

KB-1351
TR-6658



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

006698

APR 26 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: DCNA; Review of Ames Assay

TO: Lois Rossi, PM 21
Registration Division (TS-767)

FROM: Margaret L. Jones *M. L. Jones 19 April 1988*
Review Section III
Toxicology Branch
Hazard Evaluation Division

THROUGH: Marcia van Gemert, Ph.D., Head
Review Section III *M. van Gemert 4/21/88*
Toxicology Branch

and Theodore M. Farber, Ph.D., Chief
Toxicology Branch *Lois Rossi 4/21/88*

Tox. Chem: 311 Record No: 215047

Accession No: 405088-01 Tox. Project No: 8-0583

Registrant: Nor-Am

Action Requested: Review the submitted Ames Assay to support the registration of DCNA (technical dicloran).

Conclusions: DCNA (technical dicloran) was demonstrated to be mutagenic when tested to the limits of solubility in dimethylsulphoxide with and without metabolic activation.

174

006698

DATA EVALUATION REPORT

Reviewed By: Margaret L. Jones *M. L. Jones 19 April 1988* Tox. Project No. 8-0583
Review Section III
Toxicology Branch
Hazard Evaluation Division

Approval: Marcia van Gemert, Ph.D., Head
Review Section III *m. van Gemert 4/22/88*
Toxicology Branch

and Kerry Dearfield, Ph.D. *Kerry Dearfield 4.19.88*
Scientific Mission Support Staff
Toxicology Branch (TS-769C)

Chemical: DCNA; Technical dicloran Caswell No.: 311
Record No.: 215047 Guideline: 84-2
Accession No.: 405088-01 MRID: N/A
Study Type: Ames Assay CAS No.: not available

Citation: T 103 Technical Dicloran: Ames bacterial mutagenicity test

Authors: Jones, E., Fenner, L.A.

Sponsor: Nor-Am

Testing Lab: Schering Agrochemicals Limited (formerly FBC Limited)
Saffron Walden, Essex, England

Study Nos: TOX/87/199-85; SMS 44/87647; TOX 87222

Study Date: 7/87

Toxicology Branch Conclusions:

Technical dicloran was demonstrated to be mutagenic in a dose-dependent manner in Salmonella strains TA 98 and TA 1538 with and without metabolic activation and TA 100 without activation at concentrations from 500 to 5000 ug/plate in replicate assays. Positive controls demonstrated the sensitivity of the tester strains.

Recommendation: Acceptable

Detailed Review

Test Compound: Technical dicloran; Batch # CR 20642/3; yellow powder; Purity 97.5 ± 1.0% w/w (analysis no. T00202) dissolved in dimethylsulphoxide (DMSO); technical stored in dark at room temperature; Untreated group included bacteria with buffer or S-9 mix without solvent or test compound.

2

Dose Selection: Dose range finding performed using 5000, 500, 50, and 5 ug/plate. Technical dicloran was found not to be toxic to tester strains tested up to 5000 ug/plate where precipitation was observed. Therefore, 5000 ug/plate was selected as the high dose for the assay.

Procedure: The procedure was an in vitro assessment of the mutagenic potential of technical dicloran in which strains of Salmonella typhimurium (TA 1535, TA 1537, TA 1538, TA 98, TA 100, histidine-dependent auxotrophic mutants of S. typhimurium) were exposed to 0, 50, 150, 500, 1500, 5000 ug/plate of the test compound diluted in dimethyl sulphoxide. The solvent was also used as negative control.

The assays were done in the presence and absence of metabolic activation using liver extracts of rats treated with Aroclor 1254 (S-9).

In the preliminary range finding study using 5000, 500, 50, and 5 ug/plate, some precipitation was observed at the high dose. This was then selected to be the high dose for the full assay.

Untreated groups consisted of bacteria with buffer (sodium phosphate, pH 7.4) or with S-9 mix and without solvent or test compound.

Positive controls with S-9 mix were 2-aminoanthracene (AA) at 2 ug/plate for strains TA 1535 and TA 1537 and AA at 0.5 ug/plate for strains TA 1538, TA 98 and TA 100. Positive controls without S-9 mix were 2-nitrofluorene (NF) at 2 ug/plate for strain TA 1538, and NF at 1 ug/plate for strain TA 98, 9-aminoacridine (9-AC) at 80 ug/plate for strain TA 1537, N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) at 5 ug/plate for strain TA 1535 and ENNG at 3 ug/plate for strain TA 100.

Cultures of S. typhimurium (5 tester strains as indicated above) were incubated for 72 hours at 37°C with 5 concentrations of test compound between 5000 and 0 ug/plate and negative control (solvent only), with and without metabolic activation in replicate experiments (3 plates each per strain per dose per replicate with and without S-9 with additional plate as a negative control).

The authors assess as positive evidence of mutagenic potential the following observations: a. statistically significant dose-related increase in the number of revertant colonies in two separate experiments, and b. the increase in number of revertant colonies is at least twice the concurrent solvent control value.

Results: Results are summarized in the composite table which follows. At doses of 5000 ug/plate precipitation was observed. Increases in the number of revertant colonies as compared to solvent controls were seen in both replicates for strains TA 1538 and TA 98 with and without metabolic activation and for strain TA 100 without metabolic activation.

Toxicology Branch Evaluation: Technical dicloran was found to be mutagenic in a dose-dependent manner beginning at doses of 500 ug/plate and above in three of five tester strains of S. typhimurium, with and without S-9 in two strains and without S-9 in one strain. No increased mutant frequencies were seen in strains TA 1535 and TA 1537 with and without activation.

Ames Assay with Technical Dicloran

[Adapted from Tables 2,3,4,5 from Report No. SMS 44/87647]

Assay 1 Doses (ug/plate)	TA 1538		TA 98		TA 100	
	-S9	+S9	-S9	+S9	-S9	+S9
5000	33	37	48	47	185	86
1500	21	19	71	65	166	93
500	16	8	50	35	109	87
150	15	11	47	22	87	84
50	10	7	29	21	80	96
0	9	8	29	16	93	93
Solvent	11	10	29	21	74	89
Positive control	73	123	89	153	238	221
	(NF)	(AA)	(NF)	(AA)	(ENNG)	(AA)

Assay 2 Doses (ug/plate)	TA 1538		TA 98		TA 100	
	-S9	+S9	-S9	+S9	-S9	+S9
5000	51	45	84	89	199	96
1500	29	42	76	73	154	86
500	29	22	60	40	95	96
150	24	10	38	27	86	78
50	11	10	25	19	93	88
0	8	9	33	21	74	95
Solvent	10	10	34	24	84	84
Positive control	83	189	122	179	454	485
	(NF)	(AA)	(NF)	(AA)	(ENNG)	(AA)

NF = 2 nitrofluorene

AA = 2-aminoanthracene

ENNG = N-ethyl-N'-nitro-N-nitrosoguanidine

4