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Project Report 47-171
10 Pages
January 16, 1985

#5

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234

Salmonella/Microsome (Ames)

Bacterial Mutagenicity Assay

Sponsor: Union Carbide Corporation
Specialty Polymers and Composites Division

Summary

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234 was tested for potential mutagenic activity using the Salmonella/microsome bacterial mutagenicity assay (Ames test). Epoxy Resin ERL-4234 was tested with and without metabolic activation in triplicate at five concentrations, ranging from 0.1 milligrams to 10 milli-grams per plate. The highest concentration was cytotoxic in the mutagenicity tests and in a preliminary test to choose appropriate doses. Mutagenic activity was not observed with any of the five bacterial strains tested with or without metabolic activation. Epoxy Resin ERL-4234 was not mutagenic in this screening test.

Sample

Chemical Name: BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234

I.D. #: T F3-98461 4193 WB

BRRC Sample #: 47-205

BRRC Account #: 84-18-18046-46

CAS #: 3388-03-02

Chemical Synonyms: ARALDITE CY175; UP612; 2-(3,4-epoxy)cyclohexylmethyl-
5,5-spiro(3,4-epoxy)cyclohexane-m-dioxane

Molecular Formula: C₁₅H₂₂O₄

Submitted by: R. J. Cotter, Bound Brook, NJ

Sponsor: R. E. Plevan, Danbury, CT
Division: Specialty Polymers and Composites
Bound Brook, NJ

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234Salmonella/Microsome (Ames)Bacterial Mutagenicity Assay

I. Introduction and Theory

The Salmonella typhimurium/microsome mutation assay is a microbial screening test which detects the ability of chemicals to cause genetic alterations in the histidine gene of selected indicator strains of Salmonella typhimurium. The indicator strains used for this test are all histidine-requiring (his-) bacteria which carry a mutation in the histidine locus. The strains were obtained from Dr. Bruce Ames, University of California, Berkeley, CA. Histidine-independent (his+) strains can arise either spontaneously or by the mutagenic action of a chemical or physical agent. Base pair mutagens cause a base change in the DNA molecule at the site of the original mutation or at a second site in the DNA which suppresses the original mutation. Frameshift mutagens cause the addition or deletion of single or multiple base pairs in the DNA molecule. The relative frequency of induced or spontaneous reversion to his+ can be quantitated by plating the indicator strains on minimal agar and counting the number of revertant colonies, representing bacteria that were able to grow and form colonies in the absence of histidine.

To test the mutagenic potential of a test chemical, the bacterial strains are exposed to several concentrations of the chemical. Tests are done both with and without the addition of a mammalian liver homogenate (S9) because some chemicals require metabolic conversion for detection of their biological effects. Concurrent solvent and positive controls are also run with each assay to determine the responsiveness of the test system. After a suitable period of incubation (48-72 hrs), direct revertant colony counts are made and evaluated. Test chemicals which produce at least a 2-fold and dose-related increase in mutant colonies over the concurrent control value are considered to be bacterial mutagens and suspect mammalian mutagens.

II. Salmonella typhimurium Mutagenicity Assay

A. Objective

The objective was to assess the mutagenic potential of Epoxy Resin ERL-4234 as measured in the Salmonella typhimurium/microsome mutagenicity assay. A more complete discussion of the theoretical basis of this test is presented in Appendix 1.

B. Test Chemical (Available analytical and physical data is attached as Appendix 2)

1. Sample name: BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234
2. BRRRC Sample Number: 47-205
3. CAS Number: 3388-03-2
4. Purity: > 95%; IR spectra consistent with structure (see Appendix 2)
5. Density: 1.1891
6. Date Received: July 25, 1984
7. Storage Conditions: Room Temperature
8. Storage Location: Chemical Hood/Genetic Toxicology Dept.

C. Control Substances

1. Solvent: dimethylsulfoxide, Burdick & Jackson, Muskegon, MI; Lot # AD912; CAS # 67-68-5
2. Positive Controls:
 - a) 4-nitro-o-phenylenediamine; BRRRC #44-71; CAS #99-56-9
 - b) 9-aminoacridine; BRRRC #44-233; CAS #90-45-9
 - c) sodium azide; BRRRC #44-72; CAS #26628-22-8
 - d) 2-aminoanthracene; BRRRC #44-67; CAS #613-13-8

D. Metabolic Activation

S9 liver homogenate, prepared from Aroclor 1254-induced, Sprague-Dawley male rats, was purchased from Microbiological Associates, Bethesda, MD. For tests with metabolic activation, 0.5 ml of S9 mix containing 50 µl of S9 was added per plate.

E. Test Protocol

The assay was performed according to BRRRC Standard Operating Procedures 7.4.1A through 7.4.7A, 7.4.12A, and 7.4.13. A general description of the test methods is attached to this report as Appendix 1.

F. Test Dates

1. Test Initiated: October 15, 1984
2. Test Completed: October 19, 1984

G. Storage Location of Materials

1. Raw Data: BRRC Archives
2. Final Report: BRRC Archives

H. Experimental Design (detailed procedures in Appendix 1)

1. Sample Preparation:

The test substance was dissolved in dimethylsulfoxide (DMSO) to a concentration of 100 mg/ml for doses of 10 mg/plate and below. The mixture was heated to 50°C to hasten dissolution. All subsequent dilutions were made in the same solvent. Dilutions of the test substance were made fresh each day of testing. All dilutions for the mutagenicity tests were gravimetrically analyzed.

2. Dose Selection:

A preliminary toxicity test was performed using strain TA100 to determine the level of toxicity of the test substance. Ten doses were tested for toxicity with a plate assay performed in the manner used for mutagenicity determinations. Toxicity was assessed at 24 to 48 hours after treatment by either growth inhibition of the background lawn or a reduction in the number of spontaneous mutants.

3. Testing:

The test chemical was tested in triplicate at five doses chosen to span a range which included moderately toxic to relatively nontoxic concentrations. If the substance was nontoxic in the preliminary toxicity test, 100 µl of liquid or 50 mg of solid was used as the maximum dose, unless limited by solubility. Testing was performed both with and without metabolic activation. Concurrent solvent and positive controls were run in each test.

I. Quality Control

Data from each test is checked for accuracy and integrity by a second investigator not involved with the study. Compliance of testing procedures with Good Laboratory Practices is audited by the Independent Quality Assurance department at the Bushy Run Research Center.

III. Results

Epoxy Resin ERL-4234 was tested in a preliminary toxicity screen at 50, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001 milligrams per plate with strain TA100 only. The test chemical was treated as a solid because of its high viscosity. The top 2 doses of 50 and 10 mg per plate precipitated in the top agar and a slight precipitate formed at 3 mg/plate. The top dose of 50 mg per plate inhibited all growth of the background lawn and 10 mg per plate permitted only sparse growth of the background lawn. Based on these results, mutagenicity testing was done with 5 doses of 10, 3, 1, 0.3, and 0.1 milligrams per plate in triplicate. The highest dose was expected to produce some degree of cytotoxicity based on preliminary toxicity test results, observed as either a reduction in the number of revertant colonies or an inhibition of growth of the background lawn. The range of doses that was tested followed the exposure concentration recommendations of the U.S. E.P.A. Health Effects Test guidelines (HG Gene Muta-S. typhimurium, August 1982).

Gravimetric analysis of the highest test substance dilutions for each mutagenicity test indicated no more than a 1.3% error from the stated concentrations. Gravimetric analysis of the lowest two dilutions in the test without metabolic activation indicated errors > 10% from the intended concentrations. Therefore, the intended doses of 0.1 and 0.3 mg/plate are restated in Table 1 as the actual doses of 0.09 and 0.27 mg/plate. The remaining dilutions for the test without metabolic activation had no more than a 9.4% error from the stated concentrations. All subsequent dilutions for the test with metabolic activation had no more than a 1.5% error from the stated concentrations.

All plate counts and the respective means and standard deviations are shown in Table 1 (without activation) and in Table 2 (with activation). No indication of mutagenicity was observed at any of the tested doses, either by evidence of a dose-response relationship or a doubling of the number of colonies over the solvent control.

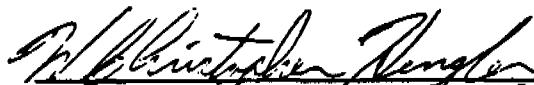
Dose selection appeared to be in a suitable range in the mutagenicity tests because some toxicity was evident in both tests. In both tests, toxicity was observed at 10 mg/plate with all strains.

All strains exhibited a positive mutagenic response with the positive controls tested both with and without S9 metabolic activation. Negative (solvent) controls were also tested with each strain, and the spontaneous reversion rates were within the historical ranges at this laboratory (see Appendix 3). All positive and negative controls were run concurrently with the test chemical. Concurrently run sterility checks showed that the S9 mix, PBS, the test chemical and all solvents and controls were sterile.

IV. Conclusion

Epoxy Resin ERL-4234 did not produce a dose-dependent mutagenic response in any of the Salmonella typhimurium strains. Under the conditions of this assay, Epoxy Resin ERL-4234 was not mutagenic in the Salmonella/microsome mutagenicity assay.

Reviewed and Approved by:

 1/2/85
W. Christopher Hengler, M.S., M.B.A. Date
Study Director

 1/2/85
Ronald S. Slesinski, Ph.D. Date
Manager, Genetic, Biochemical, & Acute Toxicology

 1/15/85
Fred R. Frank, Ph.D. Date
Director

Contributors:

Technical Assistance: Eileen R. Morabit, A.S.
Senior Technologist

WPC/rkk/0604B-2
11-01-84

TABLE 1
RESULTS OF THE SALMONELLA MUTAGENICITY ASSAYCHEMICAL: BRRC #47-205, Epoxy Resin ERL-4234 WITHOUT ACTIVATION
EXPERIMENT DATE: October 15 through October 18, 1984 SOLVENT: DMSO

TEST AGENT	DOSE PER PLATE	PLATE COUNTS			AVERAGE	STANDARD DEVIATION
		1	2	3		
STRAIN TA98						
SOLVENT	110 MG	14	19	21	18	3.6
POS: 01	0.01 MG	715	743	734	731	14.3
BRRC#	0.09 MG	14	16	13	14	1.5
47-205	0.27 MG	13	13	18	15	2.9
	1 MG	15	14	13	14	1.0
	3 MG	22	16	20	19	3.1
	10 MG	T	T	T	T	-
STRAIN TA100						
SOLVENT	110 MG	138	137	119	131	10.7
POS: 02	0.01 MG	1888	1950	1771	1870	90.9
BRRC#	0.09 MG	131	123	132	129	4.9
47-205	0.27 MG	119	121	120	120	1.0
	1 MG	150	164	139	151	12.5
	3 MG	143	132	132	136	6.4
	10 MG	T	T	54 (T)	T	-
STRAIN TA1535						
SOLVENT	110 MG	43	39	44	42	2.6
POS: 02	0.01 MG	963	951	1057	990	58.0
BRRC#	0.09 MG	39	41	33	38	4.2
47-205	0.27 MG	35	48	46	43	7.0
	1 MG	29	33	47	36	9.5
	3 MG	46	53	39	46	7.0
	10 MG	T	T	T	T	-
STRAIN TA1537						
SOLVENT	110 MG	5	1	7	4	3.1
POS: 03	0.06 MG	67	88	93	83	13.8
BRRC#	0.09 MG	1	2	6	3	2.6
47-205	0.27 MG	5	2	3	3	1.5
	1 MG	3	1	7	4	3.1
	3 MG	3	5	3	4	1.2
	10 MG	T	T	T	T	-
STRAIN TA1538						
SOLVENT	110 MG	5	7	7	6	1.2
POS: 01	0.01 MG	666	983	872	840	160.9
BRRC#	0.09 MG	5	4	9	6	2.6
47-205	0.27 MG	6	5	6	6	0.6
	1 MG	5	3	7	5	2.0
	3 MG	10	2	13	8	5.7
	10 MG	T	T	T	T	-

T: TOXIC

POS: POSITIVE CONTROLS

01: 4-NITRO-O-PHENYLENEDIAMINE

03: 9-AMINOACRIDINE

02: SODIUM AZIDE

04: 2-AMINOANTHRACENE

TABLE 2
RESULTS OF THE SALMONELLA MUTAGENICITY ASSAYCHEMICAL: BRRC #47-205, Epoxy Resin ERL-4234 WITH ACTIVATION
EXPERIMENT DATE: October 16 through October 19, 1984 SOLVENT: DMSO

TEST AGENT	DOSE PER PLATE	PLATE COUNTS			AVERAGE	STANDARD DEVIATION
		1	2	3		
STRAIN TA98						
SOLVENT	110 MG	15	28	27	23	7.2
POS: 04	0.01 MG	1106	1160	1478	1248	201.0
BRRC#	0.1 MG	17	29	15	20	7.6
47-205	0.3 MG	26	27	22	25	2.6
	1 MG	26	19	17	21	4.7
	3 MG	30	27	21	26	4.6
	10 MG	T	T	T	T	-
STRAIN TA100						
SOLVENT	110 MG	106	80	113	100	17.4
POS: 04	0.01 MG	812	824	1009	882	110.4
BRRC#	0.1 MG	108	110	79	99	17.3
47-205	0.3 MG	93	111	110	105	10.1
	1 MG	105	117	96	106	10.5
	3 MG	97	110	86	98	12.0
	10 MG	T	T	T	T	-
STRAIN TA1535						
SOLVENT	110 MG	14	13	21	16	4.4
POS: 04	0.01 MG	82	84	58	75	14.5
BRRC#	0.1 MG	10	6	9	8	2.1
47-205	0.3 MG	12	13	12	12	0.6
	1 MG	9	11	15	12	3.1
	3 MG	9	14	17	13	4.0
	10 MG	T	T	T	T	-
STRAIN TA1537						
SOLVENT	110 MG	9	5	9	8	2.3
POS: 04	0.01 MG	105	104	86	98	10.7
BRRC#	0.1 MG	5	4	8	6	2.1
47-205	0.3 MG	5	6	6	6	0.6
	1 MG	5	5	8	6	1.7
	3 MG	6	6	2	5	2.3
	10 MG	T	T	T	T	-
STRAIN TA1538						
SOLVENT	110 MG	17	21	17	18	2.3
POS: 04	0.01 MG	423	403	250	359	94.6
BRRC#	0.1 MG	9	19	8	12	6.1
47-205	0.3 MG	21	8	9	13	7.2
	1 MG	19	13	17	17	3.5
	3 MG	13	19	7	13	6.0
	10 MG	T	T	T	T	-

T: TOXIC

POS: POSITIVE CONTROLS

01: 4-NITRO-O-PHENYLENEDIAMINE

03: 9-AMINOACRIDINE

02: SODIUM AZIDE

04: 2-AMINOANTHRACENE

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12-28-84

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Good Laboratory Practices Compliance

This study was conducted in accordance with the U.S. Environmental Protection Agency's TSCA Good Laboratory Practices (GLP) Standards (40 CFR Part 792, effective December 29, 1983) with the following deviations:

1. The sponsor indicated that Epoxy Resin ERL-4234 was soluble and stable in dimethylsulfoxide and no additional analyses of stability in the test system were performed.
2. The content of the test substance and control mixtures were documented and analyzed by weight or by volume, and no additional chemical analyses were performed.

In the opinion of the study director, these deviations do not affect the validity or reliability of this study.

Prepared by:


W. Christopher Hengler, M.S., M.B.A. Date
Study Director

WPC/rkk/0604B-1
11-01-84



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Quality Assurance Unit Study Inspection Summary

Test Substance: BAKELITE[®] Cycloaliphatic Epoxy
Resin ERL-4234

Study: Ames Bacterial Mutagenicity Assay

Study Director: W. C. Hengler, M.S., M.B.A.

The Quality Assurance Unit of BRRC conducted the inspections listed below and reported the results to the study director and to management on the dates indicated. It is the practice of this Quality Assurance Unit to report the results of each inspection to both the study director and management.

<u>Date</u>	<u>Inspection</u> <u>Type</u>	<u>Date QAU Report Issued</u>	
		<u>To Study Director</u>	<u>To Management</u>
1-31-84	Standard Protocol	2-2-84	2-3-84
10-1-84	Standard Protocol Form	10-2-84	10-5-84
10-16-84	Event-Preparing Agar	10-16-84	11-28-84
1-10-85	Final Data and Final Report	1-14-85	1-16-85

Linda J. Calisti 1/16/85
L. J. Calisti, Group Leader Date
Good Laboratory Practices/Quality Assurance