



BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632

Telephone (412) 733-5200

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Project Report 47-171
10 Pages
January 16, 1985

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Background

#5

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234

Salmonella/Microsome (Ames)

Bacterial Mutagenicity Assay

Sponsor: Union Carbide Corporation
Specialty Polymers and Composit

Summary

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234 was tested for mutagenic activity using the Salmonella/microsome bacterial mutag (Ames test). Epoxy Resin ERL-4234 was tested with and without metabolic activation in triplicate at five concentrations, ranging from 0.1 to 10 milli-grams per plate. The highest concentration was cytotoxicity tests and in a preliminary test to choose appropriate mutagenic activity was not observed with any of the five bacteria 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: $C_{15}H_{22}O_4$

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

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

Bushy Run Research Center
A Joint Mellon Institute—Union Carbide Corporation Operation

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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

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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. BRRC 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; BRRC #44-71; CAS #99-56-9
 - b) 9-aminoacridine; BRRC #44-233; CAS #90-45-9
 - c) sodium azide; BRRC #44-72; CAS #26628-22-8
 - d) 2-aminoanthracene; BRRC #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 BRRC 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: BRRRC Archives
2. Final Report: BRRRC 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:

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

R. A. Slesinski 11/2/85
Ronald S. Slesinski, Ph.D. Date
Manager, Genetic, Biochemical, & Acute Toxicology

F. R. Frank 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 ASSAY

CHEMICAL: 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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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 Hengle 1/3/85
W. Christopher Hengle, M.S., M.B.A. Date
Study Director

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



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Quality Assurance Unit Study Inspection SummaryTest 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

APPENDIX 1

Standard Procedures for the Salmonella/microsome Mutagenicity Assay

A. Introduction

The strains used in this test are all Salmonella typhimurium histidine auxotrophs developed by Dr. Bruce Ames of the University of California at Berkeley. The test is designed to assay for reverse mutations in the histidine locus by plating the bacteria on minimal agar with only a trace of histidine. Bacteria that have undergone a reverse mutation in this locus can form colonies in minimal agar and are counted as mutants. The trace of histidine in the minimal agar allows the bacteria to undergo a small number of cell divisions which then form the background lawn, visible as a cloudiness in the agar. Observation of the lawn permits an estimate of toxicity. Testing with growing cultures also allows potential mutagens to act on replicating DNA which is often a more sensitive test for several classes of mutagens.

B. Indicator Organisms

Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 are used in this assay. They all lack the excision repair mechanism, which increases their sensitivity to potential mutagens. They also have a mutation affecting their lipopolysaccharide layer which allows large molecules to enter the bacteria more easily. Strains TA98 and TA100 were derived from strains TA1538 and TA1535 by the addition of an R factor which imparts ampicillin resistance and also increases sensitivity to several classes of mutagens.

Indicator organisms are stored at -80°C. Working cultures are prepared monthly by inoculating nutrient broth from the frozen cultures and incubating with agitation overnight. Bacteria are then plated onto Vogel-Bonner Medium E agar plates (master plates) with an excess of histidine and biotin (required because of the lipopolysaccharide deficiency). After incubation for 24 hours, the strains are checked for their genetic markers to verify their identity and purity (SOP #7.4.2A).

For testing, the broth cultures are prepared by inoculating from the master plates into nutrient broth and incubated overnight with agitation. The broth cultures are then kept on ice during the day of testing. Fresh cultures are made each day of testing.

C. Toxicity Testing

Sterile tubes are prepared containing 2 ml soft agar (6 g/l agar and 5 g/l NaCl) with a final concentration of 0.05 mM L-histidine and 0.05 mM D-biotin (the entire mixture is called top agar). Dilutions of the test chemical are made in an appropriate solvent so that the correct amount of chemical can be added to each tube in 100 µl amounts. If less than 100 µl amounts of the test chemical is used, phosphate-buffered saline is used to adjust the total volume to 100 µl.

At least five doses are tested with maximum doses of 50 mg (for solids) or 100 μ l (for liquids) per plate for nontoxic chemicals, unless limited by solubility. Generally, a chemical cannot be considered to be nonmutagenic unless at least 5 mg/plate has been tested, unless limited by toxicity or solubility (deSerres and Shelby, 1979; HG-Gene Muta-S. typhimurium, August 1982). A 100 μ l aliquot of an overnight broth culture of strain TA100 is added followed by the appropriate amount of test chemical. The mixture is vortexed and poured onto the surface of a Vogel-Bonner Medium E agar plate (VB-E plate). The top agar is allowed to harden and the plates are incubated at 37°C for 24 to 48 hours. The plates are examined for the condition of their background lawns and growth is recorded as either confluent, sparse, or absent. Confluency is considered an indication of nontoxicity, sparse growth indicates moderate toxicity, and lack of growth is recorded as toxic.

D. Dose Selection

For the mutagenicity test, a minimum of five half-log doses are tested within a range of moderately toxic to relatively nontoxic doses based on the results of the toxicity test. If all doses in the toxicity test were nontoxic, then a maximum dose of 50 mg or 100 μ l is used in the mutagenicity test, unless limited by solubility.

E. Controls

Concurrent solvent and positive controls are run with each test. The solvent control is the maximum amount of solvent added with the diluted test chemical. For chemicals tested without dilution (i.e., by direct addition), water is used as the solvent control. Both activation-dependent and activation-independent positive controls are used. The activation-independent controls are 4-nitro-o-phenylenediamine for TA98 and TA1538, sodium azide for TA100 and TA1535, and 9-aminoacridine for TA1537. The activation-dependent control is 2-aminoanthracene (2-anthramine) for all strains. The concentrations are determined from prior dose-response experiments on these chemicals and are listed in the tables of results in this report.

F. Metabolic Activation

Each lot of Aroclor-1254 induced, rat-liver homogenate (S9) is prescreened for activity with 7,12-dimethylbenzanthracene to determine the appropriate amount to use for testing. The S9 is added to the "S9 mix" which contains, per ml, S9 (50-250 μ l), $MgCl_2$ (8 μ moles), KCl (33 μ moles), glucose-6-phosphate (5 μ moles), NADP (4 μ moles), and sodium phosphate, pH7.4 (100 μ moles). S9 mix is prepared fresh each day of testing and kept at 0-4°C.

G. Test Chemicals and Solvents

The solvents of choice are water, DMSO or ethanol. A minimum of five concentrations of the test chemical are diluted in the appropriate solvent and tested. The chemical is diluted so that 100 μ l will deliver the required

dose. If smaller volumes are used, phosphate-buffered saline is added to adjust the total volume to 100 μ l. Test chemical solutions are prepared on the day of testing and are gravimetrically analyzed. If the analysis indicates > 10% error from the intended concentration, then either the dilution is repeated or the actual concentration is stated in the report.

H. Dosing

To a sterile tube containing 2 ml of top agar, 100 μ l aliquot of the appropriate bacterial culture is added followed by the addition of 100 μ l of the appropriate solvent, control, or test chemical solution. Either 0.5 ml of S9 mix or 0.5 ml of phosphate-buffered saline (PBS) is added for tests with and without metabolic activation, respectively. The top agar mixture is then poured onto a VB-E plate. Each dose is tested in triplicate and with all five bacterial strains. Sterility checks are done on the PBS and/or S9 mix, all solvents, and the highest concentration of each test chemical. The plates are transferred to a darkened 37°C incubator after hardening and incubated for 48-72 hours.

I. Data Collection

An Artek Model #880 Colony Counter is used to count bacterial colonies or handcounts of the plates are done. The counter is calibrated for each test to check the counting accuracy (SOP #7.4.5A). The numbers of colonies per plate are counted and recorded. An examination is also made of the background lawn on each plate. If toxicity is observed as an inhibition of growth of the background lawn, the plate is not counted, but is recorded as toxic. If the background lawn is sparse and the colony count is still recorded, that number is not used in the calculation of the mean number of colonies and its standard deviation.

J. Criteria for Test Validity and for Interpretation of Results

The spontaneous reversion for the solvent controls should be within this laboratory's historical range. The positive controls should demonstrate that the test systems are responsive with known mutagens. A test chemical is considered to be a bacterial mutagen if the number of revertant colonies is at least twice the solvent control for at least one dose level and there is evidence of a dose-related increase in the number of revertant colonies. If a test chemical produces a marginal or weak response that cannot be reproduced in a second test, the test result will be considered negative. If there is no evidence of a dose-related increase in the number of revertant colonies and the number of revertant colonies is not twice the solvent control, then the test chemical is not considered to be a bacterial mutagen.

K. Data Storage and Retrieval

Copies of the final report, statistical analyses, analytical data and raw data are stored in the BRRRC Archives.

References

1. Ames, B.N., J. McCann and F. Yamasaki, Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutation Research, 31: 347-364 (1975).
2. Vogel, H.J., and D.M. Bonner, Acetylornithase of Escherichia coli: partial purification and some properties, Journal of Biological Chemistry, 218: 97-106 (1956).
3. deSerres, F.D., and M.D. Shelby, Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay, Mutation Research, 64: 159-165 (1979).
4. Environmental Protection Agency, Health Effects Test Guidelines, HG-Gene Muta-S. typimurium, EPA Report No. 560/6-82-001, August, 1982.

WPC/rkk/0604B-1
10-24-84

REQUEST FOR STANDARD TOXICOLOGY STUDIES
AT BUSHY RUN RESEARCH CENTERBUSINESS
CONFIDENTIAL

3173

To: Mr. R.C. Myers
Bushy Run Research Center
RD 4 Mellon Road
Export, PA 15632

From: Name R. J. COTTEL
Sponsoring Division SPECIALTY POLYMERS + COMPOSITES
Dept R&D
Location (city) BUND BETH
Charge to Account No. 25-506-9440
Technology Manager G. J. KWIATKOWSKI
Study Authorization: R. J. Cotel / R. E. Plevan 7/9/84
Signature Date

COMPLETE COPIES OF THIS FORM
MUST BE SENT (BY THE SUBMITTER) TO:

- A. Dr. Bryan Ballantyne
Technical Center (3005)
South Charleston, WV 25303
- B. Dr. W.C. Kuryla
Technical Center (3005)
South Charleston, WV 25303
- C. Dr. E.R. Homan
Bushy Run Research Center
RD 4 Mellon Road
Export, PA 15632
- D. Sponsoring Division
HS & EA Manager
Name RENEVAN
Loc: DANBURY

TESTS REQUESTED (Standard procedures; other tests require specific protocols. Please contact the Corporate Applied Toxicology Dept for advice and guidance.)

RECEIVED

RANGE FINDING:

3 IN VITRO:

- ☐ Acute Oral ☐ Other _____ (specify)
- ☐ Acute Percutaneous
- ☐ Acute Inhalation
- ☐ Skin Irritancy
- ☐ Eye Irritancy

- ☒ Ames (Salmonella) Test AUG 24 1984
- ☐ Gene Mutation (CHO) W. C. HENGIE
- ☐ Chromosome Exchange (SCE)
- ☐ Chromosome Damage (Micronucleus)
- ☐ DNA Damage (UDS)
- ☐ Macrophage (RAM) test
- ☐ Other _____

KNOWN PROCEDURAL REQUIREMENT (circle):

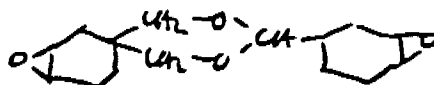
EEO, FHSA, DOT, FIFRA, TSCA, RCRA, Other _____

PLEASE PROVIDE THE FOLLOWING INFORMATION. IT IS NEEDED IN ORDER TO
EXPEDITE WORK ON THE REQUESTED STUDIES.

PRODUCT/COMPOSITION NAME: EM-4234COS NOMENCLATURE: SPIRO (1,3-DIOXANE-5,3'-(7) OXABICYCLO (4,1,0) HEPTANECAS NO.: 3388-03-2

CHEMICAL SYNONYMS/ALTERNATE/TRADE NAMES:

ARADITE CY 175; UP 612; 2-(3,4-EPOXY)CYCLOHEXYLMETHYL-5,5-SPIRO[3.4]HEPTANE

STRUCTURAL FORMULA OR COMPOSITION OF MIXTURES: CYCLOHEXANE-m-DIOXANEC₁₅H₂₂O₄

DISTRIBUTION OF REPORT: A standard divisional distribution list will be used unless otherwise requested (see page 4).

LABORATORY USE)

ACKNOWLEDGEMENT OF SAMPLE RECEIPT

Date Received: 7/25/84By: R. E. PlevanARC Sample No 47-205UCC
062493

CHEMICAL INFORMATION

1. SAMPLE PURITY/COMPOSITION: *Commercial product. Sample is probably at least 95% indicated structure. Impurities would include diene active desiccants from reaction with acetone and.*
- Analytical test method employed* _____
- *(Send copy of available data, source and location of analyses.)

Percent Composition

Amounts and identities of known impurities: _____ %

Amount of unidentified impurities: _____ %

Test material: _____ %

2. REQUIRED SAMPLE QUANTITY:

Acute Tests:

In vitro Tests: 4 ounces

- 32 ounces for full series of tests
- 16 ounces for chemicals with known high toxicity
- 2 ounces for irritancy tests only

NOTE: To reduce problems involving disposal of unused amounts of the sample, supply only the amount specified above.

3. PHYSICAL CHARACTERISTICS: (Give all which are known):Gas _____ Liquid ☒ Solid _____ Powder _____ Granular _____Molecular Wt. ~~266~~ 266

Vapor Pressure (@ 3 temp. between 15° and 150°C):

Density (liquids) 1.17 (25/25)

1. <0.01 mm @ 25° °C

pH (aqueous sol.) _____

2. _____ mm @ _____ °C

Freezing point <0 °C

3. _____ mm @ _____ °C

Boiling point >220°C 1760 mm °C

Flash point 60 °C

(Tag, closed cup)

4. SOLUBILITY:

Solvent Percent Solution

Water 42% (this may be per gram)

Acetone _____

Ethanol _____

Dimethylsulfoxide _____

Vegetable Oil _____

Other (specify) _____

5. STABILITY: (Check appropriately):Shelf Life _____ < 6 mo; ☒ > 6 mo.Light sensitive _____ yes; ☒ noTemp. sensitive _____ yes; ☒ noOxidizes _____ yes; ☒ noPolymerizes ☒ yes; _____ no

Known solvent

Incompatibility (specify) _____

* In presence of acids and bases.

HANDLING/STORAGE/DISPOSAL INFORMATION:

RECOMMENDED PRECAUTIONS FOR HANDLING*: KEEP AWAY FROM SKIN AND EYES

Enclose UCC (or other company) Material Safety Data Sheet - if available.

Any unusual fire or explosion hazards: ND

Any chemical incompatibilities (materials to avoid): STRONG ACIDS AND BASES

SPECIAL PERSONNEL PROTECTIVE EQUIPMENT NEEDED: RUBBER GLOVES

RECOMMENDED INACTIVATION/DECONTAMINATION PROCEDURE(S): WASH EXPOSED AREA WITH SOAP AND WATER

RECOMMENDED STORAGE CONDITIONS:

Temperature (Check appropriately):

Room Temp. ☒

Refrigerate < 20°C

Freeze < 0°C

Special Conditions Required (Check appropriately)

Under N₂ gas

Dessicator

Other

SAMPLE DISPOSAL: (Check appropriately):

☐ Return unused sample to:
(attach DOT shipping instructions)

☒ Disposal by BRRC is authorized in compliance with applicable regulations.

OTHER PERTINENT INFORMATION

The following information will be useful in determining or confirming the necessity of specific toxicity tests:

1. Proposed use of material (circle): (Industrial) Intermediate Home Use Experimental

Industrial Product Food Drug Cosmetic Packaging (specify Food, Drug, Cosmetic)

Other use (specify): _____

2. Potential hazard route(s) (circle): (Skin) Inhalation (Mouth) (Eye)

Other (specify): _____

3. Form shipped in commerce (circle): Solid (Liquid) Gas Suspension or Slurry

Solvent or Vehicle composition: _____

ATTACHMENTS (TO BE PROVIDED BY SUBMITTER):

- * - Analytical data for sample.
- * - Material Safety Data Sheet of sample material (if available).
- * - DOT shipping information (if applicable).

DISTRIBUTION LIST: Unless indicated below, the standard distribution list for the sponsoring division will be used.

☐ Please use the following divisional distribution list for the final study report:



MATERIAL SAFETY DATA SHEET

EFFECTIVE DATE: December 1, 1981

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I. IDENTIFICATION

PRODUCT NAME:	BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234		
CHEMICAL NAME:	2-(3,4-epoxycyclohexyl)-5,5-spiro-3,4-epoxy cyclohexane-meta dioxane	CHEMICAL FAMILY:	Epoxides
FORMULA:	C ₁₅ H ₂₂ O ₄	MOLECULAR WEIGHT:	266.33
SYNONYMS:	3-(3,4-epoxycyclohexyl)-8,9-Epoxy-2,4-Dioxaspiro [5,5]undecane		
DEPARTMENT OF TRANSPORTATION	HAZARD CLASSIFICATION	None	
	SHIPPING NAME	None	
CAS #	3388-03-2	CAS NAME	Spiro[1,3-dioxane-5,3'-(7)oxabicyclo[4.1.0]heptane], 2-(7-oxabicyclo[4.1.0]hept-3-yl)-

II. PHYSICAL DATA

BOILING POINT, 760 mm Hg	> 250 °C (> 482 °F)	POUR POINT	15 °C
SPECIFIC GRAVITY (H ₂ O = 1)	1.1891 at 20/20 °C	VAPOR PRESSURE at 20 °C	< 0.01 mm Hg
VAPOR DENSITY (air = 1)	9.2	SOLUBILITY IN WATER, % by wt. at 25 °C	4.2
PER CENT VOLATILES BY VOLUME	Nil	EVAPORATION RATE (Butyl Acetate = 1)	< 0.01
APPEARANCE AND ODOR	High viscosity liquid; faint, burnt odor.		

III. INGREDIENTS

MATERIAL	%	TLV (Units)	HAZARD
BAKELITE® Cycloaliphatic Epoxy Resin ERL-4234	100	Not established	Carcinogenic in laboratory animals. Irritant.

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT [test method(s)]	368 °F, Pensky-Martens closed cup 410 °F, Cleveland open cup		
FLAMMABLE LIMITS IN AIR, % by volume	LOWER	Not determined (Nonvolatile Material)	UPPER Not determined (Nonvolatile Material)
EXTINGUISHING MEDIA	Use water spray, carbon dioxide, dry chemical, alcohol-type or universal-type foams applied by manufacturer's recommended technique.		
SPECIAL FIRE FIGHTING PROCEDURES	Don't spray pool fires directly; a solid stream of water directed into hot burning liquid can cause frothing. Use supplied breathing air and full protective clothing.		
UNUSUAL FIRE AND EXPLOSION HAZARDS	None		

EMERGENCY PHONE NUMBER

304/744-3487

This number is available days, nights, weekends, and holidays.

While Union Carbide Corporation believes that the data contained herein are factual and the opinions expressed are those of qualified experts regarding the results of the tests conducted, the data are not to be taken as a warranty or representation for which Union Carbide Corporation assumes legal responsibility. They are offered solely for your consideration. Union Carbide Corporation and its subsidiaries disclaim any liability for damages or losses of any kind resulting from the use of these data and information must be determined by the user to be in accordance with applicable Federal, State, and local laws and regulations.

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062497

V. HEALTH HAZARD DATA

TLV AND SOURCE: None established by ACGIH or OSHA.

ACUTE EFFECTS OF OVEREXPOSURE

SWALLOWING	May cause nausea, vomiting, and diarrhea.
SKIN ABSORPTION	None currently known.
INHALATION	None currently known.
SKIN CONTACT	May cause slight redness for a short period of time.
EYE CONTACT	Causes irritation.
CHRONIC EFFECTS OF OVEREXPOSURE	Causes skin cancer in mice in lifetime skin painting studies. Skin contact and breathing of vapor should be avoided.
OTHER HEALTH HAZARDS	None currently known.

EMERGENCY AND FIRST AID PROCEDURES:

SWALLOWING	Give two glasses of water and induce vomiting by putting finger down throat.
SKIN	Remove contaminated clothing and wash skin with plenty of soap and water. Launder clothing before reuse.
INHALATION	Remove to fresh air.
EYES	Immediately flush eyes with plenty of water for at least 15 minutes. Call a physician if discomfort persists.

NOTES TO PHYSICIAN

There is no specific antidote. Toxicology studies have shown the material to be of low acute toxicity. Treatment of overexposure should, therefore, be directed at the control of symptoms and the clinical condition.

VI. REACTIVITY DATA

STABILITY		CONDITIONS TO AVOID	None
UNSTABLE	STABLE		
--	✓		
INCOMPATIBILITY (materials to avoid)		Amines, alkalies, acids, and alcohols.	
HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS		Burning can produce CO and/or CO ₂ .	
HAZARDOUS POLYMERIZATION		CONDITIONS TO AVOID	Contamination by amines, alkalies, and acids.
May Occur	Will not Occur		
✓	--		

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED	Wear suitable protective equipment; avoid skin and eye contact. Collect for disposal.
WASTE DISPOSAL METHOD	Incinerate in a furnace where permitted under appropriate Federal, State, and local regulations.

VIII. SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (specify type)	Air-supplied mask as noted below.		
VENTILATION	General (mechanical) room ventilation is satisfactory with ambient temperature conditions. To minimize exposure to vapors during processing at elevated temperatures, additional precautions should be taken, such as the use of a local ventilation system or air-supplied mask. Since this product has been shown to be a weak animal carcinogen in skin painting studies, exposure to skin should be avoided through the use of gloves and other protective clothing, as appropriate.		
PROTECTIVE GLOVES	Plastic	EYE PROTECTION	Safety goggles
OTHER PROTECTIVE EQUIPMENT	Eye bath and safety shower		

IX. SPECIAL PRECAUTIONS**PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING**

WARNING: Suspect Cancer Agent.
Do not get in eyes, on skin, or on clothing.
Avoid breathing vapor.
Keep container closed.
Wash thoroughly after handling.

FOR INDUSTRY USE ONLY**OTHER PRECAUTIONS**

This product is resistant to rapid biodegradation;
avoid discharge to natural waterways.

3180



UNION CARBIDE CORPORATION
SPECIALTY POLYMERS & COMPOSITES DIVISION

P.O. BOX 670, BOUND BROOK, N.J. 0880
TELEPHONE (201) 356-800

Report 47
Appendix
Page 8 of

RECEIVED

SEP 12 1984

W. C. HENGLER

September 6, 1984

Mr. W. C. Hengler
Bushy Run Technical Center
RD4, Mellon Road
Export, Pennsylvania 15632

Dear Mr. Hengler:

Infrared spectra have been run on the samples of cycloaliphatic epoxide that you returned to me recently. The spectra are enclosed for your files. The spectra that were obtained on these samples were compared with standard spectra for these products. In two cases, 4205 and 4299, comparisons were made with other samples of these products that are being utilized in current laboratory programs.

Comparison of these various spectra by the infrared experts in our Analytical Group (Dr. Clark and Mr. Lewis) led to the conclusion that the sample that you have are authentic samples of the UCC cycloaliphatic epoxides. Thus, the mutagenicity testing will be performed on valid samples of 4221, 4299, 4234, 4206 and 4205.

Very truly yours,

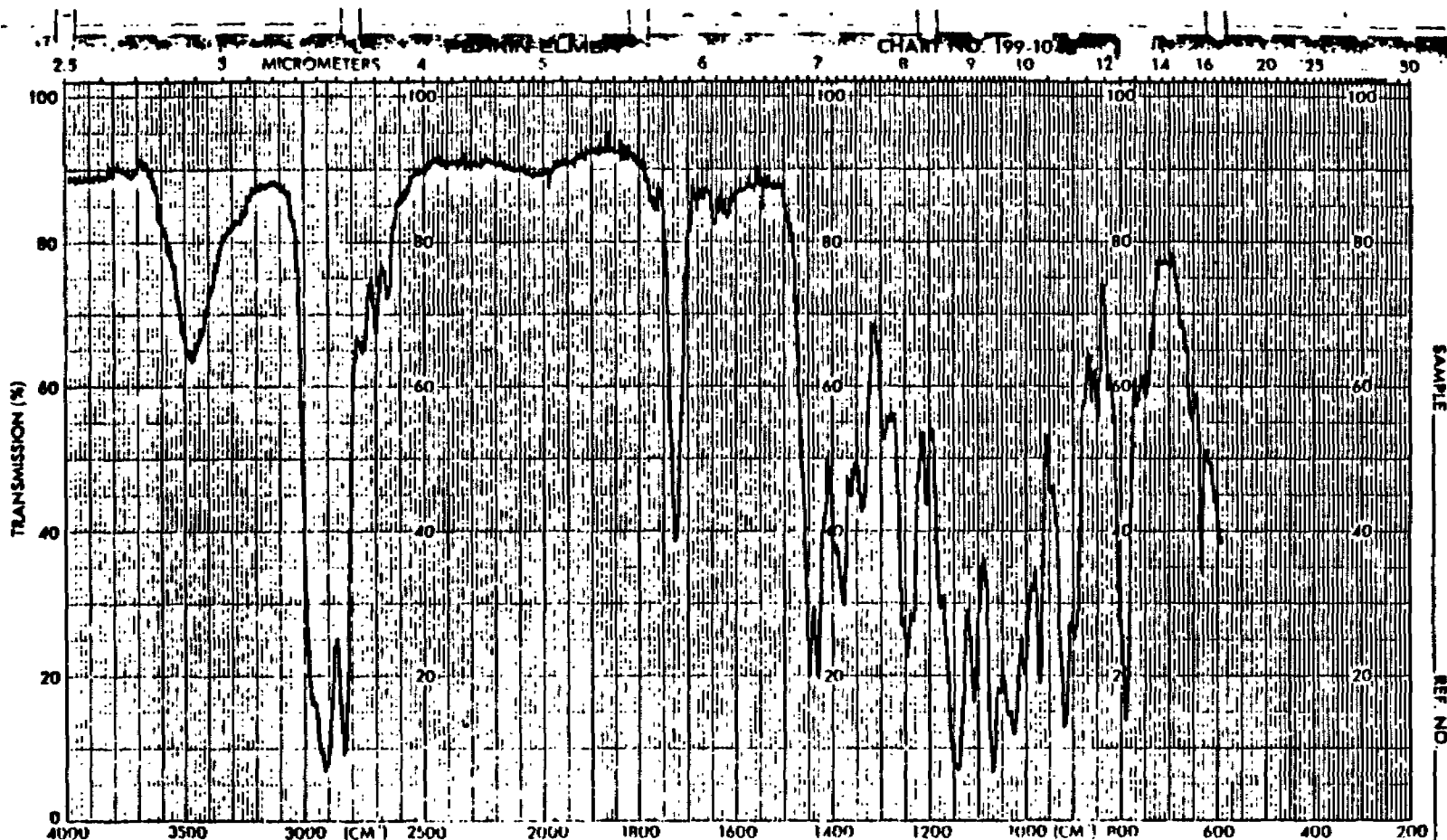
encs.

A handwritten signature in dark ink, appearing to read "R. J. Cotter".
R. J. Cotter

RJC:mjk

cc:Dr. E. M. Clark, Mr. J. Lewis, Dr. R. E. Plevan - DB

UCC
062500



ARCISSA		GRDINATE		SCAN TIME		REF. SCAN		SINGLE BEAM	
EXPANSION		EXPANSION		MULTIPLIER		TIME DRIVE			
SAMPLE <i>ERL 4224</i>		REMARKS		SLIT PROGRAM		OPERATOR <i>[Signature]</i>		DATE <i>3-1-67</i>	
SPECIM <i>Retained from isotherm</i>				SOLVENT		CELL PATH			
<i>from isotherm 6.5 sec</i>				CONCENTRATION		REFERENCE			

UCC
062501

APPENDIX 3

Salmonella typhimuriumA. Historical Data on Spontaneous Reversion Rates at BRRC
As of June 30, 1984

<u>Strain</u>	<u>+/-S9</u>	<u>N</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Median</u>	<u>Range</u>
TA98	-S9	98	18.2	5.02	18	7, 33
	+S9	98	25.6	7.40	26	14, 44
TA100	-S9	98	123.1	26.89	120	61, 187
	+S9	98	101.5	17.86	100	65, 137
TA1535	-S9	98	32.5	13.61	32	7, 60
	+S9	98	13.4	7.34	14	2, 35
TA1537	-S9	98	4.3	1.90	4	1, 9
	+S9	98	5.0	2.61	5	1, 13
TA1538	-S9	98	7.2	3.72	7	2, 19
	+S9	98	15.0	5.41	16	4, 29

B. Historical Data on Postive Control Values at BRRC
As of January 26, 1984

<u>Strain</u>	<u>Positive Control</u>	<u>+/-S9</u>	<u>N</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Median</u>	<u>Range</u>
TA98	NPD, 10 µg	-S9	84	877.1	166.73	870	532, 1301
	2AA, 10 µg	+S9	84	1434.2	327.54	1457	604, 2037
TA100	NZ, 10 µg	-S9	84	1621.8	277.37	1696	869, 2069
	2AA, 10 µg	+S9	84	1181.7	323.54	1201	475, 2225
TA1535	NZ, 10 µg	-S9	84	1622.0	253.42	1666	837, 2050
	2AA, 10 µg	+S9	84	99.3	30.84	98	37, 182
TA1537	9AA, 60 µg	-S9	84	140.6	130.70	82	28, 489
	2AA, 10 µg	+S9	84	122.5	45.52	120	36, 230
TA1538	NPD, 10 µg	-S9	84	1124.2	196.66	1168	573, 1582
	2AA, 10 µg	+S9	84	532.0	230.61	486	146, 1249

S9- rat liver homogenate; NPD- 4-nitrophenylenediamine; 2AA- 2-aminoanthracene;
NZ- sodium azide; 9AA- 9-aminoacridine.

WPC/rkk/0604B-1
10-24-84