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**INITIAL
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MISSION**

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U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460



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Re: 8(e) Report on Oral Toxicity Study of Styrene in the Mouse

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Dear Sir or Madam:

In accordance with section 8(e) of the Toxic Substances Control Act (TSCA), the Styrene Information and Research Center (SIRC) is notifying EPA of the results of an acute oral toxicity study of styrene ("benzene, ethenyl-") (CASRN 100-42-5) in the mouse conducted by Central Toxicology Laboratory in the United Kingdom (Report No. CTL/R/1410) (report attached). While this information is being submitted under section 8(e), neither SIRC, nor any individual company, has made a determination as to whether a significant risk of injury to health or the environment is actually presented by the reported information.

In this short-term (acute) study, male CD-1 mice were orally dosed with 10, 100, and 200 milligrams per kilogram body weight (mg/kg) styrene for five consecutive days. Mice dosed at the upper two levels exhibited a dose-dependent increase in cell replication in the terminal bronchioles of the lung. No increases in labeling indices were apparent in either the large bronchioles or the alveoli of these animals. There also was

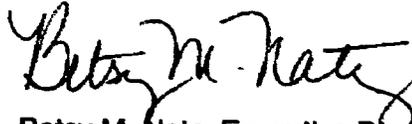
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evidence of slight focal crowding on non-ciliated cells in the epithelium of the terminal bronchiole in 3 of the 10 mice dosed at the highest level. The No- Effect Level was 100 mg/kg for morphological change and 10 mg/kg for both morphological damage and increased cell replication.

We trust that the Agency finds this information useful. Should any specific questions arise, please do not hesitate to contact me at 703-741-5010.

Sincerely,



Betsy M. Natz, Executive Director
Styrene Information and Research Center

Attachment

A 05

**CENTRAL TOXICOLOGY LABORATORY
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REPORT NO: CTL/R/1410

**THE EFFECTS OF STYRENE ON MOUSE LUNG
FOLLOWING ORAL DOSING**

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A 06

**CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK**

REPORT NO: CTL/R/1410

**THE EFFECTS OF STYRENE ON MOUSE LUNG
FOLLOWING ORAL DOSING**

AUTHOR

Dr T Green

DATE OF ISSUE

June 21st 1999

STATEMENT OF DATA CONFIDENTIALITY CLAIM

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO CEFIC.

It should not be disclosed in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of this product or any other product without the written permission of the Styrene Steering Committee, a CEFIC Sector Group.

I, the undersigned declare that this report constitutes a true record of the actions taken and the results obtained in the above study.

Dr T Green
(Study Director)

T Green

Date 21.06.99.

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
Dr T Green	Study Director
Mrs A Toghill	Study Investigator
Dr J R Foster/Mr A Soames	Pathologists

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1. SUMMARY

Male CD-1 mice orally dosed with 10, 100 and 200mg/kg styrene, daily, for five consecutive days exhibited a dose dependant increases in cell replication in the terminal bronchioles of the lung at the two higher dose levels. No increases in labelling indices were apparent in either the large bronchioles or the alveoli of the same animals. There was evidence of slight focal crowding of non-ciliated cells in the epithelium of the terminal bronchiole in 3/10 mice in the top dose group only. The 100 mg/kg dose was a no-effect level for morphological change and 10 mg/kg for both morphological damage and increased cell replication.

2. INTRODUCTION

Styrene is used in the production of polymers, copolymers and reinforced plastics. It is pneumotoxic in CD-1 mice when inhaled, with morphological changes and increases in cell division occurring after short-term exposure, and lung tumours after chronic exposure. (Green, 1999; SIRC, 1998). Whilst occupational exposure to vapours of styrene occurs mainly via inhalation, low levels of styrene may be ingested in food or drink contained in packaging made from styrene based polymers. The effects of ingested styrene on the mouse lung have not been previously assessed.

Following oral dosing of mice with styrene, very little styrene is exhaled via the lungs, even at dose levels approaching the LD₅₀ (Csanady et al. 1994). Indeed, the same authors also concluded that the amount of styrene exhaled following oral dosing could not be accurately predicted by modelling. Consequently, it is impossible to replicate, following oral dosing, the type of airway concentration seen after exposure to 40 or 160 ppm styrene by inhalation. In the absence of any clear correlation between airway exposure following oral and inhalation exposure a broad range of dose levels were chosen for the oral study. In this study, the effects of styrene on mouse lung morphology and cell replication have been assessed following oral doses of 10, 100 and 200mg/kg.

3. MATERIALS AND METHODS

3.1 Chemicals

Styrene (99%), stabilised with 10-15 ppm 4-tert-butylcatechol, was supplied by Sigma-Aldrich Co. Ltd, Poole, Dorset, UK. and assigned CTL reference number Y00115/003/009 and stored at 4°C. Corn oil was supplied by CPC UK, and assigned CTL reference number Y00790/007/008. 5-Bromo-2'-deoxyuridine (BrdU, 99%) was obtained from Sigma-Aldrich Co. Ltd, Poole, Dorset, UK.

3.2 Animals

Male CD-1 mice, 22-27g body weight, were supplied by Charles River, Margate, Kent. Prior to the study the animals were housed in stainless steel cages in rooms equipped with a 12 hour light-dark cycle. The environment of the animal room was controlled to provide conditions suitable for CD-1 mice with a temperature being maintained within a target range of 19-23 °C, a relative humidity within a target range of 40-70%, and between 25-30 air changes per hour. All conditions were monitored by the Honeywell site monitoring system and any excursions outside these ranges were noted by means of an external alarm and recorded.

Certified diet (CT1 diet, Special Diet Services, Witham, Essex, UK) and mains water were supplied *ad libitum* except during the exposure periods.

Animals were randomly assigned to treatment groups by a method based on individual bodyweights. Individual animals were identified by ear punching. All animals were observed to ensure they were normal before the start of the study and any showing adverse signs either before or during the study were killed.

3.3 Study design

Groups of 10 mice were given single oral doses of 10, 100 and 200mg/kg styrene in corn oil (10 ml/kg), daily, for 5 consecutive days. A control group of 10 mice were given single oral doses of corn oil (10ml/kg), also for 5 days. Three days prior to sacrifice each animal was fitted with an osmotic minipump (Alzet 7 day) containing 200ul 5-bromo-2'-deoxyuridine

(BrdU; 15mg/ml in 0.9% saline). The animals were killed with an overdose of halothane 24 hours after the last dose and exsanguinated. The lungs were immediately excised, infused and fixed via the trachea with 10% neutral buffered formalin and embedded in paraffin wax. Sections (5-7µm) were cut and stained with haematoxylin and eosin. The quantitation of cell replication was undertaken as described by Foster et al. (1994). Areas for evaluation of labelling indices were randomly selected from each area of the lung.

4. RESULTS

Bodyweight gains and clinical signs in animals dosed with styrene were normal and comparable to those in the control group.

4.1 Lung pathology

There were no treatment-related findings visible macroscopically following dosing with any of the three doses of styrene. Microscopically there were no effects observed in mice administered 10 or 100mg/kg styrene (Table 1). In mice dosed with 200mg/kg styrene, 3/10 animals exhibited slight focal crowding of non-ciliated cells in the epithelium of the terminal bronchiole. There was no evidence of either cellular necrosis or damage.

4.2 Cell replication

Five hundred cells were counted for each compartment of the lung. The labelling indices in the large bronchioles, terminal bronchioles and alveoli of the lungs from the control (corn oil dosed) mice were 1.2%, 1.8% and 2.8% respectively (Table 2). There was no evidence of an increase in cell replication in either the large bronchioles or the alveolar region at any of the dose levels in this study. There was, however, a marked dose dependant increase in the S-phase frequency in the terminal bronchioles at the 100 and 200 mg/kg dose levels. At 200mg/kg, the labelling index was 5-fold that measured in corn oil treated mice. A weaker, although still significant response, was seen in mice dosed 100mg/kg, while 10mg/kg proved to be a no effect level (Table 2).

5. DISCUSSION

Styrene is known to be a pulmonary toxicant in CD-1 mice when administered via the inhalation route (SIRC, 1998). Few studies have dealt with exposure to styrene via the oral route in laboratory animals, since exposure via this route was considered to be negligible when compared to occupational exposure by inhalation. In the present study, mice dosed orally with styrene at dose levels up to 200mg/kg, daily for five consecutive days, exhibited increases in cell division in the terminal bronchioles of the lung. There was a clear dose response at the two higher dose levels and a clear no-effect at the lower. Interestingly, the only region of the lung affected when styrene is administered orally, was the terminal bronchiole, whereas by inhalation, styrene also affected the epithelium of the large bronchioles (Green 1999). This difference is probably due to differences in the way styrene reaches the pulmonary epithelium following inhalation exposure or orally dosing. Following inhalation exposure all regions of the airways will be exposed to the chemical. After an oral dose very little styrene is exhaled in the mouse (Csanady et al. 1994) and hence the lung tissues are exposed to styrene primarily through the systemic circulation. The most highly perfused regions of the lung are the terminal bronchioles (Netter, 1980), which is consistent with the observed response in this study.

During inhalation exposure the cell type which is morphologically affected by exposure to styrene, and the one which undergoes an increase in cell division, appears to be the non-ciliated Clara cell. The same target cell, and a virtually identical response, is also seen after oral dosing with styrene. It can be concluded therefore that styrene has the potential to be toxic to the lungs of CD-1 mice irrespective of whether they are dosed via the inhalation or oral route.

6. REFERENCES

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- Netter, F.H. (1980). The Ciba Collection of Medical Illustrations. Volume 7. Respiratory System. Ciba Medical Education Division, NJ, USA. pp 24-30.
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TABLE 1 - MORPHOLOGICAL CHANGES IN MOUSE LUNG FOLLOWING ORAL DOSING OF STYRENE, DAILY FOR 5 DAYS

Terminal Kill Microscopic Findings	Group 1 0 m/kg	Group 2 10 mg/kg	Group 3 100 mg/kg	Group 4 200 mg/kg
Males on study	10	10	10	10
Animals completed	8*	10	10	10
Lung -				
no abnormalities detected	8	10	10	7
focal crowding in terminal bronchioles	0	0	0	3
- minimal	0	0	0	1
- slight	0	0	0	2

Fisher's exact test:- Group 1 compared with all other groups (* $p < 0.05$ ** $p < 0.01$, one sided)

* there were two intercurrent deaths in the control group

TABLE 2 - PULMONARY LABELLING INDICES IN MICE FOLLOWING ORAL DOSING OF STYRENE, DAILY FOR 5 DAYS

Dose mg/kg	Alveoli	Terminal bronchiole	Large Bronchiole
0	2.8 ± 2.1	1.8 ± 0.9	1.2 ± 0.6
10	1.3 ± 0.6	1.1 ± 0.5	0.9 ± 0.4
100	1.3 ± 0.6	3.8 ± 2.5 *	0.8 ± 0.4
200	1.5 ± 0.6	9.9 ± 2.7 **	2.9 ± 2.1

* Statistically significantly different from control values, Student's t-test, 2-sided, $p < 0.05$

** Statistically significantly different from control values, Student's t-test, 2-sided, $p < 0.01$

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