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August 28, 1989

Document Processing Center (TS-790) NM L-100
Office of Toxic Substances
Environmental Protection Agency
401 M Street S.W.
Washington, D. C. 20460

Attn: 8(e) Coordinator

Dear Sir or Madam:

The enclosed test report entitled "Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of CD-4" is being provided as follow-up information to a previous section 8(e) submission on 2-((4-amino-3-methylphenyl)-ethylamino) ethanol sulphate. The EPA Document Control Number is 8EHQ-1185-0575. Please add this report to the existing file on the subject chemical.

We have no information on this subject other than the enclosed test report.

Sincerely,

R. Hays Bell

R. Hays Bell, Ph.D.
Vice President, Corporate Relations
~~Director, Health and Environment Laboratories~~

RHB;WLN:jfal073i
Enc.

cc: Dr. J. Loranger OTS

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~~CONFIDENTIAL~~

AGF 135/89299

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AMES METABOLIC ACTIVATION TEST TO ASSESS THE POTENTIAL MUTAGENIC EFFECT OF CD-4

NOTE

This report is considered by the Study Director to be the 'final draft' and has been submitted to the HRC Quality Assurance Department for Audit.

The sponsor is requested to review this document and communicate any comments to the Study Director as soon as possible. When these comments have been received and on completion of the QA audit, the FINAL REPORT containing Study Director and QA Statements will be issued.

PLEASE NOTE

In compliance with GLP any changes to the final report after the date of issue will be in the form of a separate amendment to the report.

Date: 30 March 1989 V.1

Addressee.

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BACTERIAL REVERSE GENE MUTATION ASSAY - THE AMES TEST

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The object of this study is to assess the mutagenic potential of the test material in a bacterial system.

COMPOUND: CD-4.

Alternative name: Ethanol,2-((4-amino-3-methylphenyl) ethylamino)-Sulphate (1:1).

Batch number: Information not supplied by Sponsor.

Purity: More than 99%.

Storage conditions: Stored at room temperature.

Expiry date: Information not supplied by Sponsor.

Appearance: Grey powder.

METHOD: The method used is as described in Appendix 1.

The experiments described in this report were carried out between 20 January 1989 and 2 February 1989.

Solvent: Water.

Dose levels: Dose range finding test: 5000, 500, 50, 5 µg/plate.

Mutation tests: 1500, 500, 150, 50, 15 µg/plate.

Protocol approval signed by:

Study Director - E. Jones - 1 December 1988.

HRC Management - Dr. D.H. Christopher - 1 December 1988.

Sponsor - H.W. Koafeld 19 December 1988.

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RESULTS

The revertant colony counts for CD-4 obtained in the dose range finding test are shown in Table 1. CD-4 was toxic towards the tester strains at the highest dose level. Therefore 1500 µg/plate was chosen as the top dose level in the mutation tests.

The mean number of revertant colonies, together with the individual plate counts for CD-4 obtained in the first mutation test with tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 are shown in Table 2. Positive control mutability checks are shown in Table 3.

The mean number of revertant colonies, together with the individual plate counts for CD-4 obtained in the second mutation test with tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 are shown in Table 4. Positive control mutability checks are shown in Table 5.

In both mutation tests, a statistically significant increase in revertant colony numbers was observed with tester strain TA 100 following treatment with CD-4 both in the presence and absence of metabolic activation.

CONCLUSIONS AND COMMENTS

It is concluded that CD-4 shows reproducible evidence of slight mutagenic activity when tested in this bacterial system.

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TABLE 1

Dose range finding test on CD-4 - revertant colony numbers obtained with bacterial strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Dose level (µg/plate)	Metabolic activation	Bacterial strains				
		TA 1535	TA 1537	TA 1538	TA 98	TA 100
5000	-	NL	NL	NL	NL	NL
500	-	14	SC	12	16	189
50	-	11	17	9	17	102
5	-	13	22	15	21	99
Solvent	-	14	16	11	26	102
5000	+	IL	NL	IL	IL	NL
500	+	8	14	13	22	176
50	+	6	15	10	24	92
5	+	10	7	9	21	87
Solvent	+	10	9	8	17	77

- Absence
+ Presence
NL No bacterial lawn
IL Incomplete bacterial lawn
C Contamination

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TABLE 2

Test I

CD-4 - revertant colony numbers obtained per plate using bacterial strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Dose level (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	1500	-	7	1.2	8,6,8
	500	-	12	1.5	13,12,10
	150	-	9	3.0	6,12,9
	50	-	9	2.5	12,9,7
	15	-	10	2.0	12,8,10
	0	-	12	2.3	13,13,9
	Solvent	-	12	4.2	11,9,17
	1500	+	11	5.5	17,8,7
	500	+	9	2.1	8,7,11
	150	+	8	2.3	7,11,7
	50	+	10	0.0	10,10,10
	15	+	10	2.5	13,8,10
	0	+	9	1.0	9,10,8
	Solvent	+	12	2.0	12,10,14
TA 1537	1500	-	7	1.2	8,6,8
	500	-	7	2.1	9,5,8
	150	-	8	2.5	11,8,6
	50	-	10	3.2	12,6,11
	15	-	13	2.6	14,10,5
	0	-	10	2.1	12,8,9
	Solvent	-	11	2.5	14,11,9
	1500	+	10	1.7	9,9,12
	500	+	13	4.2	14,8,16
	150	+	10	3.5	6,12,12
	50	+	11	1.2	12,10,10
	15	+	10	4.0	8,15,8
	0	+	12	3.2	11,16,10
	Solvent	+	13	2.0	15,13,11
TA 1538	1500	-	7	0.6	7,8,7
	500	-	10	1.5	11,8,10
	150	-	8	2.0	10,6,8
	50	-	10	1.2	11,11,9
	15	-	9	3.6	13,6,8
	0	-	8	1.5	10,8,7
	Solvent	-	10	2.5	10,7,12
	1500	+	12	1.0	12,13,11
	500	+	10	2.0	10,8,12
	150	+	12	2.1	11,14,10
	50	+	11	1.7	13,10,10
	15	+	9	2.1	11,7,8
	0	+	8	0.6	7,8,8
	Solvent	+	11	2.9	13,8,13

\- Absence
\+ Presence
SD Standard deviation

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TABLE 2

(continued)

Strain	Dose level (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts
TA 98	1500	-	-	-	NL,NL,NL
	500	-	14	2.3	15,11,15
	150	-	21	4.0	17,21,25
	50	-	21	4.2	24,16,22
	15	-	21	3.5	21,18,25
	0	-	20	1.5	21,18,20
	Solvent	-	23	3.6	19,26,24
	1500	+	15	4.0	11,15,19
	500	+	19	5.9	17,26,15
	150	+	20	4.0	16,24,20
	50	+	17	1.5	18,15,17
	15	+	18	1.5	16,18,19
	0	+	18	1.2	17,17,19
	Solvent	+	19	5.8	16,26,16
TA 100	1500	-	245***	24.8	217,256,263
	500	-	185***	9.1	195,177,184
	150	-	144	4.0	145,140,148
	50	-	147	5.0	152,142,147
	15	-	128	4.0	132,124,129
	0	-	124	3.8	128,121,122
	Solvent	-	133	9.0	132,142,124
	1500	+	323***	26.0	293,339,337
	500	+	200***	21.0	184,224,193
	150	+	143	8.0	135,142,151
	50	+	132	18.2	147,138,112
	15	+	123	16.5	140,123,107
	0	+	124	2.5	121,126,124
	Solvent	+	119	14.7	136,114,108

\- Absence
\+ Presence
SD Standard deviation
NL No bacterial lawn
*** P<0.001
** 0.001<P<0.01
* 0.01<P<0.05

Student's 't'- test

TABLE 3

Test 1

Mutability tests with bacterial strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Compound	Dose level (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	ENNG	5.0	-	908	28.0	885,899,939
TA 1537	9 AC	80.0	-	1332	145.8	1239,1257,1500
TA 1538	NF	2.0	-	47	4.9	45,53,44
TA 98	NF	1.0	-	74	2.1	76,73,72
TA 100	ENNG	3.0	-	462	37.3	505,443,438
TA 1535	AA	2.0	+	83	8.3	92,80,76
TA 1537	AA	2.0	+	75	10.2	79,63,82
TA 1538	AA	0.5	+	104	10.6	112,92,108
TA 98	AA	0.5	+	113	19.0	92,118,129
TA 100	AA	1.0	+	434	18.0	442,413,446

- \- Absence
- \+ Presence
- SD Standard deviation
- ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
- 9 AC 9-aminoacridine
- AA 2-aminoanthracene
- NF 2-nitrofluorene