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FYI-93-0865

Note to: Michele Stewart
Re: Assignment of FYI numbers

All the attached documents are studies on propylene glycol mono-*tert*-butyl ether (CAS No. 57018-52-7). They were received from ARCO on October 22, 1992 through the National Toxicology Program.

Please assign FYI numbers to each of the attached documents and then return the attached documents to me for shipment to Syracuse Research Corporation.

Thanks.

John D. Walker



FYI-93-0865
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93 JAN 26 PM 12:01
ORIGINAL DOCUMENT RECEIPT 010

ARCOSOLV PTB
Product Elder.



ARCOSOLV PTB

REPORT

DELAINE CONTACT HYPERSENSITIVITY

IN THE GUINEA-PIG WITH

ARCOSOLV PTB

HUNTINGDON RESEARCH CENTRE

Huntingdon England

DELAYED CONTACT HYPERSENSITIVITY
IN THE GUINEA-PIG WITH
ARCOSOLV PTB

Addressee:

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Report issued 22 June 1988

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STUDY DIRECTOR STATEMENT

This report, which describes a study conducted at HRC under my direction, is considered to be a full and true account of the results obtained.

Sheena R Kynoch

Sheena R. Kynoch, B.Sc.,
Head, Department of Industrial Toxicology.

4.6.87

Date

QUALITY ASSURANCE STATEMENT

Acute studies are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by the HRC Quality Assurance Department. It is considered to be an accurate presentation of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

P. R. V. L.

Peter H.C.V. Richold, B.Sc.,
Systems Compliance Auditor,
Quality Assurance Department.

1.7.87

Date

3.2. Animal management

- 3.2.1. Thirty female albino guinea-pigs of the Hartley/Dunkin strain were obtained from D. Hall, Newchurch, Staffordshire, England.

The guinea-pigs selected for the study were all acclimated to the laboratory environment.

The individual bodyweights for each animal at the start and finish of the study are given in the Appendix.

- 3.2.2. Each animal was identified by ear tattoo. This number was unique within the HRC Industrial Toxicology Department throughout the duration of the study.

- 3.2.3. The animals were randomly allocated to test and control groups as follows:

	<u>Number of animals</u>	<u>Animal numbers</u>
Test animals	20	2300 to 2319
Control animals	10	2320 to 2329

- 3.2.4. The guinea-pigs were housed in suspended cages with wire mesh floors in Building R 17 Room 4. They had free access to tap water and a Vitamin C-enriched Guinea-Pig Diet F.D.1 (Special Diets Services Limited). Hay was given once weekly.

- 3.2.5. Animal room temperature was approximately 21°C and relative humidity 30-70%.

Air exchange was maintained at approximately 15 air changes per hour and lighting was controlled by means of a time switch to give 12 hours of artificial light in each 24 hour period.

- 3.2.6. All animals were observed daily for signs of ill health or toxicity.

3.3. Preliminary investigations

The intradermal and topical irritancy of a range of aqueous dilutions of ARCOSOLV PTB was investigated to identify (a) irritant test substance concentrations suitable for the induction phase of the main study and (b) non-irritant concentrations by the topical route of administration for the challenge phase.

The following concentrations of ARCOSOLV PTB were selected:

Induction

Intradermal injection: 5% v/v in water for irrigation.

Topical application: As supplied.

Challenge

30% and 20% v/v in distilled water.

3.4. Treatment procedure

The method employed in this study for the detection of delayed contact hypersensitivity was the guinea-pig maximisation test described by B. Magnusson and A.M. Kligman (1970) in "Allergic Contact Dermatitis in the Guinea-Pig : Identification of contact allergens", published by C.C. Thomas, Springfield, Illinois, U.S.A.

The procedure may be considered in two parts, (1) induction, (2) challenge.

3.4.1. Induction

Test animals

Intradermal injections

A 4 x 6 cm area of dorsal skin on the scapular region of the guinea-pig was clipped free of hair with electric clippers. Three pairs of intradermal injections were made simultaneously into this area as shown in Figure 1.

Injectables were prepared as follows:

1. Freund's complete adjuvant* was diluted with an equal volume of water for irrigation.
2. ARCOSOLV PTB, 5% v/v in water for irrigation.
3. ARCOSOLV PTB, 5% v/v in a 50 : 50 mixture of Freund's complete adjuvant and water for irrigation.

* Difco Laboratories, Detroit 1, Michigan, U.S.A.

Topical application

One week after the injections, the same 4 x 6 cm interscapular area was clipped and shaved free of hair.

A 2 x 4 cm patch of Whatman No. 3 paper was saturated with ARCOSOLV PTB, as supplied. The patch was placed on the skin and covered by a length of impermeable plastic adhesive tape (5 cm width "Blenderm"). This in turn was firmly secured by elastic adhesive bandage ("Elastoplast" 5 cm width) wound round the torso of the animal and fixed with "Sleek" impervious plastic adhesive tape. The dressing was left in place for 48 hours.

Control animals

During the induction period the control animals were treated similarly to the test animals with the exception that the test compound was omitted from the intradermal injections and topical application.

3.4.2. Challenge

The test and control animals were challenged topically two weeks after the induction period using ARCOSOLV PTB, 30% and 20% v/v in distilled water.

Hair was removed by clipping and then shaving from an area on the left flank of each guinea-pig. A 2 x 2 cm patch of Whatman No. 3 paper was saturated with approximately 0.2 ml of ARCOSOLV PTB, 30% v/v in distilled water and applied to an anterior site on the flank. ARCOSOLV PTB, 20% v/v in distilled water was applied in a similar manner to a posterior site. The patches were sealed to the flank for 24 hours under strips of "Blenderm" covered by "Elastoplast" wound round the trunk and secured with "Sleek".

3.4.3. Reading challenge reactions

The challenge sites were evaluated 24, 48 and 72 hours after removal of the patches. The numerical scores awarded to dermal reactions resulting from the challenge application are shown in Table 1.

Reactions were scored according to the following arbitrary scale:

Erythema and eschar formation:

No erythema	0
Slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

3.4.4. Interpretation of results

Dermal reactions in the test animals elicited by the challenge application were compared with the findings simultaneously obtained in the control animals.

A test animal was considered to show positive evidence of delayed contact hypersensitivity if the observed dermal reaction at challenge was definitely more marked and/or persistent than the maximum reaction seen in animals of the control group.

If the dermal reaction seen in a test animal at challenge was slightly more marked and/or persistent than (but not clearly distinguishable from) the maximum reaction seen in control animals, the result for that test animal was classified as inconclusive.

A test animal was considered to show no evidence of delayed contact hypersensitivity if the dermal reaction resulting from the challenge application was the same as, or less marked and/or persistent than the maximum reaction seen in animals of the control group.

4. ARCHIVES

All specimens, raw data and other documents generated at HRC during the course of this study, together with a copy of this Final Report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

5. RESULTS

~~The~~ numerical scores awarded to the dermal reactions elicited by the challenge application are shown in Table 1.

The dermal reactions observed in all twenty test guinea pigs were similar to those seen in the control animals.

6. CONCLUSION

In this screening test, performed in twenty albino guinea-pigs, ARCOSOLV PTB did not produce evidence of delayed contact hypersensitivity.

RECEIVED
OCT 14 1987
S. A. RIDLON

AMES METABOLIC ACTIVATION TEST TO
ASSESS THE POTENTIAL MUTAGENIC EFFECT OF
ARCOSOLV PTB

PHOTOCOPY

This photocopy is for the author's final approval and for the sponsor's early information: it has not been QAU-audited. It is requested that any comments be communicated to the Study Director as soon as possible. When these have been received and the QAU audit has been completed, copies of the official bound report containing authors' signatures and QAU statements will be despatched.

Date: 7 October 1987

JAR

Addressee:

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9

BACTERIAL REVERSE GENE MUTATION ASSAY - THE AMES TEST

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

COMPOUND: Arcosolv PTB.

Storage conditions: Stored at room temperature.

Appearance: Colourless liquid.

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

The experiments described in this report were carried out between 6 August 1987 and 22 August 1987.

Solvent: Ethanol.

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

Mutation tests: 5000, 1500, 500, 150, 50 µg/plate.

RESULTS

The revertant colony counts for Arcosolv PTB obtained in the dose range finding test are shown in Table 1. Arcosolv PTB was not toxic towards the tester strains. Therefore 5000 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 Arcosolv PTB 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 Arcosolv PTB 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.

No substantial increases in revertant colony numbers of any of the five tester strains were observed following treatment with Arcosolv PTB at any dose level, either in the presence or absence of metabolic activation (S-9 mix).

CONCLUSIONS AND COMMENTS

It is concluded that no evidence of mutagenic potential of Arcosolv PTB was obtained in this bacterial test system at the dose levels used.

TABLE 1

Dose range finding test on Arcosolv PTM - 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	-	12	2	4	28	96
500	-	13	4	8	23	95
50	-	12	9	8	28	65
5	-	9	6	14	34	91
Solvent	-	14	8	10	33	68
5000	+	8	5	8	24	80
500	+	10	5	17	32	111
50	+	14	4	17	27	100
5	+	11	8	17	20	113
Solvent	+	14	8	8	38	105

- Absence
+ Presence

APPENDIX 1

Experimental Protocol

1. MATERIALS

1.1 Bacterial strains

The following strains will be used in the Ames test:

S. typhimurium TA 1535 his G46 rfa⁻Δ uvr B⁻
S. typhimurium TA 1537 his C3076 rfa⁻Δ uvr B⁻
S. typhimurium TA 1538 his D3052 rfa⁻Δ uvr B⁻
S. typhimurium TA 98 his D3052 rfa⁻Δ uvr B⁻
S. typhimurium TA 100 his G46 rfa⁻Δ uvr B⁻

All five strains are defective in DNA repair capacity (Δ uvr B⁻) and have a defective lipopolysaccharide barrier or the cell wall (rfa⁻). These two properties confer extra sensitivity to DNA damage and also greater permeability of large molecules into the cell. Strains TA 98 and TA 100 also contain a resistance transfer factor (plasmid pKM 101). This factor, which confers resistance to ampicillin, enhances the operation of an error-prone repair system.

The strains are tested routinely for cell membrane permeability and where applicable for ampicillin resistance.

For use in tests sub-cultures are grown in Nutrient Broth (Oxoid) at 37°C for 18 hours. This culture provides approximately 2 x 10⁹ organisms per ml which is assessed by cell counting.

1.2 Positive controls

(a) With S-9 mix

2-Aminoanthracene at 2 µg/plate for strains TA 1535 and TA 1537.

~~2-Aminoanthracene at 0.5 µg/plate for strains TA 1538, TA 98 and TA 100.~~

(b) Without S-9 mix

2-Nitrofluorene at 2 µg/plate for strain TA 1538.

2-Nitrofluorene at 1 µg/plate for strain TA 98.

9-Aminoacridine at 80 µg/plate for strain TA 1537.

N-ethyl-N'-nitro-N-nitrosoguanidine at 5 µg/plate for strain TA 1535.

N-ethyl-N'-nitro-N-nitrosoguanidine at 3 µg/plate for strain TA 100.

2. PROCEDURE

2.1 Preliminary toxicity test

The following procedure is carried out on each bacterial strain:

Four concentrations of test substance are assessed for toxicity using the five tester strains. The highest concentration is usually 0.05 g of test substance dissolved in 1 ml of solvent. Three 10-fold serial dilutions of the top concentration are also tested. The chosen solvent is used as the negative control.

APPENDIX 1

(continued)

0.1 ml of an overnight bacterial culture containing approximately 2×10^9 cells/ml, and 0.5 ml S-9 mix (see Section 3) or 0.5 ml 0.1 M sodium phosphate buffer (pH 7.4) are placed in glass bijoux bottles. 0.1 ml of the test solution is added followed by 2 ml histidine deficient agar. The mixture is thoroughly shaken and overlaid onto previously prepared plates containing 20 ml minimal agar. Single petri dishes are used for each dose level. They are incubated at 37°C for 72 hours. After this period the plates are examined for the appearance of a complete bacterial lawn. Revertant colonies are counted using a Biotran Automatic Colony Counter. Any toxic effects of the test substance are detected by a substantial reduction in revertant colony counts or by the absence of a complete background bacterial lawn.

2.2 Ames test procedure

(a) Without metabolic activation

The following procedure is carried out on each tester strain.

0.1 ml aliquots of bacterial suspension and 0.5 ml of sterile 0.1 M sodium phosphate buffer (pH 7.4) are added to each of one set of sterile bijoux bottles.

0.1 ml of the test compound is added to cultures at five concentrations separated by half-log 10 intervals. The negative control is the chosen solvent. The appropriate positive control is also included. 3 bottles are used at each dose level.

2.0 ml of histidine deficient agar is added to each of the bottles, thoroughly mixed and then overlaid onto previously prepared plates containing 20 ml of minimal agar. Plates are incubated for 72 hours at 37°C.

Colonies are counted using a Biotran Automatic Colony Counter, and the mean number of revertant colonies per treatment group assessed.

(b) With metabolic activation

Methodology is as described in 2.2 (a) except that 0.5 ml of liver homogenate S-9 mix (see Section 3) is added to bijoux bottles in place of sterile buffer.

2.3 Second mutation test

The procedure outlined in Section 2.2 is repeated at a later date; though the concentrations of test substance used in the second test may be altered, if the results of the first test indicate this may be expedient.

(continued)

3. PREPARATION OF LIVER HOMOGENATE S-9 FRACTION

Species: Rat
Strain: CD (Sprague-Dawley-derived).
Source: Charles River UK Limited, Manston Road, Margate, Kent, England.
Age range: 7-8 weeks on arrival.
Weight range: 180-220 g on arrival.
Diet: Labsure's Laboratory Diet No. 1.
Number used: 7-13

3a. Stimulation of rat liver enzymes

Mixed-function oxidase systems in the rat liver are stimulated following a single i/p injection of Aroclor 1254 (diluted in Arachis oil to 200 mg/ml) at a dosage of 500 mg/kg. On the fifth day of induction, following an overnight starvation, the rats are killed and their livers aseptically removed.

3b. Preparation of liver homogenate "S-9"

i. All steps are at 0-4°C using sterile solutions and glassware. The livers are placed in beakers containing 0.15 M potassium chloride. After weighing, livers are transferred to a beaker containing 0.15 M KCl (the volume of KCl in ml is equivalent to 3 times the weight of liver in gram), minced with a sterile scalpel and homogenized in an MSE top-drive homogenizer. This homogenate is centrifuged for 10 minutes at 9000 x 'g' and the supernatant divided into 15 ml aliquots. These are frozen on dry ice and stored at -80°C, and tested with the carcinogen 7,12-dimethylbenzanthracene before use.

ii. Preparation of "S-9 mix"

S-9 mix contains: S-9 fraction (10% v/v), Mg Cl₂ (8 mM), KCl (33 mM), sodium orthophosphate buffer pH 7.4 (100 mM), se-6-phosphate (5 mM), NADP (4 mM). All the cofactors are filter-sterilized before use.

4. ASSESSMENT OF RESULTS

The mean number of revertant colonies for all treatment groups is compared with those obtained for negative and positive control groups. The effect of metabolic activation is assessed by comparing the results obtained both in the presence and absence of the liver microsomal fraction for each treatment group.

A compound is deemed to provide evidence of mutagenic potential if (1) a statistically significant dose-related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value.

APPENDIX 1

(continued)

5. MAINTENANCE OF RECORDS

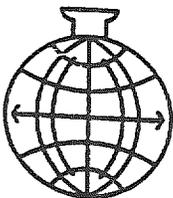
All data are kept in a loose-leaved laboratory notebook which is held in the Department of Mutagenesis and later transferred, together with a mastercopy of the final report, to the Archive Department, Huntingdon Research Centre Ltd., Huntingdon, Cambs, U.K.

6. REFERENCES

1. Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D., Proc. Nat. Acad. Sci. USA (1973) 70, 2281.
2. Ames, B.N., McCann, J. and Yamasaki, E., Mutation Res. (1975) 31, 347.
3. McCann, J., Choi, E., Yamasaki, E. and Ames, B.N., Proc. Nat. Acad. Sci. USA (1975) 75, 5135.
4. Garner, R.C., Miller, E.C. and Miller, J.A., Cancer Res. (1972) 33, 2058.
5. de Serres, F.J. and Shelby, M.D., Mutation Res. (1979) 61, 159.
6. OECD Guideline for testing of chemicals no. 471:- Genetic Toxicology : Salmonella typhimurium. Reverse Mutation Assay.

AR W. 1986

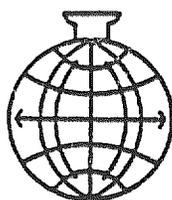
PTB-Rat
Teratology



International Research and Development Corporation

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16



International Research
and Development Corporation

MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: ARCO Chemical Company

TEST ARTICLE: 273990

TITLE: Inhalation Developmental Toxicity Study in Rats

DATE OF SUBMISSION: June 3, 1988

419-029

"credence through research"

International Research and Development Corporation

T A B L E O F C O N T E N T S
(Continued)

	<u>Page</u>
V. Results	19
A. Maternal Observations	19
1. Survival, Appearance and Behavior	19
2. Body Weights	19
3. Food Consumption	19
4. Water Consumption	20
5. Cesarean Section Observations	20
6. Organ Weights	20
B. Fetal Morphological Observations	21
1. Malformations	21
2. Developmental Variations	21
C. Exposure Concentrations	21
VI. Signatures	22
VII. Discussion and Conclusion	23
VIII. References	24

Figure No.

1. Schematic Diagram of Generation and Exposure System	35
2. Diagram Illustrating Location of Sampling Positions for Chamber Distribution Evaluations	36

Table No.

1. Summary of Maternal Antemortem and Necropsy Observations	25
2. Summary of Maternal Body Weights and Body Weight Changes	26
3. Summary of Maternal Food Consumption	27
4. Summary of Maternal Water Consumption	28
5. Summary of Maternal and Fetal Observations at Cesarean Section	29

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TABLE OF CONTENTS
(Continued)

<u>Table No. (Cont.)</u>	<u>Page</u>
6. Summary of Maternal Organ Weights	30
7. Summary of the Incidence of Fetal Malformations	31
8. Summary of the Incidence of Fetal Developmental Variations	32
9. Nominal Exposure Concentrations	33
10. Daily Average Actual Exposure Concentrations	34
APPENDIX A	
Quality Assurance Inspections	37-38
APPENDIX B	
Individual Experimental Data	39-77
APPENDIX C	
Personnel Involved in the Study	78-79

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II. SYNOPSIS

Mated Charles River CDF® female rats, consecutively assigned to one control and three treatment groups of 25 animals each, were used to determine the developmental toxicity potential of 273990. Desired concentrations of 250, 750 and 1000 ppm were administered by whole body inhalation exposure on days 6 through 15 of gestation. The control group was exposed to filtered air only on a comparable regimen. Cesarean sections were performed on all females on gestation day 20 and the fetuses were removed for teratologic evaluation.

Actual exposure concentrations were calculated as 230, 726 and 990 ppm for the study. All study animals survived to scheduled sacrifice. Approximately half of the females from the high-exposure group were noted to be pale throughout most of the exposure period; this clinical sign was attributed to the test article. The test article had no adverse effects on maternal body weight gains or food and water consumption. Absolute and relative liver weights of the mid- and high-exposure groups were significantly greater ($p < 0.05$ or 0.01) than the control values. These increases were considered exposure related.

The Cesarean section parameter values of the groups exposed to the test article were not markedly different from those of the control group. None of the fetal morphological findings noted among the exposed groups were thought to be induced by the test article.

In conclusion, the test article, 273990, did not elicit developmental toxicity at exposure levels of 990 ppm or less when administered by inhalation to gravid Charles River CDF® female rats via whole body exposure methods.

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III. INTRODUCTION

A. OBJECTIVE

The objective of this study was to determine the developmental toxicity potential of the test article in rats, in accordance with EPA/TSCA Health Effects Test Guidelines published in Federal Register 50 (188), 39397-39471, 1985.

B. TEST ARTICLE IDENTIFICATION

The test article was received from ARCO Chemical Company, Lemont, Illinois on June 9, 1986 as indicated below:

<u>Label</u>	<u>Description</u>
273990	(1 container received)

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IV. METHODS AND PROCEDURES

This study was conducted in accordance with the protocol as approved by the Sponsor and in compliance with the Standard Operating Procedures of International Research and Development Corporation (IRDC). Procedures pertinent to this study are described herein.

A. EXPERIMENTAL DESIGN

1. Animal Receipt and Maintenance

One hundred forty untreated, sexually mature, 65 day old, virgin female Charles River CDF® rats were received from Charles River Laboratories, Inc., Kingston, New York on September 25, 1986. Upon receipt, the animals were assigned temporary animal numbers and housed individually in suspended wire-mesh cages. During the 13-day acclimation period, the animals were carefully observed for changes in appearance and behavior. Prior to study initiation, a pretest viral health screen was conducted on ten selected rats in order to determine the suitability of the population of animals proposed for this study. From acquisition until sacrifice, each animal was provided with basal laboratory diet of Purina® Certified Rodent Chow® #5002 ad libitum except during actual exposures. Tap water was freely available at all times.

The basal laboratory diet was analyzed by the manufacturer for the presence of pesticides, heavy metals and aflatoxins. The drinking water at IRDC is analyzed quarterly for the presence of pesticides, heavy metals and coliforms. The results of these analyses are stored in the IRDC Archives in Mattawan, Michigan and are available upon request. The Study Director was not aware of any potential contaminants of either diet or drinking water which might have interfered with the results of this study.

Throughout the acclimation period, all animals were housed in an environmentally controlled room. Temperature ranged between 24°C and 25°C, with a mean temperature \pm standard deviation of $24.8 \pm 0.39^\circ\text{C}$;

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humidity ranged between 66% and 69%, with a mean humidity \pm standard deviation of $67.6 \pm 1.34\%$. Fluorescent lighting provided illumination 12 hours per day. During the study, all animals were housed in exposure chambers under similar environmental conditions. Rats were housed individually in suspended wire-mesh cages from receipt until sacrifice except during mating when they were cohabitated with stock males. Nesting material was not provided since the females were sacrificed prior to delivery. Each female was identified by cage, group and individually by a Monel[®] metal ear tag bearing its animal number. The individual animal number plus the IRDC study number comprised a unique identification number for each animal. The females were approximately 11 weeks old at the time of mating and weighed between 131 and 180 g on gestation day 0. Mating was initiated on October 8, 1986 and the last Cesarean section was performed on November 5, 1986.

2. Rationale for Selection of Species and Strain

The rat is an acceptable model for developmental toxicity studies. This strain is susceptible to known teratogenic agents.

3. Mating

At the end of the acclimation period, all animals were weighed and subjected to a detailed physical examination. At this time, animals considered suitable for study were cohabitated with stock males used exclusively for this purpose.

One female and one male rat of the same strain and source were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug. The day evidence of mating was detected was designated day 0 of gestation and the female was returned to an individual cage, assigned a permanent animal number and properly identified by ear tag.

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4. Organization of Test Groups

Mated females were consecutively assigned in a block design to one control and three treatment groups consisting of 25 rats each by the following procedure. The order in which the mated females were assigned corresponded to the day the copulatory plug was observed and the order in which the animal appeared on the breeding record. The first mated female on the breeding record was assigned to the first group, the second mated female assigned to the second group; all remaining animals were assigned in this manner until the required number of mated females had been placed into each group.

B. TEST ARTICLE ADMINISTRATION

1. Animal Exposure

Exposures were conducted in conjunction with IRDC Study No. 419-031 (Inhalation Developmental Toxicity Study in Rabbits). All groups were exposed simultaneously to the test material atmospheres in four stainless steel and glass exposure chambers, each with a volume of 16 cubic meters. One chamber was assigned to each group. With the exception of two short periods each day (once in the A.M. and once in the P.M.), the animals were housed in the chambers 24 hours per day during the exposure phase of the study. Exposures were conducted at approximately the same time each day. Animals were exposed for six hours a day from gestation day 6 through 15.

Prior to each exposure the animal cages were removed from the chamber, the feed and the excreta pans were removed and the cages were replaced in the chamber for the duration of exposure. Water was available at all times during the exposures. Following the exposure the cages were removed from the chamber and the chamber was cleaned. Feed and excreta pans were returned to the cages which were then replaced in the chambers. The position of the cages in the chamber was systematically rotated every three days during the exposure period.

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Chamber ventilation air was provided by an HVAC system separate from the general laboratory air-handling system. The ventilation air was filtered to remove particulates, and temperature and relative humidity were controlled. Chamber airflow rate ranged from approximately 2000 to 3200 L/min, depending on the desired exposure concentrations.

Chamber airflow rate, temperature and relative humidity were recorded for each chamber approximately every 30 minutes during the exposures. The mean (\pm S.D.) of the daily mean readings of airflow rate, temperature and relative humidity are shown in the following table:

Chamber Number	Chamber Airflow Rate (L/min)		Chamber Temperature (°F)		Chamber Relative Humidity (%)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
C-8	2131	\pm 67.4	74	\pm 0.78	37	\pm 6.8
C-7	2276	\pm 70.7	77	\pm 0.73	40	\pm 6.1
C-5	2403	\pm 86.3	80	\pm 0.66	40	\pm 7.2

S.D. - Standard deviation

2. Generation of Exposure Atmospheres

The vapor exposure atmospheres were generated with the system shown in Figure 1. The liquid test material was delivered by a fluid metering pump (FMI) from a reservoir through 3.2 mm O.D. Teflon® tubing to a glass column approximately 5 cm in diameter and 30 cm in length. The Teflon® tubing extended down into the column which was packed with glass beads (approximately 500 g of 20 mm diameter and 380 g of 5 mm diameter beads).

In house compressed air, heated by a 400 watt cartridge heater, was passed up the column counter-current to the liquid flow. The concentrated test material vapors were carried to the chamber and mixed with chamber ventilation air to dilute the vapor concentration to the desired exposure level. The generation of the desired exposure concentrations

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required the use of 1, 2 or 4 separate glass bead columns for the low, medium and high level exposure groups, respectively. The glass pipe connecting the generation system to the chamber was heated with heat tape which prevented condensation of test material within the pipe.

The operating parameters for the generation systems are shown in the following table:

	Exposure Level		
	Low	Medium	High
Chamber Number	C-8	C-7	C-5
Desired Concentration (ppm)	250	750	1000
FMI Pump Type:			
Column 1	RPG20 1/4	RPG50 1/4	RPG50 1/4
Column 2	NA	RPG50 1/4	RPG50 1/4
Column 3	NA	NA	RPG20 1/4
Column 4	NA	NA	RPG50 1/4
FMI Pump Settings:			
Column 1	8.8	5.0	3.0
Column 2	NA	4.0	3.0
Column 3	NA	NA	7.5
Column 4	NA	NA	3.5
Theoretical Flow Rate (ml/min):			
Column 1	4.5	6.7	4.2
Column 2	NA	5.9	4.2
Column 3	NA	NA	4.1
Column 4	NA	NA	4.3
Ammeter Settings (A):			
Column 1 Heater	1.7	1.8	1.9
Column 2 Heater	NA	1.8	1.9
Column 3 Heater	NA	NA	1.9
Column 4 Heater	NA	NA	1.9
Heat Tapes	1.5	1.5	1.5
Maximum Column Temp. (°C) ^a :			
Column 1	105	105	97
Column 2	NA	115	100
Column 3	NA	NA	107
Column 4	NA	NA	105
Airflow Rate (L/min):			
Columns	70	70	70
Chamber	2000-3000	2000-3000	2200-3200

^aMeasured at bottom of bead column with no liquid flow.
NA - Not applicable

2

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Prior to the start of each exposure, the generation systems, except for the FMI pumps, were turned on and the columns were allowed to heat for at least 30 minutes, at which time the FMI pumps were turned on and the exposures were started.

3. Determination of Exposure Concentrations

a. Nominal

Nominal exposure concentrations were determined for each exposure. The test material reservoir was weighed before and after the exposure, and the weight difference was divided by the total volume of air that passed through the chamber during the exposure. The resultant concentration in g/L was converted to ppm as shown below:

$$\begin{aligned} \text{ppm} &= \text{g/L} \times \frac{R}{M} \times \frac{T}{P} \times 10^6 \\ &= \text{g/L} \times 1.88 \times 10^5 \end{aligned}$$

Where:

g = weight, in grams, of test material used during the exposure

L = volume, in liters, of air passed through the chamber during the exposure

R = Universal Gas Constant, $\frac{62.36\text{L} - \text{mmHg}}{\text{mole} - ^\circ\text{K}}$

M = molecular weight of the test material, 132 g/mole

T = nominal laboratory temperature, 294°K

P = nominal laboratory barometric pressure, 740 mmHg

10⁶ = conversion factor for ppm

Any unvaporized test material left at the bottom of the glass bead column at the end of an exposure was added to a previously tared group-specific container and saved. Except for the medium level group where 40, 4, and 21 grams were found at the end of exposure days 1, 12 and 16, respectively, there was no unvaporized test material left at the end of the exposure.

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b. Actual

Actual exposure concentrations were measured with an infrared spectrophotometer (IR). Each chamber was sampled by drawing air from the chamber through Teflon® sampling lines, through a series of Teflon® solenoid valves, to the IR. Operation of the solenoid valves and collection and recording of data were controlled by a Hewlett-Packard 3388A laboratory computer. Each chamber, including the control, was sampled approximately once each hour during exposure. The operating parameters for the IR are shown in the following table:

Instrument:	Wilkes - MIRAN Model 1A-CVF
Wavelength:	9.25 microns
Range:	1 Absorbance Unit, Full-scale
Slit Width:	1 mm
Closed-Loop Volume:	5.64 L
Pathlength Setting:	1.33
Gain Switch Setting:	X10
Cell Pressure:	-1 psig
Meter Response Time:	4 seconds
Zero Gas:	Chamber supply air

The IR was calibrated with gas bags containing known vapor concentrations of the test material. The calibration concentrations were created by vaporizing the liquid test material in a known volume of air in a Tedlar® gas bag. Each bag was sampled and the resultant IR instrument response was read. The concentration of vaporized test material in the bag was calculated as follows:

$$\text{ppm} = \frac{R}{M} \times \frac{T}{P} \times \frac{V \times D}{L} \times 10^{-3} \times 10^6$$

Where: R = Universal Gas Constant, $\frac{62.36 \text{ L} \cdot \text{mmHg}}{\text{mole} \cdot ^\circ\text{K}}$

M = Molecular weight of test material, 132 g/mole

T = nominal laboratory temperature, 294°K

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- P = nominal laboratory barometric pressure, 740 mmHg
 V = liquid volume injected, mcl
 D = density of test material, 0.87 g/ml
 L = final air volume of bag, L
 10^{-3} = ml to mcl conversion factor
 10^6 = ppm conversion factor

Each calibration concentration was prepared in triplicate to the specifications shown in the following table:

<u>Amount of Test Material Injected (mcl)</u>	<u>Final Air Volume (L)</u>	<u>Prepared Concentration (ppm)</u>
50	50	160
100	50	330
190	50	620
290	50	950
380	50	1240
480	50	1570

The common log of the prepared concentration was plotted against the common log of the IR instrument response and the best fitting straight line was calculated by the method of least squares. The resultant standard curve was applied to all analytical data on the study.

Prior to each exposure the calibration of the IR was confirmed by injecting a known volume of liquid test material into the closed loop of the IR. The calibration check points and acceptable limits are shown below:

<u>Volume of Test Material Injected (mcl)</u>	<u>Actual Concentration (ppm)</u>	<u>Acceptable Range of Analyzed Concentration (ppm)</u>
8	250	220-280
24	750	680-830
42	1310	1180-1400

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Except for a few days at the beginning of the study, when all three points were checked, only one of the above points was checked on a given day.

The acceptable limits for the daily average exposure concentration were as follows:

<u>Desired Concentration (ppm)</u>	<u>Acceptable Limits for Daily Average Exposure Concentration (ppm)</u>
250	210-270
750	670-830
1000	900-1100

As further documentation of the performance of the complete analytical system, gas bags containing known concentrations of test material were attached to the end of the chamber sample line and analyzed. The bag concentrations were made up by vaporizing known amounts of liquid test material in 100 L Tedlar® gas bags. One bag was made up at approximately the desired concentration for each group, as shown in the following table:

<u>Injection Volume (mcl)</u>	<u>Volume of Air (L)</u>	<u>Actual Bag Conc. (ppm)</u>	<u>Measured Bag Conc. (ppm)^a</u>	<u>% Difference^b</u>
150	100	245	230	-6.1
450	100	735	790	+7.5
670	100	1094	1140	+4.2

^aAs measured by analytical system

^b
$$\frac{\text{Measured Bag Concentration} - \text{Actual Bag Concentration}}{\text{Actual Bag Concentration}} \times 100$$

Conc. - Concentration

The above results demonstrated that there was acceptable agreement between the actual concentration of the bag and the concentration measured by the analytical system.

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4. Analytical Development

a. Homogeneity of Exposure Atmosphere

The homogeneous distribution of the vapor exposure atmospheres was confirmed for each test material exposure chamber before the start of the study. The exposure concentration was measured at a reference location (the normal sampling position) and at five other positions which were within the animal exposure zone. The sampling positions are shown in Figure 2. The distribution determinations were performed with animal cages (without animals) in place in the chamber.

The concentrations at the various positions were then compared to the reference, with the following results:

Sample Location	% Difference from Reference ^a		
	Low-Level	Medium-Level	High-Level
Reference	NA	NA	NA
1	+4.5 ^b	0.0	+2.2
2	+4.5	-2.9	+5.6
3	+4.5	-4.3	+1.1
4	+4.5	+2.9	+2.2
5	0.0	+2.9	-1.1

^a $\frac{\text{Location Concentration} - \text{Reference Concentration}}{\text{Reference Concentration}} \times 100$

^bEach value is mean of four replicate samples
NA - Not applicable

The distribution of the vapor atmosphere in all groups was considered satisfactory for the conduct of the study.

b. Analytical Response from Test Animals

Possible response of the IR resulting from the presence of animals in the chamber was evaluated by placing 12 rabbits, in cages, in the control chamber; the other three chambers were left empty. The chambers were then monitored hourly for 6 consecutive hours using the study analytical system. The maximum IR instrument response in the chamber

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with the animals was well below the limit of detectability for the analytical system, which indicated there was no detectable IR response to animal off-gas products.

c. Presence of Aerosol in Exposure Chamber

In order to determine whether there was any aerosol formation in the exposure chambers, the atmosphere of each chamber, including the control, was monitored, under exposure conditions, with a Sibata Model P-5 digital dust indicator. Each chamber was monitored for 30 minutes, and the number of counts recorded for each test material exposure chamber was compared to the control chamber. The results showed that there was essentially the same number of counts in all four chambers. Thus, there appeared to be no indication of aerosol formation in the chambers.

C. MATERNAL OBSERVATIONS

1. Survival, Appearance and Behavior

Throughout the study, the females were observed twice daily for mortality and overt changes in appearance and behavior. The presence and duration of clinical signs of toxicity were recorded once daily on days 6 through 15 of gestation. Animals that delivered were sacrificed on the day that delivery occurred.

2. Body Weights

Individual maternal body weights were recorded on gestation days 0, 6, 8, 10, 12, 14, 16 and 20.

3. Food Consumption

Individual food consumption was measured daily from gestation days 6 to 16 and calculated as g/animal/day and/or g/kg of body weight/day for the gestation days 6 to 9, 9 to 12, 12 to 16 and 6 to 16 intervals.

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4. Water Consumption

Individual water consumption was measured daily from gestation days 6 to 16 and calculated as g/animal/day and/or g/kg of body weight/day for the gestation days 6 to 9, 9 to 12, 12 to 16 and 6 to 16 intervals.

5. Cesarean Section Observations

On gestation day 20, all surviving females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the uterus and ovaries were exposed by an abdominal incision. The number and location of viable and nonviable fetuses, early and late resorptions, and the numbers of total implantations and corpora lutea were recorded. The uterus was then excised, weighed and the fetuses removed. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded. The adrenal glands, thymus, liver, kidneys and spleen were weighed and saved in neutral buffered 10% formalin. Uteri from females that appeared nonpregnant were opened and placed in 10% ammonium sulfide solution for detection of implantations.¹

D. FETAL MORPHOLOGICAL OBSERVATIONS

Fetuses were individually weighed, sexed, tagged and examined for external malformations and variations. Approximately one-half of the fetuses were placed in Bouin's solution for subsequent soft tissue examination using the Wilson razor-blade sectioning technique². The remaining fetuses were eviscerated, fixed in alcohol, macerated with potassium hydroxide, stained with Alizarin Red S and cleared with glycerin by a method similar to that described by Dawson³ for subsequent skeletal examination. All gross, visceral and skeletal alterations observed in this study have been placed into one of two categories, malformations or developmental variations.

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Malformations are those structural anomalies that alter general body conformity, disrupt or interfere with body function, or are generally thought to be incompatible with life. Specific examples of processes that result in maldevelopment include marked/severe misshapening, asymmetry or irregularity of structure brought about by fusion, splitting, disarticulation, malalignment, hiatus, enlargement, lengthening, thickening, thinning or branching. Absence (agenesia) of parts or whole structures is also considered a malformative process.

Developmental variations are those alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal. Most examples of alterations placed in the variant category are minor variations in size and form of normally present ossification centers. While these are evaluated on a precise day of development, some variation is expected related to when conception and implantation actually occurred. Thus, differences in the pattern of ossification, manifested either as retardation or as acceleration of apparent osteogenesis, are common findings. Also included in this category are slight misshapening or misalignment of structures, processes involving continued development (bilateral skeletal centers not yet fused, incomplete maturation of renal papillae, presence of vestigial structures, etc.) and development of extra ossification sites. Several clarifications are necessary with respect to tabulation of these categories as follows: Tabulations refer to numbers of fetuses/litters affected. When a malformation exists in a particular site, a variation in the same site is also tabulated, e.g. fused ribs/14th rudimentary rib. In cases where two or more variations of the same type or structure exist in the same specimen, all are

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counted with a single exception: a unilateral rudimentary rib is not tabulated when an extra full rib is present. The kidney was evaluated according to criteria described by Woo and Hoar⁴ and only grade 0 lesions of the renal pelvis and papilla (absent papillae) were tabulated as variations. Dilatation of the ureter was also included in the tabulation, whether or not it accompanied renal lesions.

E. DATA RETENTION

All preservable specimens, raw data, a sample of the test article and copies of the final report are retained in the Archives of International Research and Development Corporation, 500 N. Main, Mattawan, Michigan 49071.

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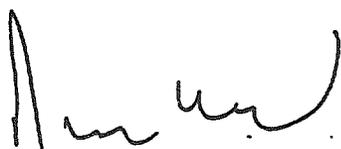
F. STATISTICAL ANALYSIS

All statistical analyses compared the values of the treated groups with those of the control group, with the levels of significance at $p < 0.05$ and $p < 0.01$. All means were accompanied by standard deviations.

Male to female fetal sex ratios and the proportions of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2 X 2 contingency tables as described by Siegel⁵ to determine the significance of differences.

The proportions of resorbed and dead fetuses, postimplantation losses, and food and water consumption were compared by the Mann-Whitney U-test as described by Siegel⁵ and Weil⁶ to determine the significance of differences.

Numbers of corpora lutea, total implantations and viable fetuses, mean fetal body weights and maternal organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variance and the appropriate t-test (for equal or unequal variance) as described by Steel and Torrie⁷ using Dunnett's⁸ multiple comparison tables to determine the significance of differences.


 Rama K. Kalra, M.B.A.
 Director of Statistics and Data
 Processing

5/31/88
 Date

419-029

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V. RESULTS

A. MATERNAL OBSERVATIONS

1. Survival, Appearance and Behavior (Table 1)

No study animal died or was sacrificed in extremis prior to scheduled sacrifice on gestation day 20. Three females, one from the control and two from the low-exposure (250 ppm) group, delivered prematurely between gestation days 16 and 19. No biological relevance was attributed to these early deliveries.

Eleven of 25 high-exposure (1000 ppm) females were noted to be pale from gestation days 7 through 15. This finding was considered related to test article exposure. All study animals, including those from the control group, had red-brown staining on the dorsal shoulders. The source of this staining is unknown, but was not considered treatment related because of its presence among the control animals. The remaining clinical findings occurred in low incidence (one to four animals per group) and were not considered induced by exposure to the test article.

Only two females had abnormal findings at necropsy. One control female had an ovarian cyst and one low-exposure female was missing an adrenal gland.

2. Body Weights (Table 2)

The body weight gains of the animals exposed to the test article were comparable with those of the control during the exposure (gestation days 6 through 15) and overall gestation (gestation days 0 to 20) periods.

3. Food Consumption (Table 3)

Exposure to the test article did not adversely affect maternal food consumption at any of the exposure levels. The g/animal/day value at the high-exposure level for the gestation day 6 to 9 interval was

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significantly less ($p < 0.05$) than the corresponding control value. The actual difference between the group mean values was only 0.5 g; this was not considered great enough to be attributed to the test article. The g/animal/day food intake values for the low- and mid-exposure groups were significantly greater ($p < 0.05$) than the control value for the overall exposure period (gestation days 6 to 16). These increases were not thought to be relevant.

4. Water Consumption (Table 4)

Maternal water consumption values calculated for the groups exposed to the test article did not indicate any adverse effects related to the test article. The g/animal/day and g/kg of body weight/day values for all exposed groups were moderately to significantly greater ($p < 0.05$ or 0.01) than those of the control group. However, as no trends were apparent with respect to exposure levels, the increases were not considered induced by the test article.

5. Cesarean Section Observations (Table 5)

None of the Cesarean section parameter values of the groups exposed to the test article indicated an adverse exposure effect. Mean corpora lutea, implantations, postimplantation loss and viable fetuses values for these groups were not markedly different from those of the control. Mean uterine and fetal weights for those groups exposed to 273990 were comparable with the corresponding control values. There were no biologically significant discrepancies with respect to fetal sex distribution.

6. Organ Weights (Table 6)

Absolute and relative liver weight values of the mid- and high-exposure levels were significantly greater ($p < 0.05$ or 0.01) than the corresponding control values. The increases were noted to become greater

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with increasing exposure levels and were considered related to the test article. The absolute and relative weights of remaining organs from the exposed animals were not markedly different from the control values.

B. FETAL MORPHOLOGICAL OBSERVATIONS

1. Malformations (Table 7)

Two fetuses from two high-exposure litters had malformations. One had a folded retina and the other had anophthalmia. Neither anomaly was considered exposure related. No malformations were observed among the control, low- or mid-exposure groups.

2. Developmental Variations (Table 8)

Developmental variations observed among fetuses from the exposed dams were not considered to have been induced by the test article. All variations occurred either in low incidence or at a rate comparable with the control group.

C. EXPOSURE CONCENTRATIONS (Tables 9 and 10)

The daily nominal and daily average actual exposure concentrations are summarized for the entire study in the following table.

Chamber Number	Desired Concentrations (ppm)	Exposure Concentration (ppm)	
		Nominal ^a	Actual ^b
C-8	250	304 ± 9.9	230 ± 12.9
C-7	750	857 ± 25.9	726 ± 28.7
C-5	1000	1105 ± 38.4	990 ± 33.6

^aMean ± S.D. of daily nominal exposure concentration

^bMean ± S.D. of daily average actual exposure concentration

S.D. - Standard deviation

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VI. SIGNATURES

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 John G. Drummond, Ph.D.
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 Kit A. Keller, Ph.D.
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 James L. Schardein, M.S., DATS
 Scientific Director, Reproduction and
 Teratology Division
 Date

419-029

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VII. DISCUSSION AND CONCLUSION

Exposure to the test article at levels of 230, 726 and 990 ppm during organogenesis elicited no developmental toxicity. The females in the mid- and high-exposure groups, however, were affected by the test article. Approximately half of the high-exposure females were noted to be pale throughout most of the exposure period. Also, the absolute and relative liver weights of the mid- and high-exposure groups were significantly greater ($p < 0.05$ or 0.01) than the control values.

In conclusion, the test article, 273990, did not result in developmental toxicity at exposure levels of 990 ppm or less when administered by inhalation to gravid Charles River CDF® female rats via whole body exposure techniques.

To the best of my knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality and integrity of the study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of the study.


James L. Schardein, M.S., DATS
Scientific Director, Reproduction and
Teratology Division
Study Director

6/2/88
Date

419-029

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VIII. REFERENCES

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8. Dunnett, C. W. (1964). New tables for multiple comparisons with a control. Biometrics, 20: 482-491.

APPENDIX C
Personnel Involved in the Study

419-029

55

The following list of people were responsible for various phases of this study:

Dale Aldridge, B.S.	Unit Supervisor, Department of Report Writing
Dixie K. Bushee, B.S.	Unit Supervisor, Test Material Control
James R. Casey, B.S.	Manager, Animal Services
Benjie A. Culp	Unit Supervisor, Inhalation Toxicology
John G. Drummond, Ph.D.	Manager of Inhalation Toxicology
Mark W. Griggs, B.S.	Manager, Test Material Control
Rama K. Kalra, M.B.A.	Director, Statistics and Data Processing
Kit A. Keller, Ph.D.	Assistant Director, Reproduction and Teratology Division
Jacqueline M. Miller, B.S.	Technical Writer, Department of Report Writing
Ronald Lindahl, B.S.	Unit Supervisor, Reproduction and Teratology Division
Gloria Nadwornik	Group Supervisor, Reproduction and Teratology Division
William Nowland	Manager, Necropsy Services
Colleen A. Prichard, B.A.	Unit Supervisor, Reproduction and Teratology Division
James L. Schardein, M.S., DATS	Scientific Director, Reproduction and Teratology Division
Charles E. Ulrich, B.S.	Scientific Director of Inhalation Toxicology



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October 14, 1986

Mr. Steve Cragg
ARCO Chemical Company
3801 West Chester Pike
Newton Square, PA 19073

Dear Mr. Cragg:

Please find enclosed your final report ABC #34076 for the
Modified Sturm Study with PTB.

Please call if you have any questions?

Sincerely,

Brian R. Bowman
Supervisor - Environmental Fate

BRB/jh

Static Acute Toxicity Report
#34254

Acute Toxicity of ARCOSOLV® PTB
to Bluegill Sunfish (Lepomis macrochirus)

SUMMARY

The acute toxicity of ARCOSOLV® PTB to bluegill sunfish (Lepomis macrochirus) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of ARCOSOLV® PTB: 100, 180, 320, 560 and 1000 mg/l. Ten fish, with a mean weight of 0.27 (± 0.062) g and a mean standard length of 22 (± 1.3) mm, were exposed to each test concentration and control.

The results of the four day static fish toxicity study using ARCOSOLV® PTB are summarized below. The 24- and 48-hour LC₅₀ values were also determined.

<u>Compound</u>	<u>96-hour LC₅₀</u>
ARCOSOLV® PTB	>1000 mg/l

Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at 1000 mg/l, which was based on the lack of mortality and abnormal effects at the highest concentration tested. There were no abnormal effects noted in any concentration tested during the 96-hour exposure period.

INTRODUCTION

The purpose of this test was to determine the 24-, 48- and 96-hour LC_{50} levels for ARCOSOLV[®] PTB to bluegill sunfish (Lepomis macrochirus).

METHODS AND MATERIALS

The procedures for static bioassay, as described in Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1) and Standard Methods for Examination of Water and Wastewater (2), were used in this experiment.

I. Test Fish

The bluegill sunfish (ABC Lot #5485) used in the test were obtained from Osage Catfisheries, Inc. in Osage Beach, Missouri. The fish were identified to species by the supplier or if necessary using the taxonomic keys developed by Eddy (3). All test fish were held in culture tanks on a 16 hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et al. (4). A daily record of fish observations during the holding period, along with any prophylactic or therapeutic disease treatments, is included in the Raw Data. During this period, the fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii (Artemia sp.) daily until 48-96 hours prior to testing at which time feeding was discontinued. The bluegill sunfish used for this experiment had a mean weight of 0.27 (± 0.062) g and a mean standard length of 22 (± 1.3) mm. This gave a test chamber loading biomass of 0.18 g/l for the definitive study. Weight and length measurements were made on the control group of fish at the termination of the test and are included in the Raw Data.

II. Test Material

The ARCOSOLV[®] PTB sample (Lot #Propylene Glycol Mono-Tertiary Butyl Ether) was received on November 20, 1985, in good condition. The sample upon receipt was observed to be a clear liquid and was stored at room temperature in the dark. Sample purity was specified as >99% in the protocol. The definitive test concentrations were obtained by transferring

appropriate weights of test compound directly to the test chambers. Before addition to the test chambers, 1.5 ml of dimethylformamide (DMF) was added to each sample weight to increase dispersion of the compound in the dilution water. All test concentrations were based on the total compound, i.e. not corrected for sample purity. The solvent control chamber received a 1.5 ml aliquot of DMF, which was equivalent to the highest amount used in any test solution. All standard weights and dilution values are listed in the Raw Data.

III. Test Water

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconstituted water composed of the following compounds in the amounts stated per liter of deionized water:

48 mg NaHCO_3
30 mg $\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$
30 mg MgSO_4
2 mg KCl

This reconstituted water was prepared to yield a total hardness of 40-45 mg/l as CaCO_3 , a total alkalinity of 30-35 mg/l as CaCO_3 and an initial pH of 7.2 to 7.6. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 mg/l and pH 7.3. The well water source from which this dilution water was prepared had the characteristics shown in Table 1.

The test vessels were kept in a water bath at 22°C (± 1.0). The test fish were acclimated to the dilution water and test temperature and held without food for 48-96 hours prior to testing. An acclimation record is included in the Raw Data.

IV. Biological Test Procedure

A range-finding test of 96-hours duration was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 1, 10 and 100 mg/l. Based on the results of preliminary testing, five concentrations of the test compound, ranging in a logarithmic series from 100 to 1000 mg/l, with ten fish per concentration were selected for the definitive bioassay. Also included was a dilution water control and a solvent control chamber. The fish were added to the test chambers by random assignment within 30 minutes after addition of test material. All test organisms were observed once every 24 hours for mortality and abnormal (sub-lethal) effects. Any dead individuals were removed from the test chambers after each 24-hour observation.

V. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC₅₀ program developed by Stephan et al. (6). This program calculated the LC₅₀ statistic and its 95-percent confidence limits using the binomial, the moving average, and the probit tests. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%) an LC₅₀ and/or its 95-percent confidence limits could not be calculated. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC₅₀ (5, 6) although all three models are valid.

RESULTS

The results of the 96-hour static toxicity test with bluegill sunfish (Lepomis macrochirus) exposed to ARCOSOLV® PTB are presented in Table 2. The 24-, 48- and 96- hour LC₅₀ values for ARCOSOLV® PTB were all >1000 mg/l. All results were based on the nominal concentrations of 100, 180, 320, 560 and 1000 mg/l. The 96-hour no-observed effect concentration was estimated to be 1000 mg/l, the highest concentration tested, based on the lack of mortality or observed abnormal (sub-lethal) effects. There were no abnormal effects noted in any concentration tested during the 96-hour exposure period. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing (Raw Data).

Nominal test concentrations, mortality rates, and water quality data are presented in Table 3. The dissolved oxygen concentrations ranged from 6.5 to 9.4 mg/l during the test. These values represented 72 and 108% saturation at 21 and 23°C, respectively, and were considered adequate for testing (1). The pH values ranged from 7.0 to 7.4.

The study was conducted following the intent of the Good Laboratory Practice Regulations (7)

Static Acute Toxicity Report
#34255

Acute Toxicity of ARCOSOLV® PTB
to Rainbow Trout (Salmo gairdneri)

SUMMARY

The acute toxicity of ARCOSOLV® PTB to rainbow trout (Salmo gairdneri) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of ARCOSOLV® PTB: 100, 180, 320, 560 and 1000 mg/l. Ten fish, with a mean weight of 0.60 (± 0.12) g and a mean standard length of 36 (± 2.0) mm, were exposed to each test concentration and control.

The results of the four day static fish toxicity study using ARCOSOLV® PTB are summarized below. The 24- and 48-hour LC₅₀ values were also determined.

<u>Compound</u>	<u>96-hour LC₅₀ (95% C.I.)</u>
ARCOSOLV® PTB	>1000 mg/l

Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at 320 mg/l, which was based on the lack of mortality and abnormal effects. Abnormal effects of mortality, surfacing, loss of equilibrium, dark discoloration and/or fish on the bottom of test chamber were observed during the 96-hour exposure period in the 560 and 1000 mg/l test chambers.

INTRODUCTION

The purpose of this test was to determine the 24-, 48- and 96-hour LC_{50} levels for ARCOSOLV[®] PTB to rainbow trout (Salmo gairdneri).

METHODS AND MATERIALS

The procedures for static bioassay, as described in Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1) and Standard Methods for Examination of Water and Wastewater (2), were used in this experiment.

I. Test Fish

The rainbow trout (ABC Lot #186) used in the test were obtained from Spring Creek Trout Hatchery in Lewistown, Montana. The fish were identified to species by the supplier or if necessary using the taxonomic keys developed by Eddy (3). All test fish were held in culture tanks on a 16 hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et al. (4). A daily record of fish observations during the holding period, along with any prophylactic or therapeutic disease treatments, is included in the Raw Data. During this period, the fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii (Artemia sp.) daily until 48-96 hours prior to testing at which time feeding was discontinued. The rainbow trout used for this experiment had a mean weight of 0.60 (± 0.12) g and a mean standard length of 36 (± 2.0) mm. This gave a test chamber loading biomass of 0.40 g/l for the definitive study. Weight and length measurements were made on the control group of fish at the termination of the test and are included in the Raw Data.

II. Test Material

The ARCOSOLV[®] PTB sample (Lot #Propylene Glycol Mono-Tertiary Butyl Ether) was received on November 20, 1985, in good condition. The sample upon receipt was observed to be a clear liquid and was stored at room temperature in the dark. Sample purity was specified as >99% in the protocol. The definitive test concentrations were obtained by transferring

65

appropriate weights of test compound directly to the test chambers. Before addition to the test chambers, 1.5 ml of dimethylformamide (DMF) was added to each sample weight to increase dispersion of the compound in the dilution water. All test concentrations were based on the total compound, i.e. not corrected for sample purity. The solvent control chamber received a 1.5 ml aliquot of DMF, which was equivalent to the highest amount used in any test solution. All standard weights and dilution values are listed in the Raw Data.

III. Test Water

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconstituted water composed of the following compounds in the amounts stated per liter of deionized water:

48 mg NaHCO_3
30 mg $\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$
30 mg MgSO_4
2 mg KCl

This reconstituted water was prepared to yield a total hardness of 40-45 mg/l as CaCO_3 , a total alkalinity of 30-35 mg/l as CaCO_3 and an initial pH of 7.2 to 7.6. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 10.0 mg/l and pH 7.2. The well water source from which this dilution water was prepared had the characteristics shown in Table 1.

The test vessels were kept in a water bath at 12°C (± 1.0). The test fish were acclimated to the dilution water and test temperature and held without food for 48-96 hours prior to testing. An acclimation record is included in the Raw Data.

IV. Biological Test Procedure

A range-finding test of 96-hours duration was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 1, 10 and 100 mg/l. Based on the results of preliminary testing, five concentrations of the test compound, ranging in a logarithmic series from 100 to 1000 mg/l, with ten fish per concentration were selected for the definitive bioassay. Also included was a dilution water control and a solvent control chamber. The fish were added to the test chambers by random assignment within 30 minutes after addition of test material. All test organisms were observed once every 24 hours for mortality and abnormal (sub-lethal) effects. Any dead individuals were removed from the test chambers after each 24-hour observation.

V. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC₅₀ program developed by Stephan et al. (6). This program calculated the LC₅₀ statistic and its 95-percent confidence limits using the binomial, the moving average, and the probit tests. However, if no mortality occurred or if a dose-response could not be demonstrated over a reasonable range (<37 to >63%) an LC₅₀ and/or its 95-percent confidence limits could not be calculated. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC₅₀ (5, 6) although all three models are valid.

RESULTS

The results of the 96-hour static toxicity test with rainbow trout (Salmo gairdneri) exposed to ARCOSOLV[®] PTB are presented in Table 2. The 24-, 48- and 96- hour LC₅₀ values for ARCOSOLV[®] PTB were all >1000 mg/l. All results were based on the nominal concentrations of 100, 180, 320, 560 and 1000 mg/l. The 96-hour no-observed effect concentration was estimated to be 320 mg/l, based on the lack of mortality or observed abnormal (sub-lethal) effects. The abnormal effects of mortality, surfacing, loss of equilibrium, dark discoloration and/or fish on the bottom of test chamber were observed in the 560 and 1000 mg/l. test concentrations during the 96-hour exposure period. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing (Raw Data).

Nominal test concentrations, mortality rates, and water quality data are presented in Table 3. The dissolved oxygen concentrations ranged from 8.8 to 10.1 mg/l during the test. These values represented 79 and 94% saturation at 11 and 12°C, respectively, and were considered adequate for testing (1). The pH values ranged from 7.0 to 7.3.

The study was conducted following the intent of the Good Laboratory Practice Regulations (7).

Static Acute Toxicity Report
#34256

Acute Toxicity of ARCOSOLV[®] PTB
to Daphnia magna

SUMMARY

The acute toxicity of ARCOSOLV[®] PTB to Daphnia magna was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured at the termination of the test and were within acceptable limits.

The results of the 48-hour static Daphnia magna toxicity study are summarized below. All reported values were based upon nominal concentrations.

<u>Compound</u>	<u>48-hour LC₅₀ (95% C.I.)</u>
ARCOSOLV [®] PTB	>1000 mg/l

The no-effect level observed for ARCOSOLV[®] PTB was 56 mg/l after 48 hours, which was based on the lack of mortality and abnormal effects.

INTRODUCTION

The purpose of this test was to determine the 24- and 48-hour LC₅₀ levels for ARCOSOLV[®] PTB to Daphnia magna.

METHODS AND MATERIALS

The procedures for static bioassay, as described in Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1) and Standard Methods for Examination of Water and Wastewater (2), were used in this experiment. The Daphnia magna used in the test were cultured at the ABC facilities. The adult Daphnia were fed algae (Selenastrum capricornutum) at least every three days prior to testing and supplemented with a suspension of Tetramin[®]/cerophyl. The daphnids were identified to species using the taxonomic key presented by Pennak (3).

The static Daphnia bioassay was conducted in 250-ml glass beakers containing 200 ml of ABC aged well water with the chemical characteristics listed in Table 1. These vessels were kept at 20 (± 2.0) °C in a temperature controlled area. The lighting was maintained at 50-70 foot-candles on a 16-hour daylight photoperiod, with 30 minute simulated dawn and dusk periods.

An initial range finding experiment was conducted using 10 Daphnia each in exposure concentrations of 0.10, 1.0, 10 and 100 mg/l. From this information, six concentrations in duplicate of the test compound with ten Daphnia (first instar less than 24-hours old) per beaker were selected for the definitive bioassay. These concentrations were a logarithmic series ranging from 56 to 1000 mg/l and included a control. All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing, clumping of the daphnids together and daphnids lying on the bottom of test chambers.

The ARCOSOLV[®] PTB (Propylene Glycol Mono-Tertiary Butyl Ether) sample was received on November 20, 1985 as a clear liquid and was stored at room temperature. Sample purity was >99%. Test concentrations were prepared based on total compound and not corrected for sample purity. All standard weights and dilutions can be found in the Appendix. Deionized water was used in the preparation of all definitive working stock solutions.

The 24- and 48-hour LC₅₀ values and corresponding 95-percent confidence limits were determined by an LC₅₀ computer program developed by Stephan et al. (4). This program calculated the LC₅₀ statistic and its 95-percent confidence limits using the binomial, moving average angle and probit methods because no one method is appropriate for all possible sets of data. The method of calculation selected was that which gave the narrowest confidence limits for each separate analysis.

78

RESULTS

Table 2 presents the predicted LC₅₀ values and 95 percent confidence intervals for ARCOSOLV® PTB. The 24- and 48-hour LC₅₀ values for ARCOSOLV® PTB were both >1000 mg/l. All results were based on the nominal concentrations of 56, 100, 180, 320, 560 and 1000 mg/l. The no-effect concentration, based on the lack of mortality and abnormal effects, was 56 mg/l after 48 hours. The abnormal effects of mortality, surfacing, quiescence and/or daphnids lying on the bottom were observed in the 100, 180, 320, 560 and 1000 mg/l test concentrations.

Table 3 presents the mortality rate and water quality parameters measured during the test. The dissolved oxygen concentrations ranged between 8.4 and 8.6 mg/l. These values represented 91- and 93-percent saturation at 20°C, respectively, and were considered adequate for testing (1). The pH values of the treated chambers were all 8.6 after 96-hours and were consistent with the control value of 8.4.

The study was conducted following the intent of the Good Laboratory Practice Regulations (5).

TABLE 2
Acute Toxicity of ARCOSOLV[®] PTB
to Daphnia magna^a

Compound	LC ₅₀ (mg/l)	
	24 hours	48 hours
ARCOSOLV [®] PTB	>1000	>1000 ^b

^aBioassay conducted at 20°C (±2.0).

^bAn LC₅₀ could be calculated for this data, but the LC₅₀ was greater than the highest test concentration (1000 mg/l). The LC₅₀ was reported as >1000 mg/l. The 95% confidence limits were not reported, since not appropriate for this data set.

The 48-hour no-effect concentration was 56 mg/l, based on the lack of mortality and abnormal effects.

All values were rounded to two significant figures following ABC S.O.P. #8.7.

LC₅₀ calculated using:

- (1) Binomial Method
- (2) Moving Average Method
- (3) Probit Method

TABLE 3
Mortality Rates and Water Quality Measurements During the
Acute Toxicity of ARCOSOLV[®] PTB to Daphnia magna

Nominal Concentration (mg/l)	Percent Mortality Hours		Water Quality					
			0 hours			48 hours		
			Temp. °C	D.O. ^a mg/l	pH ^b	Temp. °C	D.O. ^a mg/l	pH ^b
Control	0	0	20	8.5	8.2	20	8.5	8.4
56	0	0				20	8.5	8.6
100	0	5						
180	0	15						
320	0	5				20	8.4	8.6
560	0	35						
1000	0	45				20	8.6	8.6

^aDissolved oxygen concentrations - Dissolved Oxygen System (YSI Model 54).

^bpH - pH Probe (Corning Model 476182) used with a Corning Model 125 pH and mV meter.

NOTE: Dissolved oxygen saturation at the test temperature of 20°C is 9.2 mg/l.

QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for Final Report #34076, "Ready Biodegradability: Modified Sturm Test of PTB", for Dr. Steven T. Cragg, ARCO Chemical Company, Newton Square, PA.

In accordance with ABC Laboratories' intent that all studies conducted by our facility meet or exceed the criteria promulgated by the various federal agencies to assure the accuracy and precision of analytical results, the above named report was reviewed and found to be in acceptable form by a member of our Quality Assurance Unit. A procedure audit was conducted on December 31, 1985.

A final inspection of all data and records on January 20, 1986 indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

If you should have any questions concerning this statement or the function of our Quality Assurance Unit, please contact the QA Unit at your convenience.

Philip M. Buckler (p.) 1/27/86
Philip M. Buckler Date
Quality Assurance Officer

FINAL REPORT

on

DETERMINATION OF WATER AND LIPID SOLUBILITY
AND OCTANOL-WATER PARTITION COEFFICIENT OF
PROPYLENE GLYCOL MONO-TERTIARY BUTYL ETHER
(PTBE)

to

ARCO CHEMICAL COMPANY

April 4, 1988

by

Ming J. W. Chang, Bruce W. Vigon,
Louis C. Fadel, and Michael E. Placke

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505 King Avenue
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FINAL REPORT

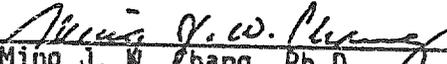
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DETERMINATION OF WATER AND LIPID SOLUBILITY
AND OCTANOL-WATER PARTITION COEFFICIENT OF
PROPYLENE GLYCOL MONO-TERTIARY BUTYL ETHER
(PTBE)

to

ARCO CHEMICAL COMPANY

April 4, 1988


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QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the study director as follows:

<u>Phase</u>	<u>Date</u>
Partition Coefficient - GC analysis of octanol/water standards; preparation of method blank; walk through of work areas, examination of study records and preliminary data	12/22/87
Audit - lab notebook No. 43018 (Pages 1-19) and supplemental 3-ring binder No. 43018-2	1/20/88
Audit - Red laboratory notebook (water and fat solubility studies), supplemental 3-ring binder and Draft Final Report	2/12/88
Audit - Final Report	4/5/88

Report to study director and management: 12/22/87, 1/20 and 2/12/88

To the best of my knowledge the methods described were the methods followed and the data presented accurately represent data generated during the study.

 4/6/88
Ramona A. Mayer, Manager
Quality Assurance Unit
Biological and Chemical Sciences

This study was conducted in compliance with the OECD Test Guidelines, final decision of the council, Paris, 1981.

Ming J. W. Chang 4-6-88
Ming J. W. Chang, Ph.D.
Chemistry Discipline Leader
Chemical Safety and Drug Development
Section

TABLE OF CONTENTS
(Continued)

	<u>Page</u>
3.2 Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Water Solubility.....	11
3.2.1 Validation for Analytical Method for PTBE Analysis...	11
3.2.2 Determination of PTBE Water Solubility.....	12
3.3 Lipid Solubility.....	12
3.4 Method Validation Using Benzoic Acid and PTBE Octanol-Water Partition Coefficient.....	13
3.4.1 Method Validation-Benzoic Acid Octanol-Water Partition Coefficient.....	13
3.4.2 Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Octanol-Water Partition Coefficient.....	14
4.0 DISCUSSION AND CONCLUSION.....	14
5.0 GLP STATEMENT/ARCHIVE.....	17
6.0 REFERENCES.....	17
7.0 STUDY PARTICIPANTS.....	18

List of Tables

Table 1. GC Analysis of PTBE Standards-Methods Validation Study.....	19
Table 2. GC Analysis of PTBE Standards Held for Eight Days - Methods Validation Study.....	20
Table 3A. GC Determination of PTBE Water Solubility at 10° and 20°C.....	21
Table 3B. GC Determination of PTBE Water Solubility at 30°C.....	22
Table 4. Gas Chromatographic Data for PTBE Fat Solubility.....	23
Table 5. Percent Relative Error and Percent Relative Recovery for GC Data of PTBE Fat Solubility Study.....	24

List of Tables
(Continued)

	<u>Page</u>
Table 6. Benzoic Acid Standards in Water for Octanol-Water Partition Coefficient Measurement.....	25
Table 7. Benzoic Acid Standards in Octanol for Octanol-Water Partition Coefficient Measurements.....	26
Table 8. PTBE Standards in Octanol for Octanol-Water Partition Coefficient.....	27
Table 9. Partition Coefficient Measurements for Benzoic Acid in Octanol-Water.....	28
Table 10. Summary of Phase Concentrations and Octanol-Water Partition Coefficients for PTBE.....	29
Table 11. Statistical Summary of Partition Coefficient Values for PTBE.....	30

FINAL REPORT

on

DETERMINATION OF WATER AND LIPID SOLUBILITY AND OCTANOL-WATER PARTITION COEFFICIENT OF PROPYLENE GLYCOL MONO-TERTIARY BUTYL ETHER (PTBE)

to

ARCO CHEMICAL COMPANY

from

BATTELLE
Columbus Division

April 4, 1988

1.0 INTRODUCTION

Propylene glycol mono-tertiary-butyl ether (PTBE) is a chemical of interest to the Atlantic Richfield Chemical Company (ARCO). This report describes a series of experiments designed to define three physical constants of PTBE. The three physical constants characterized were water solubility, lipid solubility and octanol-water partition coefficient. These studies were conducted at Battelle Columbus Division laboratories according to the OECD test guidelines (OECD, Paris, 1981, Test Guidelines, Decision of the Council). These studies were conducted under the direction of Ming J. W. Chang, Ph.D. and were performed according to the stipulations of The Good Laboratory Practices Act (GLP) for conducting non-clinical studies.

The water solubility study was designed to measure the saturation mass concentration of the test material in water at a defined temperature. The solubility in water is specified in units of mass per volume of solution.

The lipid solubility constant is defined in the mass fraction of the test material that forms a homogeneous phase with a liquid fat (oil) without giving rise to chemical reactions. The maximum of this mass fraction is defined as the saturation mass fraction and is generally a function of temperature.

844

TABLE OF CONTENTS

	<u>Page</u>
1.0 INTRODUCTION	1
2.0 MATERIALS AND METHODS	2
2.1 Test and Control Materials.....	2
2.2 Method Validation for Water Solubility Study Using a Reference Compound	3
2.2.1 Preliminary Aqueous Solubility Test for Benzoic Acid.....	3
2.2.2 Determination of Benzoic Acid Solubility in Water by Flask Method.....	3
2.3 Determination of Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Solubility in Water.....	4
2.3.1 Preliminary Aqueous Solubility Test for PTBE.....	4
2.3.2 Validation Study for Gas Chromatography Determination of PTBE.....	5
2.3.3 Stability of Aqueous PTBE Solutions.....	5
2.3.4 Determination of Water Solubility of PTBE by Flask Method.....	6
2.4 Determination of Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Lipid Solubility.....	7
2.4.1 Lipid Solubility Preliminary Test.....	7
2.4.2 Definitive PTBE Lipid Solubility Test.....	7
2.5 Octanol-Water Partition Coefficient of Propylene Glycol Mono-Tertiary Butyl Ether (PTBE).....	8
2.5.1 Test System and Reference Material Analysis.....	8
2.5.2 PTBE Octanol-Water Partition Coefficient.....	9
3.0 RESULTS.....	10
3.1 Spectrophotometric Analysis for Aqueous Benzoic Acid.....	10
3.1.1 Molar Absorptivity of Aqueous Benzoic Acid.....	10
3.1.2 Benzoic Acid Water Solubility by the Flask Method....	11

addition to two more filter cartridges that were 0.45 μ and 0.2 μ , respectively before glass distillation in a Corning Mega-Pure system.

Octanol (1-octanol, Lot No. 723549) was received on November 3, 1987 from Fisher Scientific Co. with no expiration date indicated.

2.2 Method Validation for Water Solubility Study Using a Reference Compound

A reference substance (benzoic acid) having a known water solubility was selected and analyzed to verify the performance of the method intended to be used for determining PTBE water solubility.

2.2.1 Preliminary Aqueous Solubility Test for Benzoic Acid

Benzoic acid was used as a reference compound to verify the validity of the selected analytical method for determining the water solubility of the test article.

Approximately 0.10 g of benzoic acid, the primary standard, was ground to a fine powder, placed in 100 mL graduated cylinder, and distilled, deionized water (DD H₂O) added in 10 mL increments with vigorous shaking between additions until the benzoic acid was completely dissolved. Four, 10 mL aliquots of DD H₂O were required to dissolve the 0.1 g of benzoic acid. Therefore, the estimated water solubility of benzoic acid was 2.5 g/L. The Flask Method described in the next section was used to determine the actual solubility limit of benzoic acid in water.

2.2.2 Determination of Benzoic Acid Solubility in Water by Flask Method

Based upon the preliminary test results, it was determined that ~1 g of benzoic acid would be required to saturate 20 mL of DD H₂O. Three 50 mL centrifuge bottles, fitted with glass stoppers were filled with ~1 g of

benzoic acid and 20 mL of DD H₂O were added to each bottle and agitated at 30°C for the following periods of time.

Tube A 0.99995 g 24 hrs

Tube B 1.00000 g 48 hrs

Tube C 1.00003 g 72 hrs

At the end of each incubation period, the tubes were removed and allowed to equilibrate at -23°C (room temperature) for 24 hours. Each tube contained an excess of undissolved benzoic acid and was centrifuged, the pH of the clear aqueous solutions was measured, and a 0.5 mL aliquot of the saturated aqueous solution was pipetted and diluted to 100 mL with DD H₂O in a volumetric flask. This diluted benzoic acid sample was analyzed by UV spectroscopy (228 nm) and the concentration of benzoic acid in the aqueous phase was calculated using Beer's Law (Absorbance (A) = Molar Absorptivity (E) x Light Path length (b) x Molar Concentration (C)).

The molar absorbtivity (E) of benzoic acid in water was determined by dissolving 250.00 mg of the primary benzoic acid standard in 100 mL of DD H₂O in a volumetric flask. A 0.5 mL sample was further diluted with DD H₂O to volume in a 100 mL volumetric flask yielding a final concentration of 0.0125 mg/mL which had a maximum absorbance of 0.949 at 228 nm.

2.3 Determination of Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Solubility in Water

Initially, a preliminary test was performed to decide whether the flask method or the column elution method would be used.

2.3.1 Preliminary Aqueous Solubility Test for PTBE

1.004 g of PTBE were weighed into a 10 mL graduated cylinder and DD H₂O was added 1 mL at a time to determine the solubility range. Six mL of DD H₂O were added to produce a homogeneous clear solution. The final volume was 7.1 mL and the solubility was estimated to be in the range of 100 to 200 g/L.

2.3.2 Validation Study for Gas Chromatography Determination of PTBE

The following procedure was used to validate the linearity of a standard curve of PTBE in DD H₂O by gas chromatography. A PTBE stock solution of 100 mg/mL was prepared by weighing 10.002 g of neat PTBE chemical into a 100 mL volumetric flask and diluting to volume with DD H₂O.

A 500 mg/mL dioxane solution (1,4-dioxane, Fisher Scientific, ACS certified grade, Lot No. 871725, received November 3, 1987, with no expiration date indicated) was prepared for use as an internal standard (IS) by weighing 12.5004 g of 1,4-dioxane into a 25 mL volumetric flask and diluting to volume with DD H₂O.

The following GC standards of PTBE were prepared in water:

<u>Standard (mg/mL)</u>	<u>PTBE Stock (mL)</u>	<u>IS Stock (mL)</u>	<u>mL H₂O</u>
80	8	2	0
60	6	2	2
40	4	2	4
20	2	2	6
0	0	2	8

Each standard solution was analyzed by gas chromatography using the following instrument conditions:

GC: Hewlett Packard 5840A with integrator
 Column: 20' x 1/8" stainless steel, packed with 10 percent SP-1000 on Supelcoport (80/100)
 Temperature: 135°C for 4 min, then by increased temperature 16° per minute to 220°C and held for 16 min.
 Injection: 250°C
 Detector: Flame Ionization (FID), 250°C
 Carrier: He @25 mL/min
 Sample size: 0.5 µl
 Retention time: Dioxane -4.8 min, PTBE -6.5 min

2.3.3 Stability of Aqueous PTBE Solutions

The stability of the standard solutions were determined by holding aliquots of the standard solutions in tightly sealed containers for 8 days at

room temperature and reanalyzing each sample for total PTBE concentration on both day 0 and day 8.

2.3.4 Determination of Water Solubility of PTBE by Flask Method.

Based on preliminary results, it was estimated that 18 g of PTBE would be required to saturate 30 mL of distilled deionized water.

Into each of nine 50 mL conical centrifuge tubes, approximately 18 g of PTBE were added.

<u>Tube ID</u>	<u>PTBE (g)</u>	<u>Incubation Time (hrs)</u>	<u>Equilibration Temperature (°C)</u>
1A	18.00	24	10
1B	18.02	48	10
1C	18.00	72	10
2A	18.01	24	20
2B	18.01	48	20
2C	18.01	72	20
3A	18.01	24	30
3B	18.00	48	30
3C	18.01	72	30

The final volume in each tube was raised to 30 mL with DD H₂O and the tubes screw-capped and wrapped with parafilm. The tubes were incubated in a +30°C water bath and constantly shaken for 24, 48, or 72 hours. Each tube was allowed to equilibrate at the pre-designated temperature (10°, 20° or 30°C) for 24 hours. At the end of the 24 hours equilibration, the undissolved test substance had settled down to the bottom of the conical centrifuge tube. A 2.5 mL aliquot of the saturated aqueous solution was diluted with 2 mL of internal standard and DD H₂O water to a final volume of 10 mL.

The pH of each aqueous solution was determined and the concentration PTBE was determined by the gas chromatography methods previously described in Section 2.3.2.

2.4 Determination of Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Lipid Solubility

The solubility of PTBE was determined in edible coconut oil as an estimate of the lipid solubility of the test article.

2.4.1 Lipid Solubility Preliminary Test

PTBE was added a drop at a time (~0.2 mL/drop) to a ~25 g sample of edible coconut oil. The sample was periodically stirred vigorously and allowed to stand at 37°C for approximately one hour. This process was intended to be continued until a distinguishable layer of PTBE was formed, which would indicate that an over-saturation of PTBE in the coconut oil had been reached.

Trial 1. PTBE was incrementally added to 25.0 g of coconut oil at 37°C until 12.54 g of PTBE had been added. No separation of the two layers occurred in this 33.4 percent (w/w) mixture of PTBE and coconut oil.

Trial 2. 23.83 g of coconut oil were added to 92.68 g of PTBE to give a 79.6 percent (w/w) PTBE/coconut oil solution. The mixture was immediately homogenous after about 20 seconds of shaking by hand.

Both solutions were left at room temperature for 96 hours and each remained as one homogeneous solution. It was therefore, tentatively concluded that the two components (PTBE and coconut oil) were completely miscible.

2.4.2 Definitive PTBE Lipid Solubility Test

To confirm that PTBE and edible coconut oil are completely miscible, eight samples of the following percentages of PTBE in coconut oil were prepared and analyzed by the gas chromatographic method described in Section 2.3.2: 0.00, 9.82, 33.40, 50.05, 70.27, 79.55, 90.08, and 100.00 percent (w/w) of PTBE in coconut oil.

The PTBE in the coconut oil solution was extracted with deionized water (with H₂O volumes exceeding the saturation limits of PTBE) for GC analysis. One-ml aliquots of the eight samples were diluted with 3 mL each of 200 mg/mL, 1,4-dioxane internal standard prepared in deionized water and further diluted with 96 mL of deionized water to yield a final volume of 100-mL in volumetric flasks. The extraction of PTBE into the deionized water was assisted with agitation at 37°C for 24 hours.

2.5 Octanol-Water Partition Coefficient of Propylene Glycol Mono-Tertiary Butyl Ether (PTBE)

2.5.1 Test System and Reference Material Analysis

The octanol-water partition coefficient (K_{ow}) was measured for a reference compound (benzoic acid) and the test substance, propylene glycol mono-tertiary-butyl ether (PTBE). Benzoic acid was used as the reference compound and served to check the performance of the experimental procedure used. The method used for this determination (Official Journal of the European Communities, 1984) was based on the OECD test guidelines (OECD, 1981) and involved the equilibration of the test substance with octanol and water followed by the determination of the PTBE concentration in each phase.

The phases of the octanol-water system were mutually saturated before measuring the partition coefficient. Saturation was ensured by addition of sufficient amounts of the saturating solvent to result in two phases. Subsequent to this step the pH of the water saturated with octanol measured 3.57. To prevent the ionization of benzoic acid ($pK_a = 4.2$ at 25°C), the pH of the water was adjusted to 2.45 by the addition of phosphoric acid.

To ensure accurate and reproducible data, duplicate determinations were made under three sets of test conditions. The reported solubility in water for benzoic acid was 2900 milligram/liter (mg/L) at 25°C and the log K_{ow} value was 1.87 (Verschuere, 1983).

Ultraviolet (UV) absorbance was used to quantify the benzoic acid concentration. Standard curves were developed from spectrophotometric analysis of benzoic in both octanol and water solutions at a wavelength of 227 nm.

Least squares regression analysis of the standard curves is summarized in Tables 1 and 2. The calculated detection limit of the method used was 1 microgram/milliliter (1 $\mu\text{g/mL}$). A 10:50 octanol-water ratio was determined to be optimal for the partition coefficient determination, based on the solubility of benzoic acid in the two phases and the analytical detection limit.

Following the guidelines, the three test conditions consisted of the optimal volume ratio, twice the optimal volume of octanol and half the volume of octanol. Therefore, the three ratios of octanol-water used for benzoic acid were: 5:50, 10:50 and 20:50. Fifty microliters (μL) of a 100 mg/mL benzoic acid in octanol standard was added to each duplicate sample. The six samples were shaken overnight (approximately 18 hours) and allowed to stand long enough for phase separation. The PTBE concentration in each phase was determined by UV spectrophotometry. Analyses were performed on a Shimadzu UV-160 spectrophotometer at a wavelength of 227 nm. PTBE concentrations were calculated by linear regression analysis based on a calibration curve from four standards prepared in each phase. Each phase that was measured was used as both the solvent for the standards and as the UV reference.

2.5.2 PTBE Octanol-Water Partition Coefficient

The methods used for determination of the K_{ow} for PTBE paralleled those used for benzoic acid, except that the pH of the water saturated in octanol was not adjusted, since PTBE does not ionize appreciably at a pH of 3.57. Three tests in duplicate were performed for PTBE as before.

The solubility of PTBE in water was estimated to be in a range of 100 to 200 mg/L based upon a preliminary solubility test. The detection limit for the gas chromatographic analysis was estimated from the calibration curve to be 1 mg/mL for PTBE (see Table 3). Due to the lower solubility and higher detection limit for PTBE in water, the octanol-water ratio was decreased to 10:200 and the amount of test substance was increased accordingly. The octanol-water ratios used were as follows: 5:200, 10:200 and 20:200. Fifteen mL of a 100 mg/mL PTBE standard in octanol were added to each duplicate sample. The six samples were shaken overnight (approximately 18 hours) and then centrifuged. The concentration of PTBE in each phase was determined by

gas chromatography using the GC methods described in Section 2.3.2 of this report.

The gas chromatographic separation of PTBE for determination of octanol-water partition coefficient was performed using a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (FID) and a stainless steel column (20' x 1/8", packed with 10 percent Supelcoport, 80-100 mesh). PTBE was quantitated by the internal standard techniques using 1,4 dioxane. The injector and detector temperatures were both set at 250°C. The column temperature was initially at 135°C. After 4 minutes the column temperature was increased at a rate of 16° per minute until it reached a final temperature of 220°C which was maintained for 16 minutes. The flow rates for the carrier gas (helium), air, and hydrogen were 25 mL/min, 300 mL/min and 30 mL/min, respectively. The sample injection size was 0.5 µl.

3.0 RESULTS

3.1 Spectrophotometric Analysis for Aqueous Benzoic Acid

3.1.1 Molar Absorptivity of Aqueous Benzoic Acid

The molar absorptivity (E) of primary benzoic acid was determined from a 0.0125 mg/mL benzoic acid standard. The molar concentration (C) was calculated as follows:

$$C = \frac{0.0125 \text{ mg/mL}}{122.1 \text{ mg/per mmole}} = 1.0238 \times 10^{-4} \text{ M}$$

Where 122.1 is the molecular weight (MW) of benzoic acid. The molar absorptivity (E) of the aqueous benzoic acid was calculated as:

$$E = \frac{0.949}{(1 \text{ cm})(1.0238 \times 10^{-4} \text{ M})} = 9.27 \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$$

Where 1 cm is the light path.

3.1.2 Benzoic Acid Water Solubility by the Flask Method

According to Beer's Law, i.e.

Absorbance (A) = Molar Absorptivity (E) x Light Path (b) x Molar Conc. (C)
Therefore, C (mole/L) =

$$C = \frac{A}{E \times b} = \frac{A}{9.27 \times 10^3 \times 1}$$

Where 9.27×10^3 was obtained from the above calculation (Section 3.1.1); and the light path (b) was 1 cm.

Benzoic acid concentration can be expressed as mg/mL by a conversion of c (mg/mL) = c (mole/L) x MW (g/mole) x 1000 mg/g x 1 L/1000 mL.

The following table summarizes the measured concentrations of benzoic acid in water after 24, 48, and 72 hours of constant agitation at 30°C and equilibrates to 23°C.

<u>Sample</u>	<u>pH</u>	<u>Temp.</u>	<u>A_{228 nm}</u>	<u>Obs. Conc. (mg/mL)</u>	<u>Dilution Factor</u>	<u>Corr. Conc. (mg/mL)</u>	<u>Avg. + SD (g/L)</u>
A (24 hrs)	2.77	23°C	1.210	0.0159	200	3.18	3.21 ± 0.03
B (48 hrs)	2.81	23°C	1.229	0.0162	200	3.24	
C (72 hrs)	2.73	23°C	1.215	0.0160	200	3.20	

The average solubility of 3.21 ± 0.03 g/L compares well with the values reported in The Merck Index (10th Ed.), which lists the solubility of benzoic acid in water as 2.9 g/L at 20°C and 3.4 g/L at 25°C with a pH of 2.8.

3.2 Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Water Solubility

3.2.1 Validation for Analytical Method for PTBE Analysis

The results obtained from GC analyses of PTBE standard solutions prepared for the Methods Validation Study are presented in Table 1 and Table 2.

These results indicate that PTBE water solutions were stable at room temperature for 8 days at concentrations of 20-80 mg/mL and that the GC method is suitable to determine PTBE concentrations in the range of 20 mg/mL to 80 mg/mL.

3.2.2 Determination of PTBE Water Solubility

The results from the GC analysis of PTBE-saturated water samples (at 10°C, 20°C and 30°C) are presented in Tables 3A and 3B. The data indicate that temperature changes from 10-30°C and lengths of incubation/agitation time from 24-72 hours had no significant influence on the solubility of PTBE in water. The average PTBE concentration in water at the three different temperatures tested (10°C, 20°C and 30°C) was calculated to be 182.68 ± 6.16 (g/L). The percent relative standard deviation was 3.37 percent.

3.3 Lipid Solubility

The appearance of all eight PTBE samples prepared were clear and a one-phase solution after 24 hours of incubation at 37°C. After storage at room temperature for seven days, only the 9.82 percent solution changed in appearance. The 9.82 percent sample solidified at room temperature. Aqueous extracts of 1 mL of PTBE/coconut oil samples including neat PTBE (without coconut oil) were analyzed by gas chromatography and the results shown in Table 4. The data presented in Table 4 are also shown in Table 5 with calculated relative errors and percent recovery from the lipid solution.

The retention time for PTBE remained constant (between 6.69 and 6.92 minutes) regardless of the concentration indicating that absence of any chemical reactions of PTBE with the column or the presence of method artifacts. Linearity of instrument response was good through the concentration range tested, with a correlation efficient greater than 0.999. The percent relative errors were less than 5 percent (Table 5) and the percent relative recovery was approximately 100 percent except for the two lowest samples, which had recoveries of 106-112 percent (Table 5). The percent relative GC recovery was derived as $\bar{X}/X \times 100\%$, where \bar{X} was calculated assuming that the

GC response for the diluted neat PTBE sample (1 mL PTBE to 100 mL aqueous solution) was 100 percent, since it was a true aqueous solution far from saturation and without any interference due to the presence of coconut oil. The percent relative recovery is a factor affected both by the actual content of the free PTBE in the solution as well as the water extraction efficacy. All the percent relative GC recoveries were approximately 100 percent except for the two lowest samples, which had recoveries of 106-112 percent (Table 5). The slightly higher recovery for the two lowest samples was likely due to experimental variation. Based on these observations, it was concluded that no chemical interaction occurred between the two components. It was, therefore, concluded that PTBE is completely miscible with edible coconut oil at 37°C.

3.4 Method Validation Using Benzoic Acid and PTBE Octanol-Water Partition Coefficient

Benzoic acid concentrations in each of the phases were measured and their ratio was used to compute K_{OW} . Following this step, corresponding data were obtained for the test material (PTBE). It should be noted that the octanol-water ratio changes slightly with the addition of the standard in octanol. However, results of the three different tests were corrected to the initial ratio to ensure comparable data.

3.4.1 Method Validation-Benzoic Acid Octanol-Water Partition Coefficient

The concentration found in each phase, the calculated K_{OW} values and $\log K_{OW}$ values for benzoic acid can be found in Table 9. Water phase concentrations of benzoic acid decreased from 13.6 $\mu\text{g/mL}$ to 7.1 $\mu\text{g/mL}$ with increasing octanol concentration. This difference was statistically significant at the 95 percent level. Precision of the concentration measurement was considered good, determined by the coefficient of variation (CV) for the replicate determinations at each ratio. The CV was in the range of 2.7 to 8.2 percent for these determinations.

Octanol phase concentrations also decreased with increasing octanol concentration, however, the trend was not statistically significant.

Precision of the octanol phase concentrations at ratios of 5:50 and 10:50 was poorer than at 20:50 (CV = 30 percent versus 9.6 percent).

The log K_{OW} values for benzoic acid at a 5:50 octanol-water ratio were 2.16 and 1.92. For a phase ratio of 10:50, the log K_{OW} values were 2.13 and 1.79. A ratio of 20:50 resulted in log K_{OW} values of 2.03 and 1.98. The overall mean log K_{OW} value was 2.00 with a standard deviation of 0.14.

3.4.2 Propylene Glycol Mono-Tertiary Butyl Ether (PTBE) Octanol-Water Partition Coefficient

Concentrations of PTBE measured in each phase and the calculated K_{CW} values and log K_{OW} values are summarized in Tables 10 and 11. The PTBE concentrations in the water phase were determined with a high degree of precision (CV of 1.0 to 2.9 percent). PTBE concentrations in the water at an octanol:water ratio of 20:200 were slightly lower than those determined from the other preparation with ratios of either 5:200 or 10:200 (4.2 versus 4.6 $\mu\text{g/mL}$). Octanol phase concentrations were also measured in a very precise fashion (CV of 0.9 to 2.0 percent), with slightly lower absolute concentrations (22 versus 25 $\mu\text{g/mL}$) in the 20:200 octanol:water preparation compared to the 5:200 or 10:200 ratios. The measured K_{OW} values for the phase ratio of 20:200 were 5.26 and 5.32 with a mean value 5.29 (Table 11). For a 10:200 phase ratio, the K_{OW} values were 5.41 and 5.67 with a mean value of 5.54. At a ratio of 5:200 the K_{OW} values were 5.42 and 5.44 with a mean of 5.43. The overall average K_{OW} for PTBE was determined to be 5.42.

The log K_{OW} values for the phase ratio 20:200 were 0.72 and 0.73 with a mean of 0.72. At a 10:200 phase ratio, the log K_{OW} values were 0.73 and 0.75 with a mean of 0.74. The log K_{OW} for 5:200 phase ratio was 0.73 and 0.74 for a mean of 0.73. The overall average log K_{OW} for all six samples for PTBE was 0.73 with a standard deviation of 0.01.

4.0 DISCUSSION AND CONCLUSION

The water solubility of benzoic acid determined by the flask method was found to be 3.21 ± 0.03 g per liter DD H_2O at -23°C . The solubility

obtained compared well with the values reported in literature. The Merck Index (10th Ed.) lists the solubility of benzoic acid in water as 2.9 g/L at 20°C and 3.4 g/L at 25°C with a pH of 2.8. These data indicate that the flask method was suitable for determining PTBE water solubility, provided that PTBE had a water solubility greater than 1×10^{-2} g/L, which was the case.

The same approach was used to determine the PTBE water solubility. Based on the preliminary test, a solubility of 100 to 200 g per liter was determined which suggested that the flask method would be adequate in determining an accurate measure of PTBE water solubility.

To determine the PTBE concentration, an analytical gas chromatographic method with an internal standard was validated. A good linear relationship was demonstrated between PTBE concentration (mg/mL) ranging from 20 to 80 mg/mL and the GC response (PTBE peak area/IS peak area). The reproducibility of the analysis was considered to be good. Analyses performed 8 days apart had a correlation coefficient of greater than 0.999 and an identical slope of 0.0167. The accuracy of the method was also considered good since all of the percent relative errors were less than 5.0 percent.

The PTBE water solubility was determined to be 184.61 ± 5.21 , 187.65 ± 14.70 , and 175.79 ± 5.30 g per liter at 10, 20, and 30°C, respectively. An apparent decrease in solubility occurred at 20°C as the incubation time increased from 24 hours to 48 hours to 72 hours. This was not observed at the other two temperatures studied, i.e., 10 and 30°C. Since the mean solubility at 20°C was found to be not significantly different from the mean solubilities for the other two temperatures (< 15 percent), no repeat of the test was required according to the OECD guidelines. The grand mean of the PTBE water solubility was calculated to be 182.68 ± 6.16 (g/L). The precision of the results (i.e., percent relative standard deviation) for the 10, 20, and 30°C determination was calculated to be 7.83, 2.82, and 3.01 percent, respectively.

The conclusion reached from the PTBE fat solubility study was that the two components, PTBE and coconut oil, were completely miscible at 37°C. All PTBE/coconut oil samples mixed at various weight ratios were homogeneous in appearance (clear liquid at 37°C), and had a percent relative GC recovery of about 100 percent, and an invaried GC behavior (i.e., retention time remained constant). Each of these factors indicated that the miscibility of the two components at 37°C was not due to any chemical interaction.

The OECD procedural guidelines for the determination of the octanol-water partition coefficient were successfully validated using benzoic acid as a reference substance. Although the poorer reproducibility of the concentration determinations in the octanol phase somewhat affected the confidence of the measured partition coefficient values, the overall mean value of the log K_{ow} was within 0.13 units of a reliable cited literature datum of 1.87 (Verschueren, 1983). Other values of the partition coefficient found in the literature range from 1.17 to 2.23 (Leo, et al., 1971).

The mean log K_{ow} for benzoic acid measured in this study was 2.00 with a standard deviation of 0.14. Both the mean and 90 percent confidence interval fell within the range of literature reported values. The OECD guidelines indicate that method performance is considered acceptable if the measured values fall within ± 0.3 log units of the true value. If the most probable value is taken to be 1.87, then acceptable values should range from 1.57 to 2.17. Values found in this test ranged from 1.79 to 2.16 and therefore, demonstrate acceptable method performance.

The measured concentrations of PTBE in both phases were determined with a high degree of precision as shown by the low CV values. The slightly lower concentrations in both phases at an octanol:water ratio of 20:200 are likely due to slightly less mass of test material being added and not to a procedural bias since there is no apparent systematic trend in any of the method performance statistics. The variability in the measured values of log K_{ow} as a function of phase ratio produced a very tight distribution as indicated by a standard deviation of only 0.01 (coefficient of variation approximately 1.4 percent).

As expected from the chemical formula and the preliminary solubility tests, the mean measured log K_{ow} of 0.73 for PTBE indicated that both phases had an approximate equal affinity for the compound. The average log K_{ow} value shows only a slight lyophilicity or organic phase preference.

5.0 GLP STATEMENT/ARCHIVE

All study records, raw data, and test and control material from the water and lipid solubility and octanol-water partition coefficient studies are being held at Battelle's Columbus Division and will be returned to ARCO or its designated archival facility upon acceptance of the final report.

6.0 REFERENCES

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101

7.0 STUDY PARTICIPANTS

The following staff were key participants in this study:

<u>Name</u>	<u>Study Title</u>	<u>Department</u>
Ming J. W. Chang, Ph.D.	Chemistry Discipline Leader	Toxicology and Pharmacology
Bruce Vigon, M.S.	Environmental Chemist	Environmental Sciences
Michael E. Placke, Ph.D.	Reviewed Final Report	Toxicology and Pharmacology
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Khamalil Bhatti, B.S.	Chemistry Technician	Environmental Sciences

102

1159

TABLE 1. GC ANALYSIS OF PTBE STANDARDS-METHODS VALIDATION STUDY

STD Theoretical Concentration (mg/mL)	PTBE Area	IS Area	PTBE/IS	Apparent Concentration* \hat{X}	% Relative Error**
0	0	1001000	0		
20	2947000	9448000	0.3119	20.9	+4.5
40	6179000	9964000	0.6201	39.3	-1.8
60	9750000	10320000	0.9448	58.8	-2.0
80	14130000	10730000	1.3169	81.0	+1.3

Linear regression $n = 4$

$$y = bx + a$$

$$b = 0.0167, a = 0.0365$$

$$r = 0.9990$$

*Apparent concentration (mg/mL) derived from linear equation.

**Relative Error = $(\hat{X} - \text{theoretical concentration} / \text{theoretical concentration} \times 100 \text{ percent} - 100 \text{ percent})$.

TABLE 2. GC ANALYSIS OF PTBE STANDARDS HELD FOR EIGHT DAYS -
METHODS VALIDATION STUDY

STD Theoretical Concentration (mg/mL)	PTBE Area	IS Area	PTBE/IS	Apparent Concentration* \hat{X}	% Relative Error**
0	0	11980000	0		
20	2541000	87460000	0.2905	20.7	+3.5
40	7560000	12560000	0.6019	39.4	-1.5
60	10750000	11590000	0.9275	59.0	-1.7
80	15840000	12250000	1.2931	80.9	+1.1

linear regression $n = 4$

$$y = bx + a$$

$$b = 0.0167, a = -0.0551$$

$$r = 0.9993$$

*Apparent concentration (mg/mL) derived from linear equation.

**Relative Error = $(\hat{X} - \text{theoretical concentration}) / \text{theoretical concentration} \times 100$ percent - 100 percent).

104

The Principal Investigator and Study Director of the project for Analytical Bio-Chemistry Laboratories, Inc., were Leanne Forbis, Analyst III and Brian R. Bowman, Supervisor, Environmental Fate. Supervising the conduct of the study for ARCO Chemical Company was Dr. Steven T. Cragg. The following ABC personnel assisted with various phases of the study.

<u>Name, Title</u>	<u>Signature</u>	<u>Initials</u>
Brian R. Bowman, Supervisor, Environmental Fate/ Study Director	<u>Brian R. Bowman</u>	<u>BRB</u>
Leanne Forbis, Analyst III	<u>Leanne Forbis</u>	<u>LF</u>
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TABLE OF CONTENTS

	<u>Page No.</u>
LIST OF TABLES34076-v
LIST OF FIGURES34076-v1
SUMMARY34076-1
INTRODUCTION.34076-2
METHODS AND MATERIALS34076-3
I. Test System34076-3
II. Test Materials.34076-3
A. Test Water34076-3
B. Test Activated Sludge.34076-3
C. Test Compound.34076-3
III. Test Procedure.34076-3
A. Inoculum34076-3
B. Charging of Carboys.34076-4
C. Performing the Test.34076-4
IV. Calculations.34076-5
RESULTS AND DISCUSSION.34076-5
QUALITY ASSURANCE STATEMENT34076-19
STUDY COMPLIANCE STATEMENT.34076-20
APPENDIX I - RAW DATA.34076-
APPENDIX II - PROTOCOL AND CORRESPONDENCE34076-

LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
1	Environmental Chamber Temperature Log	34076-7
2	Solutions Used in Modified Sturm Biodegradation Study with PTB.	34076-8
3	Sample Code for Ready Biodegradability: Modified Sturm Test of PTB	34076-9
4	Sample Schedule for Ready Biodegradability: Modified Sturm Test of PTB.	34076-10
5	Control Form Sheet for the Modified Sturm Test. . .	34076-11
6	Aniline Form Sheet for the Modified Sturm Test. . .	34076-12
7	PTB 10 mg C/L Form Sheet for the Modified Sturm Test.	34076-13
8	PTB 20 mg C/L Form Sheet for the Modified Sturm Test.	34076-14

LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
1	Description of Test System.	34076-15
2	Cumulative CO ₂ Evolved. Control System Subtracted	34076-16
3	Cumulative CO ₂ Evolved.	34076-17
4	Percent of Theoretical CO ₂ . Control System Subtracted	34076-18

SUMMARY

A 28-day modified Sturm test was conducted with PTB. Aniline was used to check the activity of the inoculum. By day 28 >76% of the aniline had been converted to CO₂. In the test systems, 3.7% of the PTB 10 mg C/L concentration had been converted to CO₂ and 1.2% of the PTB 20 mg C/L concentration had been converted to CO₂.

INTRODUCTION

ARCO Chemical Company contracted Analytical Bio-Chemistry Laboratories, Inc. to conduct a "Ready Biodegradability: Modified Sturm Test" with PTB. This study as described in the ABC protocol #M-8407 was authorized by Dr. Steven T. Cragg, in a letter dated November 1, 1985.

Work relating to this study was initiated on December 2, 1985. Project completion was on December 31, 1985.

METHODS AND MATERIALS

I. Test System

The test system included 4 eight liter glass carboys and 12 potassium hydroxide gas washing bottles. The outlet of each carboy was connected in series to 3 CO₂ absorber flasks (KOH Scrubbers) each filled with 100 ml of 0.5 N KOH (Figure 1).

The carboys were enclosed in an environmental chamber where temperature (Table 1) and exposure to light could be controlled. The study was conducted under dark conditions to minimize degradation by photolysis.

II. Test Materials

A. Test Water

All water used in the study was carbon-free deionized water.

B. Test Activated Sludge

Continuously aerated activated sludge was obtained for use in this study from the City of Columbia Waste Water Treatment Plant.

C. Test Compound

The test compound, 1 jar of PTE, was received on November 20, 1985 and was a clear liquid substance. Purity was >99%. The compound was stored at room temperature. Percent carbon was calculated as 63.6% of weight.

The aniline used in the study was received from J. T. Baker Chemical Company in November of 1984 and was stored at room temperature. The specified purity was 100.0%.

III. Test Procedure

A. Inoculum

The activated sludge was obtained from the Columbia Waste Water Treatment Plant. Upon arrival at the laboratory, the sludge was aerated for 4 hours. Five hundred milliliters was blended for 2 minutes and allowed to settle for 1 hour. The supernatant was filtered through glass wool and 150 ml collected. An aliquot was then taken for total plate count analysis.

B. Charging of Carboys

Each carboy was charged with 2470 ml of high quality water; 3 ml each of the ammonium sulfate, magnesium sulfate and calcium chloride stock solution; 6 ml of the phosphate buffer stock solution; 12 ml of the ferric chloride solution; and 30 ml of the activated sludge inoculum. The mixture in each carboy was then purged for 24 hours with CO₂ free air and the three CO₂ absorber flasks each filled with 100 ml of 0.5 N KOH.

Test material was then added to two of the four carboys at concentration of 10 and 20 mg carbon equivalents/liter. The amount of test material required in the carboy was calculated as follows.

$$\frac{100 \times A}{B} \times C = \text{mg PTB added to carboy}$$

where

A = mg carbon equivalents/liter
B = % TOC by weight
C = total volume carboys in liters

Sufficient weight of PTB to reach the desired test concentration, as calculated above, plus distilled water to make 473 ml was added to the appropriate carboys.

To the third carboy, used as a blank control and containing no test compound, 473 ml of high quality water was added.

To the fourth carboy, a control substance (aniline) at a concentration of 20 mg carbon equivalents/liter was added. The final volume of this system was brought to 3000 ml with high quality water.

All four systems had a final volume of 3000 ml at this point.

To confirm the nominal concentration of organic carbon of the test compound an aliquot of each of the four test systems was analyzed for total organic carbon (TOC).

C. Performing the Test

The test was started by bubbling CO₂-free air through the solution at a rate of 50-100 ml/min per carboy. The CO₂ produced was trapped as K₂CO₃ using KOH. The amount of CO₂ produced was determined with a CO₂ analyzer.

Sampling was made every other day for the first ten days and every fifth day until the 28th day (Table 4). The sample code used for identification is illustrated in Table 3. At each sampling interval, the CO₂ absorber nearest the carboy was removed for analysis and the remaining two absorbers moved one place closer to the carboy, and a new absorber filled with 100 ml of fresh trapping solution and placed at the far end of the series.

On day 27 one milliliter of concentrated HCl was added to each of the carboys to drive off inorganic carbonate. The carboys were aerated overnight and samples removed for total organic carbon (TOC) analysis and for dissolved organic carbon (DOC) analysis.

IV. Calculations

The theoretical carbon dioxide (ThCO₂) that can be generated by the test material was calculated as follows:

$$\text{ThCO}_2 = \text{mg PTB} \times \frac{\% \text{ C}}{100} \times \frac{\text{M.W. CO}_2}{\text{M.W. C}} = \text{mg CO}_2$$

The amount of carbon dioxide produced was determined by analysis of the KOH solutions using an O. I. Corporation Model 524C TOC Analyzer. A standard reference curve was obtained by injecting known quantities of Na₂CO₃ and applying linear regression to the peak area output of an HP-3390A recording integrator. This curve was used to convert the output peak areas of the samples to microgram carbon injected. Micrograms carbon was converted to microgram CO₂ by multiplying by 44/12.

The carbon evolved from the test system on any sampling day is calculated from the formula

$$\text{mg C evolved} = (\text{TF} - \text{BK}) \times \frac{V}{1000}$$

TF = concentration in µg/ml of test KOH
 BK = concentration in µg/ml of control KOH
 V = volume of KOH in ml

The percentage of theoretical CO₂ produced is calculated from the following formula:

$$\% \text{ ThCO}_2 = \frac{(\text{mg CO}_2 \text{ produced})}{\text{ThCO}_2} \times 100$$

RESULTS AND DISCUSSION

Tabulated results of KOH analysis for evolved CO₂, TOC analysis for day 0 and day 28 and DOC analysis for day 28 can be found in Tables 5,

6, 7 and 8 for the control, aniline, PTB at 10 mg C/L and PTB at 20 mg C/L systems respectively. The production of CO₂ for all test systems is shown graphically in Figure 2. Figure 3 illustrates the production of CO₂ for the aniline and two test compound systems with the control system results subtracted.

The aniline test system which was employed to demonstrate the viability of the inoculum was charged with 20 mg carbon equivalents of aniline per liter. With 100% degradation 220 mg of CO₂ would be produced. After subtraction of the control systems production of CO₂ (33.2 mg), the mg of CO₂ produced that can be attributed to aniline degradation is 168 mg or 76.4% of theoretical. This percentage conversion is well above the OECD guideline minimum of 60%, and demonstrates the viability of the inoculum which was found by plate count analysis to contain 9.3×10^5 colony forming units per milliliter.

The two test compound systems charged at levels of 10 mg C/L and 20 mg C/L would theoretically produce 110 mg CO₂ and 220 mg CO₂ if 100% degradation occurred. The actual amount of CO₂ produced from each system was 37.3 mg CO₂ and 35.8 mg CO₂. Subtracting the control amount of 33.2 mg, the mg of CO₂ produced that can be attributed to PTB is 4.1 mg CO₂ and 2.6 mg CO₂ for the low and high system respectively (Figures 2 and 3) or 3.69% of theoretical and 1.19% of theoretical CO₂ produced. This is a low level of ultimate degradation, but shows that PTB as test concentration was not microbially inhibitory.

82

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PREFACE

This report was prepared at Midwest Research Institute (MRI), 425 Volker Boulevard, Kansas City, Missouri 64110. The research was conducted in the Chemical and Biological Sciences Group, Toxicology Department, Deramus Field Station Facilities, 13100 Robinson Pike Road, Grandview, Missouri 64030.

The laboratory phase of the work was carried out between September 1, 1982 (animal arrival), and October 3, 1982 (animal sacrifice). Operation of the inhalation chambers, daily observation of the animals, body weight measurements, and data coordination were performed by Mr. Jack H. Hagensen, Inhalation Operations Supervisor and Study Director, and the staff of the Deramus Field Station Facilities. Necropsy and histologic examination of tissues were performed by Dr. James L. Carter and his staff. Sampling of the test atmospheres and analytical chemistry procedures were carried out by Mr. David Steele and Mr. Allan Chatham.

This study was conducted in compliance with Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 CFR 58) and proposed regulations of the U.S. Environmental Protection Agency (44 FR 27334, May 9, 1979). This report was reviewed by the MRI Quality Assurance Unit on October 25, 1982, and was found to accurately reflect the raw data of the study and the methods used to generate those data. All raw data, specimens, records, and reports are stored in the archives of MRI.

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140

83

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SUMMARY

Male and female rats were given a single 4-hr inhalation exposure to either HEPA-filtered air or to a concentration of $2,680 \pm 107 \text{ mg/m}^3$ of test compound A209429 and held for observation for 14 days. No mortalities occurred, no clinical signs of toxicity were observed, and group mean body weights were similar for control and exposed rats. No gross lesions were seen at necropsy (day 14), but mild extramedullary hematopoiesis was observed microscopically in two of five exposed males and two of five exposed females. The acute inhalation toxicity of A209429 is considered minimal.

(u)

I. INTRODUCTION

The purpose of this study was to evaluate the acute inhalation toxicity of A209429 in rats following a single 4-hr exposure to a target concentration of 2,000 mg/m³.

II. MATERIALS AND METHODSA. Test Atmosphere

1. Test article: A209429 was supplied by Arco Chemical Company, Newtown Square, Pennsylvania. This material was received as a clear liquid.
2. Inhalation chamber: This study employed 0.5 m³ stainless steel chambers with dynamic air flow (Young and Bertke, Cincinnati, Ohio). The flow of HEPA-filtered air through the chambers was maintained at greater than 10 air changes per hour and at slightly negative pressure with respect to room air throughout the exposure period. The temperature in the chamber during the 4-hr exposure was 21°C (70°F) and the relative humidity was 55%.
3. Generation system: The test article was precision-metered into the plenum system of the inhalation chamber using a piston pump (FMI, Oyster Bay, New York) and a specially fabricated air jet nebulizer (Rhema-Labortechnik, West Germany). Because of the high vapor pressure of the test material, the material was completely vaporized. The target concentration of A209429 was 2,000 mg/m³.
4. Concentration analysis: Samples of chamber atmosphere were drawn by vacuum pump through three serially connected mini-impingers, each containing about 25 ml of methanol. The atmosphere was sampled for 10 min at a rate of 1.0 liter/min immediately prior to the start of exposure and at 1, 2, and 3 hr thereafter. After each collection contents of the impingers were quantitatively transferred to 50 ml volumetric flasks, 1.0 ml of 2% v/v n-decane in methanol was added to each as an internal standard, and the flasks were diluted to volume with methanol. A reference standard was made up by accurately weighing out A209429 and dissolving it in methanol to a final concentration of 443.8 µg/ml. This standard also contained n-decane at the same concentration as described above for the test samples.

Analysis was by gas chromatography using a Varian 3700 instrument equipped with a flame ionization detector (Varian Instruments, Inc., Palo Alto, California). The glass column (1.8 m x 2 mm ID) contained 15% dinonylphthalate on 80/100 Chromasorb W(HF) and the nitrogen carrier gas flowed at 30 cc/min. Sample injection volume was 2.5 µl. The column was operated isothermally at 100°C, while the inlet and detector temperatures were 150°C and 250°C, respectively. These conditions produced retention times of 3.4 min for A209429 and 5.2 min for n-decane. Relative response

factors (area of the A209429 peak per area of the n-decane peak) were determined for the reference standard and the test samples. The concentration of A209429 in the chamber atmosphere in milligrams per cubic meter (C) was calculated with this equation:

$$C = \frac{\text{RRF test sample}}{\text{RRF ref. std.}} \times \frac{443.8 \mu\text{g}}{\text{ml}} \times \frac{50 \text{ ml}}{10 \text{ liter}} \times \frac{1 \text{ mg}}{10^3 \mu\text{g}} \times \frac{10^3 \text{ liter}}{1 \text{ M}^3}$$

The concentration of the reference standard, 443.8 $\mu\text{g/ml}$, corresponds to a test atmosphere concentration of 2,219 mg/m^3 . This amount was chosen because the concentration of A209429 was expected to be near 2,000 mg/m^3 . See Table 1 for actual concentration analysis.

B. Animals and Husbandry

Male and female Sprague-Dawley rats 7 to 9 weeks of age were received on September 1, 1982, from Charles River Breeding Laboratories, Inc. (Kingston, New York). The following day, the rats were ear-tagged (Gey Band and Tag Company, Norristown, Pennsylvania), weighed, stratified by weight within each sex, then randomized five per sex to either the treatment or the control group. A computer program was used for the stratification and randomization procedures. Animals were quarantined for 18 days prior to test. On the day of exposure, males weighed 284 to 330 g and females weighed 202 to 251 g. Throughout the quarantine and post-exposure observation periods, rats were housed five per cage in polycarbonate cages of the shoebox type with stainless steel tops and filter bonnets. Laboratory Rodent Chow No. 5001 (Ralston Purina Company, St. Louis, Missouri) and tap water in bottles were available ad libitum. During the 4-hr exposure period, feed and water were withdrawn and the rats were placed five per cage in stainless steel wire mesh cages specially fabricated at MRI. Lighting at all times was by an overhead fluorescent source with a fixed photoperiod of 12 hr continuous light out of every 24 hr.

C. Study Design

Five male and five female rats were exposed to the test atmosphere or HEPA-filtered room air for 4 hr in 0.5 m^3 inhalation chambers. The 4-hr exposure period included the chamber build-up time but not the chamber exhaust phase. After exposure all rats were observed daily for 14 days for signs of toxicity and any abnormal findings were recorded. Body weights were recorded immediately before the 4-hr exposure and again on days 7 and 14. On day 14 all surviving rats were killed by overdose with CO_2 and subjected to necropsy. Gross examination included nasal passages, trachea, bronchi, lungs, and any other tissues known to be affected by the test article. Lungs, liver, and kidneys were collected from all animals, placed in 10% buffered neutral formalin, and processed for histopathologic evaluation as H&E sections.

III. RESULTS~~CONFIDENTIAL~~

Analyses of the test atmosphere are shown in Table 1. Measurements after 1, 2, and 3 hr of exposure were in good agreement. The mean and standard deviation were $2,680 \pm 107 \text{ mg/m}^3$. Mean body weights are given in Table 2. Exposed males had a slightly lower group mean weight than control rats on day 14, but this difference was not statistically significant (Student's t-test, $p > 0.20$). Control and exposed females had nearly equal group mean weights at all times measured. No mortalities occurred prior to study termination, no clinical signs of toxicity were observed in any animals, and all rats appeared grossly normal at necropsy (Table 3).

Mild extramedullary hematopoiesis was seen microscopically in the livers of two of five exposed males and two of five exposed females. This lesion consisted of small foci of hematopoietic cells in some portal triads in affected livers and is considered to be compound-related. No other lesions were observed (Table 4).

IV. DISCUSSION

The histologic finding of mild extramedullary hematopoiesis in liver in this study suggests that the bone marrow might have been stressed in some of the rats exposed to A209429. Hematopoietic organs were not examined microscopically, however, and no hematologic measurements were made, so this could not be confirmed. Hematopoiesis is carried on in liver during the fetal and perinatal periods, and reversion to this state is occasionally seen in young rats. The toxicological significance of this lesion, therefore, cannot be evaluated based on results of the present study. The absence of mortalities, weight loss, and clinical signs of toxicity through 14 days of post-exposure observation supports the interpretation that the acute toxicity of A209429 to rats is minimal at most following a single 4-hr exposure by inhalation to a test atmosphere containing $2,680 \pm 107 \text{ mg/m}^3$.

90

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DIPLOMATE
AMERICAN COLLEGE
OF
VETERINARY PATHOLOGISTS

R.D. 7, WELLS ROAD
FLEMINGTON, NEW JERSEY 08822

July 30, 1983

TO: S. A. RIDLON, Ph.D.
TOXICOLOGY SUPERVISOR
ARCO CHEMICAL COMPANY
3801 WEST CHESTER PIKE
NEWTON SQUARE, PENNSYLVANIA 19073

RE: PROJECT 7450 B. ACUTE INHALATION TOXICITY STUDY
IN RATS WITH A20-9429. HISTOPATHOLOGICAL EVALUATION
OF LUNGS, LIVER, AND KIDNEYS. RFMC 78/83

Hematoxylin and eosin stained and prepared slides of the lungs, liver, and kidneys from each of 5 ♂ and 5 ♀ rats of the control and test group of the aforementioned study were submitted for microscopic examination.

Tables 2 and 3 list the rats by sex and dose group. Each of the tissues with the microscopic findings, graded where appropriate, are included in the Tables. A summary of these data is provided in Table 1 for ease of data comparison between sex and dose groups.

The microscopic examination of the tissues revealed no histological evidence of a treatment induced or related effect.

A number of tissue alterations were encountered; however, they were noted with approximately equal frequency and degree in control rats as in test animals and were judged to have been

148

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91

S. A. Ridlon, Ph.D.
Arco Chemical Company

- 2 -

July 30, 1983
Re: Project 7450 B
RFMc 78/83

unassociated with treatment. The changes encountered were characteristic of the background lesions commonly observed in laboratory rats of this age.

The only change which was slightly accentuated in test rats when compared to control animals was the accumulation of protein absorption droplets in the proximal tubule epithelial cell cytoplasm of male rats only. This renal change in male rats is not uncommon. The eosinophilic droplets have been noted to occur in control male rats as well as in treated male animals in a number of subacute rodent studies evaluated by this pathologist. They have on occasion been noted to be accentuated in a dose dependent fashion in some 30 to 90 day toxicity studies evaluated by this pathologist.

The droplets are probably a low molecular weight $\alpha_2\mu$ globulin which has been shown to be produced by the liver of male rats and has been identified both in the liver and kidneys. (1) The proximal tubule epithelium of the kidney selectively reabsorbs the protein which, due to its small size, leaks through the glomerular membrane.

Although the degree of change in this study was slightly increased in three treated male rats when compared to the controls, it was considered highly unlikely that the exacerbation was associated with treatment. The 14-day rest period from first exposure to the test material until necropsy of the

(1) Arun, R.K. and Raber, D.L. Immunofluorescent Localization of $\alpha_2\mu$ globulin In The Hepatic and Renal Tissues of Rats. J. HISTOCHEM & CYTOCHEM Vol. 20, No. 2, 89-96, 1972

149

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- 3 -

July 30, 1983
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animals was sufficient time to allow all but the most severe renal lesions to have reversed to normal. This is a major deficiency in a study of this type.

It should also be noted that the livers of control and test male rats of this study also had accumulation of proteinaceous fluid in scattered hepatocytes. This change was not observed in females. It may represent accumulations of the protein previously described.

Under the conditions of this test, it was concluded that compound A20-9429 produced no histomorphological alteration in the lungs, liver, or kidneys of male or female rats.

Respectively submitted,



ROBERT F. McCONNELL, D.V.M.

150

RFMc 78/83
7/30/83

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SUMMARY OF MICROSCOPIC FINDINGS - TABLE 1

INCIDENCE OF MICROSCOPIC FINDINGS IN INDIVIDUAL
RATS BY DOSE GROUP AND SEX
TABLES 2 and 3

LEGEND FOR TABLES:

- N = tissue within normal histological limits
- 1, 2 = degree of severity of indicated change
- 1 = minimal
- 2 = mild
- P = indicated change present, grades not applicable
- () = focal or localized change
- < > = multifocal
- = indicated change not present

151

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7/30/83

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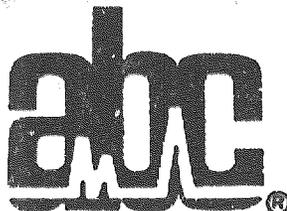
ACUTE INHALATION TOXICITY STUDY IN RATS
WITH A20-9492

INCIDENCE OF MICROSCOPIC FINDINGS
TABLE 2

GROUP SEX	A20-9429										CONTROL									
	♂					♀					♂					♀				
ANIMAL NUMBER: AT-	163	164	166	168	172	177	180	185	189	190	195	167	169	170	175	178	181	182	186	188
TISSUE/RESPONSE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LUNGS:	1	1	2	1	2	1	1	2	1	1	1	1	2	1	1	1	1	1	1	1
Parabronchial lymphoid aggregates	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Parabronchial lymphoid hyperplasia	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Congestion and/or alveolar hemorrhage (due to euthanasia method)	<1>	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Atelectasis, (artifact)	<1>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Para-arterial accumulations of heterophils	(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Interstitial pneumonitis	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>	<1>
LIVER:	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Cytoplasmic vacuolation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cytoplasmic proteinaceous fluid in scattered hepatocytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion (due to euthanasia method)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Microabscess	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Extramullary hematopoiesis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coagulation necrosis (recent)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mononuclear leucocytic infiltrates; intralobular	-	(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

94

152



ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.
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ARCO. 1986a

ABC Preliminary Report #34076

Ready Biodegradability:
Modified Sturm Test of PTB

Submitted To:

ARCO Chemical Company
Attn: Steven T. Cragg
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Newtown Square, PA 19073

October 6, 1986.

115

Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
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:dlj:8a

ARCO. 1987b

ARO 6/871093

ANALYSIS OF METAPHASE CHROMOSOMES
OBTAINED FROM CHO CELLS CULTURED
IN VITRO AND TREATED WITH

1-(1,1-DIMETHYLETHOXY)-2-PROPANOL

CAS RN 57018-52-7

(Commercially available from
Arco Chemical Co. as ARCOSOLV®

PTB solvent)

Addressee:

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ARCO Chemical Company,
3801 West Chester Pike,
Newtown Square,
PA 19073,
U.S.A.

10 November 1987.

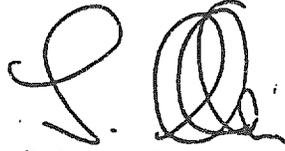
Authors:

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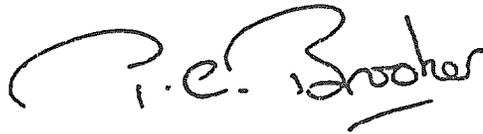
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155

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

United States Food and Drug Administration,
Title 21 Code of Federal Regulations Part 58,
Federal Register, 22 December 1978 and subsequent Amendments

Japan Ministry of Health and Welfare
Notification No. 313 Pharmaceutical Affairs Bureau
31 March 1982

Organization for Economic Co-operation and Development
ISBN 92-64-12367-9, Paris 1982



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Study Director

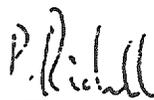
Date

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QUALITY ASSURANCE STATEMENT

Certain studies of short duration, such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC management.

This report has been audited by the HRC Quality Assurance Department. It is considered to be an accurate presentation of the procedures and practices employed during the course of the study and an accurate presentation of the findings.



Peter H.C.V. Richold, B.Sc.,
Systems Compliance Auditor,
Quality Assurance Department.

Date

21.10.87

157

CONTENTS

	Page
SUMMARY	i
INTRODUCTION	1
MATERIALS AND METHODS	2 - 4
RESULTS	
(a) Preliminary toxicity test	5
(b) Metaphase analysis	5
CONCLUSION	6
BIBLIOGRAPHY	7
TABLES	
1. Preliminary toxicity test - mitotic indices of cultured CHO cells treated with PTB	8
2. The effect of PTB on the chromosomes of cultured CHO cells	9 - 10
APPENDIX	
1. Preparation of S-9 liver homogenate fraction	11

SUMMARY

1. PTB was tested in vitro to determine whether it would cause chromosomal aberrations in a mammalian cell line derived from Chinese hamster ovary tissue. The cells were routinely grown and subcultured in tissue culture medium at 37°C in a humid atmosphere containing 5% carbon dioxide. They were incubated with the test compound both with and without supplementary metabolic activation (rat S-9 mix).
2. A preliminary toxicity test was carried out to assess the effect of the compound on the mitotic index of cultured CHO cells. The results of this test indicated that a top dose level of 1% v/v should be used for the metaphase analysis in both the absence and presence of metabolic activation.
3. In the absence of metabolic activation, cultures treated with PTB showed slight but just statistically significant increases in the incidence of chromosomal damage at the intermediate and high dose levels. However the levels of damage were on the borderline of statistical significance and were well within the historical control range for this laboratory. Therefore these increases are not considered evidence of clastogenic activity.
4. In the presence of S-9 mix, there were no statistically significant increases in the incidence of chromosomal damage at any dose level of PTB.
5. Both positive control compounds caused large statistically significant increases in the incidence of chromosomal damage, demonstrating the sensitivity of the test system and the efficacy of the S-9 mix.
6. It is concluded that PTB has shown no evidence of clastogenic activity in this in vitro cytogenetic test system.

INTRODUCTION

CHO-X₁ cells of the BH₄ subclone have an epithelial morphology with a modal chromosome number of 20 and grow as an adherent monolayer in vitro. They were originally derived from the ovary of a Chinese hamster and have frequently been used in this type of test system (6, 7, 8).

Division of the cells can be arrested at metaphase using the mitotic inhibitor colchicine (this prevents formation of the mitotic spindle). Chromosomes are examined in these metaphase cells for the presence of the following aberrations:

Gaps	}	chromatid and isochromatid
Breaks		
Chromatid exchanges		
Dicentric chromosomes		
Acentric chromosome fragments		
Chromosome rings		
Complex rearrangements.		

A gap is defined as an achromatic region (occurring in one or both chromatids) which is smaller than the width of a single chromatid. The separated regions are still aligned. A break is defined as an achromatic region, occurring in one or both chromatids, that is greater than the width of a single chromatid. The accompanying fragment is usually displaced from the rest of the chromosome.

Many authors (1, 2, 3) believe that chromatid gaps are not examples of true chromosomal aberrations. In this study, therefore, the total numbers of cells with aberrations exclusive of gap damage have been calculated. The number of cells with aberrations including gap damage has also been tabulated.

Since many compounds do not exert their mutagenic effect until they have been converted by enzyme systems not present in cultured cells, one set of cultures was incubated with test compounds in the presence of a rat liver homogenate fraction (S-9 mix) taken from an animal previously treated with a compound known to induce a high level of liver enzyme activity (4, 5, 9).

This report describes the experiments carried out between 4 May 1987 and 5 August 1987 to investigate the effects of PTB on the chromosomes of a mammalian cell line cultured in vitro. The procedure followed the guidelines of the OECD.

Study Director and HRC management approval of the GLP protocol was obtained on 3 April 1987. Sponsor approval was given on 14 April 1987.

MATERIALS AND METHODS

(a) Test compound

Identity: 1-(1,1-dimethylethoxy)-2-propanol (PTB).
Physical appearance: Clear liquid.
Solubility: Soluble in water.
Storage: Room temperature in the dark.

PTB was diluted with sterile distilled water immediately before use. The compound was used as supplied for the highest dose level in each test, being added to the culture medium at 1% v/v.

(b) Positive control compounds

Mitomycin C from Sigma London Chemical Company Limited (batch number 10SF-06551), was used as the positive control compound for the study in the absence of metabolic activation. It was prepared as a solution in sterile distilled water to give a final concentration of 0.4 µg/ml. Cyclophosphamide obtained from Sigma London Chemical Company Limited (batch number 123F-0283) was used as the positive control compound for the study in the presence of metabolic activation. It was prepared as a solution in sterile distilled water to give final concentrations of 15, 17.5 and 20 µg/ml.

(c) Cell line and culture

Chinese hamster ovary (CHO) cells, strain K₁-BH₄, were obtained from BIBRA and stored in polypropylene ampoules at -196°C in 90% foetal calf serum and 10% dimethylsulphoxide. The cells were routinely grown and subcultured in Hams F12 medium (Imperial) supplemented with 5% foetal calf serum (Gibco) at 37°C in a humid atmosphere containing 5% carbon dioxide in 175 cm² plastic tissue culture flasks (Nunc).

(d) Preliminary toxicity test

A 50 ml culture of CHO cells was harvested as follows: The supernatant medium was removed and the cells washed in 0.9% sterile saline; 20 ml of 0.25% trypsin was then added for 30 seconds. The trypsin solution was removed and the flask incubated at 37°C for 10 minutes. The cells were then resuspended in 20 ml Hams F12 + 5% FCS and diluted to give 1.0×10^5 cells/ml. Aliquots (5 ml) of cells were added to Nunc 25 cm² tissue culture flasks and the cultures incubated at 37°C in a humid atmosphere containing 5% carbon dioxide.

After 24 hours 1.25 ml of S-9 mix (see Appendix 1) was added to one set of cultures followed by 62.5 µl of various dilutions of the test compound and of the solvent. To the second set of cultures (i.e. without S-9 mix) 50 µl of the dilutions of test compound and of the solvent were added. Final concentrations of test compound in both sets were 0.02, 0.03, 0.06, 0.13, 0.25, 0.5 and 1% v/v with single flasks for each concentration and duplicate flasks for the solvent control. The cultures without S-9 mix were incubated in the presence of the test compound for 20 hours.

Two hours after the addition of the test compound to those cultures treated with S-9 mix, the medium containing the S-9 mix and test compound was carefully removed and replaced with fresh Hams F12 + 5% FCS. The cultures were returned to the incubator for a further 18 hours.

(e) Harvesting, fixation and slide preparation

Three hours before the end of the 20 hour incubation period, mitotic activity was arrested by the addition of colchicine to each culture at a final concentration of 0.25 µg/ml. After the incubation period the medium was removed and discarded. 4 ml of 0.25% trypsin solution was then added. After 45 seconds this was removed and placed in a plastic conical centrifuge tube. The flasks were then incubated for 10 minutes at 37°C after which the cells were resuspended in Hams F12 + 5% FCS. The cell suspensions were placed with the trypsin solution in the centrifuge tubes. These cell suspensions were then centrifuged for 10 minutes at 200 x 'g'. The supernatant was discarded and the cells resuspended in 2.5 ml 0.07 M KCl. After a 10 minute incubation at room temperature the cell suspensions were centrifuged for 5 minutes at 110 x 'g'. The supernatant was discarded and 4 ml of freshly prepared fixative (3 parts methanol : 1 part glacial acetic acid v/v) added. The cells were left in fixative for 2 - 3 hours, then the pellets resuspended by repeated aspiration through a 20 gauge needle, centrifuged at 200 x 'g' for 10 minutes, the supernatant discarded, and the cell pellet resuspended in about 0.5 ml of fresh fixative by repeated aspiration through a Pasteur pipette. Two drops of this cell suspension were dropped onto a cold, pre-cleaned microscope slide. The slides were left to air-dry at room temperature, then stained in 10% Giemsa. After air-drying they were mounted in DPX.

(f) Microscopical examination for mitotic index

The prepared slides were examined at a magnification of x160. The proportion of metaphase figures in each culture was recorded. From these results the EC_{50} was estimated (the EC_{50} is that concentration of test substance expected to cause a 50% reduction in the mitotic index). Where possible, this concentration, or the maximum achievable concentration, was used as the top dose in the main study. The intermediate and low doses were 50% and 10% of the top concentration.

(g) Metaphase analysis

Cultures were initiated and maintained as described in section (d). After 24 hours incubation 1.25 ml of S-9 mix was added to one set of cultures followed by 62.5 μ l aliquots of various dilutions of the test compound giving final concentrations of 0.1, 0.5 and 1% v/v. Two cultures were treated at each dose level. Four cultures were treated with 62.5 μ l aliquots of the solvent control (sterile distilled water), and two with the positive control compound, cyclophosphamide at a final concentration of 20 μ g/ml. Two additional cultures were dosed with cyclophosphamide at 15 and 17.5 μ g/ml. The cultures were then incubated at 37°C. To the remaining set of cultures (i.e. without S-9 mix) 50 μ l aliquots of the test compound were added giving final concentrations of 0.1, 0.5 and 1% v/v. 50 μ l aliquots of the solvent were added to four cultures, and mitomycin C, which was used as the positive control at a final concentration of 0.4 μ g/ml, was added to two cultures. The cultures were then incubated at 37°C. Two hours after addition of test compound those cultures treated with S-9 mix had the medium carefully removed and replaced with fresh Hams F12 + 5% FCS. The cultures were then returned to the incubator for a further 18 hours.

All cultures were treated with colchicine, harvested, fixed and slides prepared as described in section (e). The slides were stained in 10% Giemsa, mounted in DPX and coded. Metaphase spreads were identified using a magnification of x160 and examined at a magnification of x1000 using an oil immersion objective. Approximately 100 metaphase figures were examined where possible from each culture, with normally a maximum of 25 from each slide.

(h) Storage of raw data

All slides and raw data, or exact copies thereof, together with a master copy of this final report are located in the Archives of Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England.

RESULTS

(a) PRELIMINARY TOXICITY TEST

The mitotic indices of CHO cells treated with various concentrations of PTB are shown in Table 1.

In the absence of S-9 mix, a decrease in mitotic index of approximately 50% was observed at the highest concentration.

In the presence of S-9 mix, no decreases in mitotic index were observed in compound-treated cultures, compared with the negative control value.

Consequently the maximum achievable concentration, 1% v/v, was used as the highest dose for metaphase analysis in both the absence and presence of metabolic activation. Intermediate and low doses were 0.5 and 0.1% v/v.

(b) METAPHASE ANALYSIS

The effects of PTB on cultured CHO cells are shown in Table 2 in which the number and type of chromosomal aberrations are recorded.

The positive control compound in the absence of S-9 mix, mitomycin C (0.4 µg/ml) caused statistically highly significant increases in the proportion of metaphase figures containing aberrations when compared with the relevant solvent control.

Cyclophosphamide at 20 µg/ml proved extremely toxic, and few metaphase figures could be obtained from these cultures. However, two cultures included in this experiment and treated with 15 and 17.5 µg/ml cyclophosphamide showed large, statistically significant increases in chromosomal aberrations thus demonstrating the efficacy of the S-9 mix.

In the absence of S-9 mix, cultures treated with PTB showed very slight but just statistically significant increases in the incidence of chromosomal damage at the intermediate dose level ($P < 0.05$) and the highest dose level ($P < 0.05$, (including gap damage only)). However the levels of chromosomal damage observed were well within the range of historical control values for this laboratory (0-4.5%). Therefore they are not considered to be indicative of clastogenic activity.

In the presence of metabolic activation, there were no statistically significant increases in the incidence of aberrant cells at any dose level of PTB.

CONCLUSION

PTB has shown no evidence of clastogenic activity in this in vitro cytogenetic test system.

164

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65

TABLE 1

Preliminary toxicity test - mitotic indices of cultured CHO cells treated with PTB

(a) Without metabolic activation

Culture number	Test agent	Concentration (% v/v)	Mitotic index		
			Slide a	Slide b	% Mean
1	sterile distilled water	1	20/1000	18/1000	3.73
2			55/1000	56/1000	
5	PTB	0.02	32/1000	39/1000	3.55
6		0.03	45/1000	41/1000	4.30
7		0.06	32/1000	31/1000	3.15
8		0.13	40/1000	35/1000	3.75
9		0.25	39/1000	34/1000	3.65
10		0.5	36/1000	40/1000	3.80
11		1	18/1000	16/1000	1.70

(b) With metabolic activation

Culture number	Test agent	Concentration (% v/v)	Mitotic index		
			Slide a	Slide b	% Mean
19	sterile distilled water	1	30/1000	27/1000	2.48
20			20/1000	22/1000	
23	PTB	0.02	58/1000	58/1000	5.80
24		0.03	30/1000	31/1000	3.05
25		0.06	39/1000	40/1000	3.95
26		0.13	61/1000	55/1000	5.80
27		0.25	44/1000	42/1000	4.30
28		0.5	28/1000	28/1000	2.80
29		1	30/1000	24/1000	2.70

APPENDIX 1

Preparation of S-9 liver homogenate fraction

1. Animal used

Species: Rat.

Strain: CD rats of Sprague-Dawley origin.

Source: Charles River, UK Limited,
Manston Road, Margate, Kent, England.

Age range: 6-8 weeks.

Weight range: 180-220 g.

Diet: Labsure Rodent Diet LAD 1.

Number used: 10 males.

2. Stimulation of rat liver enzymes

Mixed-function oxidase systems in the rat liver were stimulated following a single i/p injection of Aroclor 1254 (diluted in Arachis oil to 200 mg/ml) at a dosage of 500 mg/kg. On the fifth day of induction, following an overnight starvation, the rats were killed and their livers aseptically removed.

3. Preparation of liver homogenate S-9

All steps were at 0-4°C using sterile solutions and glassware. The livers were placed in beakers containing 0.15 M KCl. After weighing, livers were transferred to a beaker containing 3 volumes of 0.15 M KCl and homogenised in an MSE top-drive homogeniser. This homogenate was centrifuged for 10 minutes at 9000 x 'g' and the supernatant divided into 5 ml aliquots. These were stored at -80°C and tested before use, with the carcinogen, 7,12-dimethylbenz(a)anthracene.

4. Preparation of S-9 mix

Each ml S-9 mix contained:

S-9 fraction	0.1 ml
0.4 M MgCl ₂	0.02 ml
0.2 M Na ₂ HPO ₄ (pH 7.4)	0.5 ml
1.0 M glucose-6-phosphate	0.005 ml
0.1 M NADP	0.04 ml
Distilled water	0.335 ml

All the above solutions were mixed and then filter-sterilised (apart from the S-9 fraction which was added after filter-sterilisation of the other S-9 mix components).

169

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S. A. RIDLON

AMES METABOLIC ACTIVATION TEST TO
ASSESS THE POTENTIAL MUTAGENIC EFFECT OF
ARCOSOLV PTB

PHOTOCOPY

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Date: 7 October 1987

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170

BACTERIAL REVERSE GENE MUTATION ASSAY - THE AMES TEST

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

COMPOUND: Arcosolv PTB.

Storage conditions: Stored at room temperature.

Appearance: Colourless liquid.

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

The experiments described in this report were carried out between 6 August 1987 and 22 August 1987.

Solvent: Ethanol.

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

Mutation tests: 5000, 1500, 500, 150, 50 µg/plate.

RESULTS

The revertant colony counts for Arcosolv PTB obtained in the dose range finding test are shown in Table 1. Arcosolv PTB was not toxic towards the tester strains. Therefore 5000 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 Arcosolv PTB 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 Arcosolv PTB 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.

No substantial increases in revertant colony numbers of any of the five tester strains were observed following treatment with Arcosolv PTB at any dose level, either in the presence or absence of metabolic activation (S-9 mix).

CONCLUSIONS AND COMMENTS

It is concluded that no evidence of mutagenic potential of Arcosolv PTB was obtained in this bacterial test system at the dose levels used.

APPENDIX 1

Experimental Protocol

1. MATERIALS

1.1 Bacterial strains

The following strains will be used in the Ames test:

S.typhimurium TA 1535 his G46 rfa⁻Δ uvr B⁻
S.typhimurium TA 1537 his C3076 rfa⁻Δ uvr B⁻
S.typhimurium TA 1538 his D3052 rfa⁻Δ uvr B⁻
S.typhimurium TA 98 his D3052 rfa⁻Δ uvr B⁻R⁺
S.typhimurium TA 100 his G46 rfa⁻Δ uvr B⁻R⁺

All five strains are defective in DNA repair capacity (Δ uvr B⁻) and have a defective lipopolysaccharide barrier on the cell wall (rfa⁻). These two properties confer extra sensitivity to DNA damage and also greater permeability of large molecules into the cell. Strains TA 98 and TA 100 also contain a resistance transfer factor (plasmid pKM 101). This factor, which confers resistance to ampicillin, enhances the operation of an error-prone repair system.

The strains are tested routinely for cell membrane permeability and where applicable for ampicillin resistance.

For use in tests sub-cultures are grown in Nutrient Broth (Oxoid) at 37°C for 18 hours. This culture provides approximately 2 x 10⁹ organisms per ml which is assessed by cell counting.

1.2 Positive controls

(a) With S-9 mix

2-Aminoanthracene at 2 µg/plate for strains TA 1535 and TA 1537.

2-Aminoanthracene at 0.5 µg/plate for strains TA 1538, TA 98 and TA 100.

(b) Without S-9 mix

2-Nitrofluorene at 2 µg/plate for strain TA 1538.

2-Nitrofluorene at 1 µg/plate for strain TA 98.

9-Aminoacridine at 80 µg/plate for strain TA 1537.

N-ethyl-N'-nitro-N-nitrosoguanidine at 5 µg/plate for strain TA 1535.

N-ethyl-N'-nitro-N-nitrosoguanidine at 3 µg/plate for strain TA 100.

2. PROCEDURE

2.1 Preliminary toxicity test

The following procedure is carried out on each bacterial strain:

Four concentrations of test substance are assessed for toxicity using the five tester strains. The highest concentration is usually 0.05 g of test substance dissolved in 1 ml of solvent. Three 10-fold serial dilutions of the top concentration are also tested. The chosen solvent is used as the negative control.

APPENDIX 1

(continued)

0.1 ml of an overnight bacterial culture containing approximately 2×10^9 cells/ml, and 0.5 ml S-9 mix (see Section 3) or 0.5 ml 0.1 M sodium phosphate buffer (pH 7.4) are placed in glass bijou bottles. 0.1 ml of the test solution is added followed by 2 ml histidine deficient agar. The mixture is thoroughly shaken and overlaid onto previously prepared plates containing 20 ml minimal agar. Single petri dishes are used for each dose level. They are incubated at 37°C for 72 hours. After this period the plates are examined for the appearance of a complete bacterial lawn. Revertant colonies are counted using a Biotran Automatic Colony Counter. Any toxic effects of the test substance are detected by a substantial reduction in revertant colony counts or by the absence of a complete background bacterial lawn.

2.2 Ames test procedure

(a) Without metabolic activation

The following procedure is carried out on each tester strain.

0.1 ml aliquots of bacterial suspension and 0.5 ml of sterile 0.1 M sodium phosphate buffer (pH 7.4) are added to each of one set of sterile bijou bottles.

0.1 ml of the test compound is added to cultures at five concentrations separated by half-log 10 intervals. The negative control is the chosen solvent. The appropriate positive control is also included. 3 bottles are used at each dose level.

2.0 ml of histidine deficient agar is added to each of the bottles, thoroughly mixed and then overlaid onto previously prepared plates containing 20 ml of minimal agar. Plates are incubated for 72 hours at 37°C.

Colonies are counted using a Biotran Automatic Colony Counter, and the mean number of revertant colonies per treatment group assessed.

(b) With metabolic activation

Methodology is as described in 2.2 (a) except that 0.5 ml of liver homogenate S-9 mix (see Section 3) is added to bijou bottles in place of sterile buffer.

2.3 Second mutation test

The procedure outlined in Section 2.2 is repeated at a later date; though the concentrations of test substance used in the second test may be altered, if the results of the first test indicate this may be expedient.

APPENDIX 1

(continued)

3. PREPARATION OF LIVER HOMOGENATE S-9 FRACTION

Species: Rat
Strain: CD (Sprague-Dawley-derived).
Source: Charles River UK Limited, Manston Road, Margate, Kent, England.
Age range: 7-8 weeks on arrival.
Weight range: 180-220 g on arrival.
Diet: Labsure's Laboratory Diet No. 1.
Number used: 7-13

3a. Stimulation of rat liver enzymes

Mixed-function oxidase systems in the rat liver are stimulated following a single i/p injection of Aroclor 1254 (diluted in Arachis oil to 200 mg/ml) at a dosage of 500 mg/kg. On the fifth day of induction, following an overnight starvation, the rats are killed and their livers aseptically removed.

3b. Preparation of liver homogenate "S-9"

i. All steps are at 0-4°C using sterile solutions and glassware. The livers are placed in beakers containing 0.15 M potassium chloride. After weighing, livers are transferred to a beaker containing 0.15 M KCl (the volume of KCl in ml is equivalent to 3 times the weight of liver in gram), minced with a sterile scalpel and homogenized in an MSE top-drive homogenizer. This homogenate is centrifuged for 10 minutes at 9000 x 'g' and the supernatant divided into 15 ml aliquots. These are frozen on dry ice and stored at -80°C, and tested with the carcinogen 7,12-dimethylbenzanthracene before use.

ii. Preparation of "S-9 mix"

S-9 mix contains: S-9 fraction (10% v/v), Mg Cl₂ (8 mM), KCl (33 mM), sodium orthophosphate buffer pH 7.4 (100 mM), se-6-phosphate (5 mM), NADP (4 mM). All the cofactors are filter-sterilized before use.

4. ASSESSMENT OF RESULTS

The mean number of revertant colonies for all treatment groups is compared with those obtained for negative and positive control groups. The effect of metabolic activation is assessed by comparing the results obtained both in the presence and absence of the liver microsomal fraction for each treatment group.

A compound is deemed to provide evidence of mutagenic potential if (1) a statistically significant dose-related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value.

APPENDIX 1

(continued)

5. MAINTENANCE OF RECORDS

All data are kept in a loose-leaved laboratory notebook which is held in the Department of Mutagenesis and later transferred, together with a mastercopy of the final report, to the Archive Department, Huntingdon Research Centre Ltd., Huntingdon, Cambs, U.K.

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BIO-RESEARCH LABORATORIES LTD.

RESEARCH REPORT

VOLUME I

A 4- AND 13-WEEK INHALATION TOXICITY
STUDY (WITH 3-WEEK REGRESSION) OF
ARCOSOLV™ PTB IN THE ALBINO RAT

by

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184

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SUMMARY

Five groups of Fischer-344 rats were treated by inhalation 5 days a week for 13 weeks with Arcosolv PTB at concentrations of 25, 80, 250 and 750 ppm followed by 3 weeks regression. An air control group was treated similarly in all respects except it received only room air during inhalation exposure. Subgroups were removed after 4 and 13 weeks of treatment and subjected to clinical pathology and gross and histopathological examination.

No clinical signs related to treatment were observed. Body weight gain, food consumption, ophthalmology, hematology and blood biochemistry were unaffected by treatment. A slight increase in trace amounts of urinary occult blood was observed in treated males after 13 weeks and 2 weeks regression. There was no histopathological finding in the kidney which could be correlated with this marginal finding. Furthermore, there was no change in the peripheral blood profile, bone marrow hematopoiesis or hemosiderin deposition in kidney, liver or spleen which could be related to hemolysis in the treated animals.

A significant weight increase (in terms of both absolute and relative weight) was observed in the liver and kidney of males and females and in the spleen of males treated with the test article. Liver weight change was apparent in males after 4 weeks of treatment and in both sexes after 13 weeks of treatment. The magnitude of weight gain generally increased with dose level. Kidney and spleen weight increases were apparent after 13 weeks of treatment, and only liver weight remained significantly elevated after 3 weeks regression. The weight increase in these organs occurred in the absence of any histopathologic change and may represent an adaptive response to treatment with the test article.

There was no gross or histopathological finding in any tissue which could be attributed to treatment with the test article.

In conclusion, exposure of rats to Arcosolv PTB vapor at concentrations up to 750 ppm for 13 weeks produced no evidence of toxicity. Weight increase of several organs was not associated with histopathology and may represent an adaptive response to treatment.

INTRODUCTION

The objective of this study was to investigate the potential toxicity of Arcosolv™ PTB when administered by inhalation for 6 h daily, 5 days per week for 13 weeks minimum, followed by a 3-week regression period. Test groups were exposed to concentrations of 25, 80, 250 and 750 ppm, and an air control group was treated similarly in all respects except it received only room air.

The animals arrived at Bio-Research Laboratories Ltd. on October 2, 1984. The first treatment occurred on October 22, 1984, and the last necropsy of the 3-week regression animals took place on February 20, 1985.

The study was performed at Bio-Research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec, Canada. The study was conducted in compliance with the Good Laboratory Practice Regulations of EPA TOSCA (40 CFR Part 792). All data, the protocol, reports, tissue specimens and samples collected as a result of this study have been retained in the scientific archives of Bio- Research Laboratories Ltd.

192

EXPERIMENTAL PROCEDURES1. Test Article

Arcosolv™ PTB (propylene glycol tertiary butyl ether), a clear, colorless liquid, was received from ARCO Chemical Company, Newtown Square, Pennsylvania. Shipments were received on September 4, 1984 (1 gallon), October 10, 1984 (2 x 5 gallons) and November 27, 1984 (2 x 5 gallons). Documentation accompanying the test article stated it to be stable. Gas chromatographic analysis, conducted by ARCO and accompanying the test article, verified it to be approximately 99% pure with the primary isomer comprising approximately 95% and a second isomer comprising approximately 4 to 5%.

The test article was stored at room temperature out of direct light.

Test article from the October 10, 1984 and November 27, 1984 shipments were used on this study.

2. Test Systema. Receipt

Five hundred and ninety-four (301 male and 293 female) Fischer-344 CDF (F-344) rats (Rattus norvegicus) were received from Charles River Breeding Laboratories, Kingston, N.Y., via Charles River Canada, St. Constant, Quebec. The animals were 4 to 5 weeks of age and their body weight ranged from 30 to 58 g (males) and 31 to 59 g (females) at receipt.

The animals were examined by a veterinarian or veterinary aide after receipt to ensure "normal" health. Four males and 16 females were rejected based on this examination. In addition, two female cage mates were found dead, considered due to inability to operate the watering systems, three days after receipt.

b. Housing and Maintenance

Upon receipt, animals were housed in pairs separately by sex in stainless steel mesh-bottomed metal cages equipped with an automatic watering valve and an additional water bottle. Tail bars were used to identify cage mates.



During the second week of acclimation after assignment to groups, cage mates were separated and all animals were housed individually in stainless steel mesh-bottomed metal cages equipped with an automatic watering valve. All cages were labeled with a color-coded card indicating project, group and animal numbers as well as sex, dosage level and animal identification code. Each animal was uniquely identified by a standard system of ear notches and tail bars.

Each animal had ad libitum access to tap water via an automatic watering system and certified Purina Rodent Chow #5002 except during inhalation treatment. The tap water and diet were routinely analyzed by Bio-Research Laboratories Ltd. and Purina, respectively, and there were no contaminants known to be present in either which could be reasonably expected to interfere with the purpose or quality of this study. Representative analyses of Bio-Research's tap water are presented in Appendices 1 and 2.

The animals were maintained on a 12-h light, 12-h dark photoperiod in a temperature and humidity controlled environment. The daily average temperature and relative humidity in the animal room throughout the study ranged from 19.3 to 22.7°C and 36 to 71%, respectively. On one occasion during study week 1, the average temperature in the animal room for a 12-h interval was 29.8°C, with a high of 32.9°C. This temperature elevation was a result of a brief malfunction of the air cooling system supplying the animal room. During this period, the animals were observed to be less active than usual but were otherwise normal.

c. Randomization and Assignment to Groups

Prior to assignment of animals to study groups, 10 male and 10 female rats were randomly selected from the population as a health screen and were subjected to clinical laboratory investigations (hematology, blood chemistry and urinalysis) and gross pathology.

Two hundred and fifty male and 250 female rats were randomly selected from the remaining population using a computer-based random number generator, and assigned separately by sex to 5 groups each of 50 males and 50 females. Animals at the extremes of the body weight range greater than 2 standard deviations from the mean were excluded during animal assignment. A Bartlett's homogeneity of variance test between the group body weight means of each sex after animal assignment confirmed a similar body weight distribution in the various groups.

194

<u>Group</u>	<u>Target Dose Level (ppm)</u>	<u>Animal Numbers</u>	
		<u>Males</u>	<u>Females</u>
1 Air control	0	1001-1050	1501-1550
2 Arcosolv PTB	25	2001-2050	2501-2550
3 Arcosolv PTB	80	3001-3050	3501-3550
4 Arcosolv PTB	250	4001-4050	4501-4550
5 Arcosolv PTB	750	5001-5050	5501-5550

Following the pre-study ophthalmological examination, 13 males and 9 females from the various groups were replaced by healthy spares of similar body weight. These animals assumed the same animal number and I.D. code as the animals they replaced. Twenty of the remaining spare animals (10/sex) were retained in the animal room for the duration of the study and the balance were sacrificed.

Study animals from each group were randomly selected and sacrificed throughout the study according to the following schedule:

<u>Sex</u>	<u>Number of Animals Sacrificed After</u>		
	<u>4 Weeks Rx</u>	<u>13 Weeks Rx</u>	<u>4 Weeks Regression^A</u>
Male	10/group	20/group	10/group
Female	10/group	20/group	10/group

^AInitially, the study protocol specified a 4-week regression period following 13 weeks of treatment. The animals identified for the 4-week regression sacrifice were treated for an additional 8 days in order that a possible effect of treatment on urinalysis could be more fully examined in all groups. This additional treatment reduced the regression period from 4 to 3 weeks.

The remaining 10 animals of each sex in each group were observed throughout 13 weeks of treatment for hematuria indicative of a hematological effect of treatment. No evidence of hematuria was observed throughout treatment, and these animals were discarded at the end of treatment.

3. Treatment by Inhalation

a. Inhalation Exposure Equipment

Five 36-inch cubed stainless steel and glass whole-body exposure chambers were utilized in this experiment. On each treatment day, a complete group of rats was housed in each chamber. While in the chambers, the rats were retained in stainless steel wire mesh compartmentalized cages with each compartment measuring 7 x 4 x 3 inches. Neither food nor water was available while the animals were in the inhalation chamber.

Air was drawn through the chambers by means of a low-pressure vacuum pump. Inlet air entered at the top of the chamber and was exhausted at the bottom. Flow rate through the chamber was measured in the exhaust line of each chamber by measuring on a magnehelic gauge the pressure differential across an orifice plate. This gauge was calibrated against a conventional ball-type flowmeter.

Flow rate through each inhalation chamber was set at 200 L/min to maintain a chamber environment of 20-24°C, 40-60% R.H. and at least 19% O₂ given the present animal loading conditions. Any deviation in chamber temperature and/or relative humidity was minor and was without effect on the purpose or outcome of the study. Exhaust air was drawn from the chambers and passed through an activated charcoal filtration system before being eliminated from the facility.

b. Test Atmosphere Generation

The test atmospheres of Arcosolv PTB were generated by passing dehumidified, compressed air upward through a vertical glass column 75 cm in height and 4 cm in diameter and filled with 6 mm diameter glass beads. The test article was metered into the column 8 cm above the bottom using a variable-speed peristaltic pump, and was carried upward throughout the bead bed by the generator air supply and vaporized. The vapor from the generator column was delivered to the inhalation chamber inlet port where it was mixed with the chamber air supply.

The various chamber concentrations were achieved by altering the rate of test article delivery onto the column and by adjusting the rate of airflow through the column. The air control group also received dehumidified compressed air through a glass bead column at the same rate as the treated groups, but in this case no test article was metered onto the column.

Nominal chamber concentrations were determined daily for each group by calculating the amount of test article consumed per liter of air passing through the inhalation chamber.

c. Frequency and Duration of Treatment

Exposure of the animals in the various groups to either air or the test article was conducted 5 days a week for 13 weeks minimum. All sacrificed animals were treated up to the day prior to necropsy.

Each exposure period was 6 hours in duration, with time zero being defined as that point in time at which 95% of the desired concentration of test article had been established within the chamber. Flow rates through the chamber and generating equipment were adjusted in preliminary work so that a tgs of approximately 30 minutes was attained. The generating equipment ran for a period of 6 h/day.

4. Monitoring of Experimental Atmospheres

a. Infrared Gas Analysis

Chamber test article concentration was measured on an hourly basis each day using a MIRAN 1A infrared gas analyzer set at an analytical wavelength of 8.35 microns and a pathlength of 3.75 m. Prior to the study, the instrument was calibrated by a triplicate series of injections of the test article into the gas cell. The test article concentration in the cell after each injection was calculated as follows:

$$C \text{ (ppm)} = \frac{\mu\text{L} \times d \times \text{MW}}{\text{MW} \times L}$$

where μL = test article volume injected (μL)
 d = test article density, 0.87 g/mL
 MW = test article molecular weight, 131 g/mole
 MW = molar volume of a gas at 25°C, 24,470 mL
 L = closed cell volume, 5.64 L

A calibration curve of infrared absorbance versus test article concentration was plotted.

The calibration curve was verified daily prior to the start of treatment by a single liquid injection of the test article.

Air samples were collected hourly from each chamber through a sampling manifold at a rate of approximately 5 L/min and delivered to the MIRAN analyzer. Additional samples were collected on occasion to verify the initial samples or to monitor adjustments made to the generation system. Sampling progressed sequentially from Group 1 (air control) through to Group 5 (high dose). The MIRAN analyzer and sampling manifold were purged with air from the control chamber and the analyzer was zeroed between samples from the various test chambers. The chamber concentration of test article in each group was determined from the absorbance values recorded and the calibration curve.

b. Gas Chromatographic Analysis

Gas chromatographic analysis of chamber atmospheres was performed during preliminary chamber work, daily during the first 3 weeks of treatment and weekly thereafter. Additional samples were collected on occasion to verify findings of previous samples. Air samples were collected from each chamber and passed through a sintered glass gas washing bottle containing a known volume of methanol. Aliquots of the liquid sample were assayed by gas chromatography using the procedures described in the Analytical Chemist's Report (Table 1).

Arcosolv PTB concentrations in the inhalation chambers were calculated from the GC analysis as follows:

$$C \text{ (ppm)} = \frac{A \times B \times \text{MVG}}{\text{MW} \times S}$$

where A = GC concentration of Arcosolv (mg/mL)
 B = final volume of methanol in bubbler (mL)
 MW = test article molecular weight, 131 g/mole
 MVG = molar volume of gas at 25°C, 24,470 mL
 S = air volume sampled, L

c. Chamber Conditions

Chamber airflow was monitored and recorded half-hourly during treatment, and the temperature and relative humidity in the inhalation laboratory and chambers were recorded hourly during treatment by means of Abbeon-Lufft thermohygrometers.

5. Observations During Life

a. Clinical Examinations

All animals underwent twice daily observations for mortality or morbidity as well as an individual post-treatment examination each day. Abnormal animals identified in this examination were seen by the veterinary staff weekly until they returned to normal.

White tray paper was placed under each animal's cage and was examined daily during the first 3 weeks of treatment for red staining indicative of blood in the urine. If red staining was observed, the tray paper was analyzed for blood pigment (Hematest tablet). From week 4 onward throughout treatment, white tray papers were placed under the cages of Group 1 and 5 animals only.

b. Body Weight

During the acclimatization and treatment periods, animals were individually weighed on a weekly basis. Animals were also weighed on the day of terminal sacrifice (starved body weight) and immediately prior to sacrifice at necropsy.

c. Food Consumption

Individual food consumption was determined weekly during the second week of acclimatization and during the treatment period.

d. Ophthalmology

The eyes of all animals (except the health screen) were examined with a direct ophthalmoscope and a slit lamp prior to any exposures and prior to necropsy for those animals sacrificed after 4 and 13 weeks of treatment. The assessment after 4 and 13 weeks included a corneal examination with fluorescein.

e. Clinical Laboratory Studies

Hematology, clinical chemistry and urinalysis were performed on 10 rats of each sex (health screen) during the pretreatment period and on 10 rats of each sex in Groups 1 and 5 after 4 and 13 weeks of treatment. The animals identified for the regression sacrifice (10/sex/group) had a urine analysis performed after the last inhalation exposure during the 15th treatment week and also 2 weeks into the 3-week regression period.

Urine samples from the health screen and after 4 and 13 weeks were collected by placing the animals in metabolism cages overnight (16-hour collection period), during which time they were deprived of food and water. Due to insufficient volume for analysis of some of these samples, the last urine collection in the regression animals was preceded by "water loading" the animals with 10 mL/kg body weight.

Blood samples for clinical chemistry were obtained immediately prior to termination following overnight withdrawal of food. Samples were collected from the abdominal aorta following anesthesia by intraperitoneal injection of sodium pentobarbital. Ten samples/sex/group, from Groups 1 and 5, were analyzed initially, and the remaining samples were stored frozen in the event that analysis of the lower dose levels was required.

Blood samples for hematology were obtained from the abdominal aorta at necropsy and collected using EDTA as anticoagulant.

The methods used in the laboratory investigations are presented in Appendix 32.

6. Terminal Investigations

a. Gross Pathology and Organ Weights

Prior to the commencement of treatment, 10 male and 10 female rats designated for health-screen evaluation were killed by exsanguination from the abdominal aorta after anesthesia by intraperitoneal injection of sodium pentobarbital and were then subjected to external and internal gross examination. No organ weights or tissue samples were collected.

After 4 and 13 weeks of treatment and after 3 weeks regression, animals were randomly selected (10/sex/group at the 4-week and regression kills, and 20/sex/group at the 13-week kill), sacrificed by exsanguination under anesthesia and necropsied. The sacrifices at each occasion were spread across several days, and a similar proportion of rats from each sex and group was killed on any one day. Animals awaiting necropsy were treated up to the day prior to kill. All animals were fasted overnight before scheduled sacrifice.

A complete necropsy was conducted immediately on any animal killed during the study.



For all animals, necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination.

The following organs from each animal (except the health screen) were dissected free of fat and weighed. Paired organs were weighed together.

adrenals	kidneys	pituitary
brain	liver	spleen
heart	lungs with trachea	testes

b. Tissue Preservation

On completion of the necropsy, the following tissues and organs were retained. 10% neutral buffered formalin was used for fixation and preservation unless otherwise indicated.

animal identification	liver (sample of 2 lobes)	seminal vesicles
adrenals	lungs (sample of 2 lobes)	skeletal muscle
aorta (thoracic)	lymph nodes (mesenteric and bronchial)	skin
bone (sternum with bone marrow)	mammary gland (inguinal) ⁺	spinal cord (cervical)
brain	nasal passages and sinuses ^{**}	spleen
cecum	optic nerves ^{**+}	stomach
colon	ovaries	testes*
duodenum	oviducts	thymus
epididymides*	pancreas	thyroid lobes (and parathyroids) ⁺
esophagus	pituitary	trachea
eyes*	prostate	urinary bladder
heart	rectum	uterus (body and cervix)
ileum	salivary glands	vagina
jejunum	sciatic nerve	any abnormalities as defined by the pathologist
kidneys		
lacrimal gland		
larynx		

*Fixed in Zenker's fluid (sacrificed animals only).

**Decalcified prior to sectioning and staining.

⁺Only examined histopathologically where present in routine sections of eyes (optic nerves), thyroid (parathyroids) or skin (mammary gland).

RESULTS

1. Inhalation Chamber Concentrations

a. Infrared Gas Analysis

Weekly mean chamber concentrations of Arcosolv PTB are presented in Table 7. Hourly values and the daily means are presented in Appendix 3. Daily nominal concentrations appear in Appendix 4.

Bio-Research's Time Notation System is described in Table 6.

The weekly mean concentrations were generally close to target levels. Weekly mean values during the study for the various groups were as follows: Group 2 - 23.3 to 33.4 ppm (target 25 ppm), Group 3 - 71.4 to 90.5 ppm (target 80 ppm), Group 4 - 229.2 to 257.8 ppm (target 250 ppm) and Group 5 - 658.6 to 785.5 ppm (target 750 ppm). The overall mean concentrations for the study were 28.3 ppm, 81.5 ppm, 243.2 ppm and 708.7 ppm for Groups 2, 3, 4 and 5 respectively.

On a given day, chamber concentrations were generally stable and fluctuations (more frequently experienced during the first week) were usually related to brief technical difficulties with the generation system.

Nominal chamber concentrations for each group (calculated as the quantity of test article consumed per liter of chamber air and expressed in ppm) closely paralleled the infrared chamber concentrations on a daily basis during the study. Actual concentrations were generally greater than 85-90% of the nominal concentrations.

b. Gas Chromatographic Analysis

Results of the gas chromatographic analysis of chamber samples collected during the study appear in Appendix 5. These data are presented together with the Miran concentrations measured at approximately the same timepoint during each exposure.

The primary purpose of the gas chromatographic analysis for this study was to document the proportionality of the two isomers of the vaporized test article in the chamber. This was done by comparing the relative peak areas or peak heights of the isomers in chromatograms of chamber samples with the relative peak areas or heights of chromatograms of liquid Arcosolv standards. Chamber vapor samples and liquid standards analyzed during pre-study chamber atmosphere validation contained the two isomers in the following proportions:

<u>Sample</u>	<u>Primary Isomer</u>	<u>Secondary Isomer</u>
Arcosolv Standard	96.0%	4.0%
Group 2	96.4%	3.6%
Group 3	96.6%	3.4%
Group 4	96.6%	3.4%
Group 5	96.4%	3.6%

202

Secondarily, the GC analysis served as back-up to the Miran analysis. The chamber concentrations, as measured by gas chromatography, were generally lower than the concentrations measured by infrared analysis. Efforts made during the course of the study to enhance the agreement between the two methods of analysis produced some improvement; however, the concentrations measured by GC continued to be lower than the corresponding infrared gas analyses. It is possible that factors such as the solubility of Arcosolv in the collection solvent or the effect of temperature on the scrubber collection efficiency contributed to the disagreement between the two analyses.

2. Observations During Life

a. Health Screen Assessment

Results of the health screen laboratory investigations (hematology, blood chemistry and urinalysis) on randomly selected animals prior to the start of treatment appear in Appendices 6 and 7 for males and females. The Clinical Pathologist's report appears in Table 4.

The data demonstrated no unusual findings in clinical pathology. Gross pathological examination of the animals revealed no abnormalities. Therefore, the health status of the animal population for this study was concluded to be good.

b. Clinical Examinations

The Veterinarian's report is presented in Table 2.

There were no deaths or abnormal clinical observations during the study which were considered to be related to treatment with the test article.

Male rat No. 3036 (Group 3 - 80 ppm) was sacrificed on study day 42 after having lost use of a hindlimb. No gross pathological finding was observed in this animal.

During the first week of treatment, a majority of animals from all groups including the air control group and "room spares" were observed to have reddish-brown spotting on the tray papers beneath the cages. The spotting occurred at random under the cages and was not localized in areas of urine staining, indicating that the source of the red spotting was not urinary. Representative samples of tray paper from each group were tested for occult blood using the Hematest tablet and found to be positive. The spotting disappeared in all but a few animals towards the end of the second week of the study and had completely ended by the fourth week.

During the first and second weeks of exposure, several animals also displayed mild clinical symptoms suggestive of infection with sialodacryoadenitis virus (respiratory sounds, nasal discharge and/or eye discharge or redness). Serological tests of 5 "room spares" were performed on two occasions to confirm a diagnosis, and these evaluations demonstrated significant antibody titers for rat corona virus/ sialodacryoadenitis virus (Appendix 8). The red spotting observed on tray papers was, therefore, likely related to nasal discharge symptomatic of this infection. Due to the transient nature and mild severity of the clinical symptoms, it is considered unlikely that the viral infection affected the purpose or outcome of the study.

Clinical signs observed infrequently in animals of both treated and control groups included eye opacity, urogenital fur staining, ocular discharge and moist respiratory rales, all of which were considered unrelated to treatment.

c. Body Weight

Mean body weight data are presented in Table 8, and the individual data appear in Appendices 9 and 10 for males and females, respectively.

There was no effect of treatment on body weight gain during the study.

Mean body weight for the various groups was similar during the treatment and regression periods. A statistically significant increase in body weight of Group 2 males (25 ppm) at weeks 11 to 13 was considered unrelated to treatment.

d. Food Consumption

Mean food consumption is presented in Table 9, and the individual data appear in Appendices 11 and 12 for males and females.

A trend to marginally higher food consumption in treated animals was observed from approximately week 6 onward; however, statistically significant differences were sporadic and in most instances involved several treated groups during a given week (e.g., females during weeks 6 and 8, males during weeks 9 and 11). The magnitude of increase was approximately 2 to 5%, and there was no evidence of a dose-related effect. Larger increases were occasionally seen and occurred during both the treatment and regression phases of the study (approximately ~~9% and 11% in Group 2 females at weeks 6 and 8~~, 9% in Group 5 males and Groups 3 and 4 females at week 15, 16% in Group 5 females at week 15).

204

In conclusion, the apparent marginal increase in food consumption of treated animals is of doubtful significance given the small change observed, a lack of dose response and the infrequency of statistical significance.

e. Ophthalmology

The Ophthalmologist's report is presented in Table 3, and the Veterinarian's report summarizing the corneal examination with fluorescein is presented in Table 2.

There was no effect of treatment with Arcosolv PTB on the eye.

Among the animals sacrificed after 4 weeks treatment, several from both the treated and control groups displayed minor abnormalities of the cornea consisting of small areas of opacity (8 males) or small granules, possibly protein, on the endothelium (1 male).

After 13 weeks of treatment, areas of corneal fluorescein staining were frequently observed in males of all groups; however, the highest incidence of this finding was observed in the air control group (60% of males), and the lowest incidence occurred in the high dose group (30% of males). Up to 10% of females in the various groups displayed corneal fluorescein staining. Other incidental findings included hyphema in the anterior chamber of one eye (likely related to mydriasis), of rat 1522 a corneal ulcer with neovascularization in rat 4005 and an area of peripheral corneal edema in rat 4546.

f. Clinical Laboratory Studies

i. Hematology

Mean hemograms for Groups 1 and 5 after 4 and 13 weeks of treatment are presented in Tables 10 to 13, and the individual data appear in Appendices 14 to 17. The legend for hemograms appears in Appendix 13, and the Clinical Pathologist's report is presented in Table 4.

The hemograms were unaffected by treatment with Arcosolv PTB. The mean and range of values for the various parameters were similar in treated and control animals. Statistically significant differences between the treated and control groups were observed for several parameters (mean corpuscular hemoglobin in males and hemoglobin in females after 4 weeks of treatment, mean corpuscular volume in males and white blood cell count in females

after 13 weeks of treatment). These changes were small in magnitude, were within the range of normal variation and were observed in one sex and at one assessment only. They were therefore considered to be unrelated to treatment.

ii. Blood Biochemistry

Mean values for the blood biochemical analyses after 4 and 13 weeks of treatment are presented in Tables 14 to 17, and the individual values are presented in Appendices 18 and 21.

Treatment with Arcosolv PTB had no effect on clinical chemistry. The means and range of individual values in the treated and control groups were similar. Statistically significant differences (serum chlorine in males at week 4, total protein in females at week 13) were small and within normal ranges, occurred in one sex at one occasion only and were unrelated to treatment.

iii. Urinalysis

Individual values of the urine analysis performed during the fourth and thirteenth week of treatment are presented in Appendices 23 and 26. Urine analyses performed on regression animals after 14 weeks of treatment and after two weeks of regression appear in Appendices 27 to 30. Mean values of urinary specific gravity appear in Appendix 31. A legend for urinalysis is given in Appendix 22.

The range of values for the various parameters was similar in treated and control groups.

There was a slightly higher frequency of trace quantities of occult blood in the urine of treated males, particularly in the assessments at the end of the treatment and the regression periods. This apparent effect may have been related to treatment; however, there was no histopathological change observed in the kidney, liver, spleen or bone marrow, or change in the peripheral blood profile of animals treated for 4 or 13 weeks which was suggestive of hemolysis and which could be correlated with this marginal finding.

3. Postmortem Observations

a. Organ Weights

Mean absolute organ weights of animals sacrificed after 4 and 13 weeks of treatment and after 3 weeks regression are presented in Tables 18 to 23, and mean organ weights relative to body weight appear in Tables

24 to 29. Individual, absolute and relative organ weight data are presented in Appendices 33 to 44.

Statistically significant differences were frequently seen in liver, spleen and kidney weights of treated animals compared to the control.

Liver weight was significantly increased in terms of absolute and/or relative weight in Group 4 and 5 males after 4 weeks of treatment, in Group 2 to 5 males after 13 weeks of treatment and in Group 4 and 5 males after 4 weeks regression. In females, significant differences were observed in Groups 4 and 5 at the 13 week assessment and in Group 5 at 3 weeks regression. The magnitude of increase ranged from approximately 4 to 16%, with the largest increases observed in males. Within each sex the largest increases were observed in high-dose animals after 13 weeks of treatment. Some reduction in liver weight increase was observed after 3 weeks of regression in treated animals of both sexes.

Significantly increased spleen weight was observed in Group 2, 3 and 5 males and in Group 2 and 3 females (absolute weight) after 13 weeks of treatment. A trend to increased weight was observed in Group 4 males at week 13 and in Group 2 to 5 males at 3 weeks regression. Similar trends were not seen in females. In terms of relative weight, Group 5 males were significantly different from controls after 13 weeks of treatment, and increased weight, although not significant, was seen in Group 4 males at week 13 and in Group 5 males at 3 weeks regression. The frequent spleen weight increase observed in treated males after 13 weeks is possibly related to treatment. The change observed in females of the low- and intermediate-dose groups at week 13 is unlikely related to the test article.

Absolute and/or relative kidney weight was significantly increased in Group 3, 4 and 5 males and Group 5 females after 13 of treatment. The magnitude of increase ranged from approximately 4 to 8%. Kidney weight in these groups was slightly elevated but was not statistically different from controls after 4 weeks regression.

Statistically significant differences were also observed in other organs. After 13 weeks of treatment, adrenal weight of Group 5 males was significantly higher than that of controls in terms of absolute and relative weight. Relative weights of lungs, adrenals and brain of Group 2 males were significantly lighter than those of controls. None of these changes was considered due to the test article.

b. Gross and Histopathological Findings

Gross pathological findings for individual animals appear in Appendices 45 and 46 after 4 weeks of treatment, in Appendices 47 and 48 after 13 weeks of treatment and in Appendices 49 and 50 after 4 weeks regression. Individual histopathological findings are presented in Appendices 51 and 52 for animals sacrificed at 4 weeks and in Appendices 53 and 54 for animals at the 13-week sacrifice. Hemosiderin deposition in tissues of animals sacrificed after 13 weeks of treatment is given in Appendices 55 and 56. The incidence of histopathological findings at 4 and 13 weeks is presented in Tables 30 and 31, and the incidence of hemosiderin deposition after 13 weeks of treatment is given in Table 32. The pathologist's report appears in Table 5.

Few gross or histopathological findings were observed in animals sacrificed at 4 and 13 weeks of treatment and after 3 weeks regression. The most frequent histopathological findings at the 4- and 13-week evaluations consisted of renal tubular mineralization, graded as slight, in approximately 50% of males in both the treated and control groups. This is a common finding in rats of this strain and is unrelated to treatment.

Liver, kidney and spleen of Group 1 and 5 animals after 13 weeks of treatment were examined for hemosiderin deposition. Hemosiderin granules were observed in the spleen of all males and females (moderate accumulation) and in the liver of all females (slight accumulation). Slight accumulation in the kidney was also observed in the majority of males and females in both the treated and control groups. The hemosiderin accumulation in these organs was considered to be within normal limits and unrelated to administration of the test article.

No histopathological change was observed in liver, spleen or kidney which could be correlated with increased weight of these organs in several treated groups. The liver weight increase observed, and perhaps that of the kidney as well, is likely an adaptive response to treatment, particularly in view of the regression in weight observed 3 weeks post-exposure.

Other gross and histopathological findings were infrequently seen in treated and control animals of both sexes and were considered to be agonal or incidental.

A qualitative assessment of bone marrow smears in Groups 1 and 5 revealed no effect of treatment on hematopoiesis.

208

CONCLUSION

Treatment with Arcosolv PTB by inhalation at concentrations of up to 750 ppm for 13 weeks had no effect on clinical condition, body weight gain, food consumption, clinical pathology and gross and histopathology. Weight increase in several organs was related to treatment; however, the change may represent an adaptive response to treatment since no histopathological finding was observed which could be correlated with the weight increase.



TABLE NO. 1

ANALYTICAL CHEMIST'S REPORT

PROJECT NO. 81910

ANALYSIS OF CHAMBER ATMOSPHERE SAMPLES

During the course of this study, samples of test article mixture from chamber atmospheres were received by the Analytical Chemistry Department. Upon receipt, samples were stored at room temperature for up to 4 days until analysis. In order to check the stability of Arcosolv PTB in methanol, 3 test samples were tested initially and after 30 days of storage at room temperature. No degradation was observed.

The method of assay of the test article was supplied to Bio-Research Laboratories by the Sponsor. It was satisfactorily validated in our laboratories as follows:

Gas Chromatograph	: Hewlett Packard Model No. 5750
Detector	: Flame Ionization
Column	: 20 foot X 1/8 inch stainless steel packed with 10% SP-1000 on 80/100 supelcoport
Injection Temperature	: 200°C
Oven Temperature - Initial	: 105°C (6 min)
- Rate	: 15°C/min
- Final	: 195°C (4 min)
Detector Temperature	: 240°C
Carrier Gas Flow Rate	: 2.5 mL/min
Hydrogen Flow Rate	: 50 mL/min
Air Flow Rate	: 50 mL/min
Injection Volume	: 2 uL
Recorder	: HP integrating recorder

Results of the analyses were reported to the study director. These results are considered accurate based on the excellent chromatography obtained and the linearity of the standards run on each analysis occasion.

Pamela Burnett
Pamela Burnett
Manager
Analytical Chemistry

July 31, 1985
Date



TABLE NO. 2

VETERINARIAN'S REPORT

PROJECT NO. 81910

The clinical signs exhibited during the conduct of the study included corneal opacities, staining of the urogenital region, ocular discharge and abnormal respiratory sounds (moist rales). The incidence of these clinical signs was not indicative of a treatment-related effect.

During the first and second weeks of treatment a few animals exhibited clinical signs suggestive of infection with sialodacryoadenitis virus. Serological tests demonstrated antibody titers for rat corona virus/sialodacryoadenitis virus.

Red spotting on the tray papers of several animals (treated and untreated) during the first 4 weeks of treatment was likely related to nasal discharge symptomatic of the viral infection and was not suggestive of a treatment-related effect.

The results of corneal examination using fluorescein stain were not indicative of a treatment-related effect.

M. Barker
M. Barker, B.Sc., D.V.M.
Veterinarian

11/15/56
Date

The results of the ophthalmological examination conducted after 4 and 13 weeks of treatment were not indicative of a treatment-related effect.

for *Bang S. Ostrom 3/10/85*
A. Leith, B.Sc., M.D., C.M.
Ophthalmologist

#2

217

CLINICAL PATHOLOGIST'S REPORT

The hematology, blood biochemistry and urinalysis data (Groups 1 and 5) as well as bone marrow smears (Groups 1 and 5) generated during the conduct of this study, have been reviewed for clinical significance.

The pretreatment data did not demonstrate any unusual or unexpected clinical pathological findings thereby assuring the health status of the animals for this study.

No specific changes in the various clinical pathology parameters or bone marrow smears evaluated during the course of this study can be associated with administration of Arcosolv PTB to rats up to doses of 750 ppm for thirteen weeks. Intersubject variation was present in both the treated and control animals and is, on occasion, reflected by variances in the parameter being evaluated.



Colin B. Bier, Ph.D.
Clinical Pathologist

30 July 1985
Date

ANIMALS SACRIFICED AFTER 4 WEEKS OF TREATMENT

Gross pathological examination was undertaken on all animals sacrificed after 4 weeks of treatment. Gross findings were infrequent and appeared to be either agonal or incidental.

Histopathological examination was undertaken on all animals in Group 1 (control) and Group 5 (Arcosolv PTB, 750 ppm). Renal tubular mineralization graded as slight was observed in 5 out of 20 controls and 5 out of 20 high-dose rats. This is not an uncommon finding in rats of this strain. Other changes seen in Group 1 and/or Group 5 were few and considered to be either incidental or agonal. These included focal mucosal mineralization of the larynx, laryngitis, nematode parasites in the rectum, mononuclear cell infiltration in the prostate and retinal rosettes.

ANIMALS SACRIFICED AFTER 13 WEEKS OF TREATMENT

Gross pathological examination was undertaken on all animals sacrificed after 13 weeks of treatment. Gross findings were infrequent and appeared to be either agonal or incidental. Some females had findings in the uterus consistent with normal changes associated with the estrous cycle.

Histopathological examination was undertaken on 10 males and 10 females from both Group 1 (control) and Group 5 (Arcosolv PTB, 750 ppm). Renal tubular mineralization was observed in some Group 1 and 5 male rats. This is not an uncommon finding in rats of this strain. One Group 5 male (5023) had slight degeneration of the testicular seminiferous epithelium. One other male in Group 5 (5028) sacrificed at 4 weeks of treatment had similar findings. In both animals, the lesions were focal and unilateral with the involvement of only a small number of seminiferous tubules. Based on these morphological observations and on the fact that degenerative testicular changes are reported with regularity in Fischer rats of the this age group (1), this finding was considered incidental and due to chance variation. Other changes seen in Group 1 and or Group 5 were few and appeared to be either agonal or incidental.

In addition, histopathological examination of hemosiderin-stained sections of the kidneys, liver and spleen was performed. Hemosiderin granules were seen in the spleen of all male and female animals and in the liver of all female animals. The finding was graded as moderate and slight in the spleen and liver respectively. In the kidneys, hemosiderin granules (slight accumulation in all animals) was observed in 8 out of 10 control males, 7 out of 10 control females, 8 out of 10 high-dose males and 7 out of 10 high-dose females. In conclusion, the accumulation of hemosiderin granules observed in the kidneys, liver and spleen was considered to be incidental and unrelated to administration of test article.



TABLE NO. 5
(CONT'D.)

PATHOLOGIST'S REPORT

PROJECT NO. 81910

ANIMALS SACRIFICED WEEK 17 OF STUDY

Gross pathological examination was undertaken on all animals sacrificed at 17 weeks following a 3-week regression period. Ovarian cysts observed in animals from all groups are common background spontaneous changes in female rats of this age group. Other gross findings seen in various tissues and organs were infrequent and appeared to be either agonal or incidental.

CONCLUSION

Administration of test article Arcosolv PTB during 13 weeks followed by a 3-week regression period appeared to cause no pathological effect in the rat.

Pierre Tellier, D.V.M., Dip.Path., M.Sc.
Staff Pathologist

30 July 1985

Date

- (1) Coleman, G.L., Barthold, S.W., Osbaldiston, G.W., Foster, S.J., and Jonas, A.M. Pathological changes during aging in barrier-reared Fischer 344 male rats. J. Gerontol. (1977), 32: 258-278.

Data tables excluded from this
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215