8EHQ-1202-14885

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One Houston Center
1221 McKinney
Suite 1600
P.O. Box 2583
Houston, TX 77252-2583
Telephone: 713.652.7200

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December 23, 2002

TSCA Document Control Office (7408)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington DC 20460

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Attention: TSCA 8(e) Coordinator

RE: 8EHQ Number: 8EHQ0301-14885 - Supplemental Information
2-Phenylethyl Alcohol (CAS No. 60-12-8) - Cell Proliferation in Lungs of Mice Administered Intraperitoneal Doses

Dear Sir or Madam:

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In March 2001, Lyondell Chemical Company (Lyondell) submitted information to EPA in accordance to Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide. In that correspondence, Lyondell reported the receipt of information, which to Lyondell's knowledge, was the first report of alveolar cell proliferation in the mouse lung following intraperitoneal administration of 2-Phenylethyl alcohol (CASRN 60-12-8). This submission was assigned reference number 8EHQ0301-14885. Previously, the final report for this study had been unavailable. Lyondell has now received the final report and is hereby providing a copy to EPA.

In April 2002, Lyondell submitted supplemental information in the form of an abstract of the study as well as a poster made available at the Society of Toxicology (SOT) 2002 Annual Meeting in March 2002.

Should you have any questions or require additional details, please do not hesitate to call me at 713/309-2136. I may also be reached by facsimile at 713/951-1574 or by e-mail at patrick.gibson@lyondell.com.

Sincerely,

Patrick L. Gibson

Patrick L. Gibson
Product Safety Specialist - Regulatory
Corporate TSCA Coordinator
Lyondell Chemical Company

8EHQ-01-14885

Enclosure

89030000086

ML#65871

BASF

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STUDY TITLE

Report

Styrene
1-Phenylethanol
2-Phenylethanol
Phenylacetaldehyde
Acetophenone
Phenylacetic acid

S-Phase response study in the lung of female Crl Icr: CD1® mice

AUTHORS

Dr. W. Mellert (Study Director)
Dr. W. Kaufmann
Dr. B. van Ravenzwaay

STUDY COMPLETED ON

June 3, 2002

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen/Rhein, Germany

LABORATORY PROJECT IDENTIFICATION

Project No.: 99C0284/99138

SPONSOR

Styrenics Steering Commitee (SSC) at CEFIC, Brussels, Belgium

VOLUME I OF III
(REPORT SECTION AND SUMMARY TABLES)

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GLP COMPLIANCE STATEMENT

This study was in general accordance with the OECD Principles of Good Laboratory Practice and the GLP principles of the German "Chemikaliengesetz" (Chemicals Act). However, there was the following deviation from the requirements of the above mentioned principles:

No analytical determinations of the test substance preparations were carried out.

June 3, 2002

Dr. med. vet. W. Mellert
(Study Director)

SIGNATURE PAGE

Study Director: June 3, 2002
Dr. med. vet. W .Mellert

Pathology: May 17, 2002
Dr. med. vet. W. Kaufmann

Head of Experimental Toxicology: 27. May, 2002
Dr. rer. nat. B. van Ravenzwaay

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STATEMENT

of the Quality Assurance Unit

The Quality Assurance Unit (QAU) inspected the study and reported any inspection results to the Study Director and to Management.

The final report reflects the raw data.

Phase of study	Date of inspection (mm-dd-yyyy)	Reported to Study Director and to Management (mm-dd-yyyy)
Study Plan:	10-09-2001	10-09-2001
Conduct of study:	10-09-2001 10-11-2001	10-09-2001 10-11-2001
Report:	04-30-2002	04-30-2002

Ludwigshafen, May 15, 2002

Zachmann

RheinlandPfalz

Landesanstalt
für Pflanzenbau und Pflanzenschutz

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

BASF Aktiengesellschaft
Experimentelle Toxikologie und Ökologie
D-67056 Ludwigshafen

Prüfungen nach Kategorien / Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

.....1,2,3,4,5,8,9.....

Datum der Inspektion / Date of Inspection

.....15.05.2001 und vom 21. bis 26.06.2001.....

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility/test site is included in the national GLP-Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Schiefer 24. Sept. 2001

Unterschrift, Datum / Signature, Date

(Name und Funktion der verantwortlichen Person / Name and function of responsible person)

Landesanstalt für Pflanzenbau und Pflanzenschutz Rheinland-Pfalz 1 (Siegel) Mainz

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1. SUMMARY

Styrene and its metabolites **1-Phenylethanol, 2-Phenylethanol, Phenylacetaldehyde, Acetophenone and Phenylacetic acid** were administered intraperitoneously to groups of 10 female Crl Icr: CD1® mice at a dose level of 100 mg/kg body weight for 3 days, each 3 times a day (total of 9 administrations). The control group was treated with the vehicle (doubly distilled water), only. Body weight was determined for all dose groups on day 0 and at necropsy (day 3). The animals were examined for signs of toxicity or mortality at least once a day. Osmotic minipumps containing BrdU were implanted on day 0. Cell proliferation (S-phase response) and apoptosis (TUNEL-staining) in the lungs was determined.

In the groups treated with Phenylacetaldehyde or Phenylacetic acid, each two animals died. In these groups, the terminal body weights were significantly decreased. Cell proliferation in the lungs was increased vs. controls after treatment with styrene and all tested metabolites, except acetophenone:

Test substance	Large+medium bronchi		Terminal bronchioles		Alveoli	
	%	LI	%	LI	%	LI
Control	100	2,54	100	2,85	100	3.36
Styrene	187	4,74*	173	4,93**	136	4.56*
1-Phenylethanol	114	2,90	127	3,62	157	5.26**
2-Phenylethanol	101	2,57	88	2,51	154	5.16**
Phenylacetaldehyde	44	1,11*	43	1,22*	627	21.06**
Acetophenone	74	1,87	80	2,28	92	3.08
Phenylacetic acid	52	1,32	29	0,82**	507	17.05**

% = relative to control * ≤ 0.05
LI = Labeling Index ** ≤ 0.01

Thus, the most significant induction of cell proliferation was observed in the alveoli. The highest labeling indices were found for phenylacetaldehyde (6-fold control value) and phenylacetic acid (5-fold), followed by 1-Phenylethanol (1.6-fold), 2-Phenylethanol (1.5-fold) and styrene (1.4-fold). In the large and medium bronchi as well as in the terminal bronchioles, only the styrene group exhibited a significant increase of its labeling indices with 1.8 fold and 1.7-fold, respectively.

Regarding apoptosis, an overall very low amount of positive labeled cells was present in each of the compartments. No significant changes were detected. However, a slightly higher number of apoptotic cells were found in the phenylacetaldehyde and phenylacetic acid groups in the alveoli and in the 1-phenylethanol and 2-phenylethanol groups in the terminal bronchioles, and in the phenylacetaldehyde group in the large and medium bronchi.

2. INTRODUCTION AND DOSE SELECTION

2.1. AIM OF THE STUDY

The aim of the study was to examine if the metabolites of styrene show the same mode of action with respect to cell proliferation in the lung as styrene itself.

2.2. SELECTION OF DOSES

On request of the sponsor, the test substances were tested intraperitoneously a dose level of 100 mg/kg body weight, 3 times a day for 3 days.

3. MATERIALS AND METHODS

3.1. TEST SUBSTANCES

3.1.1. Styrene

Name of test substance:	Styrene
Test substance No.:	99/284-4
CAS-No:	100-42-5
Purity:	99.96%
Batch:	Tank 21
Producer:	BASF AG
Physical state / color:	Liquid / colorless / clear
Storage conditions:	Room temperature

3.1.2. 1-Phenylethanol

Name of test substance:	1-Phenylethanol
Test substance No.:	00/0840-1
CAS-No:	98-85-1
Purity:	98%
Batch:	409497/1 21800
Producer:	Aldrich
Physical state / color:	Liquid / clear
Storage conditions:	Room temperature

3.1.3. 2-Phenylethanol

Name of test substance:	2-Phenylethanol
Test substance No.:	00/0841-1
CAS-No:	60-12-8
Purity:	99%
Batch:	67H0583
Producer:	Aldrich
Physical state / color:	Liquid / colorless / clear
Storage conditions:	Room temperature

3.1.4. Phenylacetaldehyde

Name of test substance:	Phenylacetaldehyde
Test substance No.:	00/0842-1
CAS-No:	122-78-1
Purity:	>90%
Batch:	Lot 35310-070
Producer:	Aldrich
Physical state / color:	Liquid / clear
Storage conditions:	Store cool

3.1.5. Acetophenone

Name of test substance:	Acetophenone
Test substance No.:	00/0843-1
CAS-No:	98-86-2
Purity:	99%
Batch:	48688/1 50700
Physical state / color:	Liquid
Storage conditions:	Room temperature

3.1.6. Phenylacetic acid

Name of test substance:	Phenylacetic acid
Test substance No.:	00/0839-1
CAS-No:	103-82-2
Purity:	99%
Batch:	401476/1 41100
Physical state / color:	solid
Storage conditions:	Room temperature

The analysis of styrene was carried out at the analytical department of BASF Aktiengesellschaft (see Volume III). The metabolites were used with the given specifications of the producer. No further analyses were conducted.

3.2. TEST ANIMALS

3.2.1. Species and strain

Female Crl Icr: CD1® mice were supplied by Charles River, Sulzfeld, Germany at an age of 49±2 days. Only animals free from clinical signs of disease were used for the study.

3.2.2. Animal identification

The mice were identified clearly by cage number (single housing).

3.2.3. Reason for species selection

CD1 mice were selected since this strain was also used in previous studies with styrene.

3.3. HOUSING AND DIET

The mice were housed singly in Makrolon® cages, type M I with wire cover from Becker & Co., Castrop-Rauxel, Germany (floor area about 200 cm²). Bedding was type 3/4 dustfree bedding supplied by SSNIFF, Soest, Germany. The animals were housed in a fully air-conditioned room. Central air-conditioning guaranteed a range of 20 - 24°C for temperature and of 30 - 70% for relative humidity. The day/night cycle was 12 hours (12 hours light from 06.00 a.m. - 06.00 p.m., 12 hours dark from 06.00 p.m. - 06.00 a.m.). Deviations from these ranges did not occur. The animal room was completely disinfected using a disinfector ("AUTEX", fully automatic, formalin-ammonia-based terminal disinfector). Usually, the floor and the walls were cleaned once a week. The cleansing liquid used was water containing about 0.1% Incidin perfect® (supplied by Henkel, Düsseldorf, Germany). The food used was basic maintenance diet for mice/rats/hamsters, pellets from Provimi KLIBA SA, Kaiseraugst / Switzerland. Food and drinking water (from water bottles) were available ad libitum.

3.4. TEST GROUPS AND DOSES

Dose groups	Test substance	Dose level (mg/kg body weight, 3 times a day for 3 days)	Number of animals	Cage-No.:
0	Control (doubly distilled water)	0	10	1 – 10
1	Styrene	100	10	11 – 20
2	1-Phenylethanol	100	10	21 – 30
3	2-Phenylethanol	100	10	31 – 40
4	Phenylacetaldehyde	100	10	41 – 50
5	Acetophenone	100	10	51 – 60
6	Phenylacetic acid	100	10	61 – 70

3.5. TEST SUBSTANCE PREPARATION AND ANALYSES

3.5.1. Preparation

The test substances were administered as a suspension in doubly distilled water. To prepare the suspensions, the appropriate amount of test substance was weighed, filled up to the desired volume with doubly distilled water and mixed using a magnetic stirrer or a high-speed homogenizer. During the administration the test substance preparations were kept homogenous with a magnetic stirrer. The test substance preparations were prepared daily.

3.5.2. Analyses

No analyses of the test substance preparations were carried out for this study.

3.5.3. Food analyses

The food used in the study was assayed for chemical and microbiological contaminants.

3.5.4. Drinking water analyses

The drinking water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the Technical Services of BASF Aktiengesellschaft as well as for the presence of microorganisms by a contract laboratory.

3.5.5. Bedding analyses

The bedding is regularly assayed for contaminants (chlorinated hydrocarbons and heavy metals).

3.6. EXPERIMENTAL PROCEDURE AND TIME SCHEDULE

On the day of arrival the animals were subjected to an acclimatization period during which they received ground food and drinking water ad libitum. Prior to the start of the administration the animals were distributed according to weight among the individual test groups, separated by sex. The list of randomization instructions was compiled with a computer.

The test substance preparations were administered intraperitoneously (i.p.) for 3 days, 3 times a day with 2 hours intervals. Control animals received bidistilled water. All surviving animals were sacrificed 72 h after the first administration.

The study was performed according to the following time table (due to the great number of animals, the study was started staggered):

Date	Study section / Determinations	Study day
02. Oct. 2001	Experimental starting date: arrival of the animals, start of the acclimatization period	-6 [1)] -7 [2)]
Oct. 5, 2001	Randomization	-3 [1)] -4 [2)]
Oct. 08, 2001	Implantation of BrdU minipumps and first administration for the first 5 animals of each group	0
Oct. 09, 2001	Implantation of BrdU minipumps and first administration for the second 5 animals of each group	0
Oct. 11, 2001	Necropsy (first 5 animals of each group)	3
Oct. 12, 2001	Necropsy (second 5 animals of each group)	3
Oct. 2001-Apr. 2002	Evaluation and reporting	
Apr. 17, 2002	Experimental completion date; draft report to QAU	

[1)] = first 5 animals of each group
[2)] = second 5 animals of each group

3.6.1. IMPLANTATION OF MINIPUMPS

Alzet® osmotic minipumps (model 2001´; about 20mg/ml BrdU =5-Bromo-2'-deoxyuridine in saline) were prepared 2 to 3 hours before implantation. The pumps were implanted under Metofane® anesthesia subcutaneously in the back region of the animals on day 0 and remained until day 3 (necropsy).

3.7. CLINICAL EXAMINATIONS

3.7.1. Clinical observations

The animals were examined for evident signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays.

3.7.2. Body weight data

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on day 0. At necropsy, the weight of the anesthetized animals was determined (see below).

3.7.3. Statistics of clinical examinations

For body weights, means and standard deviations of each test group were calculated. Further statistical analysis was performed according to following table:

Parameters	Statistical test	Markers in the tables	References
Body weight	A comparison of each group with the control group using DUNNETT's test (two-sided) for the hypothesis of equal means	* = 0.050 (for $p \leq 0.05$) * = 0.010 (for $p \leq 0.01$)	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 - 1121 DUNNETT, C.W. (1964): New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 - 491

3.8. PATHOLOGY AND S-PHASE RESPONSE

3.8.1. Necropsy

The animals were sacrificed by decapitation under CO_2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

3.8.2. Weight parameters

Weight assessment was carried out on all animals sacrificed at scheduled dates.
The weights of the following organs were determined:

1. Anesthetized Animals
2. Lungs

3.8.3. Organ / Tissue preservation list

The following organs / tissues were preserved in neutral buffered 4% formaldehyde solution:

1. All gross lesions
2. Lungs (fixation after initial instillation with neutral buffered 4% formaldehyde solution)
3. Brain
4. Pituitary gland
5. Thyroid glands/ parathyroid glands
6. Thymus
7. Trachea
8. Heart
9. Liver
10. Gall bladder
11. Spleen
12. Kidneys
13. Adrenal glands
14. Testes, ovaries
15. Uterus, vagina
16. Epididymides, prostate, seminal vesicle
17. Stomach (fore- and glandular stomach)
18. Duodenum
19. Jejunum
20. Ileum
21. Cecum
22. Colon
23. Rectum
24. Urinary bladder
25. Lymph nodes (mesenteric and mandibular lymph node)
26. Sciatic nerve
27. Bone marrow (femur)
28. Eyes
29. Spinal cord (cervical, thoracic and lumbar cord)

3.8.4. Histotechnical processing

Lungs and jejunum were fixed for 24 – 48 hours. Then the lungs were sliced into 5 mm thick strips (from each: the lobus pulmo sinister, lobus accessorius, lobus medius dexter, lobus cranialis resp. caudalis dexter) and afterwards stored in ethanol (70%). A section of jejunum was included as positive control.

The further histotechnical and immunohistological processing and examination by light microscopy were performed according to the following table:

Organs	Dose groups						
	0	1	2	3	4	5	6
	control	styrene	1-phenyl-ethanol	2-phenyl-ethanol	Phenylacet-aldehyde	aceto-phenone	Phenyl-acetic acid
Lungs	A10,I10	A10,I10	A10,I10	A10,I10	A10,I10	A10,I10	A10,I10
Jejunum	A10,I10	A10,I10	A10,I10	A10,I10	A10,I10	A10,I10	A10,I10

Methods and scope of examination:

A = Hematoxylin and Eosin (H&E) stain
I = Immunohistology
10 = All animals per group

The section of the jejunum was included on each slide to confirm the supply of BrdU to the tissues. Jejunum was not evaluated for determination of DNA-synthesis or histopathology. From each block five sections were nominally cut at 3 µm, one was used for H&E routine staining and the four others for immunohistology. Two of these were serving as the test slides for the calculation of the S-Phase resp. the apoptosis (BrdU stain resp. TUNEL stain). The other two served as the negative controls for each stain. The tissues were mounted on slides covered with APTS (3-aminopropyl-triethoxy-silane; Serva).

The H&E stained slides were examined and assessed and used for an estimation of the amount of mitotic and apoptotic figures in regard to their morphological features.

3.8.5. Staining Procedure - Immunohistology

BrdU – stain:

The slides were dewaxed, hydrated and covered with phosphate buffered saline (PBS). The following staining procedure was performed for all slides at room temperature in an automated cell staining system (Optimax; BioGenex, Hamburg, FRG). After DNA denaturation with HCl the slides were incubated consecutively with a primary antibody *(mouse anti-BrdU), a secondary antibody *(rabbit anti-mouse), a streptavidin enzyme label, and a chromogen substrate solution *(Fast Red). Sections were counterstained with Mayer's hematoxylin only. (* = BioGenex; Hamburg, FRG).

TUNEL stain:

The TUNEL stain was performed according to the protocol of the TUNEL kit used (Roche Diagnostics, Mannheim, FRG).

3.8.6. Quantitative Measurements of S-Phase Response and Apoptosis

Positively stained cells are characterized by a red reaction product covering the nuclei (BrdU and apoptosis). For the measurements, three lung compartments were separately evaluated: the alveoli, the terminal bronchioles, and the medium and large bronchi.

3.8.6.1. Alveoli

The LI (labeling index) was calculated in 30 measurement fields of the lungs. For that purpose the average number of labeled and unlabeled type I pneumocytes were calculated and used as a standard value for the calculation of the LI for all animals.

a) Evaluation of a *standard mean value* for the average of all labeled and unlabeled type I pneumocytes

   In a first step, 14 mice (2 from each test group) were randomly selected. In a second step, 30 measurement fields (15 in the lobus pulmo sinister and 15 in the lobus accessorius) were randomly selected using light microscopy at a magnification of 400fold in each of these animals (measurement field size: 209x152 µm). They all showed exclusively alveolar lung parenchyma without any visible attachments to terminal bronchioles or medium and large bronchi. With this procedure an average of 1108,65 unlabeled and labeled cells per animal was counted which represents the standard mean value per animal.

b) Evaluation of the LI (labeling index)

The positive labeled type I pneumocytes were counted light – microscopically at a magnification of 400fold in the same way as done for the standard mean value of all labeled and unlabeled type I pneumocytes (measurement field size: 209x152 µm, 30 randomly selected measurement fields, 15 in the lobus pulmo sinister and 15 lobus accessorius). To calculate the LI, the total count of positive labeled cells per animal was related to the standard mean value of the total count of all labeled and unlabeled cells according to the following formula:

$$LI = \frac{\text{positive labeled cells}}{\text{standard mean value}} \times 100$$

3.8.6.2. Terminal bronchioles and the medium and large bronchi

The LI (labeling index) was calculated per mm length of the bronchiolar epithelium. For that purpose the average number of labeled and unlabeled cells in the bronchiolar epithelium were calculated per mm and used as a standard value for the calculation of the LI for all animals.

a) Evaluation of a standard mean value per mm for the average of labeled and unlabeled cells in the bronchiolar epithelium

In a first step, 14 mice (2 from each test group) were randomly selected. In a second step, the unlabeled cells in the epithelium of the medium and large bronchi were counted light – microscopically at a magnification of 100fold and with total lengths from 6,982 to 9,156 mm. From these measurements the standard mean value was calculated with 133,729 cells per mm.
An identical approach was performed for the terminal bronchioles. The light – microscopical measurements at a magnification of 100fold covered total lengths from 6,856 to 9,128 mm with terminal bronchiole epithelium. From these measurements the standard mean value was calculated with 132,967 cells per mm.

b) Evaluation of the LI (labeling index)

The positive labeled cells were counted light – microscopically at a magnification of 100fold. This was carried out in a minimum length of 7,48 mm epithelium in the large and medium bronchi and 7,52 mm in the terminal bronchioles. So the measurements covered a minimum of 1000 cells as a whole for each compartment. Preferably, the measurements were performed in the lobus pulmo sinister. In the case the minimum length was not reached, the measurements were continued in the lobus accessorius, the lobus medius dexter, the lobus caudalis dexter and the lobus cranialis dexter, in that order.

To calculate the LI, all positive labeled cells were related to the standard mean value according to the following formula:

$$LI = \frac{\text{positive labeled cells}}{\text{standard mean value x measured length in mm}} \times 100$$

Light microscopy was performed at original magnifications of 100fold (terminal bronchioles and large and medium bronchi) and 400fold (alveoli) using an image analysis system (Quantimed 500, Leica or KS 400, Zeiss, Germany).

Apoptotic cells – showing a positive reaction – were differentiated from necrotic cells by morphology. Labeled and unlabeled cells were counted by genuine color detection.

3.8.7. Statistics of Pathology

Means and standard deviations of each test group were calculated for the variables of terminal body weight and of absolute and relative organ weights (related to terminal body weight) of the animals in each test group. Further statistical analyses were performed according to following tables:

Parameters	Statistical test	Markers in the tables	References
Weight parameters, data of S-Phase Response and Apoptosis	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	NIJENHUIS, A. and S. W. WILF (19710): Combinatorial Algorithms, Academic Press, New York, 32-33. HETTMANNSPERGER, T. P. (19104): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140.

3.9. RETENTION OF RECORDS

GLP-relevant records and materials are stored at BASF Aktiengesellschaft for at least the period of time specified in the GLP principles. Details concerning responsibilities or locations of archiving can be seen from the respective SOPs and from the raw data.

4. RESULTS AND ASSESSMENT OF FINDINGS

4.1. ANALYSES

4.1.1. Food analyses

On the basis of duration of use and the analytical findings with respect to chemical and microbiological contaminants the food was found to be suitable. Fed. Reg. Vol. 44, No. 91 of May 9, 1979, p. 27354 (EPA), served as a guideline for maximum tolerable chemical contaminants. The number of microorganisms did not exceed 10^5/g food. Individual results are to be found in the archives of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft.

4.1.2. Drinking water analyses

On the basis of the analytical findings the drinking water was found to be suitable. German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetzblatt, December 5, 1990) served as a guideline for maximum tolerable contaminants. Individual results are to be found in the archives of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft.

4.1.3. Bedding analyses

On the basis of the analytical findings the bedding was found to be suitable. Levels given in Lab. Animal, Nov. - Dec 1979, pp. 24 - 33, served as a guideline for maximum tolerable contaminants. Individual results are to be found in the archives of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft.

4.2. CLINICAL EXAMINATIONS

Summary tables of the results are given in the Appendix of VOLUME I; individual values are given in Part A of VOLUME II.

4.2.1. Mortality

In group 4 (Phenylacetaldehyde) and in group 6 (Phenylacetic acid) each two animals died.

4.2.2. Clinical observations
(Tables IA-001 - IA-003)

Group 4 (Phenylacetaldehyde):

- 4 animals showed a slight reduced general condition

Group 6 (Phenylacetic acid):

- 1 animal showed a severe reduced general condition
- 1 animal showed a moderate reduced general condition
- 3 animals showed a slight reduced general condition

4.2.3. Body weight data
(Tables IA-004 - IA-005)

Body weight was determined for all dose groups on day 0 to calculate the administration volume of the animals. The dose groups did not differ statistically from the control group.

For terminal body weights, see 4.3.1.

4.3. PATHOLOGY

Summary tables of the results are to be found in the Appendix of Volume I; individual tables are to be found in Part B of Volume II.

Throughout the sections of the pathology report, when intergroup differences are referred to as "significant" it implies that the differences have attained statistical significance ($p < 0.05$) when compared with the control group.

Two animals from the phenylacetaldehyde group (animal-nos. 46 and 47) and two from the phenylacetic acid group (animal-nos. 62 and 63) died prior to the scheduled termination of the study and were not used for the further examinations.

4.3.1. Weight parameters
(Tables IB-001 - IB-002)

The terminal body weights of the test groups 4 (phenylacetaldehyde) and 6 (phenylacetic acid) were significantly decreased.

There were no significant absolute or relative lung weight changes noted.

4.3.2. Gross lesions
(Table IB-003)

There were no gross lesions noted for the lungs. All other gross lesions are shown in the tables and the single animal sheets and are not further discussed.

4.3.3. Histopathological findings
(Table IB-004)

A slight or minimal "focal pneumonitis" was noted for one animal of group 1 and group 2 each. A slight and focal bronchiolo – alveolar hyperplasia was recognized for one animal in group 3. All these single findings are seen to be spontaneous or incidental in nature and not related to treatment.

4.3.4. Determination of cell proliferation (S-phase response)

Table 1 shows the group mean values of the labeling indices (LI, see also table IB-5) and their percentage from control values (control = 100%) after BrdU – staining.

Table 1:

Test substance	Test groups	Large+medium bronchi		Terminal bronchioles		Alveoli	
		%	LI	%	LI	%	LI
Control	0	100	2,54	100	2,85	100	3.36
Styrene	1	187	4,74*	173	4,93**	136	4.56*
1-Phenylethanol	2	114	2,90	127	3,62	157	5.26**
2-Phenylethanol	3	101	2,57	88	2,51	154	5.16**
Phenylacetaldehyde	4	44	1,11*	43	1,22*	627	21.06*
Acetophenone	5	74	1,87	80	2,28	92	3.08
Phenylacetic acid	6	52	1,32	29	0,82**	507	17.05**

% = relative to control
LI = Labeling Index

* = statistically significant, p – value < = 0.05
** = statistically significant, p – value < = 0.01

Treatment with styrene and a variety of metabolites led to a significant increase of cell proliferation primarily in alveoli as shown in the table above. Phenylacetaldehyde and phenylacetic acid treated animals expressed the highest labeling indices. In addition, the 1-phenylethanol and 2-phenylethanol as well as the styrene groups showed a slight but significant increase also.
No significant increase of cell proliferation was found in the large and medium bronchi and the terminal bronchioles for all test groups except the styrene group where a significant increase could be found. In contrary to these results, the labeling indices for phenylacetaldehyde and phenylacetic acid, the metabolites with the highest labeling indices in the alveoli, showed a significant decrease in these compartments of the lung parenchyma.

4.3.5. Determination of apoptosis (TUNEL – stain)

Table 2 shows the group mean values of the labeling indices (LI, see also table IB-6) and their percentage from control values (control = 100%) after TUNEL – staining.

Table 2:

Compound	Test groups	Large+medium bronchi		Terminal bronchioles		Alveoli	
		%	LI	%	LI	%	LI
Control	0	100.00	0.05	100.00	0.01	100	0.08
Styrene	1	40.00	0.02	100.00	0.01	50.00	0.04
1-Phenylethanol	2	60.00	0.03	300.00	0.03	62.50	0.05
2-Phenylethanol	3	0.00	0.00	400.00	0.04	50.00	0.04
Phenylacetaldehyde	4	200.00	0.10	100.00	0.01	212.50	0.17
Acetophenone	5	20.00	0.01	0.00	0.00	62.50	0.05
Phenylacetic acid	6	40.00	0.02	0.00	0.00	212.50	0.17

% = relative to control
LI = Labeling Index

* = statistically significant, p – value < = 0.05
** = statistically significant, p – value < = 0.01

An overall very low amount of positive labeled cells was present in each of the compartments. No significant changes were detected. However, a slightly higher number of apoptotic cells were found in the phenylacetaldehyde and phenylacetic acid groups in the alveoli and in the 1-phenylethanol and 2-phenylethanol groups in the terminal bronchioles and in the phenylacetaldehyde group in the large and medium bronchi.

5. DISCUSSION AND CONCLUSION

Styrene and its metabolites **1-Phenylethanol, 2-Phenylethanol, Phenylacetaldehyde, Acetophenone and Phenylacetic acid** were administered intraperitoneously to groups of 10 female Crl Icr: CD1® mice at a dose level of 100 mg/kg body weight for 3 days, each 3 times a day (total of 9 administrations).

In the groups treated with Phenylacetaldehyde or Phenylacetic acid, each two animals died. In these groups, the terminal body weights were significantly decreased.

Cell proliferation in the lungs was increased vs. controls after treatment with styrene and all tested metabolites, except acetophenone. The most significant induction of cell proliferation was observed in the alveoli. The highest labeling indices were found for phenylacetaldehyde (6-fold the control level) and phenylacetic acid (5-fold), followed by 1-Phenylethanol (1.6-fold), 2-Phenylethanol (1.5-fold) and styrene (1.4-fold). In the large and medium bronchi as well as in the terminal bronchioles, only the styrene group exhibited a significant increase of its labeling indices with 1.8 fold and 1.7-fold, respectively.
The lower BrdU - labeling indices of the terminal bronchioles and the large and medium bronchi for phenylacetaldehyde (44 resp. 43% of control levels) and phenylacetic acid (29 resp. 52% of control levels) do not find a sound explanation. Interestingly this is observed in both metabolites which show the most severe induction of cell proliferation in the alveoli compartment of the lung parenchyma.
Regarding apoptosis, an overall very low amount of positive labeled cells was present in each of the compartments. No significant changes were detected. However, the slightly higher number of apoptotic cells in the alveoli of the phenylacetaldehyde and phenylacetic acid groups may be considered as a reactive response on the high induction of cell proliferation. All other variations recorded are regarded to be incidental and not related to treatment.

6. APPENDIX (FIGURES AND SUMMARY TABLES)

6.1. LIST OF THE ABBREVIATIONS IN TABLES IA

g	=	weight in gram
SD	=	standard deviation
N	=	number of animals for determining Mean and SD
%dev	=	per cent deviations

6.2. LIST OF THE ABBREVIATIONS IN TABLES IB

ppm	=	parts per million
BrdU	=	2'bromo-5-deoxyuridine
H&E	=	Hematoxylin eosin
IM	=	Immunohistology
LI	=	Labeling Index
w	=	weight
mg	=	weight in milligram
approx.	=	approximately
Grp	=	group
G, g	=	weight in gram
M	=	mean
F	=	female
SD	=	standard deviation
N	=	number of animals for determining M and SD
P	=	significance level
anm	=	animal
%	=	per cent

OBSERVATIONS REPORT - INCIDENCE

tudy: 99C0284/99138
ex: Female

reatment Group ose nimal Count	0 CONTROLE 10	1 STYROL/STYRENE 10	2 1-PHENYLETHANOL 10	3 2-PHENYLETHANOL 10
eneral observation Nothing abnormal detected				
Incidence	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Observed	40	40	40	40
Mean onset (Days)	0	0	0	0
OTALS				
Incidence	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Observed	40	40	40	40
Mean onset (Days)	0	0	0	0
acrificed scheduled				
Incidence	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Observed	10	10	10	10
Mean onset (Days)	3	3	3	3
OTALS				
Incidence	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Observed	10	10	10	10
Mean onset (Days)	3	3	3	3

OBSERVATIONS REPORT - INCIDENCE

Study: 99C0284/99138
Sex: Female

Treatment Group	0	4	5	6
Dose	CONTROLE	PHENYLACETALDEHYD	ACETOPHENONE	PHENYACETIC ACID
Animal Count	10	10	10	10
General observation				
Nothing abnormal detected				
Incidence	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Observed	40	33	40	29
Mean onset (Days)	0	0	0	0
TOTALS				
Incidence	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Observed	40	33	40	29
Mean onset (Days)	0	0	0	0
Sacrificed scheduled				
Incidence	10 (100%)	8 (80%)	10 (100%)	8 (80%)
Observed	10	8	10	8
Mean onset (Days)	3	3	3	3
TOTALS				
Incidence	10 (100%)	8 (80%)	10 (100%)	8 (80%)
Observed	10	8	10	8
Mean onset (Days)	3	3	3	3
Found dead				
Incidence		2 (20%)		2 (20%)
Observed		2		2
Mean onset (Days)		3		2
TOTALS				
Incidence	0 (0%)	2 (20%)	0 (0%)	2 (20%)
Observed	0	2	0	2
Mean onset (Days)		3		2
Reduced general condition				
severe				
Incidence				1 (10%)
Observed				1
Mean onset (Days)				2
moderate				
Incidence				1 (10%)
Observed				2
Mean onset (Days)				2

OBSERVATIONS REPORT - INCIDENCE

Study: 99C0284/99138
Sex: Female

Treatment Group	0	4	5	6
Dose	CONTROLE	PHENYLACETALDEHYD	ACETOPHENONE	PHENYACETIC ACID
Animal Count	10	10	10	10
slight				
Incidence		4 (40%)		3 (30%)
Observed		5		4
Mean onset (Days)		3		3
TOTALS				
Incidence	0 (0%)	4 (40%)	0 (0%)	5 (50%)
Observed	0	5	0	7
Mean onset (Days)		3		2

	Body Weight g Day 0 D
Female, CONTROLE	
Mean	24.7
SD	1.4
N	10
%dev	
Female, STYROL/STYRENE	
Mean	24.7
SD	1.6
N	10
%dev	-0.2
Female, 1-PHENYLETHANOL	
Mean	24.8
SD	1.1
N	10
%dev	0.2
Female, 2-PHENYLETHANOL	
Mean	24.5
SD	0.9
N	10
%dev	-1.1
Female, PHENYLACETALDEHYD	
Mean	24.3
SD	1.1
N	10
%dev	-1.7
Female, ACETOPHENONE	
Mean	24.6
SD	1.3
N	10
%dev	-0.5

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

	Body Weight g Day 0 D
Female, PHENYACETIC ACID	
Mean	24.6
SD	1.0
N	10
%dev	-0.7

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

ABSOLUTE WEIGHTS - MEAN VALUES (72h)

Sacrifice group			F1						
Sex			F						
Dose group			0	1	2	3	4	5	6
Terminal body weight	g	M	25.48	25.59	25.4	25.11	23.613**	25.51	22.838**
		SD	1.319	1.164	1.208	0.918	1.034	0.825	0.761
		n	10	10	10	10	8	10	8
Lungs	mg	M	179.1	201.3	207.3	183.8	193.125	189.4	185.625
		SD	20.957	42.544	54.459	31.048	40.428	31.117	41.473
		n	10	10	10	10	8	10	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

.ATIVE WEIGHTS - MEAN VALUES (72h)

:rifice group			F1 F 0	1	2	3	4	5	6
:e group									
'minal body weight	%	M	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		n	10	10	10	10	8	10	8
;gs	%	M	0.704	0.79	0.817	0.732	0.824	0.745	0.812
		SD	0.082	0.179	0.213	0.123	0.199	0.135	0.175
		n	10	10	10	10	8	10	8

' <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
;-sided

INCIDENCE OF GROSS LESIONS (72h)

Sacrifice group	F1						
Sex	F						
Dose group	0	1	2	3	4	5	6
Animals in selected Group	10	10	10	10	8	10	8
NAD	8	9	10	8	2	10	5
Jejunum	.	.	.	.	.	.	.
- Dilation	.	.	.	.	6	.	3
Gallbladder	.	.	.	.	.	.	.
- Discoloration	.	.	.	.	5	.	1
Kidneys	.	.	.	.	.	.	.
- Discoloration of contents	.	.	.	1	.	.	.
- Organ size reduced	1	.	.	.	.	.	.
Ovaries	.	.	.	.	.	.	.
- Paraovarian cyst(s)	2	1	.	2	.	.	.

INCIDENCE OF MICROSCOPIC FINDINGS: LUNGS

Sacrifice group	F1						
Sex	F						
Dose group	0	1	2	3	4	5	6
Animals in selected Group	10	10	10	10	8	10	8
Lungs	10	10	10	10	8	10	8
- Pneumonitis, focal	.	1	1	.	.	.	.
- Hyperplasia, bronch.	.	.	.	1	.	.	.

BrdU – Labeling indices (LI) – Mean values

		Large and medium bronchi	Terminal bronchioles	Alveoli (pneum. I)
Compound		LI	LI	LI
Control	M	2.54	2.85	3.36
	SD	1.82	2.34	0.89
	n	10	10	10
Styrene	M	4.74*	4.92**	4.56*
	SD	2.96	1.79	1.31
	n	10	10	10
1-Phenylethanol	M	2.90	3.62	5.26**
	SD	2.13	3.14	1.52
	n	10	10	10
2-Phenylethanol	M	2.57	2.51	5.16**
	SD	1.24	1.01	1.11
	n	10	10	10
Phenylacetaldehyde	M	1.11*	1.22*	21.06**
	SD	0.70	0.77	4.88
	n	8	8	8
Acetophenone	M	1.87	2.28	3.08
	SD	0.83	0.84	0.79
	n	10	10	10
Phenylacetic acid	M	1.32	0.82**	17.05**
	SD	1.17	0.86	5.77
	n	8	8	8

* : $p <= 0.05$
** : $p <= 0.01$: Wilcoxon-test (two-sided)

TUNEL – Labeling indices (LI) – Mean values

		Large and medium bronchi	Terminal bronchioles	Alveoli (pneum. I)
Compound		LI	LI	LI
Control	M	0.05	0.01	0.08
	SD	0.08	0.03	0.09
	n	10	10	10
Styrene	M	0.02	0.01	0.04
	SD	0.06	0.03	0.08
	n	10	10	10
1-Phenylethanol	M	0.03	0.03	0.05
	SD	0.09	0.09	0.06
	n	10	10	10
2-Phenylethanol	M	0	0.04	0.04
	SD	0	0.09	0.06
	n	10	10	10
Phenylacetaldehyde	M	0.10	0.01	0.17
	SD	0.17	0.03	0.17
	n	8	8	8
Acetophenone	M	0.01	0	0.05
	SD	0.03	0	0.05
	n	10	10	10
Phenylacetic acid	M	0.02	0	0.17
	SD	0.04	0	0.10
	n	8	8	8

* : $p <= 0.05$
** : $p <= 0.01$: Wilcoxon-test (two-sided)

STUDY TITLE

Report

Styrene
1-Phenylethanol
2-Phenylethanol
Phenylacetaldehyde
Acetophenone
Phenylacetic acid

S-Phase response study in the lung of female Crl Icr: CD1® mice

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen/Rhein, Germany

LABORATORY PROJECT IDENTIFICATION

Project No.: 99C0284/99138

VOLUME II OF III
(TABLES SECTION, INDIVIDUAL VALUES)

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List of abbreviations used in Tables IIA

g	=	weight in gram
F	=	Female

List of abbreviations used in Tables IIB

ppm	=	parts per million
BrdU	=	2'bromo-5-deoxyuridine
H&E	=	Hematoxylin eosin
IM	=	Immunohistology
LI	=	Labeling Index
w	=	weight
mg	=	weight in milligram
approx.	=	approximately
Grp	=	group
G, g	=	weight in gram
M	=	mean
F	=	female
SD	=	standard deviation
N	=	number of animals for determining M and SD
P	=	significance level
anm	=	animal
%	=	per cent

OBSERVATIONS REPORT - SUMMARY

Study: 99C0284/99138

Sex	Group	Animal	Observation	Days
F	0	1	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	2	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	3	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	4	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	5	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	6	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	7	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	8	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	9	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	0	10	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3

Study: 99C0284/99138

OBSERVATIONS REPORT - SUMMARY

Sex	Group	Animal	Observation	Days
F	1	11	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	12	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	13	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	14	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	15	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	16	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	17	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	18	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	19	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	1	20	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3

Study: 99C0284/99138

OBSERVATIONS REPORT - SUMMARY

Sex	Group	Animal	Observation	Days
F	2	21	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	22	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	23	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	24	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	25	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	26	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	27	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	28	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	29	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	2	30	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3

Study: 99C0284/99138

OBSERVATIONS REPORT - SUMMARY

Sex	Group	Animal	Observation	Days
F	3	31	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	32	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	33	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	34	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	35	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	36	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	37	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	38	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	39	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	3	40	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3

Study: 99C0284/99138

OBSERVATIONS REPORT - SUMMARY

Sex	Group	Animal	Observation	Days
F	4	41	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	4	42	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	4	43	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	4	44	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	4	45	General observation. Nothing abnormal detected	0-1
			Reduced general condition. slight	2-3
			Sacrificed scheduled	3
F	4	46	General observation. Nothing abnormal detected	0-2
			Found dead	3
F	4	47	General observation. Nothing abnormal detected	0-3
			Found dead	3
F	4	48	General observation. Nothing abnormal detected	0-2
			Reduced general condition. slight	3
			Sacrificed scheduled	3
F	4	49	General observation. Nothing abnormal detected	0-2
			Reduced general condition. slight	3
			Sacrificed scheduled	3
F	4	50	General observation. Nothing abnormal detected	0-2
			Reduced general condition. slight	3
			Sacrificed scheduled	3

Study: 99C0284/99138

OBSERVATIONS REPORT - SUMMARY

Sex	Group	Animal	Observation	Days
F	5	51	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	52	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	53	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	54	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	55	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	56	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	57	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	58	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	59	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	5	60	General observation. Nothing abnormal detected	0-3
			Sacrificed scheduled	3

OBSERVATIONS REPORT - SUMMARY

tudy: 99C0284/99138

ex	Group	Animal	Observation	Days
F	6	61	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	6	62	General observation, Nothing abnormal detected	0-1
			Reduced general condition, severe	2
			Found dead	3
F	6	63	General observation, Nothing abnormal detected	0
			Found dead	1
F	6	64	General observation, Nothing abnormal detected	0-1
			Reduced general condition, moderate	2-3
			Sacrificed scheduled	3
F	6	65	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	6	66	General observation, Nothing abnormal detected	0-1
			Reduced general condition, slight	2-3
			Sacrificed scheduled	3
F	6	67	General observation, Nothing abnormal detected	0-2
			Reduced general condition, slight	3
			Sacrificed scheduled	3
F	6	68	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	6	69	General observation, Nothing abnormal detected	0-3
			Sacrificed scheduled	3
F	6	70	General observation, Nothing abnormal detected	0-2
			Reduced general condition, slight	3
			Sacrificed scheduled	3

Study: 99C0284/99138

	Body Weight g Day 0
Female, CONTROLE	
1	24.4
2	24.4
3	25.5
4	25.8
5	25.2
6	23.2
7	21.7
8	26.5
9	25.2
10	25.5
Female, STYROL/STYRENE	
11	24.3
12	23.0
13	27.9
14	24.0
15	26.8
16	25.7
17	24.6
18	23.4
19	23.5
20	23.8
Female, 1-PHENYLETHANOL	
21	25.6
22	23.5
23	26.7
24	26.3
25	24.7
26	24.0
27	24.3
28	23.5
29	24.8
30	24.6
Female, 2-PHENYLETHANOL	
31	23.8
32	22.9
33	25.3
34	24.4
35	25.9
36	24.9
37	23.8
38	23.7
39	24.8
40	25.1

tudy: 99C0284/99138

	Body Weight g Day 0
emale, PHENYLACETALDEHYD	
1	22.7
2	24.1
3	26.2
4	25.2
5	25.0
6	23.2
7	23.7
8	23.1
9	24.5
0	25.4
emale, ACETOPHENONE	
1	25.5
2	23.9
3	27.2
4	25.8
5	25.2
6	23.9
7	24.5
8	22.4
9	23.8
0	23.9
emale, PHENYACETIC ACID	
1	25.4
2	23.0
3	25.3
4	25.2
5	24.6
6	23.2
7	23.8
8	24.6
9	24.7
0	25.9

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	0	
	Term. body weight	Lungs
	g	mg
M	25.48	179.1
SD	1.319	20.957
n	10	10
1	24.4	186.
2	24.4	164.
3	24.9	188.
4	25.2	177.
5	25.2	168.
6	25.	187.
7	24.1	148.
8	27.8	167.
9	26.1	227.
10	27.7	179.

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group F1
Sex F
Dose group 1

	Term. body weight	Lungs
	g	mg
M	25.59	201.3
SD	1.164	42.544
n	10	10
11	25.5	205.
12	25.4	203.
13	24.9	177.
14	24.1	192.
15	27.9	155.
16	26.3	218.
17	27.	169.
18	25.4	281.
19	24.7	153.
20	24.7	260.

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	2	
	Term. body weight	Lungs
	g	mg
M	25.4	207.3
SD	1.208	54.459
n	10	10
21	25.2	176.
22	24.3	191.
23	28.2	211.
24	25.5	260.
25	25.7	323.
26	24.	176.
27	24.5	251.
28	25.1	171.
29	26.4	156.
30	25.1	158.

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	3	
	Term. body weight	Lungs
	g	mg
M	25.11	183.8
SD	0.918	31.048
n	10	10
31	24.6	198.
32	24.2	190.
33	27.	171.
34	24.4	205.
35	25.5	244.
36	25.8	204.
37	25.3	154.
38	25.2	177.
39	25.3	158.
40	23.8	137.

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	4	
	Term. body weight	Lungs
	g	mg
M	23.613	193.125
SD	1.034	40.428
n	8	8
41	21.9	236.
42	22.7	215.
43	24.6	151.
44	24.1	155.
45	22.9	252.
48	24.2	180.
49	23.6	148.
50	24.9	208.

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	5	
	Term. body weight	Lungs
	g	mg
M	25.51	189.4
SD	0.825	31.117
n	10	10
51	25.1	223.
52	24.6	257.
53	26.	199.
54	25.8	185.
55	26.3	174.
56	25.8	144.
57	26.2	185.
58	23.7	178.
59	26.1	177.
60	25.5	172.

ABSOLUTE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	6	
	Term. body weight	Lungs
	g	mg
M	22.838	185.625
SD	0.761	41.473
n	8	8
61	22.8	173.
64	22.8	167.
65	22.1	188.
66	22.	220.
67	24.2	176.
68	22.4	137.
69	22.7	155.
70	23.7	269.

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group		F1
Sex		F
Dose group		0
	Term. body weight	Lungs
	%	%
M	100.	0.704
SD		0.082
n	10	10
1	100.	0.762
2	100.	0.672
3	100.	0.755
4	100.	0.702
5	100.	0.667
6	100.	0.748
7	100.	0.614
8	100.	0.601
9	100.	0.87
10	100.	0.646

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group		F1
Sex		F
Dose group		1
	Term. body weight %	Lungs %
M	100.	0.79
SD		0.179
n	10	10
11	100.	0.804
12	100.	0.799
13	100.	0.711
14	100.	0.797
15	100.	0.556
16	100.	0.829
17	100.	0.626
18	100.	1.106
19	100.	0.619
20	100.	1.053

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	2	
	Term. body weight	Lungs
	%	%
M	100.	0.817
SD		0.213
n	10	10
21	100.	0.698
22	100.	0.786
23	100.	0.748
24	100.	1.02
25	100.	1.257
26	100.	0.733
27	100.	1.024
28	100.	0.681
29	100.	0.591
30	100.	0.629

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	3	
	Term. body weight	Lungs
	%	%
M	100.	0.732
SD		0.123
n	10	10
31	100.	0.805
32	100.	0.785
33	100.	0.633
34	100.	0.84
35	100.	0.957
36	100.	0.791
37	100.	0.609
38	100.	0.702
39	100.	0.625
40	100.	0.576

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group		F1
Sex		F
Dose group		4
	Term. body weight	Lungs
	%	%
M	100.	0.824
SD		0.199
n	8	8
41	100.	1.078
42	100.	0.947
43	100.	0.614
44	100.	0.643
45	100.	1.1
48	100.	0.744
49	100.	0.627
50	100.	0.835

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group F1
Sex F
Dose group 5

	Term. body weight	Lungs
	%	%
M	100.	0.745
SD		0.135
n	10	10
51	100.	0.888
52	100.	1.045
53	100.	0.765
54	100.	0.717
55	100.	0.662
56	100.	0.558
57	100.	0.706
58	100.	0.751
59	100.	0.678
60	100.	0.675

RELATIVE WEIGHTS - INDIVIDUAL VALUES

Sacrifice group	F1	
Sex	F	
Dose group	6	
	Term. body weight	Lungs
	%	%
M	100.	0.812
SD		0.175
n	8	8
61	100.	0.759
64	100.	0.732
65	100.	0.851
66	100.	1.
67	100.	0.727
68	100.	0.612
69	100.	0.683
70	100.	1.135

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	0
Animal	1

General Information

Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 2

General Information

Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 3

General Information

Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 4

General Information

Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	0
cont. Animal	4

Macroscopy
Kidneys
- Organ size reduced, right side, moderate.
Ovaries
- Paraovarian cyst(s), right side and left side, diameter 4.0 mm.
All other organs without macroscopic findings.

Microscopy
Kidneys
- Histopathologic evaluation of gross lesion(s) will not be performed.
Ovaries
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 5

General Information
Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 6

General Information
Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 7

General Information
Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	0
cont. Animal	7

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 8

General Information
Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 9

General Information
Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 10

General Information
Sex : Female
Dose group : 0 (0 mg/kg)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Ovaries
- Paraovarian cyst(s), left side, diameter 4.0 mm.
All other organs without macroscopic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	0
cont. Animal	10

Microscopy
Ovaries
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	1
Animal	11

General Information

Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 12

General Information

Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 13

General Information

Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 14

General Information

Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	1
cont. Animal	14

Macroscopy
Animal without particular findings.
Microscopy
Lungs
- Focal pneumonitis, grade 2.
No other organs examined.

Animal 15

General Information
Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Ovaries
- Paraovarian cyst(s), left side, diameter 4.0 mm.
All other organs without macroscopic findings.
Microscopy
Ovaries
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 16

General Information
Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 17

General Information
Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group F1
Sex F
Dose group 1
cont. Animal 17

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 18

General Information
Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 19

General Information
Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 20

General Information
Sex : Female
Dose group : 1 (100 mg/kg Styrol/Styrene)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	2
Animal	21

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 22

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
Lungs
- Focal pneumonitis, grade 1.
No other organs examined.

Animal 23

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 24

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	2
cont. Animal	24

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal	25

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal	26

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal	27

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	2
Animal	28

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	29

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	30

General Information
Sex : Female
Dose group : 2 (100 mg/kg 1-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	3
Animal	31

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Ovaries
- Paraovarian cyst(s), diameter 5.0 mm, content bloody.
All other organs without macroscopic findings.

Microscopy
Ovaries
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal	32

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	33

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	3
Animal	34

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
Lungs
- Hyperplasia, bronchiolo-alveolar, grade 2.
No other organs examined.

Animal 35

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Kidneys
- Discoloration of contents, left side, diameter 2.0 mm.
Ovaries
- Paraovarian cyst(s), right side, diameter 4.0 mm.
All other organs without macroscopic findings.

Microscopy
Kidneys
- Histopathologic evaluation of gross lesion(s) will not be performed.
Ovaries
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 36

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	3
cont. Animal	36

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 37

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 38

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal 39

General Information
Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	3
Animal	40

General Information

Sex : Female
Dose group : 3 (100 mg/kg 2-Phenylethanol)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	4
Animal	41

General Information
Sex : Female
Dose group : 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Jejunum
- Dilation, slight, content liquid.
Gallbladder
- Discoloration, dark green.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
Gallbladder
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 42

General Information
Sex : Female
Dose group : 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Jejunum
- Dilation, slight, content liquid.
Gallbladder
- Discoloration, dark green.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
Gallbladder
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 43

General Information
Sex : Female
Dose group : 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	4
cont. Animal	43

Macroscopy
Jejunum
- Dilation, slight, content liquid.
Gallbladder
- Discoloration, dark green.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
Gallbladder
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 44

General Information

Sex	: Female
Dose group	: 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group	: Final sacrifice group
Necropsy status	: Planned sacrifice
Date of death	: Oct/11/2001
	3 days after start of exposure
	1 day after end of exposure

Macroscopy
Jejunum
- Dilation, slight, content liquid.
Gallbladder
- Discoloration, dark green.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
Gallbladder
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 45

General Information

Sex	: Female
Dose group	: 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group	: Final sacrifice group
Necropsy status	: Planned sacrifice
Date of death	: Oct/11/2001
	3 days after start of exposure
	1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	4
cont. Animal	45

Macroscopy
Jejunum
- Dilation, slight, content liquid.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 48

General Information
Sex : Female
Dose group : 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 49

General Information
Sex : Female
Dose group : 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Jejunum
- Dilation, slight, content liquid.
Gallbladder
- Discoloration, dark green.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
Gallbladder
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	4
Animal	50

General Information

Sex : Female
Dose group : 4 (100 mg/kg Phenylacetaldehyd)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy

Animal without particular findings.

Microscopy

All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	5
Animal	51

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	52

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	53

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	54

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	5
cont. Animal	54

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal	55

General Information
Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal	56

General Information
Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Animal	57

General Information
Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	5
Animal	58

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	59

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal	60

General Information

Sex : Female
Dose group : 5 (100 mg/kg Acetophenone)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	6
Animal	61

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Jejunum
- Dilation, slight, content liquid.
Gallbladder
- Discoloration, dark green.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
Gallbladder
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 64

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Jejunum
- Dilation, slight, content liquid.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 65

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/11/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

Sacrifice group	F1
Sex	F
Dose group	6
cont. Animal	65

Macroscopy
Jejunum
- Dilation, slight, content liquid.
All other organs without macroscopic findings.

Microscopy
Jejunum
- Histopathologic evaluation of gross lesion(s) will not be performed.
All other organs examined without microscopic findings

Animal 66

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 67

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.

Microscopy
All organs examined without pathologic findings.

Animal 68

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

SINGLE ANIMAL SHEET

	Sacrifice group	F1
	Sex	F
	Dose group	6
cont.	Animal	68

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

	Animal	69

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

	Animal	70

General Information
Sex : Female
Dose group : 6 (100 mg/kg Phenylacetic acid)
Sacrifice group : Final sacrifice group
Necropsy status : Planned sacrifice
Date of death : Oct/12/2001
3 days after start of exposure
1 day after end of exposure

Macroscopy
Animal without particular findings.
Microscopy
All organs examined without pathologic findings.

Single Animal Data of the Lung: BrdU - stain

Group	Animal no	Alveoli LI
Group 0	1	3,70
	2	2,71
	3	4,60
	4	4,96
	5	2,07
	6	3,16
	7	3,07
	8	3,52
	9	2,53
	10	3,25
	M	3,36
	SD	0,89
	n	10
Group 1	11	5,77
	12	4,78
	13	5,23
	14	4,15
	15	5,05
	16	3,43
	17	5,77
	18	1,62
	19	4,06
	20	5,77
	M	4,56
	SD	1,31
	n	10
Group 2	21	7,85
	22	4,51
	23	6,31
	24	4,33
	25	7,31
	26	5,59
	27	3,16
	28	5,41
	29	3,88
	30	4,24
	M	5,26
	SD	1,52
	n	10
Group 3	31	5,95
	32	4,42
	33	5,05
	34	6,22
	35	6,31
	36	4,69
	37	5,59
	38	5,50
	39	5,32
	40	2,53
	M	5,16
	SD	1,11
	n	10

Group	Animal no	Alveoli LI
Group 4	41	22,82
	42	20,02
	43	26,97
	44	22,73
	45	17,23
	46	animal died
	47	animal died
	48	11,55
	49	21,74
	50	25,44
	M	21,06
	SD	4,88
	n	8
Group 5	51	3,34
	52	3,34
	53	4,69
	54	2,98
	55	2,80
	56	2,53
	57	3,43
	58	3,07
	59	1,53
	60	3,16
	M	3,08
	SD	0,79
	n	10
Group 6	61	20,48
	62	animal died
	63	animal died
	64	12,00
	65	22,91
	66	16,69
	67	12,00
	68	11,37
	69	14,16
	70	26,79
	M	17,05
	SD	5,77
	n	8

Single Animal Data of the Lung: BrdU - stain

Group	Animal no	Terminal bronchioles LI
Group 0	1	9,15
	2	2,23
	3	3,29
	4	1,31
	5	1,60
	6	2,71
	7	3,31
	8	2,07
	9	1,20
	10	1,63
	M	2,85
	SD	2,34
	n	10
Group 1	11	5,25
	12	3,79
	13	3,44
	14	8,84
	15	2,65
	16	3,45
	17	6,46
	18	5,28
	19	5,09
	20	5,00
	M	4,92
	SD	1,79
	n	10
Group 2	21	7,10
	22	4,28
	23	1,47
	24	5,56
	25	0,79
	26	1,41
	27	1,29
	28	2,81
	29	1,27
	30	10,22
	M	3,62
	SD	3,14
	n	10
Group 3	31	1,37
	32	3,85
	33	1,93
	34	1,32
	35	1,98
	36	2,38
	37	3,45
	38	1,73
	39	3,07
	40	4,01
	M	2,51
	SD	1,01
	n	10

Group	Animal no	Terminal bronchioles LI
Group 4	41	1,34
	42	0,69
	43	0,59
	44	0,66
	45	1,30
	46	animal died
	47	animal died
	48	2,96
	49	1,35
	50	0,88
	M	1,22
	SD	0,77
	n	8
Group 5	51	1,15
	52	0,84
	53	3,08
	54	2,24
	55	2,96
	56	3,52
	57	2,47
	58	2,53
	59	2,15
	60	1,82
	M	2,28
	SD	0,84
	n	10
Group 6	61	0,18
	62	animal died
	63	animal died
	64	0,26
	65	0,47
	66	0,64
	67	2,57
	68	1,67
	69	0,09
	70	0,65
	M	0,82
	SD	0,86
	n	8

Single Animal Data of the Lung: BrdU - stain

Group	Animal no	Large/Medium bronchi LI
Group 0	1	7,01
	2	1,12
	3	3,05
	4	1,53
	5	2,66
	6	1,29
	7	3,60
	8	2,03
	9	0,66
	10	2,45
	M	2,54
	SD	1,82
	n	10
Group 1	11	4,47
	12	3,77
	13	2,37
	14	12,74
	15	2,26
	16	3,81
	17	4,66
	18	4,67
	19	3,64
	20	5,05
	M	4,74
	SD	2,96
	n	10
Group 2	21	1,94
	22	2,23
	23	1,81
	24	4,93
	25	0,37
	26	1,51
	27	2,38
	28	5,11
	29	1,46
	30	7,21
	M	2,90
	SD	2,13
	n	10
Group 3	31	0,82
	32	2,25
	33	2,46
	34	0,99
	35	5,12
	36	3,22
	37	3,08
	38	2,52
	39	1,94
	40	3,28
	M	2,57
	SD	1,24
	n	10

Group	Animal no	Large/Medium bronchi LI
Group 4	41	0,48
	42	1,15
	43	0,76
	44	1,06
	45	0,35
	46	animal died
	47	animal died
	48	2,48
	49	1,76
	50	0,83
	M	1,11
	SD	0,70
	n	8
Group 5	51	0,82
	52	3,51
	53	1,24
	54	1,62
	55	2,16
	56	2,07
	57	2,97
	58	1,61
	59	1,41
	60	1,27
	M	1,87
	SD	0,83
	n	10
Group 6	61	0,41
	62	animal died
	63	animal died
	64	0,19
	65	0,09
	66	3,05
	67	2,58
	68	2,07
	69	0,45
	70	1,71
	M	1,32
	SD	1,17
	n	8

Single Animal Data of the Lung: TUNEL - stain

Group	Animal no	Alveoli LI
Group 0	1	0,09
	2	0,18
	3	0,00
	4	0,18
	5	0,18
	6	0,18
	7	0,00
	8	0,00
	9	0,00
	10	0,00
	M	0,08
	SD	0,09
	n	10
Group 1	11	0,00
	12	0,00
	13	0,00
	14	0,00
	15	0,00
	16	0,18
	17	0,00
	18	0,00
	19	0,00
	20	0,18
	M	0,04
	SD	0,08
	n	10
Group 2	21	0,00
	22	0,18
	23	0,09
	24	0,00
	25	0,09
	26	0,00
	27	0,09
	28	0,00
	29	0,09
	30	0,00
	M	0,05
	SD	0,06
	n	10
Group 3	31	0,00
	32	0,00
	33	0,18
	34	0,00
	35	0,09
	36	0,09
	37	0,00
	38	0,00
	39	0,00
	40	0,00
	M	0,04
	SD	0,06
	n	10

Group	Animal no	Alveoli LI
Group 4	41	0,46
	42	0,27
	43	0,00
	44	0,00
	45	0,27
	46	animal died
	47	animal died
	48	0,00
	49	0,18
	50	0,18
	M	0,17
	SD	0,17
	n	8
Group 5	51	0,09
	52	0,00
	53	0,00
	54	0,00
	55	0,09
	56	0,09
	57	0,09
	58	0,09
	59	0,00
	60	0,00
	M	0,05
	SD	0,05
	n	10
Group 6	61	0,37
	62	animal died
	63	animal died
	64	0,09
	65	0,09
	66	0,18
	67	0,09
	68	0,27
	69	0,18
	70	0,09
	M	0,17
	SD	0,10
	n	8

Single Animal Data of the Lung: TUNEL - stain

Group	Animal no	Terminal bronchioles LI
Group 0	1	0,00
	2	0,09
	3	0,00
	4	0,00
	5	0,00
	6	0,00
	7	0,00
	8	0,00
	9	0,00
	10	0,00
	M	0,01
	SD	0,03
	n	10
Group 1	11	0,00
	12	0,09
	13	0,00
	14	0,00
	15	0,00
	16	0,00
	17	0,00
	18	0,00
	19	0,00
	20	0,00
	M	0,01
	SD	0,03
	n	10
Group 2	21	0,00
	22	0,00
	23	0,00
	24	0,00
	25	0,00
	26	0,00
	27	0,00
	28	0,00
	29	0,00
	30	0,27
	M	0,03
	SD	0,09
	n	10
Group 3	31	0,00
	32	0,09
	33	0,00
	34	0,00
	35	0,00
	36	0,00
	37	0,00
	38	0,09
	39	0,27
	40	0,00
	M	0,04
	SD	0,09
	n	10

Group	Animal no	Terminal bronchioles LI
Group 4	41	0,00
	42	0,00
	43	0,00
	44	0,00
	45	0,00
	46	animal died
	47	animal died
	48	0,00
	49	0,08
	50	0,00
	M	0,01
	SD	0,03
	n	8
Group 5	51	0,00
	52	0,00
	53	0,00
	54	0,00
	55	0,00
	56	0,00
	57	0,00
	58	0,00
	59	0,00
	60	0,00
	M	0,00
	SD	0,00
	n	10
Group 6	61	0,00
	62	animal died
	63	animal died
	64	0,00
	65	0,00
	66	0,00
	67	0,00
	68	0,00
	69	0,00
	70	0,00
	M	0,00
	SD	0,00
	n	8

Single Animal Data of the Lung: TUNEL - stain

Group	Animal no	Large/Medium bronchi LI
Group 0	1	0,00
	2	0,19
	3	0,00
	4	0,00
	5	0,00
	6	0,00
	7	0,19
	8	0,00
	9	0,00
	10	0,09
	M	0,05
	SD	0,08
	n	10
Group 1	11	0,00
	12	0,00
	13	0,00
	14	0,18
	15	0,00
	16	0,00
	17	0,00
	18	0,00
	19	0,00
	20	0,00
	M	0,02
	SD	0,06
	n	10
Group 2	21	0,00
	22	0,27
	23	0,00
	24	0,00
	25	0,00
	26	0,00
	27	0,00
	28	0,00
	29	0,00
	30	0,00
	M	0,03
	SD	0,09
	n	10
Group 3	31	0,00
	32	0,00
	33	0,00
	34	0,00
	35	0,00
	36	0,00
	37	0,00
	38	0,00
	39	0,00
	40	0,00
	M	0,00
	SD	0,00
	n	10

Group	Animal no	Large/Medium bronchi LI
Group 4	41	0,00
	42	0,00
	43	0,00
	44	0,00
	45	0,30
	46	animal died
	47	animal died
	48	0,08
	49	0,44
	50	0,00
	M	0,10
	SD	0,17
	n	8
Group 5	51	0,09
	52	0,00
	53	0,00
	54	0,00
	55	0,00
	56	0,00
	57	0,00
	58	0,00
	59	0,00
	60	0,00
	M	0,01
	SD	0,03
	n	10
Group 6	61	0,09
	62	animal died
	63	animal died
	64	0,00
	65	0,00
	66	0,00
	67	0,00
	68	0,00
	69	0,08
	70	0,00
	M	0,02
	SD	0,04
	n	8

STUDY TITLE

Report

Styrene
1-Phenylethanol
2-Phenylethanol
Phenylacetaldehyde
Acetophenone
Phenylacetic acid

S-Phase response study in the lung of female Crl Icr: CD1® mice

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen/Rhein, Germany

LABORATORY PROJECT IDENTIFICATION

Project No.: 99C0284/99138

VOLUME III OF III
(SUPPLEMENT)

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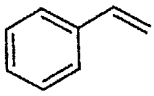
Analytical Report

Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

Test substance:	Styrene
Chemical name:	Styrene
Chemical structure:	
Batch identification:	tank 21
Date of production:	July 20 and 28, 1999
ZHT no.:	99/284-1
Origin of test substance:	Dr. Mitulla, KSS/SQ, H 700
Sponsor:	M. Lange, ZHT/ES, Z 470
Testing facility:	BASF Aktiengesellschaft, ZAX Analytik, D-67056 Ludwigshafen
Date of receipt of test substance:	August 13, 1999
Study director:	Kl. Schwinn, ZAX/CG, E 210
Study period:	August 20 to September 20, 1999
Storage conditions:	Room temperature
Retention of records:	GLP archives ZAX, E 210

Summary:

The area fraction of styrene is 99.9 area-% determined by gas chromatography with flame ionization detection and corrected with the content of water (0.02 g/100 g). Content of the sum of styrene oligomers is 60 mg/kg. Identity was confirmed by ^{1}H-NMR spectroscopy.

Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

1. Appearance and homogeneity

Method:	By visual inspection
Result:	The test substance is a clear, colourless liquid. It appears homogeneous.
Date of test:	August 27, 1999
Head of laboratory:	Dr. Güntner, ZAX/CG (GC), E 210

2. Identity

Method:	^{1}H-NMR spectroscopy (400 MHz) as solution in $CDCl_3$
Result:	The spectrum exhibits the signals expected for the chemical structure of the test substance. See ^{1}H-NMR spectrum on page 7.
Date of test:	August 20, 1999
Head of laboratory:	Dr. Dembowski, ZAX/CS (NMR), E 210

3. Mass fraction of water

Method:	Karl-Fischer titration in "Hydranal-Methanol"
Result:	w (H_2O)= 0.02 g/100 g
Date of test:	August 23, 1999
Head of laboratory:	Dr. Schmidt, ZAX/EO (CPK), M 320

Characterization of "Styrene" before start of toxicological studies
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4. GC purity

Method:	Capillary gas chromatography, evaluation by the area-% method, corrected with the content of water
Apparatus:	Gas chromatograph equipped with autosampler, split injector and flame ionization detector (FID) and adapted to a chromatography data system
Sample preparation:	Direct injection

Chromatographic conditions:

Column:	fused silica capillary column:	DB-1
	length:	30 m
	internal diameter:	0.25 mm
	film thickness:	1.0 µm
Carrier gas:	Nitrogen	
	column head pressure:	0.56 bar
	split:	40 ml/min
	septum purge:	5 ml/min
Temperatures:	oven:	hold 5 min at 60°C 60 - 260°C at 5 K/min hold 15 min at 260°C
	injector:	250°C
	detector (FID):	280°C
Injection volume:	0.2 µl	

Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

Result:

Styrene 99.9 area-% [1)]

[1)] Mean value of two individual injections, corrected with the content of water. The determination on a second column (CP-Wax 52) shows the same result.

See gas chromatogram on page 8.

Date of test: August 20 to 23, 1999

Head of laboratory: Dr. Güntner, ZAX/CG (GC), E 210

5. Mass fraction of oligomers

Method: OnColumn capillary gas chromatography, evaluation by the standard addition method

Apparatus: Gas chromatograph equipped with programable pressure unit, split injector and flame ionization detector (FID) and adapted to a chromatography data system

Reagents: Isooctane
Styrene oligomer mixture
(in the main dimer and trimer styrene), (BASF, ZAX reference material no. A 2121/02/GC_731)

Standard solutions: By weighing and diluting two solutions of the styrene oligomer mixture in isooctane were prepared.

Sample preparation: Two portions of each about 1 g of test substance were weighed to the nearest 0.1 mg. Then each 10 ml of isooctane (containing c. 0.02 mg of eicosane, not used for calculations) were added.

Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

Standard addition:	Two portions of each about 1 g of test substance were weighed to the nearest 0.1 mg. Then each 5 ml of one of the standard solutions and 5 ml of isooctane (containing c. 0.02 mg of eicosane, not used for calculations) were added.

Chromatographic conditions:

Pre-Column:	"Phe-Sil" retention gap (Machery & Nagel), length: c. 50 cm, i.d.: 0.53 mm	
Column:	fused silica capillary column:	CP-Sil 5 CB ("Low Bleed")
	length:	25 m
	internal diameter:	0.32 mm
	film thickness:	0.25 µm
Carrier gas:	Helium	
	column head pressure:	hold 2 min at 20 kPa 20 - 110 kPa at 3 kPa/min hold 15 min at 110 kPa
	split:	OnColumn
	septum purge:	c. 14 ml/min
Temperatures:	oven:	hold 2 min at 80°C 80 – 300°C at 10 K/min hold 20 min at 300°C
	injector:	off (oven track: on)
	detector (FID):	320°C
Injection volume:	2.0 µl	

Analytical Report

Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

Result:

w (styrene oligomers)= 60 mg/kg [1)]

[1)] sum of dimer and trimer styrenes according to the comparison of retention time with the reference material (A 2121/02/GC_731); according to the GC/MS coupling further compounds of molecular weights of 206, 222 and 224 were detected:

comp. no.	molecular weight
0.5	206
1	224 + 206
2	206
3	222

See gas chromatogram on page 9.

Date of test: August 25 to September 20, 1999 (including GC/MS coupling)

Head of laboratory: Dr. Güntner, ZAX/CG (GC), E 210
Dr. Hutmacher, ZAX/CS (MS), E 210

GLP-Statement

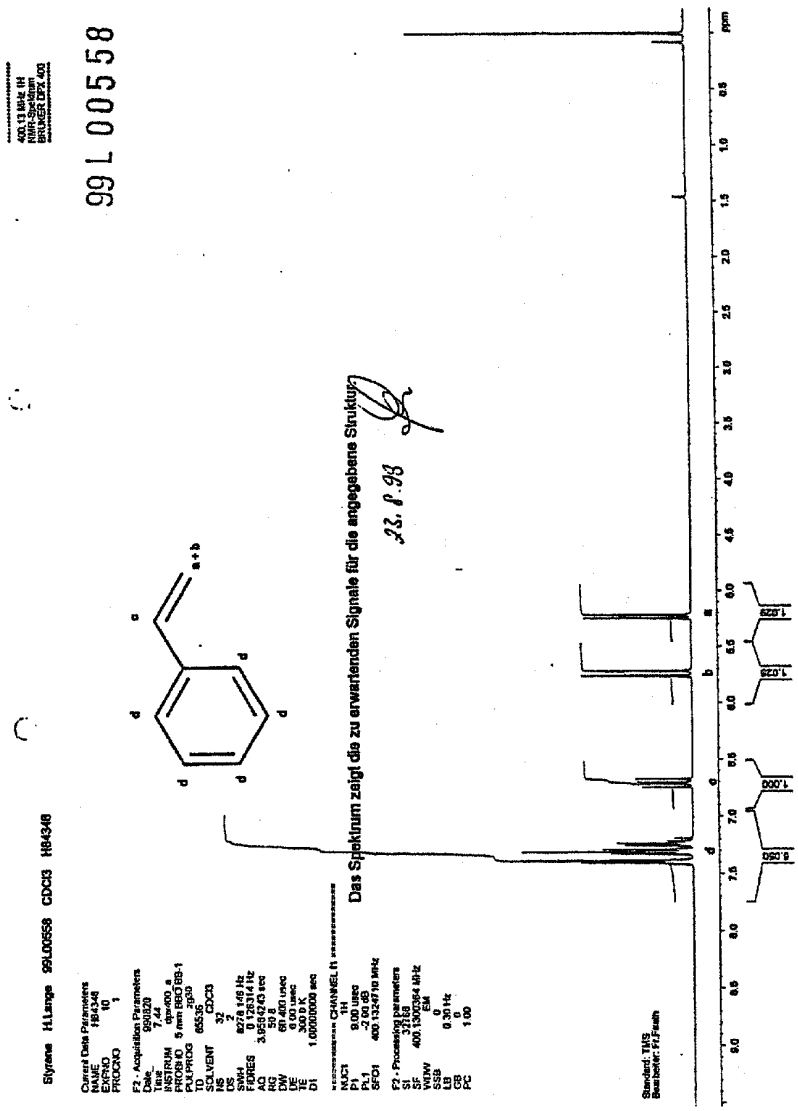
The tests reported in this Analytical Report were conducted in compliance with the principles of Good Laboratory Practice according to Appendix 1 of the German "Chemikaliengesetz".

Study director (Kl. Schwinn)

10. November 1999
Date

Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

^{1}H-NMR spectrum



Analytical Report

Characterization of "Styrene" before start of toxicological studies
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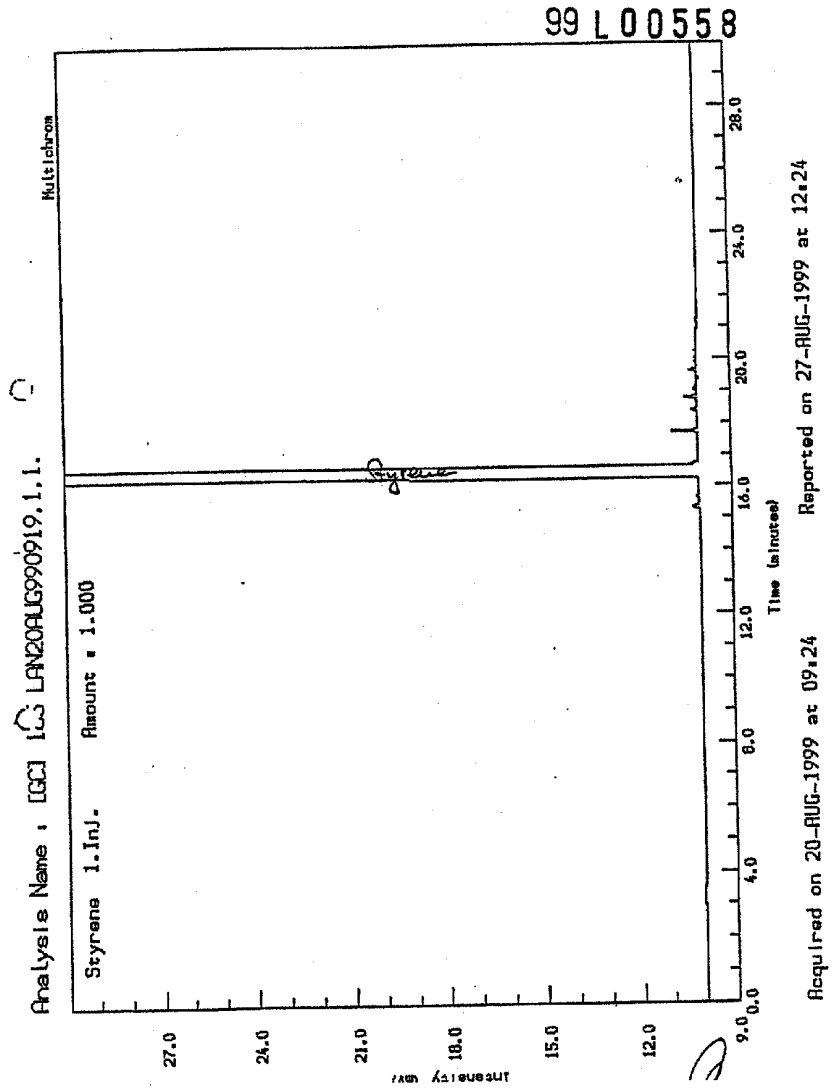
Gas chromatogram (purity)



Analytical Report

Characterization of "Styrene" before start of toxicological studies
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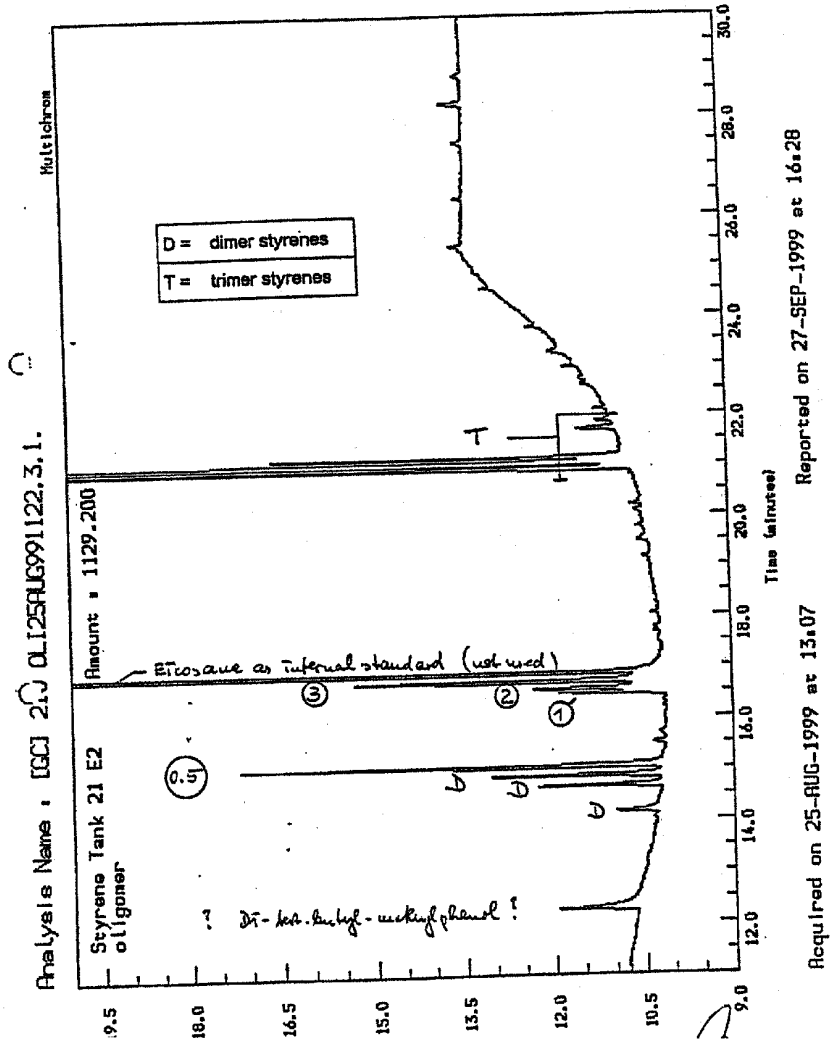
Gas chromatogram (styrene oligomers)

99 L 00558



Characterization of "Styrene" before start of toxicological studies
ZAX study no. 99L00558

STATEMENT OF THE QUALITY ASSURANCE UNIT

The laboratories and the conduct of analytical studies are inspected in regular intervals. Besides these general inspections we inspected the following items concerning this special study. - Findings are reported to analytical study director and his management.

Inspection of		Date of inspection	Reported to study director and management
Conduct of study:	Capillary gas chromatography	Aug. 20, 1999	Aug. 20, 1999
Raw data:		Nov. 10, 1999	Nov. 10, 1999
Analytical report: final:		Nov. 10, 1999	Nov. 10, 1999

Ludwigshafen

QAU: Meyer-Schlieker

Nov. 10, 1999
Date