Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

2-Phenylpropene

EC Number: **202-705-0**

CAS Number: **98-83-9**

Index Number: **601-027-00-6**

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1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

1.1.1 NTP, 2007 and De Costa *et al.*, 2001

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

De Costa, Kristi S., et al. "Metabolism and disposition of 2-phenylpropene in rats." Drug metabolism and disposition 29.2 (2001): 166-171.

ECHA dissemination page: 001 Key | Experimental results

Test type

Toxicokinetic study, similar to OECD TG 417

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- Lot #: BNW 13871-4, BNW 13871-54
- *Physicochemical properties that may be important when assessing toxicokinetics:*
    - colourless, volatile liquid
    - vapour pressure of 2.3 mm Hg (20° C)
    - boiling point 165°C
    - soluble in organic solvent
    - log *K*ow: 3.48 (25°C)

Detailed study summary and results:

[Please provide the test material identity, a detailed study and results transparently and objectively as in the original data source without subjective interpretations.]

Material and methods

Experimental animals:

- *test animals:* rats, F344/N, male (age / weight at dosing: 79 – 85 days / 241 – 263 g)
- *test material:* [^{14}C]2-phenylpropene (radiolabelled with carbon-14 in the phenyl ring)
- *intravenous study:*
    - single dose (10.8 mg/kg bw) intravenous administration

  - sacrificed 72 h post dosing
  - urine, faeces, exhaled air (incl. CO2) analysed for cumulative excretion of radioactivity 6, 12, 24, 48, and 72 h after intravenous administration of [^{14}C]2-phenylpropene (n = 4)
  - tissue distribution of radioactivity (2-phenylpropene equivalents in ng/g tissue) examined 72 h after intravenous administration of [^{14}C]2-phenylpropene (n = 4)
  - urinary profile of 2-phenylpropene metabolites determined in urine collected up to 48 h after intravenous administration of [^{14}C]2-phenylpropene (n = 1)
- *oral study:*
  - intragastric gavage (1000 mg/kg bw) administration
  - sacrificed 48 h post dosing
  - urine and faeces analysed for cumulative excretion of radioactivity 6, 24, and 48 h after oral administration of [^{14}C]2-phenylpropene (n = 1)
  - urinary profile of 2-phenylpropene metabolites determined in urine collected 6 to 24 h after oral administration of [^{14}C]2-phenylpropene (n = 1)
- *inhalation study:*
  - nose only inhalation (300 and 900 ppm) administration for 6 h
  - sacrificed 6, 24, and 72 h after initiation of exposure
  - 300 / 900 ppm: urine, faeces, volatile breath analysed for excretion of radioactivity 6, 12, 24, 48, and 72 h after initiation of exposure (6 h exposure with [^{14}C]2-phenylpropene; n = 4 / dose)
  - 300 / 900 ppm: tissue distribution of radioactivity (2-phenylpropene equivalents in µg/g tissue) examined 6 h, 24 h (only 300 ppm), and 72 h after initiation of exposure (6 h exposure with [^{14}C]2-phenylpropene, n = 3 - 5)
  - urinary profile of 2-phenylpropene metabolites determined in urine collected between 6 and 48 h following initiation of exposure (6 h exposure with [^{14}C]2-phenylpropene; n = 1)
  - analysis of blood concentrations in serial blood sample collected from 5 to 24 h following initiation of exposure (6 h exposure with [^{14}C]2-phenylpropene; n = 5)
  - analysis of 2-phenylpropene metabolites determined in blood
  - toxicokinetic data generated from the 24 h sampling time
- *tissues analysed:* adipose tissue, muscle, skin (ear or leg), kidney, liver, spleen, lung, testes, bladder, heart, brain, stomach, small intestine, cecum, large intestine)

Human liver slices:

- 2-phenylpropene metabolites determined following incubation of human liver slices from one donor (45-year old male, fatally wounded, medicated, under the influence of a controlled substance, renal cancer patient) with 2-phenylpropene

Results

Experimental animals:

- *intravenous Study:*
    - excretion following a single intravenous administration of 10.8 mg/kg bw radioactively labelled 2-phenylpropene (given as percent of dose recovered)
        - **mainly via urine** (post dosing: 76.4 ± 2.1 % [24 h] and 86 ± 1.4 % [72 h])
        - little excretion via faeces (post dosing: 1.2 ± 0.3 % [24 h] and 1.9 ± 0.7 % [72 h]) and breath (post dosing: 2.1 ± 0.8 % [24 h] and 2.2 ± 0.8 % [72 h]; CO_2: 0.02 %)
    - **low tissue concentration (0.3 %)** of recovered radioactivity at 72 h
    - urinary metabolic profiling revealed:
        - 82.4 % of the initial dose was excreted within 48 h
        - **2-phenyl-1,2-propanediol glucuronide** (~41 % of the initial dose; i.e. 50 % of total urinary radioactivity) and **atrolactic acid** were found to be the most abundant metabolites (~22 % of the initial dose; i.e. 27 % of total urinary radioactivity)
        - Minor metabolites were identified as 2-phenyl-1,2-propanediol (~3 % of the initial dose) and S-(2 hydroxy-2-phenylpropyl)-N-acetylcysteine (~11 % of the initial dose) and presumably 2-phenyl propionic acid (~1 %)
        - S-(2 hydroxy-2-phenylpropyl)-N-acetylcysteine may constitute an early metabolite as the highest peak intensity was detected at the first sampling time (6 h after initiation of exposure)
- *oral study:*
    - excretion following a single oral administration of 1000 mg/kg radioactively labelled 2-phenylpropene
        - **mainly via urine** (cumulative levels post dosing: 3.28 % [6 h], 57.8 % [24 h], and 74.6 % [48 h])
        - little excretion via faeces (cumulative levels post dosing: 0 % [6 h], 1.51 % [24 h], and 2.96 % [48 h])
    - urinary metabolic profiling was similar as compared to intravenous administration
- *inhalation study:*
    - excretion following a 6 h exposure to 300 ppm (130.8 mg/kg) and 900 ppm (340.1 mg/kg) radioactively labelled 2-phenylpropene (given as percent of dose recovered)

      300 ppm

- **total recovered dose** in all excreta, residual carcass, and tissues (1.6 ± 1.6 % [6 h[1]], 33 ± 5.6 % (34.6 %[2]) [12 h], and 43.3 ± 4.9 % (77.9 %) [24 h], 13.2 ± 3.4 % (91.4 %) [48 h], and 3.1 ± 0.7 % (100 %) [72 h]
- **mainly via urine** (1.3 ± 1.3 % [6 h], 30.1 ± 6.4 % [12 h], 42.1 ± 5.0 % [24 h], 12.4 ± 3.8 % [48 h], and 2.3 ± 0.7 % [72 h]; **cumulative total: 88.2 ± 3.9 %**)
- little excretion via faeces (0.3 ± 0.3 % [6 h[1]], N/A [12 h], 1.0 ± 0.2 % [24 h], 0.7 ± < 0.05 % [48 h], and 0.1 ± < 0.05 % [72 h]; cumulative total: 2.2 ± 0.3 %) and exhalation (3.0 ± 0.8 % [12 h] and 0.2 ± 0.1 % [24 h]; cumulative total: 3.1 ± 0.9 %)

900 ppm

- **total recovered dose** in all excreta, residual carcass, and tissues (1.6 ± 1.6 % [6 h], 33 ± 5.6 % (34.6 %) [12 h], 43.3 ± 4.9 % (77.9 %) [24 h], 13.2 ± 3.4 % (91.4 %) [48 h], and 3.1 ± 0.7 % (100 %) [72 h]
- **mainly via urine** (3.6 ± 3.6 % [6 h[1]], 27.0 ± 4.5 % [12 h], 43.5 ± 5.1 % [24 h], 15.8 ± 2.9 % [48 h], and 2.5 ± 0.6 % [72 h]; **cumulative total: 92.4 ± 1.0 %**)
- little excretion via faeces (<0.05 ± <0.05 % [6 h[1]], N/A [12 h], 1.1 ± 0.2 % [24 h], 1.3 ± 0.2 % [48 h], and 0.2 ± 0.1 % [72 h]; cumulative total: 2.6 ± 0.2 %) and exhalation (2.1 ± 0.4 % [12 h], 1.1 ± 0.2 % [24 h], 0.2 ± < 0.05 % [48 h], and < 0.05 ± < 0.05 % [72 h]; cumulative total: 2.5 ± 0.4 %)

- concentration of radioactivity recovered from the **residual carcass and tissues** following 6 h exposure to 300 ppm (130.8 mg/kg) and 900 ppm (340.1 mg/kg) radioactively labelled 2-phenylpropene
  - at 72 h after initiation of the 6 h exposure: 5.9 ± 3.8 % (300 ppm; range: 2.6 – 10.1 %) and 1.6 ± 0.6 % (900 ppm; range 1.1 – 2.4 %) of the initial dose was recovered from analysed the **residual carcass and tissues**
  - highest concentrations of radioactivity were found in adipose tissue, bladder, liver, kidney and skin at 6, 24 (300 ppm only), and 72 h following initiation of exposure
- urinary metabolic profiling revealed:
  - 96 - 100 % of the initial dose was excreted
  - **2-phenyl-1,2-propanediol glucuronide** (~47 % [300 ppm] and ~30 % [900 ppm] of the initial dose) and **atrolactic acid** were found to be the most abundant metabolites (~38 % [300 ppm] and ~51 % [900 ppm] of the initial dose)
  - The level of atrolactic acid excretion was nearly twice as much as compared with intravenous administration

[1] immediately analysed following 6 h exposure

[2] cumulative percent of total radiolabelled dose excreted (all excreta, residual carcass, and tissues)

- Minor metabolites were identified as S-(2 hydroxy-2-phenylpropyl)-N-acetylcysteine (~10 % [300 ppm] and ~10 % [900 ppm] of the initial dose), 2-phenyl-1,2-propanediol (~2 % [300 ppm] and ~1 % [900 ppm] of the initial dose), and 2-phenyl propionic acid (~1 % [300 ppm] and ~1 % [900 ppm] of the initial dose)

- blood concentrations:
  - Recovery: 74 ± 10 % (300 ppm), 82 ± 9 % (900 ppm)
  - Radioactively labelled 2-phenylpropene concentrations dropped shortly after cessation of exposure following by a steady decrease at a slow rate
- blood metabolites:
  - four metabolites were found (2-phenyl-1,2-propanediol, atrolactic acid, 2-phenylpropionic acid, and unidentified metabolite)
  - 2-phenyl-1,2-propanediol was the most abundant metabolite
- toxicokinetic analysis (noncompartmental analysis of blood 2-phenylpropene concentration versus time data; n = 5):
  - elimination half-life: $t_{1/2}$ = 4.99 ± 1.14 h (300 ppm) and 2.81 ± 0.54 h (900 ppm)
  - area under the blood concentration-time curve extrapolated to time infinity: AUC_{INF} (hours × mg/L) = 26.8 ± 4.9 h (300 ppm) and 132.6 ± 33.5 h (900 ppm)
  - volume of distribution based on the terminal phase: V_z(L/kg) = 38.6 ± 15.1 h (300 ppm) and 11.2 ± 4.1 h (900 ppm)
  - clearance: Cl (L/hours per kg)= 5.3 ± 0.9 h (300 ppm) and 2.7 ± 0.7 h (900 ppm)

Proposed metabolic pathways:

1. epoxidation to yield α-methylstyrene oxide
2a. epoxid hydrolase to yield 2-phenyl-1,2-propanediol
2a1. glucuronidation to yield 2-phenyl-1,2-propanediol glucuronide
2a2. oxidation to yield atrolactic acid
2b. conjugation with glutathione to yield S-(2 hydroxy-2-phenylpropyl)-N-acetylcysteine
2c. 1,2 hydride shift and oxidation to yield
2-phenyl propionic acid

Human liver metabolites:

- **2-phenyl-1,2-propanediol** was the primary metabolite in the media of human liver slices
- The profile of metabolites was similar to what had been observed in the urine of rats

1.1.2 **Bardodej *et al.*, 1970**

Study reference:

Bardodej, Zdenek, and Eva Bardodejova. "Biotransformation of ethyl benzene, styrene, and alpha-methylstyrene in man." American Industrial Hygiene Association Journal 31.2 (1970): 206-209.

ECHA dissemination page: 002 Other | Experimental results

Test type

- Non-guideline study to determine the biotransformation of 2-phenylpropene in humans
- Not GLP compliant
- Method: 2-phenylpropene metabolites were analyses within the expired air and urine of study participants subjected to 2-phenylpropene by inhalation in a gas chamber for eight hours. The study mainly focused on ethyl benzene and styrene.
- Study details regarding the applied methodology and presentation of the results are insufficiently reported

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* not specified

Detailed study summary and results:

Material and methods

- Analytical methods:
    - Expired air: spectrophotometrical analysis following absorption in ethanol or direct measurement using a Hendrey mercury-vapor meter
    - Urine: paper chromatography

Results

- atrolactic acid was identified as a metabolite of 2-phenylpropene in the urine of exposed subjects

2 HEALTH HAZARDS

2.1 Skin sensitisation

2.1.1 Animal data

2.1.1.1 Study report Skin Sensitisation, 2016

Study reference:

Unpublished study report concerning skin sensitisation (2016)

ECHA dissemination page: 001 Key | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

Local Lymph Node Assay (LLNA)

Similar to OECD Guideline 429 and EU Method B.42

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* 99,783% (clear colourless liquid)
- *Batch number:* 011080116F

Test animals

- *Species, strain, sex:* mice, CBA/Ca (CBA/CaOlaHsd), females
- *No. of animals per sex per dose:* 5 mice / dose
- *Age and weight at the study initiation:* 8 to 12 weeks old; 15 to 23 g

Administration/exposure

- *Control group and treatment:*
  - *Control:* vehicle
  - *Treatment:* 2-phenylpropene (undiluted or diluted)
- *Route of induction and challenge induction*
  - *Topical:* dorsal surface of the ear
- *Induction*
  - *Concentration(s) and volume of test substance*
    - concentration: 100%, 50%, 25% v/v in acetone/olive 4:1
    - volume: 25µL / ear (50 µl in total)
  - *Induction vehicle:* acetone/olive oil (4:1 v/v)
  - *Note whether more than one dose was given:* topical application for three consecutive days (day 1, 2, 3)
  - *The spacing between doses:* five days following the first application (day 6), animal were injected with radioactive labelled thymidine

Results and discussion

- *Endpoint to measure effect:* proliferation of lymph nodes (stimulation index [SI], disintegrations per minute [DPM])
- *Statistical methods*: to determine statistical significance, parametric one way analysis of variance (ANOVA) and Dunnett's multiple comparison procedure were used for normally distributed and homogeneity of variances; otherwise, non-parametric Kruskal-Wallis Rank Sum and Mann-Whitney U test procedures were used
- *Results:* (1) SI (mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group):

Concentration (%v/v)	SI	Results
Vehicle	n.a.	n.a.

25	2.35	Negative
50	3.13	Positive
100	4.50	Positive
Positive control[3]	6.08	Positive

EC3 value: 46 % (concentration expected to cause a 3-fold increase in radioactive incorporation)

(2) DPM (mean DPM/animal ± SD)

Concentration (%v/v)	DPM
Vehicle	661.04 ± 262.06
25	1550.37 ± 630.17
50	2071.57 ± 478.40
100	2971.66 ± 288.36

2.1.1.2 Secondary source, 1983

Study reference:

Secondary source (study in Russian without detailed information on individual test design and results)

Akhmetov VM & Maksimov GG (1983)
Sb Nauch Trudov Ryazanskogo Med In-ta 80, 56-59.
Cited in: Sheftel VO (1990) Toxic Properties of Polymers and Additives, RAPRA Technology Ltd, Shrewsbury

ECHA dissemination page: 002 other | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- not specified, in vivo (non-LLNA)

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* not specified

Test animals

- *Species/strain/sex:* guinea pig
- *No. of animals per sex per dose:* not specified
- *Age and weight at the study initiation:* not specified

Administration/exposure

[3] hexyl cinnamic aldehyde (concentration: 25 % (v/v))

- no information

Results and discussion

- 2-phenylpropene was judged as skin sensitizer in guinea pigs

2.1.2 Human data

2.1.2.1 Unpublished human study, 1986

Study reference:

Unpublished study without detailed information on individual test design and results

ECHA dissemination page: 003 Other | Other result type

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- medical examination

Results

- possibly sensitising in humans

2.1.2.2 Ishii, et al. 2009

Study reference:

Ishii S, Ishii K, Imatanaka N, Fujino Y, Sasaki K & Nakadate M (2009): Evaluation for skin sensitization based on published literatures (existing information) of major PRTR designated chemical substances in Japan, Reg Toxicol Pharmacol 55, 43-51

ECHA dissemination page: 004 Other | Other result type

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- review of sensitisation data

Results

- sensitising in humans

2.1.2.3 NIOSH/OSHA - Occupational Health Guidelines for Chemical Hazards

Study reference:

Secondary source without detailed information on individual test design and results

Mackison FW, Stricoff RS & Partridge L (1981): NIOSH/OSHA - Occupational Health Guidelines for Chemical Hazards, DHHS(NIOSH) Publication No. 81-123 (3 VOLS), Washington

ECHA dissemination page: 005 Other | No specified result type

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- medical data

Results

- "overexposure" may cause sensitisation in humans

2.2 Germ cell mutagenicity

2.2.1 In vitro data

2.2.1.1 Study report mutagenicity, 1997

Study reference:

Unpublished study report concerning in vitro gene mutation study in bacteria (1997) in Japanese

ECHA dissemination page: 001 Key | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type:* in vitro gene mutation study in bacteria
- *Guideline:* equivalent or similar to OECD TG 471/472 (Bacterial Reverse Mutation Assay)
- *Limitation:* full study report is not available (limiting reporting)
- *Number of replicates:* two and three plates/test
- *Number of doses:* seven doses
- *Positive and negative control groups and treatment:*
    - positive control without S9:
      TA 100, TA 98, E. coli WP2 uvrA: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
      TA 1535: sodium azide
      TA 1537: 9-aminoacridine
    - positive control with S9:
      all strains: 2-aminoanthracene
    - negative control group: dimethylsulfoxide (DMSO)
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *GLP compliant:* yes

Test substance

- *Test material:* 2-phenylpropene
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* 99,6%

Administration/exposure

- *Strain: S. typhimurium* TA 1535, TA 1537, TA 98 and TA (Test 1)
  E. coli WP2 uvr A (Test 2)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 mix from rat liver
  - *quantity:* not specified
  - *induced or not induced:* induced
  - *chemicals used for induction:* phenobarbital and 5,6-benzoflavone
  - *co-factors used:* not specified
- *Test concentrations:* 0, 12.5, 25, 50, 100, 200, 400 µg/plate
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Statistical methods:* not specified

Results and discussion

- *Justification should be given for choice of tested dose levels:* not specified but cytotoxicity observed at ≥ 200 µg/plate (+/- S9)
- *Cytotoxic concentrations with and without metabolic activation:* yes, at ≥ 200 µg/plate (+/- S9)
- *Genotoxic effects:* **negative** (in all tested strains, at all tested concentrations, with and without metabolic activation)
- *Concurrent negative (solvent/vehicle) and positive control data:* valid
- *Confounding factors:* not specified

2.2.1.2 NTP, 2007 (bacterial gene mutation test)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: 004 Supporting | Experimental results

Detailed study summary and results:

Test type

- *Test type:* bacterial gene mutation test

- *Guideline:* equivalent or similar to OECD TG 471, performed according to the protocol established by Zeiger *et al.*, 1992 [Zeiger, Errol, et al. "Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals." Environmental and molecular mutagenesis 19.S21 (1992): 2-141.]
- *Number of replicates:* three
- *Number of doses:* five doses
- *Positive and negative control groups: s*olvent (negative control), positive control (+S9: 2-aminoanthracene (all strains); -S9: sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-o-phenylenediamine (TA98))
- *Deviations from OECD TG 471:*
    - strains detecting certain oxidising mutagens, cross-linking agents and hydrazines not included (*E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102)
    - solvent not specified including justification for choice
- *GLP compliant:* not specified

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Administration/exposure

- *Strain: Salmonella typhimurium* TA97, TA98, TA100, or TA1535
- *Type and composition of metabolic activation system:*
    - *species and cell type:* Sprague-Dawley rat or Syrian hamster liver
    - *quantity:* 10 % or 30 %
    - *induced or not induced:* induced
    - *chemicals used for induction:* aroclor 1254
    - *co-factors used:* cofactor mix
- *Test concentrations:* five doses (1 - 3333 µg/plate) tested in triplicates
- *Vehicle:* Solvent (not specified)
- *Statistical methods:* not specified

Results and discussion

- *Justification for choice of tested dose levels:* high dose limited by cytotoxicity
- *Cytotoxic concentrations with and without metabolic activation:* observed at the highest dose (333 – 3333 µg/plate)
- *Genotoxic effects:* **negative** (in all tested strains, at all tested concentrations, with and without metabolic activation)
- *Confounding factors:* not reported

2.2.1.3 Study report mutagenicity, 1991

Study reference:

Unpublished study report concerning in vitro gene mutation study in bacteria (1991)

ECHA dissemination page: 005 Supporting | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type: b*acterial gene mutation test
- *Guideline:* equivalent or similar to OECD TG 471
- Full study report is not available (limiting reporting)
- *Number of replicates:* three
- *Number of doses*: five doses
- *Positive and negative control groups:*
    - positive controls without S9: 2-nitrofluorene (TA 98, TA 1538), sodium azide (TA 100, TA 1535), ICR-191 (TA 1537)
    - positive control with S9: 2-aminoanthracene (all strains)
    - negative control: DMSO (solvent)
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *GLP compliant:* yes

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* 99%
- *Lot No:* 07726 LW

Administration/exposure

- *Strain:* S. typhimurium, other: TA 98, TA 100, TA 1535, TA 1537, TA 1538e
- *Type and composition of metabolic activation system:*
    - *species and cell type :* adult male SD rat liver
    - *quantity:* not specified
    - *induced or not induced:* induced
    - *chemicals used for induction:* aroclor 1254
    - *co-factors used:* not specified
- *Test concentrations :* 5 doses (0 - 5000 µg/plate)
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Statistical methods:* not specified

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies):* Dose-range finding study
- *Cytotoxic concentrations with and without metabolic activation:* observed at ≥ 100 µg/plate
- *Genotoxic effects:* **negative** (in all tested strains, at all tested concentrations, with and without metabolic activation)
- *Concurrent negative (solvent/vehicle) and positive control data:* valid
- *Confounding factors:* not reported

2.2.1.4 Study report mutagenicity, 1989

Study reference:

Unpublished study report concerning in vitro gene mutation study in bacteria (1989)

ECHA dissemination page: 006 Supporting | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type: b*acterial gene mutation test
- *Guideline:* similar to OECD TG 471, performed according to the protocol by Ames et al. (1975) Mutat Res 31, 347 – 364
- *Limitation:* full study report is not available (limiting reporting)
- *Number of replicates:* two
- *Number of doses:* not specified
- *Positive and negative control groups:* not specified
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *GLP compliant:* not specified

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* not specified

Administration/exposure

- *Strain : Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538
- *Type and composition of metabolic activation system:*
  - *species and cell type:* rat liver
  - *quantity:* not specified
  - *induced or not induced:* induced
  - *chemicals used for induction:* aroclor
  - *co-factors used:* not specified

- *Test concentrations:* 0 - 5000 µg/plate
- *Vehicle:* acetone
- *Statistical methods:* not specified

Results and discussion

- *Justification should be given for choice of tested dose levels:* not specified
- *Cytotoxic concentrations with and without metabolic activation:* observed at ≥ 100 µg/plate
- *Genotoxic effects:* **negative** (in all tested strains, at all tested concentrations, with and without metabolic activation)
- *Concurrent negative (solvent/vehicle) and positive control data:* not specified
- *Confounding factors:* not specified

2.2.1.5 Study report mutagenicity, 1997

Study reference:

Unpublished study report concerning in vitro cytogenicity / chromosome aberration study in mammalian cells (1997) in Japanese

ECHA dissemination page: 002 Key | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type:* in vitro cytogenicity / chromosome aberration study in mammalian cells
- *Guideline:* equivalent or similar to OECD TG 473
- *Limitation:* full study report is not available (limiting reporting)
- *Number of replicates:* two
- *number of doses*: four doses
- *Positive and negative control groups:*
    - positive controls without S9 mix: mitomycin C;
    - positive controls with S9 mix: Cyclophosphamide
    - negative control: untreated control and solvent control
- *GLP compliant:* yes

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* 99,6%

Administration/exposure

- *Cell line:* Chinese hamster lung cells (CHL/IU)
- *Type and composition of metabolic activation system:*
    - *species and cell type:* rat liver

  - *quantity:* not specified
  - *induced or not induced:* induced
  - *chemicals used for induction:* phenobarbital and 5,6-benzoflavone
  - *co-factors used:* not specified
- *Test concentrations:*
  - without S9 mix (continuous treatment): 0, 0.04, 0.09 and 0.17 mg/mL
  - without S9 mix (short-term treatment): 0, 0.04, 0.09 and 0.17 mg/mL
  - with S9 mix (short-term treatment): 0, 0.06, 0.12 and 0.23 mg/mL
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Treatment time:*
  - continuous treatment: 24 h and 48 h
  - short-term treatment: 6 h
- *Sampling time (after beginning of treatment):* not specified
- *Statistical methods:* not specified

Results and discussion

- *Justification should be given for choice of tested dose levels:* not specified
- *Cytotoxic concentrations with and without metabolic activation:* observed at $\geq$ 0.17 mg/mL
- *Genotoxic effects:* **negative** (with or without an exogenous metabolic activation system)
- *Concurrent negative (solvent/vehicle) and positive control data:* valid
- *Confounding factors:* not specified

2.2.1.6 NTP, 2007 (*in vitro* chromosomal aberration)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: 007 Supporting | Experimental results

Detailed study summary and results:

Test type

- *Test type:* in vitro cytogenicity / chromosome aberration study in mammalian cells
- *Guideline:* equivalent or similar to OECD TG 473, performed according to the protocol established by Galloway *et al.*, 1987 [Galloway, S. M., et al. "Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals." Environmental and molecular mutagenesis 10.S10 (1987): 1-35.]
- *Number of doses:* three to four

- *Positive and negative control groups:*
    - positive control with S9: cyclophosphamide
    - positive control without S9: mitomycin-C
    - negative control: solvent control (DMSO)
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *Number of metaphases analysed:* 200 first-division metaphase cells were/dose (characterised by good morphology and completeness of karyotype
- *GLP compliant:* not specified

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Administration/exposure

- *Cell line:* Chinese hamster ovary cells (CHO-W-B1)
- *Type and composition of metabolic activation system:*
    - *species and cell type:* rat liver S9
    - *induced or not induced:* induced
    - *chemicals used for induction: Aroclor 1254*
    - *co-factors used:* cofactor mix
- *Test concentrations:*
    - 1st trail: 0, 100.5, 150, 200 μg/mL
    - 2nd trail +/-S9: 0, 33.7, 125.7, 251.3 μg/mL
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Treatment time:*
    - -S9: 10 h (8 h 2-phenylpropene + 2 h colcemid)
    - +S9: 2 h
- *Sampling time (after beginning of treatment):*
    - -S9: 10 h
    - +S9: 12 h (8 h + 2 h colcemid)
- *Statistical methods:* $P \leq 0.05$ considered statistically significant for pairwise comparison and $P \leq 0.015$ for the trend test

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* 251.3 μg/mL (+/-S9)
- *Genotoxic effects:* **negative** (at all tested concentrations, with and without metabolic activation)

- *Confounding factors:* no confounding factor reported

2.2.1.7 Study report mutagenicity, 1991

Study reference:

Unpublished study report concerning in vitro cytogenicity / chromosome aberration study in mammalian cells (1991)

ECHA dissemination page: 008 Supporting | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type:* in vitro cytogenicity / chromosome aberration study in mammalian cells
- *Guideline:* Equivalent or similar to OECD TG 473
- *Limitation:* full study report is not available (limiting reporting)
- *Number of replicates:* not specified
- *Number of doses, justification of dose selection:* five doses
- *Positive and negative control groups and treatment:*
    - positive control with S9: cyclophosphamide
    - positive control without S9: triethylenemelamine
    - negative control: untreated negative control (complete medium or S9 reaction mixture) and solvent control (DMSO)
- *Number of metaphases analysed:* not specified
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *GLP compliant:* yes

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* 99%
- *Batch number:* 07726 LW

Administration/exposure

- *Cell line:* Chinese hamster Ovary (CHO)
- *Type and composition of metabolic activation system:*
    - *species and cell type:* male SD rats (S9)
    - *quantity:* not specified
    - *induced or not induced:* induced
    - *chemicals used for induction:* Aroclor 1254

  - *co-factors used:* not specified
- *Test concentrations:*
  - preliminary study: 0 – 5 µl/mL
  - main study: 0 – 0.15 µl/mL (0.019, 0.025, 0.05, 0.1 and 0.15 µL/mL).
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Treatment time*: not specified
- *Sampling time (after beginning of treatment):*
  - -S9: 18 h
  - +S9: 20 h
- *Statistical methods:* Fischer's exact test

Results and discussion

- *Justification should be given for choice of tested dose levels:* preliminary study
- *Cytotoxic concentrations with and without metabolic activation:* observed at ≥ 0.1 µL/mL
- *Genotoxic effects:* **negative** (at all tested concentrations, with and without metabolic activation)
- *Concurrent negative (solvent/vehicle) and positive control data:* valid
- *Confounding factors:* no confounding factor reported

2.2.1.8 Study report mutagenicity, 1991

Study reference:

Unpublished study report concerning in vitro gene mutation study in mammalian cells (1991)

ECHA dissemination page: 009 Supporting | Experimental results

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type:* in vitro gene mutation study in mammalian cells
- *Guideline:* equivalent or similar to OECD TG 476
- *Limitation:* full study report is not available (limiting reporting)
- *Number of replicates:* not specified
- *Number of doses:* five
- *Positive and negative control groups:*
  - positive control without S9: EMS
  - positive control with S9: B(a)P
  - negative control: untreated negative control (growth medium); solvent control (DMSO)
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified

- *GLP compliant*: yes

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* 99%
- *Batch number:* 07726 LW

Administration/exposure

- *Cell line:* Chinese hamster Ovary (CHO)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* male SD rat liver (S9)
  - *quantity:* not specified
  - *induced or not induced:* induced
  - *chemicals used for induction:* aroclor
  - *co-factors used:* not specified
- *Test concentrations:*
  - Initial assay: 0.15, 0.125, 0.1, 0.075 and 0.05 µL/mL +/- S9
  - Confirmatory assay: 0.115, 0.1, 0.085, 0.075 and 0.05 µL/mL (-S9) and at 0.135, 0.125, 0.1, 0.075 and 0.05 µL/mL (+S9)
- *Treatment time*: not specified
- *Sampling time (after beginning of treatment):* not specified
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Statistical methods*: not specified

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* as measured by cloning efficiencies relative to the solvent controls (highest to lowest dose)
  - Without S9
    Initial assay: 0, 0, 21, 78 and 92 %
    Confirmatory assay: 8, 38, 97, 100 and 90 %
  - With S9
    Initial assay: 0, 47, 80, 102 and 97 %
    Confirmatory assay: 4, 30, 106, 106 and 96 %
- *Genotoxic effects:* **negative** (at all tested concentrations, with and without metabolic activation)
- *Concurrent negative (solvent/vehicle) and positive control data:* valid
- *Confounding factors:* no confounding factor reported

2.2.1.9 NTP, 2007 (*in vitro* sister chromatid exchange assay in mammalian cells)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: 003 Key | Experimental results

Detailed study summary and results:

Test type

- *Test type:* in vitro mammalian sister chromatid exchange test (in vitro DNA damage and/or repair study)
- *Guideline:* equivalent or similar to OECD TG 479 (deleted) to OECD Guideline 479
- *Number of doses, justification of dose selection:* three to four
- *Positive and negative control groups and treatment*
    - positive control without S9: mitomycin-C
    - positive control with S9: cyclophosphamide
    - negative control: solvent control (DMSO)
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *Criteria for evaluating results:* positive response defined as ≥ 20% increase over the solvent control
- *GLP compliant*: yes

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Administration/exposure

- *Cell line:* Chinese hamster ovary (CHO) cells
- *Type and composition of metabolic activation system:*
    - *species and cell type:* rat liver S9
    - *induced or not induced:* induced
    - *chemicals used for induction:* aroclor 1254
    - *co-factors used:* cofactor mix
- *Test concentrations:*
    - without S9: 5, 16.7, 50, 166.7 µg/mL
    - with S9: 5, 16.7, 50, 166.7 µg/mL (trial 1); 50, 124.4, 149.9 µg/mL (trial 2)
- *Treatment time:*
    - without S9: 25.5 h

  - with S9: 2 h
- *Sampling time*
  - without S9: 2 h
  - with S9: 25.2 – 25.5 h
- *Number of total cells scored:* 50
- *Vehicle:* dimethylsulfoxide (DMSO)
- *Statistical methods*: linear regression trend test

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* observed at 166.7 µg/mL +/-S9
- *Genotoxic effects*: **positive**
- *Concurrent negative (solvent/vehicle) and positive control data: valid*
- *Confounding factors:* not reported
- *Statistical results:*
  - significantly increased frequency of sister chromatid exchanges (SCEs) in cells exposed to 50, 124.4, 149.9 µg/mL with metabolic activation (+S9)
  - negative without metabolic activation (-S9)

Concentration	SCEs/Chromosome (%[#])
Trial 1 +S9	
DMSO	0.36
5 µg/mL	0.38 (5.2 %)
16.7 µg/mL	0.36 (-1.8 %)
50 µg/mL	0.46 (28.4 %)*
166.7 µg/mL	cytotoxic
Trial 2 +S9	
DMSO	0.34
50 µg/mL	0.47 (39.6 %)*
124.4 µg/mL	0.51 (49.2 %)*
149.9 µg/mL	0.62 (82.8 %)*

[#]relative change of SCEs / chromosome
*positive response (≥ 20 % of the ctrl)

2.2.1.10 Norppa *et al.*, 1983

Study reference:

Norppa, Hannu, and Harri Vainio. "Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes." Mutation Research/Genetic Toxicology 116.3-4 (1983): 379-387.

ECHA dissemination page: 010 Supporting | Experimental results

Detailed study summary and results:

Test type

- *Test type:* in vitro sister chromatid exchange test in human lymphocytes (DNA damage and/or repair study)
- *Guideline:* no guideline followed
- *Limitations:* study details are insufficiently reported
- *Number of replicates:* two
- *Number of doses, justification of dose selection:* not specified
- *Positive and negative control groups and treatment*
    - positive control: no
    - negative control: solvent control (acetone)
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *GLP compliant*: no

Test substance

- *Test material:* 2-phenylpropene
- *Degree of purity:* 98 – 99 %

Administration/exposure

- *Cell type*: human lymphocytes (derived from a healthy male donor) in a whole blood culture
- *Type and composition of metabolic activation system:*
    - *cell type:* presumably erythrocyte-mediated
    - *induced or not induced:* not induced
- *Test concentrations:* not specified
- *Treatment time:* 48 h (first 24 h without treatment; total culture time: 72 h)
- *Sampling time*: immediate after treatment (72 h)
- *Number of total cells scored:* 50
- *Vehicle:* acetone
- *Statistical methods:* t-test (1-tailed) and the determination of a dose-response by testing the significance of the coefficient of linear regression in the t test (2-tailed)

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* not specified
- *Genotoxic effects:* weakly positive
- *Statistical results*
    - 0.33 mM: statistically significantly different (week effect: less than twice as many SCEs/cell as compared to the control)
    - 1 mM: not statistically significantly different
- According to the authors of the study, the test substance is presumably converted to a reactive metabolite due to erythrocyte-mediated activation

2.2.1.11 Norppa *et al.*, 1984

Study reference:

Norppa, H and Tursi F. "Erythrocyte-mediated metabolic activation detected by SCE." (1984): 547 - 559.

ECHA dissemination page: 011 Other | Experimental results

Detailed study summary and results:

Test type

- *Test type:* in vitro sister chromatid exchange test in human lymphocytes (DNA damage and/or repair study)
- *Guideline:* no guideline followed
- *Limitations:* study details are insufficiently reported
- *Number of replicates:* not specified
- *Number of doses, justification of dose selection:* not specified
- *Positive and negative control groups and treatment*
    - positive control: no
    - negative control: solvent control
- *Justification for choice of vehicle:* not specified
- *Solubility and stability of the test substance in vehicle if known:* not specified
- *GLP compliant*: no

Test substance

- *Test material:* 2-phenylpropene

Administration/exposure

- *Cell type:* human lymphocytes cultured isolated (< 1000 erythrocytes/ml) or with whole-blood ($2x10^8$)
- *Type and composition of metabolic activation system:*
    - *cell type:* erythrocyte-mediated
    - *induced or not induced:* not induced
- *Test concentrations:* isolated culture and whole-blood culture: 1 and 2 mM
- *Treatment time:* 48 h (first 24 h without treatment; total culture time: 72 h)
- *Sampling time*: immediate after treatment (72 h)
- *Number of total cells scored:*
    - whole blood: 50
    - isolated lymphocytes: 33-50
- *Vehicle:* not specified
- *Statistical methods:* t-test (1-tailed)

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* not specified
- *Genotoxic effects:* positive (treatment-related induction of SCEs in isolated and whole-blood cultures)
- *Statistical results:*

Concentration	SCEs/cell ± S.E.
Whole blood	
Control	7.6 ± 0.4
1 mM	8.9 ± 0.4*
2 mM	11.4 ± 0.6***
Isolated lymphocytes	
Control	10.1 ± 0.5
1 mM	11.1 ± 0.6
2 mM	12.1 ± 0.6**

*P < 0.05; **P < 0.01;***P < 0.001

2.2.2 Animal data

2.2.2.1 NTP, 2007 (mouse peripheral blood micronucleus test)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: key study

Detailed study summary and results:

Test type

- *Test type:* in vivo micronucleus test in peripheral blood erythrocyte integrated in the 90-day subchronic inhalation toxicity study
- *Guideline:* similar to OECD TG 474 according to a protocol published by Witt *et al.*, 2000 (Witt, Kristine L., et al. "Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program." Environmental and Molecular Mutagenesis 36.3 (2000): 163-194.) and MacGregor *et al.*, 1990 (MacGregor, James T., et al. "The in vivo erythrocyte micronucleus test: measurement at steady state increases assay efficiency and permits integration with toxicity studies." Toxicological Sciences 14.3 (1990): 513-522.)
- *Deviations from OECD TG 474:*
    - no positive control
    - number of analysed polychromatic erythrocytes (PCEs) less than recommended
- *GLP compliant:* yes (21 CFR, Part 58)

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Test animals

- *Species, strain, sex:* mice, $B6C3F_1$, male/females
- *No. of animals per sex per dose:* 10 (8 ♀ in the high-dose group)
- *Age and weight at the study initiation:* approx. 6 weeks, approx. ♂ 23 g (av.) and ♀ 19 g (av.)

Administration/exposure

- *Doses/concentration levels:* 0, 75, 150, 300, 600, 1000 ppm
- *Route of administration:* whole-body inhalation (vapour)
- *Duration of study:* 3 month exposure (91 days)
- *Frequency of treatment:* 6 h/d plus T_{90} (12 minutes), 5 d/w (except holidays), 65 treatments in total
- *Sampling time (time interval between final treatment and cell sampling):* 24 h
- *Control group and treatment:* yes
- *Positive and negative (vehicle/solvent) control data:* chamber control (negative), no positive control
- *Methods of slide preparation:* methanol-fixed slides stained with acridine orange
- *Criteria for scoring and number of cells analysed per animal:*
    - Smears of peripheral blood samples obtained at the end of the 90-day exposure duration prepared and fixed yield methanol-fixed slides (slide-based approach)
    - number of micronuclei determined in normochromatic erythrocytes (NCEs) from animals of all exposure groups – 10000 NCE scored per sample (Witt et al., 2000)
    - number of micronuclei determined in polychromatic erythrocytes (PCEs) of control and 1000 ppm mice – 1000 PCEs scored per sample (Witt et al., 2000)
    - the proportion of PCEs among erythrocytes was determined as a marker for bone marrow toxicity – number of PCEs among ~5000/10000 erythrocytes (Witt et al., 2000)
- *Statistical methods:* one-tailed Cochran-Armitage trend test followed by pairwise comparison of every exposure group with the control

Results and discussion

- *Genotoxic effects:* negative in ♂ (PCEs/NCEs); negative in ♀ (PCEs), positive in ♀ (NCEs)

Number of micronuclei in peripheral blood erythrocytes of mice (as presented in the NTP report):

Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated Cells / 1000 Cells				PCEs (%) (mean ± SE)
		PCEs (mean ± SE)	P Value	NCEs (mean ± SE)	P Value	
Males						
0	10	3.90 ± 0.66		5.30 ± 0.50		3.70 ± 0.17

75	10			5.80 ± 0.44	0.3171	3.36 ± 0.09
150	10			5.80 ± 0.63	0.3171	3.12 ± 0.10
300	10			5.00 ± 0.65	0.6165	3.20 ± 0.15
600	10			4.60 ± 0.45	0.7597	3.18 ± 0.17
1000	10	5.00 ± 0.58	0.1213	6.30 ± 1.02	0.1759	3.27 ± 0.17
Trend test:				P = 0.346		
Females						
0	10	4.10 ± 0.59		5.10 ± 0.46		3.76 ± 0.19
75	10			2.40 ± 0.43	0.9991	3.19 ± 0.10
150	10			2.90 ± 0.90	0.9931	3.42 ± 0.15
300	10			3.60 ± 0.48	0.9465	3.45 ± 0.14
600	10			5.30 ± 0.42	0.4221	3.27 ± 0.14
1000	8[#]	4.75 ± 0.59	0.2561	**9.13 ± 0.77**	**0.0006**	3.53 ± 0.29
Trend test:				**P ≤ 0.001**		

[#] *two death occurred on day 3 of treatment*

- *Concurrent positive control data:* no positive control
- *Statistical results:*
  - positive trend in the frequency of micronuclei in NCEs of ♀ mice with statistical significance
  - increased number of micronuclei in NCEs of ♀ mice at 1000 ppm with statistical significance
- *Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:*
  - no treatment-related effect on the proportion of PCE among 1000 erythrocytes, indicating absence of bone marrow toxicity
  - slightly decreased erythron (lower values of haemoglobin and erythrocytes counts) in ♀ at 1000 ppm with statistical significance
- *Mortality at each dose level by sex:* **two** ♀ **died** on day 3 in the highest dose group
- *Clinical signs:*
  - sedation (moderate to severe) in ♂ at 1000 ppm
  - ataxia in ♂ and ♀ at 1000 ppm
- *Body weight changes:*
  - reduced final mean body weight in ♂ and ♀ with statistical significance
    - ♂: 600 ppm (- 13 %), 1000 ppm (- 17 %)
    - ♀: 75 ppm (- 9 %), 300 ppm (- 9 %), **1000 ppm (- 11 %)**
  - reduced final mean body weight gains in ♂ and ♀ at ≥ 300 ppm with statistical significance
    - ♂: 300 ppm (- 14 %), 600 ppm (- 34 %), 1000 ppm (- 43 %)
    - ♀: 300 ppm (- 23 %), 600 ppm (- 12 %), **1000 ppm (- 32 %)**
- *Food/water consumption:* No changes reported
- *Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable:* systemic toxicity observed

2.2.2.2 Rim et al., 2012

Study reference:

Kyung-Taek, Rim, et al. "A study of micronucleus induction with isopropenyl benzene and trimellitic anhydride in bone marrow cells of Institute for Cancer Research (ICR) mice." Journal of Environmental Chemistry and Ecotoxicology 4.6 (2012): 110-115.

No included on ECHAs dissemination page

Detailed study summary and results:

Test type

- *Test type:* in vivo micronucleus test in bone marrow cells
- *Guideline:* similar to OECD TG 474
- *Deviations from OECD TG 474:*
    - no evidence of bone marrow exposure
    - number of analysed PCEs and total erythrocytes less than recommended
    - no historical control data reported
- *GLP compliant:* yes

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* > 99 %

Test animals

- *Species, strain, sex:* mice, Institute for Cancer Research (ICR) mice, males
- *No. of animals per sex per dose:* 6
- *Age and weight at the study initiation:* 7 weeks, 36-39 g

Administration/exposure

- *Doses/concentration levels:* 0, 500, 1000, 2000 mg/kg
- *Vehicle:* olive oil
- *Route of administration:* oral
- *Treatment:* single application
- *Sampling time:* 24 h following oral administration
- *Control groups and treatment:* yes
- *Positive and negative (vehicle/solvent) control data*
    - *negative control:* solvent control (olive oil)
    - *positive control:* mitomycin C (MMC) 0.5 mg/kg b.w.
- *Criteria for scoring and number of cells analysed per animal*
    - no. of MN determined in at least 2000 PCEs

- bone marrow toxicity determined by the no. of PCEs in 500 erythrocytes

- *Statistical methods:* One Way ANOVA ($P < 0.001$) test and the Dunnett's method ($P < 0.05$)

Results and discussion

- *Effect on PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte) ratio:* not altered with statistical significance
- *Genotoxic effects:* negative
- *Concurrent positive control data:* valid
- *Statistical results*

Dose (mg/kg)	MNPCE [%]*
Ctrl	0.08 ± 0.07
500	0.06 ± 0.05
1000	0.06 ± 0.05
2000	0.20 ± 0.15
Positive ctrl	0.62 ± 0.62

* frequency of micronucleated polychromatic erythrocytes (MNPCE) per 2000 cells (mean ± SD)

- *Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable:* uncertain as no signs of treatment-related toxicity in the bone marrow was observed

2.3 Carcinogenicity

2.3.1 Animal data

2.3.1.1 NTP, 2007 (rats)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: 001 Key | Experimental results

Detailed study summary and results:

Test type

- *Test type:* 2-years Carcinogenicity study
- *Guideline:* Similar to OECD TG 451 (NTP standards)
- *GLP compliant:* yes (21 CFR, Part 58)

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*

- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Test animals

- *Species, strain, sex:* rats, F344/N, male/females
- *No. of animals per sex per dose:* 50
- *Age and weight at the study initiation:* approx. 6 weeks, approx. 75 g (av.)

Administration/exposure

- *Route of administration:* whole-body inhalation (vapour)
- *Duration of test/exposure period:* 2 years (105 weeks)
- *Doses/concentration levels, rationale for dose level selection:* 0, 100, 300, 1000 ppm (based on the preliminary 90-day subchronic toxicity study)
- *Frequency of treatment:* 6 h/d plus T_{90} (12 minutes), 5 d/w (except holidays)
- *Control group and treatment:* yes
- *Historical control data:* available
- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* 2-phenylpropene vapour was generated and transported to the exposure chamber, vapour concentration and test substance stability was monitored (online GC), T_{90} of 12 minutes

For inhalation studies:

- *Type of inhalation exposure and test conditions:* inhalation exposure chamber
- *Method of exposure:* whole-body
- *Analytical verification of test atmosphere concentrations:* online GC

Results and discussion

- *Mortality:* no significant difference between control and treatment groups
- *Clinical signs:* no clinical signs observed
- *Body weight gain:* reduced mean body weight in ♂ and ♀ at the highest dose level (5 – 10 %) during the 2nd year of the study
- *Food/water consumption:* not specified
- *Ophthalmoscopic examination:* n/a
- *Clinical chemistry:* n/a
- *Haematology:* n/a
- *Urinalysis:* n/a
- *Organ weights:* n/a
- *Necropsy findings:* performed on all animals
- *Histopathological findings:*

histopathology performed on the following tissues: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

Kidney:

- increased incidence of **hyperplasia of the renal tubules** in ♂ rats at 1000 ppm without statistical significance

  no. of animals with lesions (percentage, severity: 1 minimal, 2 mild, 3 moderate, 4 marked):
  ♂ rats (0, 100, 300, 1000 ppm): 1/50 (2 %, 1.0), 0/50, 1/50 (2 %, 1.0), 4/50 (8 %, 2.3)

- increased incidence of **mineralization of the renal papilla** (commonly associated with α2u-globulin nephropathy) in ♂ rats at 1000 ppm and ♀ rats at 300 and 1000 ppm with statistical significance. Mineralization in ♂ rats was mostly linear and in ♀ rats characterized by laminated concretions

  no. of animals with lesions (percentage, severity: 1 minimal, 2 mild, 3 moderate, 4 marked):
  ♂ rats (0, 100, 300, 1000 ppm): 12/50 (24 %, 1.1), 16/50 (32 %, 1.0), 10/50 (20 %, 1.0), 33/50** (66 %, 1.4)
  ♀ rats (0, 100, 300, 1000 ppm): 1/49 (2 %, 1.0), 6/50 (12 %, 1.0), 8/50* (16 %, 1.0), 7/50* (14 %, 1.0)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

Nose:

- increased incidence of **basal cell hyperplasia** of the olfactory epithelium in ♂ and ♀ rats of all treatment groups with statistical significance.

  no. of animals with lesions:
  ♂ rats (0, 100, 300, 1000 ppm): 0/50 (0 %), 17/50** (34 %), 18/50** (36 %), 43/49** (88 %)
  ♀ rats (0, 100, 300, 1000 ppm): 0/49 (0 %), 14/49** (29 %), 30/50** (60 %), 49/50** (98 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

- increased incidence of **degeneration of the olfactory epithelium** in ♂ rats at 1000 ppm and ♀ rats at 300 and 1000 ppm with statistical significance

  no. of animals with lesions:
  ♂ rats (0, 100, 300, 1000 ppm): 1/50 (2 %), 3/50 (6 %), 3/50 (6 %), 16/49** (33 %)
  ♀ rats (0, 100, 300, 1000 ppm): 1/49 (2 %), 1/49 (2 %), 7/50* (14 %), 24/50** (48 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

- *Tumour incidence data by sex, dose and tumour type:*

  Kidney:

- increased incidence of **renal tubule adenoma and carcinoma** (combined)[4] in ♂ rats at 1000 ppm with statistical significance

  no. of animals with lesions:

  ♂ rats (0, 100, 300, 1000 ppm): 1/50 (2 %), 2/50 (4 %), 3/50 (6 %), 7/50* (14 %); *p = 0.006*†

  (* p ≤ 0.05, ** p ≤ 0.01, †trend test)

  historical control incidence (inhalation): 4/399 (1.0 % ± 1.1 %; range 0 % - 2 %)

- **renal tubule adenoma** (includes multiple)

  no. of animals with lesions:

  ♂ rats (0, 100, 300, 1000 ppm): 1/50 (2 %), 2/50 (4 %), 2/50 (4 %), 5/50 (10 %)

  historical control incidence (inhalation): 3/399 (0.8 % ± 1.0 %; range 0 % - 2 %)

- **renal tubule carcinoma**

  no. of animals with lesions:

  ♂ rats (0, 100, 300, 1000 ppm): 0/50 (0 %), 0/50 (0 %), 1/50 (2 %), 2/50 (4 %)

  historical control incidence (inhalation): 1/399 (0.3 % ± 0.7 %; range 0 % - 2 %)

- **secondary neoplasms:** renal carcinoma metastasised to the lung: ♂ rats (1000 ppm): 2/50 (4 %)

Testis:

- increased incidence of **interstitial cell adenoma** in all treatment groups with statistical significance

  no. of animals with lesions:
  ♂ rats (0, 100, 300, 1000 ppm): 33/50 (66 %), 44/50* (88 %), 41/50 (82 %), 44/50* (88 %); *p = 0.007*†

  (* p ≤ 0.05, ** p ≤ 0.01, †trend test)

  historical control incidence (inhalation): 316/399 (79.2 % ± 3.9 %; range 72 % - 84 %)

All Organs:

- increased incidence of **mononuclear cell leukaemia** in ♂ rats at 1000 ppm with statistical significance

  no. of animals with lesions :

  ♂ rats (0, 100, 300, 1000 ppm): 26/50 (52 %), 32/50 (64 %), 29/50 (58 %), 38/50* (76 %); *p = 0.018*†

  (* p ≤ 0.05, ** p ≤ 0.01, †trend test)

  historical control incidence (inhalation): 188/399 (47.1 % ± 10.3 %; range 32 % - 66 %)

[4] data of the standard and extended evaluations together (single and step section combined)

- *Local or multi-site responses:* multi-site response in male rats
- *Progression of lesions to malignancy:* proliferative lesions (hyperplasia) were observed (incidence not significant) in the kidney of male rats presumably progressing to adenoma and carcinoma (morphologic continuum)
- *Gender and/or species-specific responses:* neoplastic effects only observed in male rats
- *Mode of action (genotoxic, non-genotoxic):* uncertain
- *Tumour latency:* n/a
- *Statistical methods:* survival analyses using Cox's method, incidences of neoplasms or nonneoplastic lesions using the poly-k test

2.3.1.2 NTP, 2007 (mice)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

Detailed study summary and results:

Test type

- *Test type:* 2-years Carcinogenicity study
- *Guideline:* Similar to OECD TG 451 (NTP standards)
- *GLP compliant:* yes (21 CFR, Part 58)

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #:* BNW 13871-4, BNW 13871-54

Test animals

- *Species, strain, sex:* mice, $B6C3F_1$, male/females
- *No. of animals per sex per dose:* 50
- *Age and weight at the study initiation:* approx. 6 weeks, approx. 21 g (av.)

Administration/exposure

- *Route of administration:* whole-body inhalation (vapour)
- *Duration of test/exposure period:* 2 years (105 weeks)
- *Doses/concentration levels, rationale for dose level selection:* 0, 100, 300, 600 ppm (based on the preliminary 90-day subchronic toxicity study)
- *Frequency of treatment:* 6 h/d plus T_{90} (12 minutes), 5 d/w (except holidays)

- *Control group and treatment:* yes
- *Historical control data:* available
- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* 2-phenylpropene vapour was generated and transported to the exposure chamber, vapour concentration and test substance stability was monitored (online GC), T_{90} of 12 minutes

For inhalation studies:

- *Type of inhalation exposure and test conditions:* inhalation exposure chamber
- *Method of exposure:* whole body
- *Analytical verification of test atmosphere concentrations:* online GC

Results and discussion

- *Mortality:* no significant difference between control and treatment groups
- *Clinical signs:* no clinical signs observed
- *Body weight gain:* reduced mean body weight in ♂ and ♀ (after week 13) at the highest dose level
    - reduced final mean body weight in ♂ (- 8 %) and in ♀ (- 9 %) at 600 ppm
    - reduced final mean body weight gains in ♂ (- 12 %) and in ♀ (- 20 %) at 600 ppm
- *Food/water consumption:* not specified
- *Ophthalmoscopic examination:* n/a
- *Clinical chemistry:* n/a
- *Haematology:* n/a
- *Urinalysis:* n/a
- *Organ weights:* n/a
- *Necropsy findings:* performed on all animals
- *histopathological findings:*

  Histopathology performed on the following tissues: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

  Nose:

  - increased incidence of **metaplasia of the olfactory epithelium** in ♂ and ♀ mice in all treatment groups with statistical significance

    no. of animals with lesions:
    ♂ mice (0, 100, 300, 600 ppm): 6/50 (12 %), 47/50** (94 %), 49/50** (98 %), 49/50** (98 %)
    ♀ mice (0, 100, 300, 600 ppm): 2/49 (4 %), 49/49** (100 %), 47/50** (94 %), 50/50** (100 %)
    (* $p \leq 0.05$, ** $p \leq 0.01$)

- increased incidence of **hyperplasia of the submucosal glands (olfactory epithelium)** in ♂ and ♀ mice in all treatment groups with statistical significance

  no. of animals with lesions:
  ♂ mice (0, 100, 300, 600 ppm): 4/50 (8 %), 50/50** (100 %), 50/50** (100 %), 50/50** (100 %)
  ♀ mice (0, 100, 300, 600 ppm): 3/49 (6 %), 49/49** (100 %), 50/50** (100 %), 50/50** (100 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

- increased incidence of **atrophy of the olfactory epithelium** in ♂ mice at 300 and 600 ppm with statistical significance

  no. of animals with lesions:
  ♂ mice (0, 100, 300, 600 ppm): 0/50 (0 %), 2/50 (4 %), 8/50** (16 %), 12/50** (24 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

Forestomach:

- increased incidence of **hyperplasia of the epithelium** in ♂ mice at 300 and 600 ppm with statistical significance

  no. of animals with lesions:
  ♂ mice (0, 100, 300, 600 ppm): 1/50 (2 %), 4/49 (8 %), 7/49* (14 %), 11/48** (23 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

- increased incidence of **inflammation** in ♂ mice at 600 ppm with statistical significance

  no. of animals with lesions (percentage):
  ♂ mice (0, 100, 300, 600 ppm): 0/50 (0 %), 2/49 (4 %), 1/49 (2 %), 5/48* (10 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

Kidney:

- increased incidence[5] and severity[6] of **renal nephropathy** in ♀ mice at 600 ppm with statistical significance

  no. of animals with lesions (percentage, severity: 1 minimal, 2 mild, 3 moderate, 4 marked):
  ♀ mice (0, 100, 300, 600 ppm): 16/50 (32 %, 1.1), 21/49 (43 %, 1.3), 12/50 (24 %, 1.0), 26/50* (52 %, 1.6)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

Liver:

- increased incidence of **eosinophilic foci** in ♀ mice at 600 ppm with statistical significance

  no. of animals with lesions:
  ♀ mice (0, 100, 300, 600 ppm): 2/50 (4 %), 5/50 (10 %), 7/50 (14 %), 12/50** (24 %)
  (* $p \leq 0.05$, ** $p \leq 0.01$)

[5] focal to multifocal cortical tubules with cytoplasmic basophilia, nuclear crowding, and thickened basement membranes

[6] thickening and hypercellularity of the glomerular tufts and infiltration of mononuclear cells around affected tubules

- *Tumour incidence data by sex, dose and tumour type:*

Liver:

- increased incidence of **hepatocellular adenoma or carcinoma (combined)** in ♂ mice at 100 and 600 ppm and ♀ mice in all treatment groups with statistical significance

  no. of animals with lesions:
  ♂ mice (0, 100, 300, 600 ppm): 28/50 (56 %), 36/50* (72 %), 33/50 (66 %), 37/50* (74 %); *p = 0.093*†

  historical control incidence (inhalation): 196/350 (56.0 % ± 6.2 %; range 50 % - 68 %)

  ♀ mice (0, 100, 300, 600 ppm): 13/50 (26 %), 26/50** (52 %), 24/50* (48 %), 33/50** (66 %); *p = 0.014*†

  historical control incidence (inhalation): 108/347 (31.1 % ± 6.8 %; range 22 % - 39 %)

  (* p ≤ 0.05, ** p ≤ 0.01; † trend test)

- **hepatocellular adenoma** (includes multiple)**:**

  no. of animals with lesions:
  ♂ mice (0, 100, 300, 600 ppm): 24/50 (48 %), 27/50 (54 %), 27/50 (54 %), 25/50 (50 %); *p = 0.453*†

  historical control incidence (inhalation): 134/350 (38.3 % ± 6.3 %; range 30 % - 46 %)

  ♀ mice (0, 100, 300, 600 ppm): 10/50 (20 %), 20/50* (40 %), 21/50** (42 %), 23/50** (46 %); *p < 0.001*†

  historical control incidence (inhalation): 78/347 (22.5 % ± 8.1 %; range 12 % - 35 %)

  (* p ≤ 0.05, ** p ≤ 0.01; † trend test)

- **hepatocellular carcinoma** (includes multiple)**:**

  no. of animals with lesions:
  ♂ mice (0, 100, 300, 600 ppm): 10/50 (20 %), 12/50 (24 %), 11/50 (22 %), 17/50 (34 %); *p = 0.081*†

  historical control incidence (inhalation): 85/350 (24.3 % ± 4.8 %; range 18 % - 32 %)

  ♀ mice (0, 100, 300, 600 ppm): 3/50 (6 %), 9/50 (18 %), 6/50 (12 %), 18/50** (36 %); *p < 0.001*†

  historical control incidence (inhalation): 37/347 (10.7 % ± 1.8 %; range 8 % - 12 %)

  (* p ≤ 0.05, ** p ≤ 0.01; † trend test)

- **secondary neoplasms:** hepatocellular carcinoma metastasised to the lung

  no. of animals with lesions:
  ♀ mice (0, 100, 300, 600 ppm): 1/50 (2 %), 5/50 (10 %), 3/50 (6 %), 13/50 (26 %)

- *Local or multi-site responses:* local response (liver)
- *Progression of lesions to malignancy:* proliferative lesions were especially seen in the olfactory epithelium (hyperplasia, metaplasia) without progression to malignancy
- *Gender and/or species-specific responses:* neoplastic effects observed in both sex

- *Mode of action (genotoxic, non-genotoxic):* uncertain
- *Tumour latency:* n/a
- *Statistical methods:* survival analyses using Cox's method, incidences of neoplasms or nonneoplastic lesions using the poly-k test,

2.4 Specific target organ toxicity – repeated exposure

2.4.1 Animal data - Inhalation

2.4.1.1 NTP, 2007 (rats)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: 001 Key | Experimental results

Detailed study summary and results:

Test type

- 90-day subchronic inhalation toxicity study
- Similar to OECD TG 413 (NTP standards)
- GLP compliant (21 CFR, Part 58)

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* > 99 %
- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Test animals

- *Species, strain, sex:* **rats**, F344/N, ♂/♀
- *No. of animals per sex per dose:* 10 (core study), 10 (clinical pathology study)
- *Age and weight at the study initiation:* approx. 6 weeks, approx. 90 g (av.)

Administration/exposure

- *Route of administration:* whole-body inhalation (vapour)
- *Duration of test/exposure period:* 14 weeks (core study), 23 days (clinical pathology study)
- *Doses/concentration levels, rationale for dose level selection:* 0, 75, 150, 300, 600, and 1000 ppm *Frequency of treatment:* 6 h/d plus T_{90} (12 minutes), 5 d/w (except holidays)
- *Control group and treatment:* yes

- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* 2-phenylpropene vapour was generated and transported to the exposure chamber, vapour concentration and test substance stability was monitored (online GC), T_{90} of 12 minutes

For inhalation studies:

- *Type of inhalation exposure and test conditions:* inhalation exposure chamber
- *Method of exposure:* whole-body
- *Analytical verification of test atmosphere concentrations:* online GC

Results and discussion

- *Body weight and body weight changes:* no effect on body weight gains and final mean body weight
- *Food/water consumption:* not specified
- *Clinical signs:* no clinical signs observed
- *Haematological findings:* slightly decreased erythron (lower values of haematocrit, haemoglobin, and erythrocytes counts) in ♂ at ≥ 150 ppm (no effects in ♀) with statistical significance
- *Clinical biochemistry findings:*
    - **increased bile acid concentration** in ♂ and ♀ at 1000 ppm; no other effect associated with hepatocellular toxicity (alkaline phosphatase, alanine aminotransferase and sorbitol dehydrogenase activities unaffected or decreased) with statistical significance
    - increased parameter indicative of **adverse effects on renal tubular epithelium** (increased ratios of protein/creatinine, alkaline phosphatase/creatinine, aspartate aminotransferase/creatinine, lactate dehydrogenase/creatinine, γ-glutamyltransferase/creatinine, and N-acetyl-β-glucosaminidase/creatinine) in ♂ at ≥ 300 ppm and ♀ at ≥ 600 ppm with statistical significance
- *Gross pathology findings:*
    - no gross lesions
    - **increased absolute/relative liver weight** in ♂ at ≥ 150 ppm and ♀ at ≥ 600 ppm with statistical significance
    - **increased absolute/relative kidney weight** in ♂ at 1000 ppm and ♀ at ≥ 600 ppm with statistical significance
- *Histopathology findings*[7]*:*

[7] tissues examined to the no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with mainstem bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus

- **hyaline droplet accumulation** slightly more severe (minimal to mild) in ♂ at ≥ 600 ppm (altered morphology of hyaline droplets (larger more variable in shape) without changes in hyaline droplet accumulation incidences)

  No. of animals with lesions (severity: 1 minimal, 2 mild, 3 moderate, 4 marked):

  ♂ rats (0, 75, 150, 300, 600, 1000 ppm): 9/10 (1.1), 10/10 (1.2), 10/10 (1.3), 10/10 (1.1), 10/10 (1.8), 10/10 (1.7)
- **increased renal cell proliferation** (labelling index) **and elevated α2u-globulin concentrations**[8] in the kidney of ♂ at ≥ 150 ppm with statistical significance

Conc. (ppm)	renal cell proliferation (labelling index [%])	α2u-globulin (ng/µg soluble protein)
0	2.339 ± 0.110	81.32 ± 8.87
75	3.032 ± 0.316 [+30 %]	115.46 ± 16.81 [+42 %]
150	3.083 ± 0.226* [+32 %]	119.29 ± 11.28* [+46 %]
300	3.353 ± 0.230** [+43 %]	160.82 ± 16.51** [+98 %]
600	3.050 ± 0.159** [+30 %]	176.02 ± 26.18** [+117 %]
1000	3.935 ± 0.307** [+68 %]	167.42 ± 20.50** [+106 %]

- no evidence of granular casts within the renal tubules

- *Mortality and time to death:* no mortality, all animals survived

2.4.1.2 NTP, 2007 (mice)

Study reference:

NTP (2007): Toxicology and carcinogenesis studies of alpha-methylstyrene (Cas No. 98-83-9) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser (543), 1-210

ECHA dissemination page: 002 Supporting | Experimental results

Detailed study summary and results:

Test type

- 90-day subchronic inhalation toxicity study
- Similar to OECD TG 413 (NTP standards)
- GLP compliant (21 CFR, Part 58)

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* > 99 %

[8] α2u-globulin concentration was determined in the supernatant of whole kidney homogenate using a competitive indirect enzyme-linked immunosorbent assay (ELISA)

- *Impurities:* sec-butylbenzene (0.21 %)
- *Lot #*: BNW 13871-4, BNW 13871-54

Test animals

- *Species, strain, sex:* **mice**, B6C3F$_1$, ♂/♀
- *No. of animals per sex per dose:* 10 (core study), 10 (clinical pathology study)
- *Age and weight at the study initiation:* approx. 6 weeks, approx. ♂ 23 g (av.) and ♀ 19 g (av.)

Administration/exposure

- *Route of administration:* whole-body inhalation (vapour)
- *Duration of test/exposure period:* 14 weeks (core study), 23 days (clinical pathology study)
- *Doses/concentration levels, rationale for dose level selection:* 0, 75, 150, 300, 600, and 1000 ppm
- *Frequency of treatment:* 6 h/d plus T_{90} (12 minutes), 5 d/w (except holidays)
- *Control group and treatment:* yes
- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* 2-phenylpropene vapour was generated and transported to the exposure chamber, vapour concentration and test substance stability was monitored (online GC), T_{90} of 12 minutes

For inhalation studies:

- *Type of inhalation exposure and test conditions:* inhalation exposure chamber
- *Method of exposure:* whole-body
- *Analytical verification of test atmosphere concentrations:* online GC

Results and discussion

- *Body weight and body weight changes:*
  - **reduced final mean body weight** in ♂ and ♀ with statistical significance
    - ♂: 600 ppm (- 13 %), 1000 ppm (- 17 %)
    - ♀: 75 ppm (- 9 %), 300 ppm (- 9 %), 1000 ppm (- 11 %)
  - **reduced final mean body weight gains** in ♂ and ♀ at ≥ 300 ppm with statistical significance
    - ♂: 300 ppm (- 14 %), 600 ppm (- 34 %), 1000 ppm (- 43 %)
    - ♀: 300 ppm (- 23 %), 600 ppm (- 12 %), 1000 ppm (- 32 %)
- *Food/water consumption:* not changes reported
- *Clinical signs:*
  - sedation (moderate to severe) in ♂ at 1000 ppm
  - ataxia in ♂ and ♀ at 1000 ppm
- *Haematological findings:* slightly decreased erythron (lower values of haemoglobin and erythrocytes counts) in ♀ at 1000 ppm with statistical significance
- *Clinical biochemistry findings:* not specified
- *Gross pathology findings:*
  - no gross lesions

- **increased absolute liver weight** in ♀ at ≥ 600 ppm with statistical significance and in ♂ at 1000 ppm without statistical significance
- **decreased epididymal weights** in ♂ at 1000 ppm with statistical significance but no effects on other reproductive endpoints (epididymal sperm concentration and motility, spermatid heads/testis) or histopathology in the reproductive tract were noted
- **prolonged oestrous cycle** in ♀ at ≥ 600 ppm with statistical significance

- *Histopathology findings[9]:*
  - minimal to mild **centrilobular hypertrophy** in the **liver** of ♂ and ♀ at ≥ 600 ppm which contributed to the increased liver weight
  - **high incidences numerous nasal lesions** in ♂ and ♀ at ≥ 75 ppm (atrophy and metaplasia of the olfactory epithelium, atrophy and hyperplasia of Bowman's glands)

	Ctrl	**75 ppm**	**150 ppm**	**300 ppm**	**600 ppm**	**1000 ppm**
Male						
Atrophy of the Bowman's glands	0/10	7/10**(1.0)$^{\#}$	10/10** (1.3)	10/10** (1.9)	10/10** (2.0)	10/10** (2.0)
Hyperplasia of the Bowman's glands	0/10	9/10** (1.1)	10/10** (1.6)	10/10** (2.3)	10/10** (2.9)	10/10** (2.7)
Atrophy of the olfactory epithelium	0/10	10/10** (1.1)	10/10** (1.4)	10/10** (2.0)	10/10** (2.0)	10/10** (2.1)
Metaplasia of the olfactory epithelium	0/10	5/10* (1.2)	10/10** (1.4)	10/10** (2.0)	10/10** (2.0)	10/10** (2.0)
Hyaline degeneration of the respiratory epithelium	0/10	1/10 (1.0)	2/10 (1.0)	1/10 (1.0)	2/10 (1.0)	0/10
Females						
Atrophy of the Bowman's glands	0/10	8/10** (1.0)	9/10** (1.3)	10/10** (2.0)	10/10** (2.0)	8/8** (2.5)
Hyperplasia of the Bowman's glands	0/10	5/10* (1.0)	10/10** (1.7)	10/10** (2.3)	10/10** (2.6)	8/8** (2.6)
Atrophy of the olfactory epithelium	0/10	10/10** (1.0)	10/10** (1.6)	10/10** (2.0)	10/10** (2.0)	8/8** (2.0)
Metaplasia of the olfactory epithelium	0/10	4/10* (1.0)	9/10** (1.7)	10/10** (2.0)	10/10** (2.0)	8/8** (2.0)
Necrosis of the olfactory epithelium	0/10	0/10	0/10	0/10	0/10	2/10 (3.0)
Hyaline degeneration of the respiratory epithelium	0/10	2/10 (2.0)	6/10** (1.3)	9/10** (1.6)	8/10** (1.4)	4/8* (1.0)

* $p \leq 0.05$, ** $p \leq 0.01$ (number of animals with lesions / number of animal examined), $^{\#}$ average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

- *Mortality and time to death:* all ♂ survived, two ♀ died on day 3 in the highest concentration group

[9] (tissues examined to the no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with mainstem bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus)

2.4.1.3 Morgan *et al.*, 1999

Study reference:

Morgan, Daniel L., et al. "Characterization of inhaled alpha-methylstyrene vapour toxicity for B6C3F1 mice and F344 rats." Toxicological sciences: an official journal of the Society of Toxicology 47.2 (1999): 187-194.

ECHA dissemination page: 003 Supporting | Experimental results

Detailed study summary and results:

Test type

- Non-guideline repeated dose toxicity study via the inhalation route in B6C3F1 mice and F344 rats
- Not GLP compliant
- Method:
    - *Sub-study 1*: performed in ♂/♀ mice at 0, 125, 250, or 500 ppm; body weight monitored; blood samples taken for haematology and clinical biochemistry[10]; weight recorded und histopathology performed on lung, kidney, spleen, and liver; liver glutathione measured
    - *Sub-study 2*: performed in ♂/♀ mice at 0, 600, 800, 1000 ppm and ♂/♀ rats at 0, 600, 1000 ppm; body weight monitored; blood samples taken for haematology and clinical biochemistry[3]; weight recorded und histopathology performed on lung, liver, kidney, spleen, nasal cavity, brain, stomach, heart, thymus, and adrenal glands; liver glutathione measured
    - *Sub-study 3*: performed in ♂/♀ rats at 0, 125, 250, or 500 ppm in addition to ♂ NBR rats (α2u-globulin deficient strain); body weight monitored; histopathology performed on the kidney to elucidate the role of α2u-globulin

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* 99 % (purchased from Aldrich Chemical Co.)

Test animals

- *Species/strain/sex:*
    - **mice**, B6C3F1, ♂/♀
    - **rats**, Fischer 344, ♂/♀
    - **rats**, NCI Black-Reiter (NBR), ♂ (α2u-globulin deficient strain, only employed as negative control in sub-study 3)
- *No. of animals per sex per dose per time point:*
    - *sub-study 1:* 6 mice / sex / concentration / time point (72 ♂ and ♀ mice in total)

[10] serum creatinine, urea nitrogen (UN), sorbitol dehydrogenase (SDH), or alanine aminotransferase (ALT)

- *sub-study 2:* 6 mice / sex / concentration / time point (78 ♂ and ♀ mice in total (18 / sex / 0, 600, 800 ppm + 24 / sex / 1000 ppm)) and 5 rats (F344) / sex / concentration (15 ♂/♀ rats)
- *sub-study 3:* 4 rats (F344) / sex / concentration + 4 ♂ rats (NBR) / concentration

- *Age at the study initiation:* 6-7 weeks

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:*
  - *sub-study 1:* 3, 5, 12 days
  - *sub-study 2:* 6 h, 5 and 12 days (mice); 12 days (rats)
  - *sub-study 3*: 9 days
- *Doses/concentration levels:*
  - *sub-study 1:* ♂/♀ mice at 0, 125, 250, or 500 ppm
  - *sub-study 2:* ♂/♀ mice at 0, 600, 800, 1000 ppm and ♂/♀ rats at 0, 600, 1000 ppm
  - *sub-study 3:* ♂/♀ rats at 0, 125, 250, or 500 ppm
- *Control group and treatment:* conditioned air
- *Actual dose:*
  - *sub-study 1:* 133 ± 3, 245 ± 5, 489 ± 19 ppm
  - *sub-study 2:* 596 ± 29, 799 ± 17, 1056 ± 7 ppm
- *Statistical methods:* statistical significance determined by one-way ANOVA and Dunnett's multiple comparison test

For inhalation studies:

- *Type of inhalation exposure and test conditions:* vapour was mixed with air and delivered to the inhalation chamber
- *Method of exposure:* whole body
- *Analytical verification of test atmosphere concentrations:* yes, using Fourier transform infrared spectrophotometers

Results and discussion

Sub-study 1 (♂/♀ B6C3F1 mice):

- *Body weight and body weight changes:* no effect observed
- *Clinical biochemistry findings:* no effect observed
- *Gross pathology findings:* no effect on organ weight observed
- *Histopathology findings:* no effect observed
- *Mortality:* no effect observed

Sub-study 2 (♂/♀ B6C3F1 mice and ♂/♀ F344 rats):

Mice

- *Body weight and body weight changes:*
  - **reduced body weight** in ♂ at 600, 800, 1000 ppm after 5 and 12 days with statistical significance
- *Clinical signs:*
  - sedated appearance after 6 h exposure at 600, 800, 1000 ppm (effect disappeared after prolonged exposure)
  - hyperactivity and unresponsive to noise
- *Clinical biochemistry findings:*
  - no effect on serum creatinine, urea nitrogen (UN), sorbitol dehydrogenase (SDH), or alanine aminotransferase (ALT)
  - **decreased liver glutathione** in ♀ at ≥ 600 ppm after 1 or 5 days and in ♂ at ≥ 600 ppm after 1 day of exposure and ≥ 800 ppm after 5 days of exposure with statistical significance (the depletion was concentration-depended after day 5 in both sexes)
- *Gross pathology findings:*
  - **decreased relative spleen weight** in ♂ and ♀ at all exposure levels with statistical significance
  - **increased relative liver weight** in ♂ at 600 ppm after 5 days of exposure and at ≥ 800 ppm after 1, 5, and 12 days with statistical significance; in ♀ at ≥ 600 ppm after 1, 5, and 12 days with statistical significance (except for ♀ at 600 ppm after short-term, 1 day, treatment)
- *Histopathology findings:* **no effect observed** in spleen, liver, lung, kidney, nasal cavity, brain, stomach, heart, thymus, adrenal glands
- *Mortality:* cause of death obscure
  - no mortality in ♂
  - 1/18 ♀ at 600 ppm
  - 10/18 ♀ at 800 ppm
  - 5/24 ♀ at 1000 ppm

Rats

- *Body weight and body weight changes:* no effect
- *Clinical signs:* no effect observed
- *Clinical biochemistry findings:* no effect on serum creatinine, urea nitrogen (UN), sorbitol dehydrogenase (SDH), or alanine aminotransferase (ALT)
- *Gross pathology findings:*
  - **increased relative liver weight** in ♂ and ♀ at all exposure levels with statistical significance (concentration-dependent)
  - **increased relative lung weight** in ♂ at 1000 ppm with statistical significance

- **increased relative kidney weight** in ♂ at 600 ppm with statistical significance but not at 1000 ppm

- *Histopathology findings:*
  - no effect observed in liver, spleen, lung, nasal cavity, brain, stomach, heart, thymus, adrenal glands
  - abnormal accumulation[11] of cytoplasmic eosinophilic granules (**hyaline droplets**) in the renal tubules in ♂ at all exposure levels (indicative of hyaline droplet nephropathy; mild to moderate severe) but not in ♀ rats (♂ Ctrl: 5/5[12] (1.0)[13], 600 ppm: 5/5 (3.0), 1000 ppm: 5/5 (3.0); ♀ Ctrl: 0/5, 600 ppm: 0/5, 1000 ppm: 0/5)
  - no granular casts in the renal tubules
  - no exacerbation of chronic nephropathy[14] due to hyaline droplet accumulation (no. of regenerative renal tubule foci)
- *Mortality:* no mortality observed

Sub-study 3 (♂/♀ F344 rats and ♂ NBR rats):

- *Body weight and body weight changes:* no effect observed
- *Gross pathology findings:*
  - no effect on kidney weight
- *Histopathology findings:*
  - **hyaline droplet accumulation** (Mallory-Heidenhain staining method) confirmed in ♂ at ≥ 250 ppm
  - no other related effects in the kidney
  - no signs of hyaline droplet nephropathy in ♀ or ♂ NBR rats
- *Mortality:* no effect observed

2.4.1.1 Wolf *et al.*, 1956

Study reference:

Wolf, M. A., et al. "Toxicological Studies of Certain Alkylated Benzenes and Benzene. Experiments on Laboratory Animals." Arch. Indust. Health 14.4 (1956): 387-398.

ECHA dissemination page: 004 - 007 Supporting | Experimental results

Detailed study summary and results:

[11] described as *"accumulation of inclusions that were larger and more variable in shape, with globular or angular, crystalline forms predominating"*
[12] no. of animals affected / no. of animals examined
[13] Semi-quantitatively score based on size and shape on a scale from 1 to 4
[14] common observation in control F344 rats

Test type

- Non-guideline repeated dose toxicity study via the inhalation route in Wistar rats, albino guinea pigs, rabbits and rhesus monkeys
- Not GLP compliant
- Method: general clinical observation recorded; food consumption monitored; weight gain and mortality recorded; gross pathology performed on lungs, heart, liver, kidneys, spleen and testes (including weight); histopathology performed on lungs, heart, liver, kidneys, spleen, testes, portions of adrenals, pancreas and femoral bone marrow; blood urea nitrogen examined; bone marrow counts performed

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* 98.6 – 99.1 %

Test animals

- *Species/strain/sex:*
    - Wistar **rats**
    - albino **guinea pigs**
    - **rabbits**
    - **rhesus monkeys**
- *No. of animals per sex per dose*
    - rat: 10 – 25 ♂/♀ + control animals
    - guinea pig: 5 – 10 ♂/♀ + control animals
    - rabbit: 1 – 2 ♂/♀ + control animals
    - rhesus monkey: 1 – 2 (♂/♀ low dose, ♀ high dose) + control animals
- *Age and weight at the study initiation:*

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* up to six month (7 – 8 h, 5 days a week) depending on the concentration
- *Doses/concentration levels:*
    - rat: 0, 200, 600, 800, 3000 ppm
    - guinea pig: 0, 200, 600, 800, 3000 ppm
    - rabbit: 0, 200, 600 ppm
    - rhesus monkeys: 0, 200, 600 ppm
- *Post exposure observation period:* sacrificed 18 to 22 hours following their last treatment

- *Control group:* control animals exposed to air in exposure chamber
- *Statistical methods:* Fisher t-test for comparison of mean values ($P < 0.05$ considered significant)

For inhalation studies:

- *type of inhalation exposure and test conditions:* exposure chamber
- *method of exposure exposure data:* whole body
- *analytical verification of test atmosphere concentrations:* analytical verification of the vapour concentration performed
- *type or preparation of particles (for studies with aerosols):* liquid solution of the test substance vaporised into the in-flowing air

Results and discussion

Table: Results as reported within the publication

Species	Concentration (ppm)	Sex	No. of 7 h exposures	Duration of experiment (days)	Effect: growth depression	Effect: organ weight	Mortality
Rat	3000	♂/♀	3-4	3-4			severe
	800	♂/♀	28	38	slight	liver and kidney, slight	
	600	♂/♀	149	212		liver and kidney, slight	
	200	♂/♀	139	197		no effect	
Guinea pig	3000	♂/♀	3-4	3-4			severe
	800	♂/♀	27	38	slight	liver and kidney, slight	
	600	♂/♀	144	212		liver, slight	
	200	♂/♀	139	197		no effect	
Rabbit	600	♂/♀	152	212	slight		slight
	200	♂/♀	139	197		no effect	
Rhesus monkey	600	♀	149	212		no effect	
	200	♂/♀	139	197		no effect	

- *Body weight and body weight changes:*
  - rat: slight growth depression at 800 ppm
  - guinea pig: slight growth depression at 800 ppm
  - rabbit: slight growth depression at 600 ppm
  - rhesus monkey: no effects
- *Food/water consumption:* monitored but data not given
- *Clinical signs:* no clinical signs reported
- *Haematological findings:* no findings reported
- *Gross pathology findings:*
  - rat: slightly increased average liver and kidney weight at 600 and 800 ppm
  - guinea pig: slightly increased average liver weight at 600 and 800 ppm; slightly increased average kidney weight at 800 ppm
  - rabbit: no findings reported

  - rhesus monkey: no findings reported
- *Histopathology findings:* no findings reported
- *Mortality and time to death:*
  - rat: severe mortality at 3000 ppm following 3 – 4 days of exposure
  - guinea pig: severe mortality at 3000 ppm following 3 – 4 days of exposure
  - rabbit: slight mortality at 600 ppm
  - rhesus monkey: no findings reported

2.4.2 Animal data - Oral

2.4.2.1 Study report RDT, 1997

Study reference:

Unpublished study report concerning repeated dose toxicity (1997)

ECHA dissemination page: key study (specific target organ toxicity – repeated oral exposure)

Detailed study summary and results as reported on ECHAs dissemination page:

Test type

- *Test type:* Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test
- *Guideline:* Equivalent or similar to OECD TG 422
- *GLP compliant:* yes

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* 99.6 %
- *Lot/batch #*: 33041

Test animals

- *Species, strain, sex:* **rats**, Crj: CD(SD), ♂/♀
- *No. of animals per sex per dose:* 10
- *Age at the study initiation:* 8 weeks
- *Weight at the study initiation:* ♂ 309 – 337 g, ♀ 181 – 225 g

Administration/exposure

- *Route of administration:* oral (gavage)
- *Duration of test/exposure period:*
  - ♂: 43 days (14 days prior mating)

- ♀: approximately 53 days (14 days prior mating until post-partum day 3)[15]

- *Doses/concentration levels, rationale for dose level selection:* 0, 40, 200 or 1000 mg/kg bw/d based on a 10-day dose range finding study with suppression of body weight gain and increase of liver size observed in male rats at 1000 mg/kg bw/d
- *Frequency of treatment:* once a day
- *Vehicle:* olive oil (2-phenylpropene was dissolved in olive oil and administrated at a volume of 5 ml/kg)
- *Control group and treatment:* olive oil
- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* the test solution was stored in the fridge; stability and concentrations were confirmed prior to use
- *Statistical methods:* Bartlett's test, one-way analysis of variance, Kruskal-Wallis test. Dunnett's test / Dunnett-type multiple comparison test and Scheffe / Scheffe type multiple comparison, Fisher's exact probability test; significance was set at 5 % for all analyses

Results and discussion

- *Body weight and body weight changes:* reduced body weight gain in ♂ at 1000 mg/kg bw/d (from day 7 until the end of the study)
- *Food/water consumption:* decreased food consumption on day 7 in ♂ at 1000 mg/kg bw/d
- *Clinical signs:*
  - slight or moderate level of salivation in ♂ at ≥ 200 mg/kg bw/d and ♀ at 1000 mg/kg bw/d
  - intermittent slight level of salivation in a small number of ♂ at 40 mg/kg bw/d and ♀ at 200 mg/kg bw/d
- *Haematological findings:* no statistically significant effect observed
- *Clinical biochemistry findings:*
  - increased GPT (glutamate pyruvate transaminase; alanine transaminase ALT) levels in the serum of ♂ at ≥ 200 mg/kg bw/d
  - increased urea nitrogen and potassium in ♂ at 1000 mg/kg bw/d
  - decreased triglyceride in ♂ at 1000 mg/kg bw/d
  - changes in serum levels of additionally analysed enzymes such as GOT (glutamic oxaloacetic transaminase; aspartate transaminase AST), ALP (alkaline phosphatase, AP), gamma-GTP (gamma-glutamyl transpeptidase, GGT) not specified
- *Gross pathology findings:*
  - enlarged liver in ♂/♀ at 1000 mg/kg bw/d (dark reddish change in ♂ at ≥ 200 mg/kg bw/d)

[15] 14 days prior mating, up to 14 days mating*, 22 days gestation*, 3 days lactation (* information taken from OECD TG 422)

- enlarged kidney in ♂/♀ at 1000 mg/kg bw/d (discoloration of the cortico-medullary junction in ♀ at ≥ 200 mg/kg bw/d)
- yellow micro-granular calculi in the urinary bladder of ♂ at 1000 mg/kg bw/d
- enlarged adrenals (greyish colour) and thymus atrophy individually found in ♀ at 1000 mg/kg bw/d
- **increased** absolute and relative **liver weight** in ♂ at 1000 mg/kg bw/d and ♀ at ≥ 200 mg/kg bw/d
- **increased** absolute and relative **kidney weight** in ♂/♀ at 1000 mg/kg bw/d (relative kidney weight increased in ♀ at 200 mg/kg bw/d already)
- **decreased** absolute and relative **thymus weight** in ♀ at 1000 mg/kg bw/d

- *Histopathology findings:* no statistics available
  - liver:
    - acidophilic change of the hepatocytes in ♂/♀ at ≥ 200 mg/kg bw/d
      - micro-granular acidophilic cells were diffusely spread in the entire small lobules, loss of fatty droplets in ♂
      - micro-granular acidophilic cells distinctively around the centre of the lobules and enlarged hepatocytes in ♀
  - kidney:
    - increased hyaline droplets in the renal tubular epithelium of ♂ at 200 mg/kg bw/d accompanied in most cases by basophilic changes of the renal tubular epithelium
    - vacuolation in the renal tubular epithelium and infiltrated lymphocytes in adjacent areas in some animals (vacuoles identified as lipid droplets by oil red O staining) in ♀ at ≥ 200 mg/kg bw/d
  - adrenals:
    - increased number of lipid droplets in the fascicular zone of ♂/♀ at 1000 mg/kg bw/d
  - urinary bladder:
    - hyperplasia of the mucosal epithelium in ♂ at 1000 mg/kg bw/d including thickened mucosal epithelium layer, tissue erosion and infiltration of inflammatory cells
  - thymus:
    - atrophy in ♀ at ≥ 200 mg/kg bw/d (unclear boundary between the cortex and medulla)

- *Mortality and time to death (if occurring):* one ♂ (day 23) at 1000 mg/kg bw/d which exhibited anaemia, haematuria, decreased locomotor activity, and bradypnea on day 22 (necropsy/histopathology findings: 7-8 calculi in the urinary bladder, distended bladder, blood-like discoloration of urine, enlarged kidneys, expanded urinary duct, renal papillary necrosis, distinct bleeding in renal pelvis, urinary ducts, urinary bladder and epididymides and prostate gland adjacent to the urinary bladder, ischuria, pulmonary oedema, spleen atrophy)

2.4.2.2 Gagnaire *et al.*, 2005

Study reference:

Gagnaire, François, and Cristina Langlais. "Relative ototoxicity of 21 aromatic solvents." Archives of Toxicology 79.6 (2005): 346-354.

Detailed study summary and results:

Test type

- *Test type:* Non-guideline repeated dose toxicity study (Sprague-Dawley rats were examined for ototoxicity following 14 days of exposure by gastric intubation)
- *Guideline:* no guideline followed
- *GLP compliant:* no

Test substance

- *Test material:* 2-phenylpropene *(test material used in the study is equivalent to the substance identified in the CLH dossier)*
- *Degree of purity:* 99 %

Test animals

- *Species, strain, sex:* rats, Sprague–Dawley, ♂
- *No. of animals per sex per dose:* 7-8
- *Age at the study initiation:* 8 weeks
- *Weight at the study initiation:* 317 ± 6 g

Administration/exposure

- *Route of administration:* oral (gavage)
- *Duration and frequency of test/exposure period:* 14 days (5 days / week)
- *Doses/concentration levels, rationale for dose level selection:* 8.47 mmol/kg bw/d (~1000 mg/kg bw/d); based on preliminary range-finding studies with toluene
- *Post exposure observation period:* 10 days
- *Vehicle:* olive oil (administration volume 2 ml/kg)
- *Control group and treatment:* not specified

Results and discussion

- *Body weight and body weight changes:* no effect observed
- *Food/water consumption:* not specified
- *Clinical signs:* no effect observed
- *Histopathology findings:*
    - mild ototoxicity
    - lesions of the organ of Corti

- loss of outer hair cells (OHC) in the area of the cochlea that is responsive to medium frequencies (third row)

- *Mortality and time to death:* not observed, all animals survived