UNION CARBIDE

BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 733-5200

Project Report 47-183
10 Pages
February 4, 1985

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4206

Salmonella/Microsome (Ames)

Bacterial Mutagenicity Assay

Sponsor: R. E. Plevan, HS&EA Manager
Union Carbide Corporation
Specialty Polymers & Composites Division

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Summary

Epoxy Resin ERL-4206 was tested for potential mutagenic activity using the Salmonella/microsome bacterial mutagenicity assay (Ames test). Epoxy Resin ERL-4206 was tested with and without metabolic activation in triplicate at five concentrations, ranging from 0.3 to 30 milligrams per plate. The highest concentration was cytotoxic in the mutagenicity test and in a preliminary test to choose appropriate doses. Positive, dose-related increases of the number of colonies above the solvent control levels were observed with strains TA98, TA100, TA1535 and TA1537 without metabolic activation and with strains TA98, TA100, and TA1535 with activation. The results of this test indicate that the test chemical caused frameshift and base-pair substitution mutations. Epoxy Resin ERL-4206 was an active mutagen in this in vitro screening test.

Sample

Chemical Name: BAKELITE® Cycloaliphatic Epoxy Resin ERL-4206
I.D. #: TF3-96521 3083 HKT
BRRC Sample #: 47-202
BRRC Account #: 84-18-18050-46
CAS #: 106-87-6
Chemical Synonyms: 7-oxabicyclo[4.1.0]heptane,3-oxiranyl; UNOX 206; 4-vinylcyclohexene diepoxide; EP-206
Molecular Formula: $C_8H_{12}O_2$

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

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

BAKELITE® Cycloaliphatic Epoxy Resin ERL-4206

Salmonella/Microsome (Ames)

Bacterial Mutagenicity Assay

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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 supresses 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 quantititated 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-4206 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-4206
2. BRRC Sample Number: 47-202
3. CAS Number: 106-87-6
4. Purity: > 97%; IR spectra consistent with structure (see Appendix 2)
5. Density: 1.0986
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 29, 1984
2. Test Completed: November 2, 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 300 mg/ml for doses of 30 mg/plate and below. 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-4206 was tested in a preliminary toxicity screen at 109.9, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.003 milligrams per plate with strain TA100 only. The top dose of 109.9 milligrams per plate inhibited all growth of the background lawn, while 30 milligrams per plate allowed only sparse growth of the background lawn. Based on these results, mutagenicity testing was done with 5 doses of 30, 10, 3, 1, and 0.3 milligrams per plate tested with triplicate cultures. 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 initial dilutions of the test substance for both tests indicated no more than a 0.6% error from the stated concentration. All subsequent dilutions had no more than a 5.8% 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). Dose-related mutagenic activity was observed in both tests. In the test without metabolic activation, the ratios of the average number of revertant colonies to the average number of colonies observed in the solvent control cultures increased with increasing dose as follows: 1.0, 1.2, 1.3, and 2.5-fold increases with TA98; 1.4, 2.2, 4.3, and 12.7 with TA100; 1.6, 2.7, 4.1, and 10.2 with TA1535; and 1.2, 1.2, 1.2, and 2.8 with TA1537. No evidence of mutagenic activity was observed with TA1538. Strains TA98 and TA1537 detect frameshift mutagens, while TA100 and TA1535 detect base-pair substitution mutagens.

In the test with metabolic activation, the ratios of the average number of revertant colonies to the average number from the solvent control increased with increasing dose as follows: 1.1, 1.1, 1.4, and 4.7-fold increases with TA98; 1.6, 3.0, 6.3, and 15.9 with TA100; and 2.3, 6.7, 15.9, and 40.8 with TA1535. No evidence of mutagenic activity was observed with strains TA1537 and TA1538. The data from the 30 mg/plate dose were not used in the above calculations because of the observed cytotoxicity.

The mutagenic effects of Epoxy Resin ERL-4206 appear to be caused by both frameshifts and base-pair substitutions. A greater degree of mutagenic activity was observed with a metabolic activation system than without metabolic activation, although strain TA1537 was not reverted in the test with activation. The increased mutagenic activity with metabolic activation could be due either to the production of active metabolites from the parent compound or to reduced cytotoxicity caused by metabolic conversion of the test agent, thus allowing more revertant bacteria to survive the tested doses.

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 the maximum dose of 30 mg/plate with all strains.

3352

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-4206 produced a dose-dependent mutagenic response with the Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 without metabolic activation and with strains TA98, TA100, and TA1535 with metabolic activation. Under the conditions of this assay, Epoxy Resin ERL-4206 was an active mutagen in the Salmonella/microsome mutagenicity assay.

Reviewed and Approved by:

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

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

Fred R. Frank, Ph.D. — Date 1/4/85
Director

Contributors:

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

WPC/rkk/0620B-2
11-16-84

TABLE 1
RESULTS OF THE SALMONELLA MUTAGENICITY ASSAY

CHEMICAL: BRRC #47-202, Epoxy Resin ERL-4206 WITHOUT ACTIVATION
EXPERIMENT DATE: October 29 through November 1, 1984 SOLVENT: DMSO

TEST AGENT	DOSE PER PLATE	PLATE COUNTS 1	2	3	AVERAGE	STANDARD DEVIATION
STRAIN TA98						
SOLVENT	110 MG	22	29	27	26	3.6
POS: 01	0.01 MG	819	953	884	885	67.0
BRRC#	0.3 MG	33	20	26	26	6.5
47-202	1 MG	41	22	31	31	9.5
	3 MG	48	27	26	34	12.4
	10 MG	68	67	62	66	3.2
	30 MG	T	T	T	T	-
STRAIN TA100						
SOLVENT	110 MG	134	138	129	134	4.5
POS: 02	0.01 MG	2112	2340	2179	2210	117.2
BRRC#	0.3 MG	184	186	184	185	1.2
47-202	1 MG	245	287	341	291	48.1
	3 MG	629	542	563	578	45.4
	10 MG	1735	1711	1669	1705	33.4
	30 MG	T	T	T	T	-
STRAIN TA1535						
SOLVENT	110 MG	46	31	28	35	9.6
POS: 02	0.01 MG	2095	2383	2290	2256	147.0
BRRC#	0.3 MG	52	64	56	57	6.1
47-202	1 MG	92	85	108	95	11.8
	3 MG	148	172	115	145	28.6
	10 MG	385	356	329	357	28.0
	30 MG	T	T	T	T	-
STRAIN TA1537						
SOLVENT	110 MG	4	7	4	5	1.7
POS: 03	0.06 MG	78	102	93	91	12.1
BRRC#	0.3 MG	7	3	7	6	2.3
47-202	1 MG	7	7	4	6	1.7
	3 MG	3	9	5	6	3.1
	10 MG	15	14	13	14	1.0
	30 MG	T	T	T	T	-
STRAIN TA1538						
SOLVENT	110 MG	10	7	9	9	1.5
POS: 01	0.01 MG	1016	1023	1058	1032	22.5
BRRC#	0.3 MG	14	5	16	12	5.9
47-202	1 MG	13	5	9	9	4.0
	3 MG	15	6	9	10	4.6
	10 MG	7	7	9	8	1.2
	30 MG	T	T	T	T	-

T: TOXIC
POS: POSITIVE CONTROLS
01: 4-NITRO-O-PHENYLENEDIAMINE
02: SODIUM AZIDE
03: 9-AMINOACRIDINE
04: 2-AMINOANTHRACENE

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TABLE 2
RESULTS OF THE SALMONELLA MUTAGENICITY ASSAY

CHEMICAL: BRRC #47-202, Epoxy Resin ERL-4206 WITH ACTIVATION
EXPERIMENT DATE: October 30 through November 2, 1984 SOLVENT: DMSO

TEST AGENT	DOSE PER PLATE	PLATE COUNTS 1	2	3	AVERAGE	STANDARD DEVIATION
STRAIN TA98						
SOLVENT	110 MG	20	22	22	21	1.2
POS: 04	0.01 MG	1314	1342	1814	1490	280.9
BRRC#	0.3 MG	26	18	27	24	4.9
47-202	1 MG	16	29	26	24	6.8
	3 MG	28	32	30	30	2.0
	10 MG	98	100	100	99	1.2
	30 MG	T	T	160	160(T)	-
STRAIN TA100						
SOLVENT	110 MG	113	100	87	100	13.0
POS: 04	0.01 MG	1268	1217	1183	1223	42.8
BRRC#	0.3 MG	169	174	149	164	13.2
47-202	1 MG	237	315	353	302	59.1
	3 MG	716	617	567	633	75.8
	10 MG	1652	1588	1522	1587	65.0
	30 MG	T	T	1967	1967(T)	-
STRAIN TA1535						
SOLVENT	110 MG	11	10	7	9	2.1
POS: 04	0.01 MG	40	95	89	75	30.2
BRRC#	0.3 MG	19	21	22	21	1.5
47-202	1 MG	41	61	78	60	18.5
	3 MG	156	152	120	143	19.7
	10 MG	381	407	313	367	48.5
	30 MG	T	T	213	213(T)	-
STRAIN TA1537						
SOLVENT	110 MG	5	8	6	6	1.5
POS: 04	0.01 MG	79	32	79	63	27.1
BRRC#	0.3 MG	12	7	3	7	4.5
47-202	1 MG	6	8	5	6	1.5
	3 MG	4	4	2	3	1.2
	10 MG	8	9	7	8	1.0
	30 MG	T	T	17	17(T)	-
STRAIN TA1538						
SOLVENT	0.11 MG	17	7	20	15	6.8
POS: 04	0.01 MG	184	209	226	206	21.1
BRRC#	0.3 MG	8	13	17	13	4.5
47-202	1 MG	9	27	21	19	9.2
	3 MG	9	5	13	9	4.0
	10 MG	31	17	18	22	7.8
	30 MG	15	T	T	15(T)	-

T: TOXIC
POS: POSITIVE CONTROLS
01: 4-NITRO-O-PHENYLENEDIAMINE
02: SODIUM AZIDE
03: 9-AMINOACRIDINE
04: 2-AMINOANTHRACENE

3355

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Good Laboratory Practices Compliance

This study was conducted in accordance with the U.S. Environmental Protection Agency's 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-4206 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 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/0620B-1
11-08-84

UNION CARBIDE

BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 733-5200

Quality Assurance Unit Study Inspection Summary

Test Substance: BAKELITE® Cycloaliphatic Epoxy Resin ERL-4206

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.

Inspection Date	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
1-31-84	Standard Protocol	2-2-84	2-3-84
10-3-84	Standard Protocol Form	10-3-84	10-3-84
10-30-84	Event-Preparing Agar	11-2-84	11-28-84
1-15 to 1-17-85	Final Data and Final Report	1-18-85	2-1-85

Linda J. Calisti 2/4/85

Linda 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 µl (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 µl 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 µl 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 in 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 µl), $MgCl_2$ (8 µmoles), KCl (33 µmoles), glucose-6-phosphate (5 µmoles), NADP (4 µmoles), and sodium phosphate, pH7.4 (100 µmoles). 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 µl will deliver the required

dose. If smaller volumes are used, phosphate-buffered saline is added to adjust the total volume to 100 µl. 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 µl aliquot of the appropriate bacterial culture is added followed by the addition of 100 µl 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 BRRC 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/0620B-1
11-08-84

REQUEST FOR STANDARD TOXICOLOGY STUDIES AT BUSHY RUN RESEARCH CENTER

UNION CARBIDE BUSINESS CONFIDENTIAL

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

From: Name R. J. Cotter

Sponsoring Division SPECIALTY POLYMERS & COMPOSITES

Dept R+D

Location (city) BOUND BROOK

Charge to Account No. 25-506-9440

Technology Manager B. T. KWIATKOWSKI

Study Authorization: R. J. Cotter (Signature) R C Myers 7/9/84 (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: L.E. PUEHAN
Loc.: DANBURY

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

1. RANGE FINDING:
- ☐ Acute Oral
- ☐ Other _____ (Specify)
- ☐ Acute Percutaneous
- ☐ Acute Inhalation
- ☐ Skin Irritancy
- ☐ Eye Irritancy

2. KNOWN PROCEDURAL REQUIREMENT (circle):
OECD, FHSA, DOT, FIFRA, TSCA, RCRA, Other_____

3. IN VITRO:
- ☒ Ames (Salmonella) Test
- ☐ Gene Mutation (CHO)
- ☐ Chromosome Exchange (SCE)
- ☐ Chromosome Damage (Micronucleus)
- ☐ DNA Damage (UDS)
- ☐ Macrophage (RAM) test
- ☐ Other _____

RECEIVED
AUG 24 1984
W. C. HENGLER

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

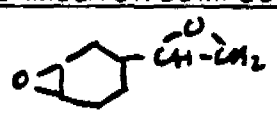
PRODUCT/COMPOSITION NAME: ERL-4206

IUPAC NOMENCLATURE: 7-OXABICYCLO(4.1.0)HEPTANE, 3-OXIRANYL-

CAS NO.: 106-87-6

CHEMICAL SYNONYMS/ALTERNATE/TRADE NAMES:
HALOCITE RD 4; UNOX 206; 4-VINYLCYCLOHEXENE DIOXIDE; ERL-206

STRUCTURAL FORMULA OR COMPOSITION OF MIXTURES:



$C_8H_{12}O_2$

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

(BARC USE) ACKNOWLEDGEMENT OF SAMPLE RECEIPT

Date Received July 25, 1984 By: B. C. Myers

BARC Sample No 47-202

UCC
062517

3362

CHEMICAL INFORMATION

1. SAMPLE PURITY/COMPOSITION: Commercial product. Product is > 97% of indicated structure by gc. Impurities include acetophenone and benzaldehyde.

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:
1. 32 ounces for *full series* of tests
2. 16 ounces for chemicals with known high toxicity
3. 2 ounces for *irritancy tests only*

In vitro Tests: 4 ounces

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. 140.18

Density (liquids) 1.09 - 1.10 (25/25°)

pH (aqueous sol.) ____

Freezing point -55 °C °C

Boiling point 227°C/760 mm °C

Flash point 107 °C
(Tag, closed cup)

Vapor Pressure (@ 3 temp between 15° and 150°C):
1. 0.1 mm @ 20°C °C
2. ____ mm @ ____ °C
3. ____ mm @ ____ °C

4. SOLUBILITY:

Solvent	Percent Solution
Water, % by wt. @ 25°C	1.8
Acetone	
Ethanol	
Dimethylsulfoxide	
Vegetable Oil	
Other (specify)	

5. STABILITY: (Check appropriately):

Shelf Life ____ < 6 mo; ✓ > 6 mo.

Light sensitive ____ yes; ✓ no

Temp. sensitive ____ yes; ✓ no

Oxidizes ____ yes; ✓ no

Polymerizes* ✓ yes; ____ no

Known solvent Incompatibility (specify) ____

In presence of acids and bases

UCC
062518

3363

HANDLING/STORAGE/DISPOSAL INFORMATION:

1. 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: NV

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

2. SPECIAL PERSONNEL PROTECTIVE EQUIPMENT NEEDED: RUBBER GLOVES

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

4. RECOMMENDED STORAGE CONDITIONS:

Temperature (Check appropriately):		Special Conditions Required (Check appropriately)	
Room Temp.	✓	Under N_2 gas	
Refrigerate	< 20°C	Dessicator	
Freeze	< 0°C	Other	

5. SAMPLE DISPOSAL: (Check appropriately):

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

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

UCC
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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

(Approved by U.S. Department of Labor "Essentially Similar" to Form LSB-OOS-4)

CHEMICAL NAME: BAKELITE® CYCLOALIPHATIC EPOXY RESIN ERL-4206

SYNONYMS:	Vinyl-3-Cyclohexene Diepoxide; Epoxyethyl-3, 4-Epoxycyclohexane	CHEMICAL FAMILY:	Epoxy Resins
FORMULA:	$C_8H_{12}O_2$	MOLECULAR WEIGHT:	140.18

TRADE NAME AND SYNONYMS: BAKELITE Cycloaliphatic Epoxy Resin ERL-4206

I. PHYSICAL DATA

BOILING POINT, 760 mm. Hg	227°C. (440.6°F.)	FREEZING POINT	Sets to glass below -55°C.
SPECIFIC GRAVITY (H_2O = 1)	1.0986 at 20/20°C.	VAPOR PRESSURE AT 20°C.	<0.1 mm. Hg
VAPOR DENSITY (air = 1)	4.86	SOLUBILITY IN WATER, % by wt. at 20°C.	18.3
PERCENT VOLATILES BY VOLUME	Nil	EVAPORATION RATE (Butyl Acetate = 1)	0.01
APPEARANCE AND ODOR	Low viscosity liquid; characteristic odor.		

II. HAZARDOUS INGREDIENTS

MATERIAL	%	TLV (Units)
Vinyl Cyclohexene Diepoxide	100	Not established by ACGIH or OSHA
(See Sections III through VIII)		

III. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (Test method)	225°F., Pensky-Martens closed cup ASTM D 93 230°F., Cleveland open cup ASTM D 92	AUTOIGNITION TEMPERATURE	747°F.
FLAMMABLE LIMITS IN AIR, % by volume	Not determined (Nonvolatile material)		

EXTINGUISHING MEDIA	Use carbon dioxide or dry chemical for small fires. Use alcohol-type foam for large fires.
SPECIAL FIRE FIGHTING PROCEDURES	Water in a fine spray can be used. Do not use a solid stream of water; it may cause frothing.
UNUSUAL FIRE AND EXPLOSION HAZARDS	Hazardous polymerization may occur, due to heat generated by exposure to a fire.

IV. EMERGENCY PHONE NUMBER

304/744-3487

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

THRESHOLD LIMIT VALUE	10 ppm. ACGIH notice of intended changes (1975).
EFFECTS OF OVEREXPOSURE	Contact with skin or eyes may be irritating, with eyes most severely affected. Absorption through skin may cause nausea, vomiting, and other signs of illness. Skin painting tests in animals caused skin cancers, but no cancers have been reported in humans.
EMERGENCY AND FIRST AID PROCEDURES	In case of contact immediately flush eyes with plenty of water for at least 15 minutes and wash skin thoroughly with plenty of soap and water. Call a physician for eyes. Remove contaminated clothing at once and wash underlying skin. Wash clothing before reuse.

V. REACTIVITY DATA

STABILITY			
UNSTABLE	STABLE	CONDITIONS TO AVOID	Avoid storage at temperatures above 100°F.
✓	—		

INCOMPATIBILITY (materials to avoid)	Avoid amines, acids, alkalies.
HAZARDOUS DECOMPOSITION PRODUCTS	Thermal decomposition or burning may produce carbon monoxide and/or carbon dioxide.

HAZARDOUS POLYMERIZATION			
May Occur	Will not Occur	CONDITIONS TO AVOID	Avoid amines, acids, alkalies.
✓	—		

VI. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED	Small spills can be flushed with large quantities of water. Larger spills should be collected for disposal.
WASTE DISPOSAL METHOD	Dilute with an inert solvent, and incinerate in a furnace where permitted under appropriate Federal, State, and local regulations.

RESPIRATORY PROTECTION (specify type)		Air-supplied mask in confined areas		
VENTILATION	LOCAL EXHAUST	Preferred	SPECIAL	—
	MECHANICAL (general)	—	OTHER	—
PROTECTIVE GLOVES		Rubber	EYE PROTECTION	Coverall goggles
OTHER PROTECTIVE EQUIPMENT		Protective creams on arms and face; eye bath, and safety shower.		

VIII. SPECIAL PRECAUTIONS

PRECAUTIONARY LABELING	BAKELITE® CYCLOALIPHATIC EPOXY RESIN ERL-4206 WARNING! PROLONGED AND REPEATED CONTACT OF LIQUID OR BREATHING OF VAPORS OR MISTS MAY CAUSE DELAYED AND SERIOUS INJURY. ABSORPTION THROUGH SKIN HARMFUL. LIQUID CAUSES EYE INJURY. Do not get on skin, in eyes, or on clothing. Avoid inhalation of vapors or mists. FIRST AID: In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. For eyes, call a physician. Remove contaminated clothing and shoes at once. Wash thoroughly before reuse. FOR INDUSTRY USE ONLY
OTHER HANDLING AND STORAGE CONDITIONS	Avoid storage temperature above 100°F.

3368

UNION CARBIDE CORPORATION
SPECIALTY POLYMERS & COMPOSITES DIVISION

P.O. BOX 670, BOUND BROOK, NJ 08805
TELEPHONE (201) 356-8000

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 ot samples of the these products that are being utilized in current laboratory progra

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, th mutagenicity testing will be performed on valid samples of 4221, 4299, 4234, 4206 and 4205.

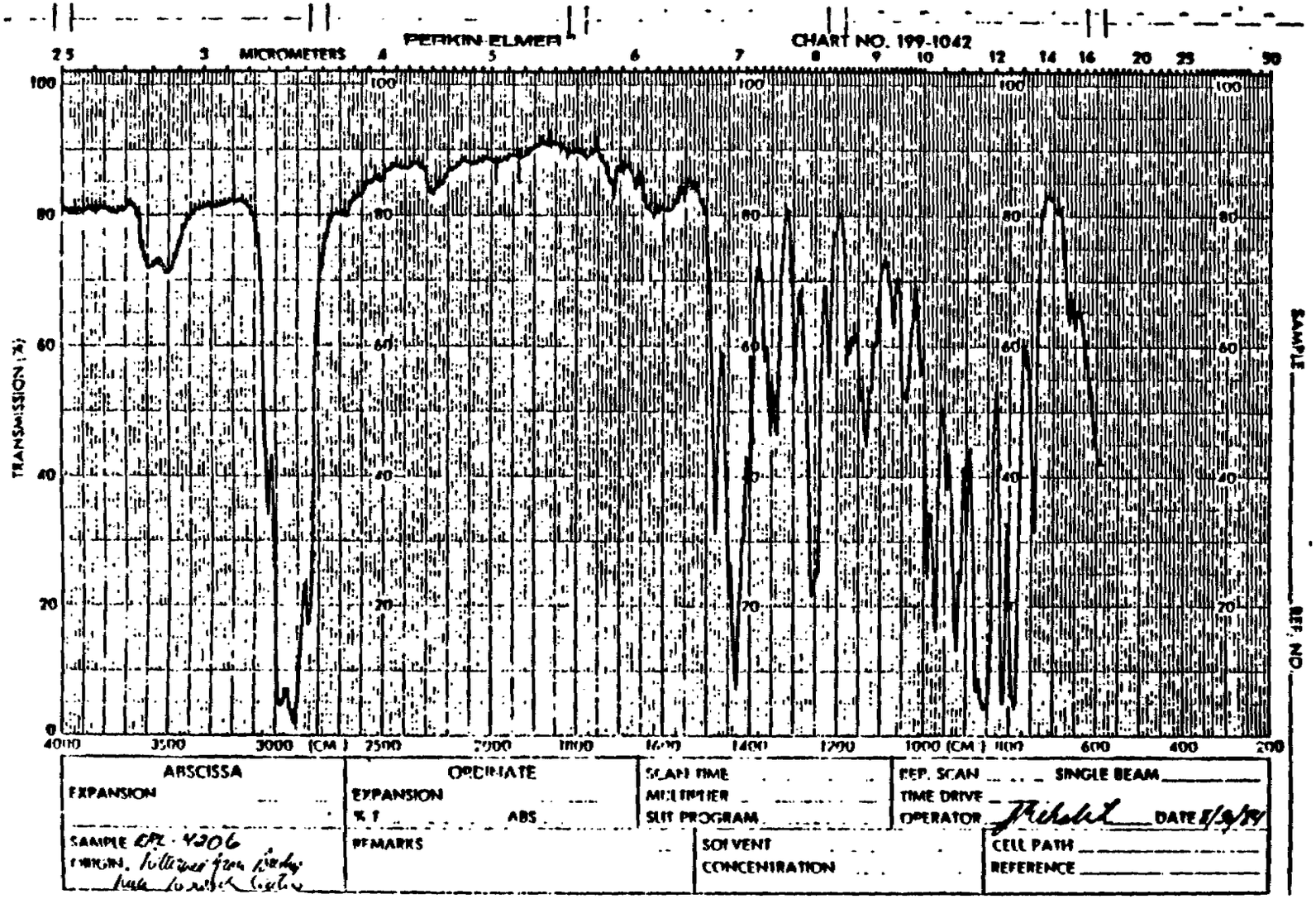
Very truly yours,

encls.

R. J. Cotter

RJC:mjk

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



Appendix 4
Page 9 of 9

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062525

APPENDIX 3

Salmonella typhimurium

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

Strain	+/-S9	N	Mean	Standard Deviation	Median	Range
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

Strain	Positive Control	+/-S9	N	Mean	Standard Deviation	Median	Range
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/0620B-1
11-08-84