) at ≥25 µg/plate (TA1537) and at 50 µg/plate (TA98, TA100, TA1535, and TA1538). No marked increases in the number of revertants were observed at any concentration in any strain in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigator concluded that under the conditions of this study, Chlorothalonil technical did not induce mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 when tested up to a toxic dose level in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - Chlorothalonil technical was tested up to cytotoxic concentrations (12.5-25 µg/mL, -S9) and (25-50 µg/mL,+S9). No precipitation was observed in either trial. No marked increases in the number of revertants were observed at any concentration in any strain in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.** It should be noted that the study was conducted in 1988, when *S. typhimurium* strain TA1538 was commonly used instead of strain TA102 or *Escherichia coli* strain WP2.

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not change the conclusions of this DER:

- The dose formulations were not analyzed for actual concentrations.
- The evaluation criteria for assay validity were not provided.

ATTACHMENT

LSR-RTC Department of Genetic Toxicology
Reverse Mutation in S. typhimurium

Test Substance : CHLOROTHALONIL TECHNICAL
Strain : TA 1537 Titre : 2.00
Experiment No. : 1 Solvent: DMSO
Experiment date: 21-Jan-88 S9: 87/28

Dose-level [ug/pl]	Without metabolic activation Plate counts			Mean	S.E.	With metabolic activation Plate counts			Mean	S.E.
Untreated	23	18	30	24	3.5	30	27	35	31	2.3
0.00	16	26	23	22	3.0	24	24	30	26	2.0
1.56	26	22	19	22	2.0	-	-	-	-	-
3.13	19	17	22	19	1.5	27	27	31	28	1.3
6.25	26	19	26	24	2.3	28	25	30	28	1.5
12.5	15TT	20TT	25TT	20	2.9	19	29	24	24	2.9
25.0	MTTT	MTTT	MTTT	-	-	26T	26T	25T	26	0.3
50.0	-	-	-	-	-	23T	27T	26T	25	1.2

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.699	-0.0762	-0.25865	0.7084	0.50158
1 - 4	-	4.571	0.0279	0.16788	0.5385	0.60200
1 - 5	-	4.654	-0.0098	-0.10527	0.3817	0.70886
1 - 3	+	5.142	0.0259	0.27839	0.7669	0.46822
1 - 4	+	5.258	-0.0220	-0.30926	1.0284	0.32800
1 - 5	+	5.195	-0.0077	-0.22859	0.8466	0.41253
1 - 6	+	5.164	-0.0035	-0.21145	0.8654	0.39963

Positive and negative controls

Treatment		S9	Plate counts			Mean	S.E.
DMSO	100 ul/pl	-	16	26	23	22	3.0
9-Aminoacridine	50 ug/pl	-	151	113	357	207	75.8
DMSO	100 ul/pl	+	24	24	30	26	2.0
2-Aminoanthracene	1 ug/pl	+	132	104	C	118	14.0

ARTEK calibration factors: A = 0.5716 B = 1.1220

LSR-RTC Department of Genetic Toxicology
Reverse Mutation in S. typhimurium

Test Substance : CHLOROTHALONIL TECHNICAL
Strain : TA 1537 Titre : 2.11
Experiment No. : 2 Solvent: DMSO
Experiment date: 8 APRIL 88 S9: 88/2

Dose-level [ug/pl]	Without metabolic activation Plate counts			Mean	S.E.	With metabolic activation Plate counts			Mean	S.E.
Untreated	13	22	18	18	2.6	23	28	18	23	2.9
0.00	12	10	27	16	5.4	20	29	31	27	3.4
1.56	25	18	20	21	2.1	-	-	-	-	-
3.13	13	15	10	13	1.5	26	19	15	20	3.2
6.25	26	25	14	22	3.8	26	24	24	25	0.7
12.5	17TT	16TT	16TT	16	0.3	23	18	28	23	2.9
25.0	MTTT	MTTT	MTTT			18	26	22	22	2.3
50.0	-	-	-	-	-	7T	12T	10T	10	1.5

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.210	-0.1213	-0.21540	0.5836	0.57780
1 - 4	-	3.974	0.0706	0.22268	0.7223	0.48665
1 - 5	-	4.117	0.0055	0.03646	0.1315	0.89736
1 - 3	+	4.935	-0.0269	-0.13787	0.3683	0.72354
1 - 4	+	4.899	-0.0123	-0.11697	0.3724	0.71733
1 - 5	+	4.884	-0.0089	-0.16832	0.6157	0.54873
1 - 6	+	5.077	-0.0348	-0.76569	4.7617	0.00021

Positive and negative controls

Treatment		S9	Plate counts			Mean	S.E.
DMSO	100 ul/pl	-	12	10	27	16	5.4
9-Aminoacridine	50 ug/pl	-	106	126	189	140	25.0
DMSO	100 ul/pl	+	20	29	31	27	3.4
2-Aminoanthracene	1 ug/pl	+	135	135	103	124	10.7

ARTEK calibration factors: A = 0.5716 B = 1.1220

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