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January 11, 2005

FYI-0105-01489

Mr. Charlie Auer
Director
TSCA Document Control Office (7407)
EPA East Building, Room 6428
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Avenue, NW
Washington, DC 20460-0001

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Dear Mr. Auer:

The American Chemistry Council makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following reports that the American Chemistry Council's Olefins Panel recently conducted are enclosed:

"1-Butene: Combined Repeated-Exposure Toxicity, Reproduction and Neurotoxicity Screening in Rats via Whole-Body,"

"Isoprene: Acute Toxicity to *Daphnia Magna*,"

"Isoprene: Acute Toxicity to Rainbow Trout (Semi-Static Exposure Conditions),"

"Isoprene: Algal Growth Inhibition Assay,"

"Isoprene: Assessment of Biodegradability Using the Closed Bottle Method," and

"Isoprene: Identification and Determination of Purity."

The enclosed reports do not include confidential information.

If you have any questions, please call the Olefins Panel Manager of my staff at 301-924-

2006



Sincerely yours,

Enclosure



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STUDY NO. 02-4224
SPONSOR STUDY NO. OLF-83.0-HPV2-HLS

1-BUTENE:

COMBINED REPEATED-EXPOSURE TOXICITY, REPRODUCTION
AND NEUROTOXICITY SCREENING IN RATS VIA WHOLE-BODY
INHALATION EXPOSURES

Final Report

Volume I of II

Performed by: Huntingdon Life Sciences
Mettlers Road
East Millstone, New Jersey 08875-2360

Submitted to: American Chemistry Council
1300 Wilson Boulevard
Arlington, VA 22209

Attn: Elizabeth J. Moran, Ph.D., D.A.B.T.

Date: 01 August 2003

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SUMMARY

This study was designed to assess the potential toxicity, including neurotoxicity and reproductive performance, in male and female rats when 1-Butene was administered as a gas by whole-body inhalation exposures. Reproductive effects were assessed by histological examination of the reproductive organs, mating behavior, conception, development of the conceptus, parturition, and pup survival to Lactation Day 4.

The test substance was administered to Sprague Dawley rats (12/sex/Main Study group and 12 females/Satellite group) at target concentrations (based on results of prior testing with similar chemicals and the lower explosion limits for the test article) of 500, 2000 and 8000 ppm for 6 hours/day, 7 days/week for 2 weeks before mating initiation. Main Study females were for subchronic evaluations and Satellite females were for reproductive evaluations only. Exposure of Main Study males (12/group) continued for a minimum exposure of 28 days (during mating and post-mating until they were euthanized), while Main Study females (12/group) were exposed once daily (6 hours/day), 7 days/week for 28 days. Satellite females (12/group) continued to be treated once daily (6 hours/day) during mating, and then once daily (6 hours/day) during gestation (Days 0-19). Those Satellite animals without evidence of mating (but which were actually pregnant) continued treatment (6 hours/day) following completion of the mating period until their estimated Gestation Day 19. In addition, a control group (12 males and 24 females) received nitrogen enriched air only while in chamber. Exposure levels were determined using an on-line gas chromatograph 4 times per chamber per day. Particle size distribution measurements were also made once per chamber per week using a TSI Aerodynamic Particle Sizer.

Viability checks were performed twice daily to check for mortality and signs of severe toxic or pharmacologic effects. Physical observations and body weight measurements were made once pretest and at least weekly during the study. Animals were also observed once during each exposure. Satellite female rats had a detailed physical observation performed weekly during the pre-mating period and on Gestation Days 0, 7, 14, 20 and Lactation Days 0, 1 and 4. Mated Satellite female rats were weighed on Gestation Days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on Lactation Days 1 and 4. Feed consumption measurements were obtained beginning the week prior to treatment initiation and at least weekly during the study with the exception of during the mating period for males and Satellite females. For pregnant or confirmed mated Satellite female rats, feed consumption was recorded on Gestation Days 0-7, 7-14, and 14-20 and on Lactation Days 1-4. Neurobehavioral (Functional Observational Battery and Locomotor Activity) examinations were performed at pretest and during the last week of exposure (on a non-exposure day) on all Main Study male rats and all Main Study female rats from all exposures. Hematology, coagulation, and clinical chemistry were performed

SUMMARY

on 12 animals/sex/main study group at study termination. After completion of exposures, all parental animals (P₀ generation: Main Study males and Satellite females) were sacrificed. Selected organs were weighed and organ/body weight and organ/brain weight ratios calculated. Complete macroscopic postmortem examinations were performed on all parental animals. Histopathological evaluations of selected tissues were conducted on selected parental animals.

Pups (F₁ generation) were observed as soon as possible after parturition for their sex, the number of live and dead pups and pup abnormalities. Thereafter, litters were observed twice daily (morning and afternoon) and gross physical examinations were performed on Lactation Days 0 and 4. Pups were sexed on Lactation Day 0 and sex verified on Lactation Day 4. Individual pup body weights were recorded on Lactation Days 1 and 4. Pups surviving until Lactation Day 4 were euthanized followed by a macroscopic postmortem examination (external), in which any unusual abnormalities were noted and then the carcasses were discarded.

The test article, purchased from MG Industries, was assayed by GC versus an analytical standard, purchased from Aldrich Chemicals, before and after the study to demonstrate the purity and stability of the test article. The test article was determined to be 100% 1-Butene before the study and 99.84% 1-Butene (with the balance Isobutane) after the study demonstrating the purity and the stability of the test article. Chamber distribution analyses showed that the test article was evenly distributed within each chamber. The mean (\pm standard deviation) analytical (GC) concentrations for the control and the exposure groups were as follows: 0 ± 0 , 524 ± 40 , 2062 ± 126 and 8271 ± 683 . The analytically measured exposure levels of the airborne test article were reasonably close to the targeted exposure levels. Chamber environmental conditions averaged 23°C temperature and 57% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test article chambers and the air control chambers.

There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes or feed consumption in the test article exposed animals compared to the Air Control animals. There was no apparent exposure-related effect on motor activity or functional observational battery parameters for either sex in this study. There were no exposure-related differences in hematology or coagulation values or clinical chemistry values in test article exposed animals compared to the Air Control animals at the terminal interval.

SUMMARY

There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test article exposed animals compared to the Air Control animals. There were no microscopic findings considered to be related to exposure to 1-Butene.

All mated female animals (except one animal in the 2000 ppm group) were found pregnant and delivered live pups. Mating indices for the male rats treated with the test article were comparable to the Air Control group. Mating, fertility and gestation indices for the female rats treated with the test article were comparable to the Air Control group. Most of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the Air Control group. There were no treatment-related differences in all parturition parameters including the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the number of implantation sites and corpora lutea per dam, the pup sex ratio and the number of live pups/litter, when compared to the Air Control group. The pups were unremarkable during the lactation period. There were no exposure-related differences in body weights or weight gains in the pups feeding from test article exposed animals compared to the pups feeding from Air Control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test article exposed animals compared to the pups feeding from Air Control animals.

In conclusion, exposure of male and female rats to target concentrations of 500, 2000 and 8000 ppm of 1-Butene resulted in no general systemic effects or effects on reproductive performance. Therefore, a no observed effect level (NOEL) of 8000 ppm was determined.

ISOPRENE

ACUTE TOXICITY TO *DAPHNIA MAGNA*

ACC Reference Number: OLF-63.0-HPV3-HLS

Data requirement: EC Directive 92/69, Part C2
OECD Procedure No. 202, Part I

Huntingdon Life Sciences Limited
Project Identity: CSS/033

Study completed on: 17 November 2003

Sponsor

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USA

Research Laboratory

Huntingdon Life Sciences Ltd.
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ENGLAND

Study Director: C.A. Jenkins

SUMMARY

The acute toxicity of isoprene to *Daphnia magna* was assessed under static exposure conditions.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 1992) Part C, Method 2 - Acute Toxicity For *Daphnia* and the OECD Guideline for Testing of Chemicals No. 202, Part I - *Daphnia* Acute Immobilisation Test (1984).

The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of isoprene. The test media were prepared, either directly or by dilution, from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 22 hours. After being allowed to stand for approximately 1.5 hours, to obtain an equilibrium concentration of isoprene, aliquots of the medium were removed mid-vessel and used to fill replicate vessels at each concentration.

Groups of twenty *Daphnia*, less than 24 hours old, were exposed for 48 hours to a control solution (Elendt M4 medium) or to isoprene, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored using a GLC method of analysis. Although lower than intended, the measured concentrations of isoprene at the start (between 30 and 48% of their nominal values) were adequately maintained during the test, giving measured levels of between 30 and 51% of nominal after 48 hours. Based on an arithmetic mean, the overall mean measured levels of isoprene were 0.648, 1.55, 3.52, 9.47 and 24.6 mg/l.

Observations of the *Daphnia* in each control and test vessel were made after 24 and 48 hours. After 48 hours, the lowest measured concentration resulting in 100% immobility was 9.47 mg/l and the highest measured concentration at which no immobilisation occurred was 3.52 mg/l.

Based on these findings the following values have been calculated:

48-hour EC₅₀ value[#] : 5.77 mg/l (95% confidence limits of 3.52 and 9.47 mg/l).

[#] medium effect concentration

ISOPRENE
ACUTE TOXICITY TO RAINBOW TROUT
(SEMI-STATIC EXPOSURE CONDITIONS)
ACC Reference Number: OLF-63.0-HPV3-HLS

Data requirement: EC Directive 92/69, Part C1
OECD Procedure No. 203

Huntingdon Life Sciences Limited
Project Identity: CSS/032

Study completed on: 17 November 2003

Sponsor

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Study Director: C.A. Jenkins

SUMMARY

The acute toxicity of isoprene to rainbow trout (*Oncorhynchus mykiss*) was assessed under semi-static exposure conditions.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 29 December 1992) Part C, Method 1 - Acute Toxicity For Fish and the OECD Guideline for Testing of Chemicals No. 203 - Fish, Acute Toxicity Test (July 1992).

The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of isoprene. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for approximately 24 hours. After being allowed to stand for at least 60 minutes to obtain an equilibrium concentration of isoprene, aliquots of medium were removed from the middle of the vessel and used to fill duplicate test vessels at each concentration.

Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, *ca.* 150-200 mg/l as CaCO₃) or to isoprene at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored using a GLC method of analysis.

The measured concentrations of isoprene ranged between 42 and 105% of their nominal values in samples of freshly prepared media and between 44 and 84% of their nominal values in samples of expired (24-hour-old) media (between 68 and 106% of their starting values). Based on a geometric mean, the overall mean measured levels of isoprene were 1.68, 3.57, 6.71, 15.0 and 28.7 mg/l.

Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.

After 96 hours, the highest measured concentration at which no mortality had occurred was 3.57 mg/l and the lowest at which there was 100% mortality was 15.0 mg/l. Treatment-related effects were exhibited at 6.71 mg/l and higher concentrations.

Based on these findings the following values have been estimated:

96-hour LC₅₀[#] value : 7.43 mg/l (95% confidence limits of 6.71 and 15.0 mg/l)

[#] median lethal concentration.

ISOPRENE
ALGAL GROWTH INHIBITION ASSAY
ACC Reference Number: OLF-63.0-HPV3-HLS

Data requirement: EC Directive 92/69, Part C3
OECD Procedure No. 201
US EPA TSCA 797.1050 & 797.1060

Huntingdon Life Sciences Limited
Project Identity: CSS/029

Study completed on: 17 November 2003

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Study Director: C A Jenkins

SUMMARY

The effect of isoprene on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* was assessed under non-axenic (non-sterile) conditions.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 29 December 1992) Part C, Method 3 - Algal Inhibition Test and the OECD Guideline for Testing of Chemicals No. 201 - Alga, Growth Inhibition Test (7 June 1984) with the following exceptions so that the requirements of the US EPA Code of Federal Regulations, Part 797 - Environmental Effects Testing Guidelines under the Toxic Substances Control Act (1987) could be met: the duration of the test was 96 hours, the test temperature was $24 \pm 2^\circ\text{C}$ and the light intensity of the test area was approximately 4000 lux. To accommodate the testing of a volatile substance, the study design deviated from each of these test guidelines by altering the composition and pH of the culture medium; these amendments were necessary to compensate for the lack of headspace for gaseous exchange and to minimise the pH increase during the test.

The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of isoprene. The test media were prepared, either directly or by dilution, from an aqueous preparation in which the test substance was stirred in a sealed vessel for approximately 21 hours in the dark. After being allowed to stand for approximately 1.5 hours to obtain an equilibrium concentration of isoprene, a portion of the medium was removed from the middle of the vessel and after dilution and inoculation with algal cells, it was used to fill the test vessels. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.4 to 24.0°C for 96 hours.

Replicate algal cultures, with an initial cell density of $1 \times 10^4/\text{ml}$, were exposed to algal medium or isoprene at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l. The exposure levels were monitored using a GLC method of analysis. The measured concentrations of isoprene ranged between 14 and 39% of their nominal values at the start of the test and between 20 and 32% of nominal after 96 hours. Based on an arithmetic mean, the overall mean measured levels of isoprene were 0.846, 1.68, 6.00, 10.3 and 35.2 mg/l.

Cell densities (the number of cells per ml) were counted daily to monitor growth. The test results are expressed in terms of the area under the growth curve (the increase in cell density during the test period) and growth rate (the increase in cell density per unit time) using the mean measured concentrations.

The following values were derived from the data:

Area under the growth curve

E_bC_{50} (72 h)	: 15.3 mg/l (95% confidence limits, 12.9 & 18.6 mg/l)
E_bC_{50} (96 h)	: 15.5 mg/l (95% confidence limits, 13.3 & 18.4 mg/l)
No observed effect concentration (NOEC)	: 1.68 mg/l

ISOPRENE

**ASSESSMENT OF BIODEGRADABILITY
USING THE CLOSED BOTTLE METHOD**

ACC Reference Number: OLF-63.0-HPV3-HLS

Data requirement: EC Directive 92/69, C.4-E
OECD Procedure 301D
OPPTS Method 835.3110 (o).

Huntingdon Life Sciences Limited
Project Identity: CSS/036

Study completed on: 12 November 2003

Sponsor

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Study Director: Dr. S. P. Barnes

SUMMARY

The objective of this study was to assess the biotic degradability of isoprene. It was based on methods outlined in Procedure C.4-E, "Determination of Ready Biodegradability - Closed Bottle Test", of the Annex to European Commission Directive 92/69/EEC, published 29 December 1992, Method 301D of the OECD Guidelines for Testing of Chemicals, adopted 17 July 1992 and US Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances (OPPTS) Method 835.3110 (method "o"), adopted January 1998.

The organic carbon content of isoprene was determined before the start of the main test using a CEC Model 440 Elemental Analyser. The measured carbon content (89.0%) was equivalent to 101.0% of the theoretical value (88.1%), which was calculated using the empirical formula.

In the main test, eighteen modified bottles were filled with mineral salts medium that had been inoculated with sewage effluent (1 ml/l), and isoprene was added at a nominal concentration of 2 mg/l. A further four bottles were established for a concurrent five-day microbial inhibition assay, in which the degradation of the readily degradable reference substance sodium benzoate was examined in the presence of the test substance. Groups of eighteen control and reference bottles were filled with inoculated mineral salts medium, with and without sodium benzoate (5 mg/l). The concentrations of dissolved oxygen (DO) in duplicate bottles from each group were measured at the start of the test and after five days of incubation at $22 \pm 2^\circ\text{C}$ in darkness. DO concentrations in the control and reference groups and in bottles containing isoprene alone were measured also after 7, 11, 14, 18, 21, 25 and 28 days.

Sodium benzoate had been degraded to 79% of its ThOD (1.67 mgO₂/mg) after five days of incubation and 86% by Day 28; in the presence of isoprene, sodium benzoate had been degraded by 78% after five days. These results confirm that the inoculum was viable and that the test substance was not inhibitory to the activity of the microbial inoculum.

Oxygen consumption by Day 28 in control bottles containing inoculated medium (maximum = 0.97 mgO₂/l on Day 28) was acceptable for this assay system (recommended maximum value = 1.5 mgO₂/l).

Oxygen consumption in bottles containing isoprene had achieved, at most, 0.18 mgO₂/mg or 5% of its ThOD (3.29 mgO₂/mg) on or before Day 14 of the test. However, the level of biodegradation on or after Day 18 ranged between 0.14 mgO₂/l or 2% and 4.90 mgO₂/l or 60% of its ThOD in single bottles. The extent of biodegradation was substantial ($\geq 54\%$) in three culture bottles on or after Day 18, but was negligible ($\leq 13\%$) in five others.

This indicated that isoprene was biodegradable after a period of acclimatisation. Further confirmation of this was sought in a supplementary investigation, which was performed to assess the extent of degradation after seven days with pre-exposed microorganisms. The culture medium from a bottle that had been incubated for 28 days (isoprene degradation = 58%) was added at a rate of 10 ml/l to mineral salts medium. Four modified and four standard bottles were then filled with the medium and isoprene was added at a nominal concentration of 2 mg/l to four modified BOD bottles. The concentration of dissolved oxygen, the pH and temperature of four bottles (two with and two without isoprene) were determined on Day 0. The remaining four bottles were used for measurements after seven days of incubation, in darkness at $22 \pm 2^\circ\text{C}$.

Biodegradation of isoprene had achieved $\geq 45\%$ (45 and 64% in replicate bottles) after seven days of incubation, which confirmed that the material was biodegradable in the presence of microorganisms that had been pre-exposed to the test substance for a relatively short period.

INTRODUCTION

The objective of this study was to assess the biotic degradability of isoprene. It was based on methods outlined in procedure C.4-E, "Determination of Ready Biodegradability - Closed Bottle Test" of Directive 92/69 (formerly method C6 of EC Directive 84/449), OECD Procedure 301D, Closed Bottle test, and US Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances, (OPPTS) Method 835,3110 (method "o"), adopted January 1998.

The protocol was approved by Huntingdon Life Sciences Management on 13 February 2002, by the Sponsor on 20 February 2002, and by the Study Director on 13 March 2002.

The experimental start and termination dates were 18 March and 28 May 2002, respectively.

A preliminary investigation to determine the carbon content of the test substance using elemental analysis was carried out by MEDAC Ltd., (Appendices 2 and 3).

The Closed Bottle test was preceded by a formulation trial.

The test substance was considered to be a volatile compound that could be lost from Biochemical Oxygen Demand (BOD) bottles during the preparation of mixtures in the standard 28-day Closed Bottle test. Therefore, mixtures containing isoprene were established in modified BOD bottles that contained a side-limb fitted with a Teflon-coated rubber septum. The test substance was injected directly through the septum using a microsyringe into bottles that were pre-filled with inoculated medium.

Two tests were conducted, both of which are reported here. In the first test, a sewage effluent inoculum that had been collected at the beginning of the test was used to prepare test mixtures. In the second test, an inoculum derived from the same sample of sewage effluent was used, that had been pre-exposed to the test substance for 28 days.

The study was conducted at Huntingdon Life Sciences Ltd., Eye Research Centre, Eye, Suffolk, IP23 7PX.

ISOPRENE
IDENTIFICATION AND DETERMINATION OF PURITY
(ACC Reference Number OLF-63.0-HPV3-HLS)

Sponsor

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Report issued: 5 November 2003

SUMMARY

The study was designed to characterise isoprene using a gas chromatographic method to determine the area percent concentration and identification by retention time and to assess its stability for the duration of toxicity testing performed at Huntingdon Life Sciences.

The results from the tests are as follows:

Component	Initial purity (area %) (16 November 2001)	Final purity (area %) (23 July 2002)
Isoprene	98.6	98.5

The identical GC retention times obtained for the reference standard and test sample confirms the identity of the test material as isoprene.