4/20/86

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

005040

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: H. Jacoby, PM # 21
Fungicide-Herbicide Branch
Registration Division TS-767C

FROM: D. Ritter, Acting Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C

DR 4-20-86

Subject: Chlorothalonil: EPA ID # 50534-7; Review of additional toxicity data submitted in response to the Registration Standard.

Pilot studies for a 21 day dermal toxicity study in rabbits were submitted. These will be reviewed when the completed study is received. We noted no unusual or unreasonable adverse effects in these studies.

Two mutagenicity studies were reviewed by Dr. Chen; these are attached. There was no evidence of mutagenic activity in either of these Ames bacterial assays.

A pilot study designed to elucidate the effects of probencid on the renal tubular excretion was submitted. We will await receipt of the full study before commenting fully; however, the results suggest that probenicid inhibits active urinary excretion of chlorothalonil metabolites. We have attached the sponsor's abstract of this study.

Attachments

Study 1: Salmonella/Mammalian-Microsomal Plate Incorporation Assay With and Without Renal Activation With 2,5-Dichloro-4,6-Bismercaptoisophthalonitrile (SDS-3939) SDS Biotech Corp. Document No. 694-5TX-85-0042-002 (Authors: M. Mizens, J.C. Killeen, and J.A. Ignatoski), October 22, 1985. Accession No. 260841

Objective:

To evaluate the mutagenicity of SDS-3939 (a potential metabolite of Chlorothalonil) in the Ames test with and without renal metabolic activation. From the previous toxicology studies, one of the target organs for Chlorothalonil has been shown to be the kidney. Thus, rat kidney microsomes were used to better relate the results with this target organ. Although this is not required by the Chlorothalonil Registration Standard, this was conducted to better understand the potential effects associated with metabolites.

Procedure:

The mutagenic activity of SDS-3939 dissolved in acetone at predetermined concentrations (i.e. 100, 500, 2500, 5000, and 10,000 ug/plate under the activated assay system and 40, 200, 1000, 2000, and 4000 ug/plate under the nonactivated assay system) was evaluated by the Ames test (Mutation Res. 31:347-364, 1975) in the presence and absence of exogenous metabolic activation derived from Fischer 344 rat kidneys. Five histidine-requiring strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, and TA100) were obtained directly from Dr. Bruce Ames. All cultures were harvested by monitoring optical density prior to testing (1 to 2 x 10^9 cells/mL). The in vitro mammalian metabolic activation system consisted of kidney microsomal enzymes (S9 homogenate) from the Aroclor 1254 induced male Fischer rats and cofactor solution described by Ames. Mutations were quantified on triplicate plates for each strain by counting His^+ revertant colonies after 48 hours of incubation at 37 °C on a histidine deficient agar. If the compound is mutagenic, it would demonstrate at least twofold increase over the control value and also exhibited a dose-related increase in the number of histidine-independent colonies. Positive controls and solvent control were run concurrently with the test compound in this study.

Results:

(A) Preliminary Cytotoxicity Determination of SDS-3939

Treatment		W/Renal Activation TA100 Colonies/ Plate	W/O Renal Activation TA100 Colonies/ Plate
Acetone	50 ul/plate	106	85
SDS-3939	10 ug/plate	113	98
	33 do.	88	102
	67 do.	85	95
	100 do.	92	94
	333 do.	99	84
	667 do.	82	91
	1000 do.	102	85
	3333 do.	109	60*
	6667 do.	116	34
	10,000 do.	90	0

*Starting point of toxicity to the strain TA100.

Cytotoxicity Findings: Based on the results of this preliminary toxicity study with various concentrations of SDS-3939 with and without metabolic activation, the concentrations of 4000 ug/plate and 10,000 ug/plate were selected as the maximum concentrations for the nonactivated assay and the activated assay, respectively.

(B) Mean Number of His^+ Revertant Colonies per Plate

Treatment	Per Plate Conc.	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA1535 -S9	TA1535 +S9	TA1537 -S9	TA1537 +S9	TA1538 -S9	TA1538 +S9
Acetone	50 ul	16	30	88	104	15	14	8	13	7	11
Positive Controls											
2-Nitrofluorene	5 ug	687*	-	-	-	-	-	-	-	956*	-
Sodium Azide	5 "	-	-	1051*	-	1001*	-	-	-	-	-
9-Aminoacridine	75 "	-	-	-	-	-	-	506*	-	-	-
2-Aminoanthracene	4 "	-	2642*	-	3237*	-	1281*	-	410*	-	1973*
SDS-3939											
do.	40 "	24	-	92	-	17	-	-		5	-
do.	200 "	17	-	97	-	20	-	8	-	10	-
do.	1000 "	19	-	102	-	17	-	8	-	9	-
do.	2000 "	18	-	90	-	16	-	6	-	8	-
do.	4000 "	19	-	85	-	13	-	8	-	8	-
do.	100 "	-	33	-	102	-	9	-	19	-	15
do.	500 "	-	34	-	104	-	14	-	20	-	13
do.	2500 "	-	25	-	110	-	13	-	21	-	14
do.	5000 "	-	31	-	110	-	17	-	16	-	13
do.	10,000 "	-	32	-	111	-	12	-	11	-	2

*Significantly different from the solvent control: greater than twofold increase over the solvent control value.

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Findings:

1) The spontaneous revertant colonies for each of these five strains of Salmonella typhimurium were found within the normal range of His^+ revertant colonies recommended by the Ames test (Mutation Res. 31:347-364, 1975).

2) The strain-specific control compounds (2-nitrofluorene, sodium azide, and 9-aminoacridine) and the positive control compound to ensure the efficacy of the activation system (2-aminoanthracene) have given the strongly positive responses as expected.

3) No significant increases in the number of revertant colonies for any test strain were observed following exposure to the test compound (SDS-3939) in either the presence or absence of metabolic activation.

Evaluation:

Under the test conditions reported, the assay was conducted in a manner to generate valid results. SDS-3939 (90.5% purity) was not mutagenic in the Ames test either with or without renal metabolic activation at the concentrations tested. This study is acceptable.

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Study 2: Salmonella/Mammalian-Microsomal Plate Incorporation Assay With and Without Renal Activation with 5-(2,4-Dicyano-3,5,6-trichlorophenyl)glutathione (SDS-66382) SDS Biotech Corp. Document No. 694-5TX-85-0043-002 (Authors: M. Mizens, J.O. Killeen, and J.A. Ignatoski), June 24, 1985. Accession No. 260841.

Objective:

To evaluate the mutagenicity of SDS-66382 (a potential metabolite of Chlorothalonil) in the Ames test with and without renal metabolic activation. (Also, see the reasons for using renal activation in this study described in the Ames test with SDS-3939.)

Procedure:

The procedure used in this study was identical to that described in the Ames test with and without renal activation with SDS-3939. The following five concentrations of SDS-66382 were selected for the mutation assay both with and without activation: 100, 500, 2500, 5000, 10,000 ug/plate.

Results:

(A) Preliminary Cytotoxicity Determination of SDS-66382

Treatment		W/Renal Activation TA100 Colonies/ Plate	W/O Renal Activation TA100 Colonies/ Plate
Acetone	10 ul/plate	82	83
SDS-66382	10 ug/plate	80	84
	33 do.	106	100
	67 do.	94	93
	100 do.	83	101
	333 do.	82	99
	667 do.	83	95
	1000 do.	83	100
	3333 do.	79	77
	6667 do.	70	104
	10000 do.	72	72

Findings: Because of a lack of toxicity, the maximum dose level of the mutagenicity assay was selected to be 10,000 ug of SDS-66382 per plate with and without metabolic activation.

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(B) Mean Number of His^+ Revertant Colonies Per Plate

Treatment	Conc. Per Plate	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA1535 -S9	TA1535 +S9	TA1537 -S9	TA1537 +S9	TA1538 -S9	TA1538 +S9
Acetone	10 ul	17	25	80	95	29	16	5	12	9	19
Positive Controls											
2-Nitrofluorene	5 ug	820*	-	-	-	-	-	-	-	1374*	-
Sodium Azide	5 do.	-	-	1095*	-	1058*	-	-	-	-	-
9-Aminoacridine	75 do.	-	-	-	-	-	-	-	-	-	-
2-Aminoanthracene	4 do.	-	228!*	-	2466*	-	244*	-	560*	-	2295*
SDS-66382	100 do.	17	29	86	92	22	13	8	11	10	19
	500 do.	14	27	75	86	22	14	10	16	13	17
	2500 do.	12	27	78	88	21	18	8	11	9	11
	5000 do.	18	19	56	70	24	13	10	7	8	15
	10,000 do.	10	23	57	58	27	20	5	9	10	11

*Significantly different from the solvent control: greater than twofold increase over the solvent control value.

Findings:

1) The spontaneous revertant colonies for each of these five strains of Salmonella typhimurium were found within the normal range of His^+ revertant colonies recommended by the Ames test (Mutation Res. 31:347-364, 1975).

2) The strain-specific control compounds (2-nitrofluorene, sodium azide, and 9-aminoacridine) and the positive control compound to ensure the efficacy of the activation system (2-aminoanthracene) have given the strongly positive responses as expected.

3) No significant increases in the number of revertant colonies for any test strain were observed following exposure to the test compound (100 through 10,000 ug/plate) in either the presence or absence of metabolic activation.

Evaluation:

The assay was conducted in a manner to generate valid results. SDS-66382 (97.5% purity) was not mutagenic at the concentrations tested either with or without renal metabolic activation. This study is acceptable.

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