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:

Cumene

EC Number: **202-704-5**

CAS Number: **98-82-8**

Index Number: **601-024-00-X**

Version number: 01 **Date: 15.8.2019**

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2.1.1 [Study 1]

Study reference:

Chen, L.-J.; Wegerski, C.J.; Kramer, D.J.; Thomas, L.A.; McDonald, J.D.; Dix, K.J.; Sanders, J.M., Disposition and metabolism of cumene in F344 rats and B6C3F1 mice, *Drug Metabolism and Disposition*, 39, 498-509, 2011 (Chen et al., 2011)

Test type

The absorption, distribution, metabolism and excretion of [^{14}C]cumene was studied in male rats and male and female mice after oral and intravenous administration. No guideline is indicated in the publication and no information on GLP compliance is available.

Test substance

- [Ring-U-^{14}C]cumene (specific activity 54 mCi/mmol with a radiochemical purity of 98%)
- Unlabelled cumene with a purity of 99%

Detailed study summary and results:

Material and methods

- Test animals
    - Male F344 rats (177 - 214 g, 9 weeks old)
    - Male bile duct-cannulated (BDC) F344 rats (248 – 275 g, 9 weeks old)
    - Male B6C3F1 mice (25.2 – 28.9 g, 9 weeks old)
    - Female B6C3F1 mice (17.1 – 22.2 g, 9 weeks old)

- Animals were housed individually in all-glass metabolism cages from 1 day before dosing until sacrifice. Food and water were provided ad libitum.

- Oral application
  - Gavage
  - Vehicle: corn oil
  - Rats: 5 mL/kg, mice: 10 mL/kg
  - Ratio of unlabelled cumene to [^{14}C]cumene for rats 34:1 and for mice 2808:1
  - Target doses in rats: 1.4, 14, or 140 mg/kg bw
  - Target doses in male mice: 10, 50, 100, or 1000 mg/kg bw
  - Target doses in female mice: 10, 150, or 1000 mg/kg bw
  - All dose groups consisted of 4 animals
- Intravenous application
  - Injection into the tail vein of the animals
  - Vehicle: water-ethanol-Alkamuls-EL 620 (8:1:1, v/v/v)
  - Rats: 1 mL/kg, mice: 4 mL/kg
  - Ratio of unlabelled cumene to [^{14}C]cumene for rats 51:1 and for mice 111:1
  - Target dose in rats: 1.4 mg/kg bw
  - Target doses in male and female mice: 10 mg/kg bw
  - All dose groups consisted of 3-4 animals
- Sample collection and analysis
  - Single dose studies
    - Urine collected 6, 12, 24, 48 and 72 h after administration
    - Faeces collected after administration, and 12, 24, 48 and 72 h after administration
  - Repeated dose studies
    - Urine collected at 24 h intervals
    - Faeces collected at 24 h intervals
  - Bile was collected from BDC rats at 0.25, 1, 2, 3, 4, 5, 6, 12, and 24 h
  - Expired air was collected by passing air from the metabolism cages through a series of traps
  - Blood was collected before sacrifice
  - Adipose tissue and skin were collected after sacrifice
  - The following organs were collected and weighted: brain, lung, heart, spleen, kidneys, urinary bladder, uterus, liver, thyroid, stomach, small intestine, cecum, remaining large intestine
  - Duplicate or triplicate aliquots of all samples were analysed for ^{14}C content
- Anaesthesia and euthanasia
  - Animals were administered a sodium pentobarbital-based solution by i.p. administration
  - Animals were euthanized by exsanguination and sectioning of the diaphragm

- Metabolite isolation
  - Metabolite isolation performed on excreta collected during 24 h of dosing
  - Metabolites were isolated by HPLC
- In vitro microsomal incubations
  - Microsomes were prepared from liver and lung of four female F344 rats and 10 female B6C3F1 mice.
  - For in vitro experiments [^{14}C]cumene was incubated with the prepared microsomes for 30 min. Metabolites in the supernatant were detected by HPLC

Results

Distribution (see Table 2-1)

- Highest concentration of ^{14}C was found in blood, liver, kidney and lung.
- Rats: Increases of ^{14}C were proportional to the dose; in mice these data were more variable.

Table 2-1: Distribution of ^{14}C in selected tissue of male rats and male and female mice after single oral or intravenous administration. Values are given as mean ± SD. Results taken from Chen et al. (2011), table 3

Route	Dose [mg/kg bw]	Time [h]	Blood Conc. [nmol-Eq/g]	Liver		Kidney		Lung	
				Conc. [nmol-Eq/g]	T/B	Conc. [nmol-Eq/g]	T/B	Conc. [nmol-Eq/g]	T/B
Male rat									
i.v.	1.4	24	0.17 ± 0.03	1.1 ± 0.3	6.2 ± 1.5	1.3 ± 0.3	7.9 ± 0.9	0.24 ± 0.02	1.4 ± 0.3
Oral	1.4	24	0.37 ± 0.10	2.2 ± 0.6	5.9 ± 0.5	2.8 ± 1.2	7.8 ± 3.2	0.33 ± 0.09	0.91 ± 0.22
Oral	14	24	3.8 ± 0.3	22 ± 3	5.9 ± 1.4	33 ± 3	8.6 ± 1.7	6.1 ± 2.6	1.6 ± 0.7
Oral	140	24	35 ± 6	146 ± 38	4.1 ± 0.6	279 ± 30	8.0 ± 0.7	26 ± 6	0.74 ± 0.05
Oral	14	72	0.30 ± 0.07	1.1 ± 0.3	3.8 ± 0.5	1.4 ± 0.5	4.5 ± 0.8	0.48 ± 0.05	1.6 ± 0.4
Male mouse									
i.v.	10	24	0.13 ± 0.03	0.67 ± 0.11	5.2 ± 0.5	0.66 ± 0.03	5.2 ± 1.1	1.1 ± 0.1	9.1 ± 2.5
Oral	10	24	0.40 ± 0.04	2.6 ± 0.8	6.5 ± 1.6	3.2 ± 1.2	8.1 ± 3.4	1.4 ± 0.9	3.6 ± 2.5
Oral	50	24	0.61 ± 0.18	4.5 ± 0.8	7.8 ± 2.6	3.0 ± 1.1	5.3 ± 2.3	2.5 ± 0.6	4.4 ± 1.8
Oral	100	24	1.8 ± 0.2	7.4 ± 4.6	4.3 ± 2.8	7.9 ± 5.2	4.6 ± 3.0	5.8 ± 3.2	3.3 ± 1.9
Oral	100	24	9.4 ± 2.1	41 ± 9	4.4 ± 0.4	71 ± 35	7.3 ± 2.2	21 ± 9	3.3 ± 1.3
Female mouse									
i.v.	10	24	0.24 ± 0.08	1.3 ± 0.4	5.3 ± 0.7	0.58 ± 0.20	2.4 ± 0.1	1.7 ± 0.6	6.9 ± 0.9
Oral	10	24	0.44 ± 0.04	2.4 ± 0.0	5.5 ± 0.4	0.93 ± 0.13	2.1 ± 0.3	0.85 ± 0.18	2.0 ± 0.5
Oral	150	24	1.8 ± 0.5	7.6 ± 1.7	4.7 ± 2.1	4.0 ± 0.9	2.3 ± 0.4	7.9 ± 1.2	4.6 ± 0.8
Oral	100	24	38 ± 15	101 ± 32	2.7 ± 0.4	139 ± 109	4.0 ± 2.6	101 ± 57	2.6 ± 0.6

T/B: tissue/blood ratio

Metabolisms (see Table 2-2 and Table 2-3)

- The following metabolites were identified:
  - [M1] unknown
  - [M2] 2-(2-hydroxy-2-propyl) phenylsulfate
  - [M3] 4-(2-hydroxy-2-propyl) phenylsulfate
  - [M4] unknown
  - [M5] 2-hydroxy-2-phenylpropylsulfate
  - [M6] 2-phenyl-1,2-propandiol-2-glucuronide
  - [M7] 2-phenyl-1,2-propandiol-1-glucuronide
  - [M8] 2-hydroxy-2-phenylproprionic acid
  - [M9] 2-phenyl-2-propanol glucuronide
  - [M10] 2-phenylpropionyl glucuronide
  - [M11] 2-phenylpropionyl glycine
  - [M12] S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine
  - [M13] 2-phenyl-1-propanol glucuronide
  - [M14] 2-phenyl-2-propanol
  - [M15] 2-phenyl-1-propanol
  - [M16] 2-phenylpropionic acid
- Metabolites M9, M6, M7, M13 and M16 were detected in bile of BDC mice with M9 at the highest concentration.
- Three metabolites were detected in the supernatant of the in vitro microsomal incubations: Methylstyrene (AMS), 2-phenyl-2-propanol [M14], 2-phenyl-1-propanol [M15], whereas mouse lung microsomes metabolised more cumene than microsomes from mouse liver, rat lung, or rat liver. See Table 2-4.

Table 2-2: Percentage of dose of cumene metabolites in cumulative 0-24 h male rat urine and 0-6 h male rat bile. Values are given as percentage of all radiolabelled peaks, mean ± SD, n = 4. Results taken from Chen et al. (2011), table 5

Metabolite	Normal Male Rat Urine				BDC Male Rat Bile (1.4 mg/kg i.v.)
	140 mg/kg p.o.	14 mg/kg p.o.	1.4 mg/kg p.o.	1.4 mg/kg i.v.	
M1	N.D	N.D	N.D	N.D	N.D
M2	Trace[#]	Trace	Trace	Trace	N.D
M3	11.4 ± 1.6	8.2 ± 0.9	7.0 ± 0.9	4.9 ± 1.1	N.D
M4	5.6 ± 0.5	5.2 ± 0.7	5.6 ± 1.1	5.5 ± 0.7	N.D
M5	2.6 ± 0.3	2.5 ± 0.4	2.2 ± 1.5[##]	3.0 ± 0.3	N.D
M6	1.6 ± 0.0	N.D	N.D	N.D	4.5 ± 0.6*
M7	17.8 ± 1.0	20.1 ± 0.6	19.3 ± 0.8	17.4 ± 0.4	11.8 ± 2.8*
M8	16.4 ± 2.0	12.1 ± 0.8	12.1 ± 0.3	10.8 ± 1.6	N.D
M9 + M10**	38.1 ± 2.2	47.0 ± 1.3	48.4 ± 3.6	49.6 ± 3.0	73.2 ± 2.6*
M11	N.D	N.D	N.D	N.D	N.D
M12 + M13	4.8 ± 0.5	4.9 ± 0.5	4.0 ± 1.0	6.3 ± 1.7	5.4 ± 0.4*
M14	1.6***	Trace	1.8***	Trace	N.D

M15	N.D	N.D	N.D	N.D	N.D
M16	2.1 ± 0.5	Trace	Trace	2.3 ± 0.2##	2.9***

N.D.: not detected, #: trace amount observed but not quantified, ##: n = 2, *: n = 3, **: M10 is a minor metabolite coeluted with M9, ***: n = 1

Table 2-3: Percentage of dose of cumene metabolites in cumulative 0-24 h mouse urine. Values are given as percentage of all radiolabelled peaks, mean ± SD, n = 4. Results taken from Chen et al. (2011), table 6

Metabolite	Male Mouse Urine			Female Mouse Urine		
	1000 mg/kg p.o.	100 mg/kg p.o.	10 mg/kg p.o.	1000 mg/kg p.o.	150 mg/kg p.o.	10 mg/kg p.o.
M1	Trace#	N.D	N.D	3.0 ± 0.3	2.9 ± 1.0	1.8 ± 0.3
M2	Trace	N.D	N.D	4.4 ± 1.0	3.4 ± 0.2	N.D
M3	N.D	N.D	N.D	Trace	Trace	N.D
M4	N.D	N.D	N.D	Trace	N.D	N.D
M5	3.0 ± 0.1	6.3 ± 0.6	8.4 ± 0.3	5.8 ± 1.3	16.7 ± 1.9	19.1 ± 1.4
M6	4.4 ± 0.4	2.9 ± 0.4	3.1 ± 0.8	4.2 ± 0.5	2.6 ± 0.7	2.5 ± 0.5
M7	16.9 ± 0.5	11.9 ± 0.4	8.6 ± 0.9	16.5 ± 1.3	9.0 ± 1.8	6.1 ± 0.5
M8	12.8 ± 2.2	14.4 ± 0.8	15.7 ± 0.9	20.4 ± 0.8	12.9 ± 0.7	11.4 ± 0.9
M9	42.8 ± 2.4	39.3 ± 3.4	33.5 ± 2.2	36.8 ± 2.1	35.1 ± 2.1	29.8 ± 0.9
M11	11.0 ± 0.5	6.4 ± 1.3	5.1 ± 0.9	2.8 ± 0.2	2.9 ± 0.4	3.7 ± 0.4
M12* + M13	5.8 ± 1.5	2.1 ± 0.2	1.6 ± 0.1	2.1c	2.3 ± 0.4	1.5 ± 0.2
M14	N.D	1.5**	N.D	N.D	N.D	N.D
M15	N.D	1.6**	N.D	N.D	N.D	N.D
M16	Trace	N.D	N.D	Trace	N.D	N.D

N.D.: not detected, #: trace amount observed but not quantified, *: only a trace amount of M12 was observed in mouse urine, **: n = 1

Table 2-4: Percentage of cumene and metabolites in microsomal incubations. Values are given as percentage of all radiolabelled peaks, mean ± SD, n = 4. Results taken from Chen et al. (2011), table 7

	Female mouse		Female rat	
Metabolite	Lung Microsomes	Liver Microsomes	Lung Microsomes	Liver Microsomes
2-Phenyl-2-proponal	37.7 ± 2.3	15.5 ± 3.3	10.5 ± 2.0	7.7 ± 0.8
2-Phenyl-1-proponal	3.4 ± 0.8	N.D	4.4 ± 1.4	N.D
AMS	6.2 ± 0.5	3.4 ± 0.4	0.7 ± 0.9*	1.3 ± 0.4
Cumene	52.8 ± 2.4	81.2 ± 3.4	84.3 ± 3.1	91.0 ± 0.7

N.D.: not detected, *: AMS was detected in two of four incubations

Excretion (see Table 2-5)

- Rats
  - 24 h after dosing approximately 70 – 80% of ^{14}C was excreted in urine, 1% in faeces
  - Most of non-excreted dose was located in the GI tract (after 24 h)
  - 72 h after dosing only small amounts of ^{14}C were located in the GI tract

- Small amounts of ^{14}C were excreted as VOCs 24 h after single administration
- Excretion as $^{14}CO_2$ was negligible
- In BDC rats 37% ± 14% of an intravenous dose of 1.4 mg/kg bw was excreted in bile within 24 h.

- Mice
  - ^{14}C was mainly excreted in the urine
  - Only small amounts remained in the GI tract 24 h after dosing
  - Mice excreted significantly more ^{14}C as VOCs in higher dose groups
  - Excretion as $^{14}CO_2$ was negligible

Table 2-5: Recovery of ^{14}C after intravenous and oral administration to male rats and male and female mice. Values are given as mean ± SD, Results taken from Chen et al. (2011), table 2

Route	Dose [mg/kg bw]	Time [h]	% Dose in				Total Recovery [%]
			Urine	Faeces	VOC	GI Tract	
Male rat							
i.v.	1.4	24	90.1 ± 5.9	1.2 ± 0.1	4.6 ± 0.9	17.0 ± 5.7	116.0 ± 3.0
Oral	1.4	24	77.4 ± 10.1	1.2 ± 0.5	1.3 ± 0.9	21.9 ± 2.0	105.0 ± 12.0
Oral	14	24	70.2 ± 3.4	1.2 ± 0.8	1.4 ± 0.3	22.0 ± 4.6	97.4 ± 6.6
Oral	140	24	69.8 ± 8.9	0.9 ± 0.1	8.7 ± 5.0	13.8 ± 1.8	96.9 ± 3.5
Oral	14	72	85.6 ± 4.0	5.3 ± 1.8	N.D	0.6 ± 0.1	91.2 ± 2.6
Oral	14 x 3	24	73.9 ± 2.7	2.0 ± 0.7	N.D	4.4 ± 1.6	82.2 ± 4.1
Oral	14 x 7	24	81.3 ± 5.6	1.5 ± 1.1	N.D	1.4 ± 0.7	85.4 ± 4.6
Male mouse							
i.v.	10	24	47.4 ± 6.5	1.8 ± 1.8	2.2 ± 1.0	0.3 ± 0.1	51.7 ± 9.1
Oral	10	24	105.0 ± 5.0	4.5 ± 1.0	3.0 ± 3.5	0.5 ± 0.1	113.0 ± 5.0
Oral	50	24	90.2 ± 6.1	5.3 ± 4.0	4.5 ± 5.6	0.2 ± 0.1	100.0 ± 9.0
Oral	100	24	79.3 ± 9.9	4.0 ± 2.3	0.6 ± 0.2	0.2 ± 0.0	84.0 ± 10.0
Oral	1000	24	80.9 ± 6.1	5.2 ± 4.4	13.9 ± 3.7	0.2 ± 0.1	102.0 ± 5.0
Female mouse							
i.v.	10	24	74.0 ± 8.4	1.7 ± 0.6	3.0 ± 0.4	0.4 ± 0.0	79.0 ± 8.3
Oral	10	24	93.7 ± 3.4	3.6 ± 1.9	5.2 ± 5.6	0.3 ± 0.1	103.0 ± 1.0
Oral	150	24	86.5 ± 5.0	2.5 ± 1.3	2.9 ± 0.8	0.2 ± 0.1	92.1 ± 5.8
Oral	1000	24	79.2 ± 5.7	2.0 ± 0.2	21.9 ± 3.6	1.7 ± 0.6	105.0 ± 5.0
Oral	150 x 3	24	80.1 ± 11.1	4.7 ± 1.4	N.D	0.2 ± 0.1	85.0 ± 10.3
Oral	150 x 7	24	77.2 ± 7.8	4.7 ± 1.4	N.D	0.1 ± 0.0	87.7 ± 7.7

ND: not determined

2.1.2 [Study 2]

Study reference:

Metabolism, disposition and pharmacokinetics of cumene in F-344 rats following oral, IV administration or nose-only inhalation exposure. Report RTI/4353-01F. CMA Reference No. CU-5.0-PK-RTI. NTIS/OTS0522880, Research Triangle Park, NC, USA (Research Triangle Institute, 1989)

Test type

Distribution, elimination metabolism, and pharmacokinetics of cumene were studied in male and female rats after inhalation exposure. No guideline is indicated in the publication and no information on GLP compliance is available. (Study also describes effects after oral and inhalation exposure, which are not described in this annex.)

Test substance

- [Phenyl-^{14}C]cumene with a radiochemical purity of greater than 98%
- Unlabelled cumene with a purity of at least 99%

Detailed study summary and results:

Material and methods

- Test animals
    - Male and female F344 rats (100 - 225 g, 7 - 9 weeks old)
    - Animals were housed individually during inhalation exposure in polycarbonate restrainers
    - During post exposure phase animals were housed in metabolism cages
    - Food and water were provided ad libitum.
    - Rats were quarantined in polycarbonate cages for at least 7 days prior to initiation of each study.
- Application
    - Inhalation exposure “nose-only”
    - No vehicle used
    - Duration of treatment: 6 h
    - All dose groups consisted of 4 male and 4 female animals
    - Exposure concentration: 0, 100, 500, 1200 ppm (nominal), 104 ± 6, 494 ± 3, 1194 ± 67 ppm (measured)
- Sample collection and analysis
    - Pharmacokinetic studies
        - Tissue and body fluids sampled: urine, faeces, blood, expired breath, cage rinse
        - Tissues sampled: adipose tissue (3 sample locations), bones, brain, heart, spleen, kidney, liver, lung, skeletal muscle (3 sample locations), gonads, and residual carcass.
        - Time and frequency of sampling: 8, 16, 24, 48 and 72 h after start of exposure
        - Blood was sampled 0, 5, 10, 15 and 30 minutes and at 1, 2, 4, 8, 16, 24 and 48 h after initiation of inhalation exposure.
    - Distribution studies
        - Tissues and body fluids sampled: adipose tissue, femur, brain, heart, kidney liver, lung, spleen, skeletal muscle, gonads, carcass

        - Time and frequency of sampling: 72 h after start of exposure
    - Metabolite characteristics studies
        - Tissues and body fluids sampled: urine, expired breath
        - Time and frequency of sampling: pooled samples 0-24 h and 24-48 h
        - From how many animals: pooled samples by sex and concentration
        - Method type(s) for identification: HPLC, Liquid scintillation counting, Comparison of retention times with known substances, Enzymatic treatment (ß-glucuronidase, sulfatase)
        - - Limits of detection and quantification: Not reported
- Anaesthesia and sacrifice
    - Appropriate anaesthetics (i.p. injection of ketamine/xylazine 7:1, 60 mg/kg bw) were used to avoid pain or distress
    - Animals were killed by intracardiac injection of T-61 euthanasia solution
- Generation of test atmosphere (chamber description)
    - Exposure apparatus:
    - Cumene was evaporated at 60°C in a glass tube with glass beads. The cumene vapour was delivered via teflon and stainless steel tubing to individual nose-ports
    - Method of holding animals in test chamber: Batelle adjustable rat restrainer
    - Source and rate of air: Room air
    - Method of conditioning air: Not reported except that air was heated up to 60°C

Results

Details on absorption

- Cumene was absorbed rapidly from the lung, with detectable levels found in the blood within 5 min after start of exposure (see Table 2-6).
- Cumene concentration in blood reached a peak after 6 hours of exposure and decreased after the end of exposure (first analysed 2h after). The overall absorption is about 100%
- Half-life of disappearance from the blood at:
    - 100 ppm: 3.9 ± 0.7 h
    - 500 ppm: 4.6 ± 0.7 h
    - 1200 ppm: 6.6 ± 1.3 h

Table 2-6: Concentration of cumene in blood, measured over 24 h

Time	100 ppm		500 ppm		1200 ppm	
	Male [µg/g blood]	Female [µg/g blood]	Male [µg/g blood]	Female [µg/g blood]	Male [µg/g blood]	Female [µg/g blood]

5 min	0.6	0.6	2.9	4.8	3.6	5.8
10 min	1.0	0.8	2.7	6.8	7.1	9.8
15 min	0.9	0.9	5.1	4.6	11.1	12.8
30 min	1.0	1.1	4.3	4.3	17.8	17.2
1 h	1.8	1.5	6.0	5.8	21.9	25.9
2 h	1.6	1.9	11.0	8.4	40.7	50.0
4 h	1.7	1.9	12.4	16.0	41.4	63.4
6 h	4.3	4.4	17.1	20.9	59.5	82.4
8 h	1.1	2.4	4.8	5.7	38.0	41.1
16 h	1.5	1.9	0.9	1.0	2.5	2.2
24 h	1.9	1.7	0.6	0.7	0.9	0.9
48 h	1.4	0.9	0.4	0.4	0.5	0.5

Details on distribution in tissues

- Generally, concentrations in the tissues are low since >90% were excreted.
- Adipose tissues was observed to have slightly elevated concentrations at all doses, followed by liver and kidney (see Table 2-7)
- Further, elevated levels were detected in bone and muscle.

Table 2-7: Distribution of radiolabelled ^{14}C to tissue

	100 ppm		**500 ppm**		**1200 ppm**	
	Male [% of dose in total tissue]	**Female [% of dose in total tissue]**	**Male [% of dose in total tissue]**	**Female [% of dose in total tissue]**	**Male [% of dose in total tissue]**	**Female [% of dose in total tissue]**
Adipose	0.04	0.03	0.08	0.06	0.05	0.02
Blood	0.03	0.02	0.01	0.01	0.01	<0.005
Bone	0.03	0.07	0.01	0.01	0.02	0.01
Brain	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Heart	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Kidney	<0.005	0.01	<0.005	<0.005	<0.005	<0.005
Liver	0.03	0.02	0.02	0.01	0.01	0.01
Lung	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Muscle	0.12	0.15	0.13	0.12	0.10	0.07
Plasma	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Spleen	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Testis	<0.005	-	<0.005	-	<0.005	-

Ovary	-	<0.005	-	<0.005	-	<0.005
Carcass	2.68	3.64	0.87	2.93	1.57	0.98

Details on excretion

- Urine was found to be the predominant route of excretion (see Table 2-8).
- Excretion was rapid with the majority of cumene being excreted within 24 h (78.6 to 84.6%) and after 72 h nearly complete excretion was observed (96.0 to 98.9%).
- Urine was the major route of elimination (76.2 to 93.2%).
- Elimination via faeces was only of minor importance with 1.5 to 4.8%.
- At the highest concentration of 1200 ppm excretion via expired breath increases and in male and female resulted in 8.4 and 17.5% of the absorbed cumene, respectively.
- Half-life for excretion:
    - 100 ppm: could not be determined
    - 500 ppm: 17 h
    - 1200 ppm: 30 h

Table 2-8: Excretion of radiolabelled ^{14}C after 72 h

	Total [% of absorbed]	**Urine [% of absorbed]**	**Faeces [% of absorbed]**	**CO_2 Breath [% of absorbed]**	**Volatile Breath [% of absorbed]**
100 ppm					
Male	97.1	93.1	1.5	0.2	2.3
Female	96.0	91.1	2.0	0.2	2.8
500 ppm					
Male	98.9	93.2	2.0	0.2	3.4
Female	96.9	86.4	3.2	0.2	7.0
1200 ppm					
Male	98.2	87.2	2.5	0.2	8.4
Female	98.9	76.2	4.8	0.2	17.3

Details on metabolites

- Six unknown radiolabelled metabolites were detected by HPLC.
- After treatment with glucuronidase and sulfatase, the following metabolites were identified:
    - The major metabolite of urinary excretion (50% and more) was 2-phenyl-2-propanol and its glucuronide and/or sulphate conjugates (Metabolites 6).
    - Metabolites 1, 4, and 5 were converted to free 2-phenyl-1,2-propanediol.
    - Metabolite 2 was unaffected by deconjugating enzymes.

- For metabolite 3 also no change was observed. However, this metabolite occurred only in a minimal extent. Afterwards an in-depth analysis using ^{13}C- and ^{1}H-NMR spectroscopy resulted in two compounds. One of the two was identified to be phenylmalonic acid. The identity of the second compound could not be verified.
- Based upon retention time the metabolites were the same after i.v., oral and inhalation exposure.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

Evaluation not performed for this substance

3.2 Acute toxicity - dermal route

Evaluation not performed for this substance

3.3 Acute toxicity - inhalation route

Evaluation not performed for this substance

3.4 Skin corrosion/irritation

Evaluation not performed for this substance

3.5 Serious eye damage/eye irritation

Evaluation not performed for this substance

3.6 Respiratory sensitisation

Evaluation not performed for this substance

3.7 Skin sensitisation

Evaluation not performed for this substance

3.8 Germ cell mutagenicity

3.8.1 In vitro data

No data presented here

3.8.2 Animal data

3.8.2.1 [Study 1]

Study reference:

NTP, Toxicology and Carcinogenesis Studies of Cumene in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 542, 2009 (NTP, 2009).

Detailed study summary and results:

Test type

Acute *in vivo* bone marrow micronucleus test in rats, no explicit mentioning of OECD-TG, GLP compliance according to NTP's laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations is given.

F334/N male rats (n=5/dose) were exposed to cumene at 78.13, 156.25, 312.5, 625, 1250, 2500 mg/kg (Trial 1) or 312, 625, 1250, 2500 mg/kg (Trial 2) intraperitoneally three times at 24-hour intervals. Concurrent treatment with a vehicle and positive control was also performed. Afterwards, blood smears from bone marrow cells were prepared and micronucleus induction in erythrocytes in exposed animals compared to controls were analysed.

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- In the 2-week, 3-month, and 2-year studies details on test substance are given (purity: 99.9%, no impurities with an area percent greater than 0.1% determined by GC-chromatography, and Lot number: 200556852). It may be possible that the same test substance was used for this bone marrow micronucleus test, but no clear indication is given.

Test animals

- Rat/F344/N/male
- 5 male rats per dose
- Age and weight at the study initiation: no data given

Administration/exposure

- Two trials were performed; Trial 1: 78.13, 156.25, 312.5, 625, 1250, and 2500 mg/kg; Trial 2: 312, 625, 1250, and 2500 mg/kg
- Rational for dose selection was based on chemical solubility and toxicity and the extent of cell cycle delay induced by cumene exposure, as highest dose 2500 mg/kg was selected based on toxicity

- Vehicle: corn oil (no further information available)
- Route of administration: intraperitoneal injection
- Duration of test/exposure period: three times at 24-hour intervals
- Total exposure time: 72 h
- Animals were killed 24 h post final administration and blood smears were prepared
- Control group: yes, concurrent treatment, 5 male rats were administered corn oil intraperitoneally (vehicle control)
- Positive control data: yes, concurrent treatment, 5 male rats were administered cyclophosphamide intraperitoneally (25 mg/kg)
- Methods of slide preparation:
    - Blood smears were prepared from bone marrow cells obtained from femurs
    - Air-dried smears were fixed and stained
- Criteria for scoring and number of cells analysed per animal:
    - 2000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in up to five rats per dose group
    - Percentage of PCEs among the total erythrocyte population in bone marrow was scored for each dose group in order to determine the cumene-induced bone marrow toxicity
- Statistical methods:
    - Results were presented in tabular form as the mean of pooled results from all animals within a treatment group and the standard error of the mean was given
    - Analysis of frequency of micronucleated cells among normochromatic erythrocytes (NCEs) was performed by a one-tailed Cochran-Armitage trend test testing for increasing trend over exposure groups, subsequently values from each exposed group and the control group (vehicle control) were compared pairwise
    - If presence of excess binomial variation was detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation
    - A positive result for an individual trial of the micronucleus test was achieved if the trend test P value was less than or equal to 0.025 or if the P value for any single exposed group was less than or equal to 0.025 divided by the number of exposed groups
    - The final call of a positive result is preferably based on reproducibly positive trials or made by scientific staff

Results and discussion

- Trial 1 and Trial 2: increase in the induction of micronuclei in polychromatic erythrocytes in bone marrow at the 1250 mg/kg dose (for trial 2 also at 312 mg/kg dose) were observed

- At the highest test dose considerable toxicity and mortality was observed, 3 of 5 male rats died in Trial 1 at this dose level, thus a statistical evaluation with two data points was not feasible
- Percentage of PCEs in bone marrow fluctuated independently to dose level and probably represented variations within the normal range of 40% to 60% PCEs among total erythrocytes in bone marrow
- Genotoxic effects: positive
- Concurrent positive control data: yes, valid
- Statistical results:
    - Trial 1: statistically significant positive results obtained at 1250 mg/kg dose level (P<0.005) and in the trend test (P<0.001)
    - Trial 2: at all four doses micronucleated erythrocytes were elevated, statistically significant positive results obtained at 312 and 1250 mg/kg dose levels (P<0.006), but not for the trend test (P=0.085)

The results are listed in the following Table 3-1.

Table 3-1: Results of micronucleus test in male rats after oral exposure to cumene. Results taken from NTP (2009).

Compound	Dose (mg/kg)	Number of rats with erythrocytes scored	Micronucleated PCEs/ 1000 PCE's[a]	Pairwise P Value[b]	PCE[a] (%)
Trial 1					
Corn oil[c]	0	5	0.50 ± 0.16		50.2 ± 2.9
Cumene	78.13	5	1.20 ± 0.25	0.0447	59.4 ± 5.1
	156.25	5	1.20 ± 0.34	0.0447	64.8 ± 4.2
	312.5	5	1.30 ± 0.54	0.0296	54.6 ± 3.1
	625	5	0.80 ± 0.41	0.2026	45.1 ± 1.7
	1250	5	2.60 ± 0.29	0.0001	46.6 ± 4.8
	2500	2[d]	1.25 ± 0.25		49.3 ± 2.8
			P < 0.001[e]		
Cyclophospha-mide[f]	25	5	17.30 ± 2.32	0.0000	50.3 ± 4.3
Trial 2					
Corn oil	0	5	0.50 ± 0.27		53.2 ± 3.8
Cumene	312	5	1.70 ± 0.20	0.0052	50.2 ± 1.0
	625	5	1.40 ± 0.33	0.0194	47.6 ± 3.1
	1250	5	1.80 ± 0.34	0.0033	44.5 ± 3.0
	2500	3	1.50 ± 1.00	0.0192	54.3 ± 2.1
			P = 0.085		
Cyclophospha-mide	25	5	7.80 ± 1.63	0.0000	38.7 ± 2.7

[a] mean ± standard error, [b] Pairwise comparison with the vehicle control, dosed group values are significant at P ≤ 0.005 (trial 1) or P ≤ 0.006 (trial 2), positive control values are significant at P ≤ 0.05; [c] Vehicle control; [d] Statistical tests not performed due to high mortality; [e] Significance of micronucleated PCEs/1000 PCEs tested by the one-tailed trend test, significant at P ≤ 0.025; 2500 mg/kg group excluded due to high mortality; [f] Positive control.

3.8.2.2 [Study 2]

Study reference:

NTP, Toxicology and Carcinogenesis Studies of Cumene in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 542, 2009 (NTP, 2009).

Detailed study summary and results:

Test type

Acute *in vivo* peripheral blood micronucleus test in mice, no explicit mentioning of OECD-TG, GLP compliance according to NTP's laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations is given.

B6C3F1 mice (n=10/concentration) were exposed to cumene concentrations at 0, 62.5, 125, 250, 500, and 1000 ppm (only male mice) in air for 6 hours plus T90 (12 minutes) per day, 5 d/w, for a period of 14 weeks. Afterwards, blood smears from peripheral blood were prepared and evaluated if micronucleus induction in erythrocytes compared to the chamber control group is observed.

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- Degree of purity: 99.9%, (blood from animals of the 3-month study was used)
- Impurities: no impurities with an area percent greater than 0.1% determined by GC-chromatography
- Lot/Batch number: 200556852

Test animals

- Mice/B6C3F1/male and female
- 10 male and female animals/concentration group
- Age at study initiation: 5 to 6 weeks and weight at first day of exposure: 23.4 ± 0.3 g (males) and 19.3 ± 0.3 g (females)

Administration/exposure

- Nominal concentration levels: 62.5, 125, 250, 500, and 1000 ppm, dose selection rationale was based on results from a 14-d study in which exposure to cumene (0, 250, 500, 1000, 2000 and 4000 ppm) led in males and females to: mortalities at 2000 and 4000 ppm and signs of central nervous system effects, thus lower concentrations were used in the subchronic study)
- Route of administration: inhalation (vapour)
- Duration of test/exposure period: 6 hours plus T90 (12 minutes) per day

- Frequency of treatment: 5d/w, 14 weeks
- Control group: animals were chamber exposed (air)
- Positive control data: no information available
- Methods of slide preparation:
    - Peripheral blood samples after 3 months exposure were immediately used for preparing blood smears and fixed in absolute methanol
    - Fixed slides were stained with acridine orange and coded
- Criteria for scoring and number of cells analysed per animal:
    - Frequency of micronucleated cells in 1000 normochromatic erythrocytes (NCEs) in each of 9 or 10 mice per exposure group was determined
    - percentage of PCEs in a population of 1000 erythrocytes was determined as a measure of bone marrow toxicity
- Statistical methods:
    - Results were presented in tabular form as the mean of pooled results from all animals within a treatment group and the standard error of the mean was given
    - Analysis of frequency of micronucleated cells among normochromatic erythrocytes (NCEs) was performed by a one-tailed Cochran-Armitage trend test testing for increasing trend over exposure groups, subsequently values from each exposed group and the control group were compared pairwise
    - If presence of excess binomial variation is detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation
    - A positive result for an individual trial of the micronucleus test is achieved if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups
    - The final call of a positive result is preferably based on reproducibly positive trials or made by scientific staff

Results and discussion

- Induction of micronuclei: no increase in the frequency of micronucleated NCEs was seen in peripheral blood samples from male or female mice exposed to cumene for 3 months by inhalation
- Ratio of PCE/NCE: Chemical exposure had no effect on the ratio of PCEs to NCEs in peripheral blood, indicating no toxicity to the bone marrow by cumene
- Genotoxic effects: negative
- Concurrent positive control data: no data available
- Mortality: one female mice of the lowest concentration group died

- Body weight: mean body weights of exposed animals were similar to chamber controls

The results are listed in the following Table 3-2.

Table 3-2: Results of micronucleus test in mice after inhalation exposure to cumene. Results taken from NTP (2009).

Compound	Dose (mg/kg)	Number of rats with erythrocytes scored	Micronucleated NCEs/ 1000 NCE's[a]	Pairwise P Value[b]	PCEs[a] (%)
Male					
Air[c]	0	10	2.40 ± 0.69		2.7 ± 0.1
Cumene	62.5	10	2.20 ± 0.66	0.6161	2.6 ± 0.1
	125	10	2.10 ± 0.48	0.6728	2.6 ± 0.1
	250	10	1.80 ± 0.36	0.8230	2.8 ± 0.1
	500	10	2.00 ± 0.26	0.7270	2.9 ± 0.1
	1000	10	2.20 ± 0.42	0.6161	2.9 ± 0.2
			P = 0.553[d]		
Female					
Air	0	10	2.30 ± 0.40		3.3 ± 0.1
Cumene	62.5	9	1.33 ± 0.37	0.9396	2.3 ± 0.1
	125	10	1.70 ± 0.30	0.8289	3.1 ± 0.2
	250	10	2.10 ± 0.53	0.6186	3.3 ± 0.2
	500	10	2.10 ± 0.35	0.6186	3.4 ± 0.1
			P = 0.329		

[a] mean ± standard error, [b] Pairwise comparison with the chamber controls, significant at P≤0.005 (males) or P ≤ 0.006 (females); [c] Chamber control; [d] Significance of micronucleated NCEs/1000 NCEs tested by the one-tailed trend test, significant at P ≤ 0.025.

3.8.2.3 [Study 3]

Study reference:

Gulf Oil Corporation, Micronucleus Test of Cumene. Gulf Project No. 84-2129. EPA/OTS878216015

Gulf Oil Corporation, Pittsburgh, PA, 1985 (Gulf Oil Corporation, 1985).

Detailed study summary and results:

Test type

Acute *in vivo* peripheral blood micronucleus test in mice, no explicit mentioning of OECD-TG, GLP compliance is given (including certificate).

Crl:CDR-l (ICR) BR Swiss mice (n=10/dose (for cumene exposed and vehicle control group, n=4/positive control) were exposed to cumene doses at 0.25, 0.5, and 1.0 g/kg by gavage for two days. Afterwards, blood smears from bone marrow were prepared and evaluated if micronucleus induction in erythrocytes compared to the negative control group is observed.

A reliability of 2 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- Degree of purity: 2.5 g cumene in 50 mL paraffin oil (5% w/v)
- Impurities: no information available
- Lot/Batch number: no information available

Test animals

- Mice/ Crl:CDR-l (ICR) BR Swiss /male and female
- 10 animals per sex and dose (also for negative control); positive control: 4 animals per sex and dose, 15 animals per sex were given the maximum dose only once
- Age and weight at study initiation: 10 weeks, 30 - 38 g (males) and 23 - 31 g (females)

Administration/exposure

- 0.25, 0.5, and 1.0 g/kg, dose selection was based on observed mortalities in a range-finding study (1.25, 2.5, and 5.0 g cumene/kg)
- Vehicle: paraffin oil
- Doses were calculated individually using day 1 body weights
- Route of administration: oral, gavage (test substance and negative control), intraperitoneal injection (positive control)
- Duration of test/exposure period: daily exposure for 2 consecutive days, except for animals receiving the maximum dose only once
- Control groups: yes, concurrent treatment, negative control: paraffin oil (20 mL/kg)
- Positive control: cyclophosphamide (75 mg/kg)
- Sampling times:
    - Half of the animals receiving 2 doses (test substance or control) were sacrificed on day 3 and the remaining on day 4
    - Animals receiving the maximum dose were sacrificed on day 2, 3, or 4
    - Animals receiving cyclophosphamide were sacrificed on day 3
- Methods of slide preparation:
    - Blood smears were prepared from bone marrow cells
    - Staining of bone marrow smears was performed with stained May-Grunwald and Giemsa stains
- Criteria for scoring and number of cells analysed per animal:

- Microscopically examination (400 – 1000x) revealed polychromatic erythrocytes (PCE's) as bluish-gray, nonnucleated cells of the same approximate size as the mature erythrocytes and micronuclei as blue-purple particles, 1/20 – 1/5 of the cell size
- Per animal: 1000 PCE's and all mature erythrocytes in the scan path were analysed

- Statistical methods:
  - From the microscopic examinations of slides total PCE's, total normochromic erythrocytes (NORM's), PCE' s with micronuclei, and NORM's with micronuclei were determined
  - Calculation of group means and standard deviation for PCE' s with micronuclei and for the ratio of PCE's to NORM's was performed
  - Values from treated groups versus negative control group were pairwise compared by applying the Student's t-test
  - Determination of significance ($p<0.05$) in increased micronucleated PCE's at any dose level or a dose-dependent response lead to a positive test result
  - If no criterion is fulfilled or only one, the test result is negative or equivocal, respectively

Results and discussion

- Induction of micronuclei: no significant increase in the frequency of micronucleus formation was seen in bone marrow samples from male or female mice exposed to cumene
- Genotoxic effects: negative
- Concurrent positive control data: yes, valid; concurrent negative control data: yes, valid
- Mortality: one female mouse of the negative control group died on or before day 4
- Body weight: mean body weights of exposed animals were similar to controls

The results of the micronucleus test are listed in Table 3-3 and Table 3-4.

Table 3-3: Results of the ratio of polychromatic to normochromatic erythrocytes in mice after oral exposure to cumene. Results taken from Gulf Oil Corporation (1985).

Treatment (g/kg)	Sacrificed on day 2		Sacrificed on day 3		Sacrificed on day 4	
	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.
Males						
Paraffin oil (20 mL/kg)		NA	5	0.8 ± 0.06	5	0.9 ± 0.05
Cumene						
0.25		NA	5	0.8 ± 0.04	5	0.8 ± 0.09
0.50		NA	5	0.8 ± 0.04	5	0.8 ± 0.06
1.00		NA	5	0.8 ± 0.05	5	0.8 ± 0.02
1.00 (one dose)	5	0.9 ± 0.05	5	0.8 ± 0.07	5	0.8 ± 0.09
Cyclophosphamide (75 mg/kg)		NA	4	0.4 ± 0.05*		NA

Female						
Paraffin oil (20 mL/kg)		NA	5	0.8 ± 0.05	5	0.9 ± 0.07
Cumene						
0.25		NA	5	0.8 ± 0.05	5	0.8 ± 0.04
0.50		NA	5	0.8 ± 0.07	5	0.8 ± 0.04
1.00		NA	5	0.8 ± 0.05	5	0.8 ± 0.09
1.00 (one dose)	5	0.8 ± 0.02	5	0.9 ± 0.02	5	0.8 ± 0.02
Cyclophosphamide (75 mg/kg)		NA	4	0.4 ± 0.05*		NA

No. : Number of animals surviving until sacrifice; S.D.: standard deviation; * Significantly different from control (P≤0.05).

Table 3-4: Results of micronucleated polychromatic erythrocytes (%) in mice after oral exposure to cumene. Results taken from Gulf Oil Corporation (1985).

Treatment (g/kg)	Sacrificed on day 2		Sacrificed on day 3		Sacrificed on day 4	
	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.
Males						
Paraffin oil (20 mL/kg)		NA	5	0.02 ± 0.04	5	0.04 ± 0.05
Cumene						
0.25		NA	5	0.8 ± 0.04	5	0.00 ± 0.00
0.50		NA	5	0.8 ± 0.04	5	0.14 ± 0.05
1.00		NA	5	0.10 ± 0.07	5	0.06 ± 0.05
1.00 (one dose)	5	0.10 ± 0.07	5	0.8 ± 0.04	5	0.18 ± 0.13
Cyclophosphamide (75 mg/kg)		NA	4	5.25 ± 0.79*		NA
Female						
Paraffin oil (20 mL/kg)		NA	5	0.12 ± 0.16	5	0.02 ± 0.05
Cumene						
0.25		NA	5	0.12 ± 0.11	5	0.14 ± 0.05
0.50		NA	5	0.00 ± 0.00	5	0.10 ± 0.07
1.00		NA	5	0.04 ± 0.05	5	0.10 ± 0.10
1.00 (one dose)	5	0.02 ± 0.04	5	0.06 ± 0.13	5	0.00 ± 0.00
Cyclophosphamide (75 mg/kg)		NA	4	4.25 ± 0.59*		NA

No.: Number of animals surviving until sacrifice; S.D.: standard deviation; * Significantly different from control (P≤0.05).

3.8.3 Human data

No data presented here.

3.8.4 Other data

No data presented here.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 [Study 1]

Study reference:

NTP, Toxicology and Carcinogenesis Studies of Cumene in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 542, 2009 (NTP, 2009).

Detailed study summary and results:

Test type

2-year carcinogenicity study in rats, similar to OECD TG 451, GLP compliance according to FDA Good Laboratory Practice Regulations (21 CFR, Part 58) is given.

Male and female rats (n=50/sex/concentration) were exposed to cumene concentrations at 0, 250, 500, and 1000 ppm in air for 6 hours plus T_{90} (12 minutes) per day, 5 d/w, for a period of 105 weeks. Survival, clinical signs, and body weights were observed during the study. Surviving animals were sacrificed at study end and necropsy performed. Appearances of neoplasms and non-neoplastic lesions in exposed animals compared to controls were analysed.

A reliability of 2 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- Degree of purity: 99.9%
- Impurities: no impurities with an area percent greater than 0.1% determined by GC-chromatography
- Lot/Batch number: 200556852
- Stability under test conditions: stable over the test period, no degradation of test substance was detected

Test animals

- Rats/F344/N/ male and female
- 50 male and female animals /concentration group
- Age at study initiation: 5 to 6 weeks, weight at first day of exposure: 105-107 g (males) and 88-89 g (females)

Administration/exposure

- Route of administration: inhalation (vapour)
- Duration of test/exposure period: 6 hours plus T_{90} (12 minutes) per day
- Nominal concentration levels: 0, 250, 500, and 1000 ppm, analytical concentration levels: 0, 250 ± 5, 502 ± 11, and 1005 ± 23 ppm, dose selection rationale was based on results from a 90-d study in which exposure to cumene (0, 62.5, 125, 250, 500, and 1000 ppm) had effects on renal tubules (granular casts of the medulla, 250 ppm and greater; increase in α2u-globulin in right kidneys at 125 ppm or greater) and caused accumulation of hyaline droplets in the cortex (250 ppm or greater) in male rats, no mortalities or body weight effects or lesions in other tissues were observed, minimal organ weight changes (males: kidney and liver weights at 250 ppm and greater; females: liver 1,000 ppm) were noticed.
- Frequency of treatment: 5d/w, 105 weeks
- Control group animals were chamber exposed
- Historical control data is available for the study
- No post exposure time
- Vehicle: unchanged (no vehicle)
- Type of inhalation exposure and test conditions: an inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) was used. Vapour was generated by evaporating the substance in glass column with glass beads. Test conditions in chamber were: Temperature (°C): 24 ± 2 (75 ± 2° F), Humidity (%): 55 ± 15, Air changes (per h): 15 ± 2, Photoperiod (hrs dark / hrs light): 12/12. Each chamber had a total active mixing volume of 1.7 m^3.
- Method of exposure: whole body, animals were hold individually in exposure chambers
- Analytical verification of test atmosphere concentrations: Every 20 minutes, the concentrations in exposure chambers were monitored by on-line gas chromatography (GC-FID).
- Particle size: A condensation particle counter (Model 3022A, TSI, Inc., St. Paul, MN) was used. Particle counts greater than 200 particles/cm^3 were not detected.

Examinations:

- Cage side observations: yes, twice daily
- Detailed clinical observations: yes, every 4 weeks through week 93, afterwards every 2 weeks and at the end of the study
- Body weight: yes, initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter and at the end of the study
- Food efficiency: No data
- Water consumption: No data

- Ophthalmoscopic examination: No data
- Clinical pathology: No
- Haematology: No
- Clinical chemistry: No
- Gross pathology: yes, all animals were examined for grossly visible lesions on all organs and tissues
- Histopathology: Yes, adrenal gland, bone with marrow, brain, clitoral gland, oesophagus, eyes, 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

Results and discussion

Incidences of concentrations are given in the following order: controls, 250, 500, and 1000 ppm.

- Survival rates at the end of the study of exposed male (26/50, 23/50, 27/50, 24/50) and female (21/50, 27/50, 31/50, 32/50) rats are similar in comparison to controls.
- Clinical signs: no effects
- Body weights of exposed rats were similar to controls throughout the study. However, body weights of females exposed to the highest concentration were slightly decreased compared to controls during the second year of the study, which was compensated at the end of the study.
- Organ weights: no effects
- Necropsy findings: no effects
- Statistical methods: Statistical methods were used for estimating the probability of survival, identifying treatment-related effects on survival, calculating incidences, determining incidences of neoplasm and non-neoplastic lesions, determining the significance of pairwise comparisons between exposed and control groups and are described in details in the study on pages 31 to 32.

HISTOPATHOLOGY:

Histopathological findings in form of incidences of neoplastic and non-neoplastic lesions are given in Table 3-5 for male and in Table 3-6 for female rats.

NON-NEOPLASTIC

NOSE:

Incidences of non-neoplastic lesions (hyperplasia) of basal cells in olfactory epithelium were significantly increased in all exposed rats (male: 0/50, 19/50, 27/49, 26/50; female: 0/50, 14/48, 25/50, 31/50). A

significant increase in hyperplasia of the respiratory epithelium was seen in males of all exposed groups (0/50, 15/50, 16/49, 23/50) and in females of the highest concentration group (0/50, 0/48, 4/50, 6/50).

KIDNEY:

Male rats exposed to 500 and 1000 ppm had significantly higher incidences of hyperplasia of renal tubule (0/50, 3/50, 8/50, 6/50) and transitional epithelium of renal pelvis (3/50, 5/50, 14/50, 15/50) compared to controls. In all exposed groups of males, mineralisation of renal papilla (5/50, 35/50, 44/50, 41/50) was significantly severe compared to controls. For females, only nephropathies of the kidney were observed, see Table 3-5.

The non-neoplastic lesions of the kidney observed in rats after cumene exposure are characteristic for an accumulation of α2u-globulin.

HISTOPATHOLOGY: NEOPLASTIC

NOSE:

Incidences of adenoma of the respiratory epithelium were significantly increased in males of all exposed groups (0/50, 7/50, 18/49, 10/50) and in females of the lowest concentration (0/50, 5/48, 4/50, 3/50), for further details see Table 3-5 and Table 3-6.

KIDNEY:

The combined incidence of adenoma or carcinoma of renal tubule (2/50, 5/50, 8/50, 7/50) was increased in all exposed groups of males, but a significance compared to controls was only seen at 500 ppm in males. For females, no neoplastic effects of kidneys were seen.

TESTES:

Neoplastic effects observed in testes were equivocal. Male rats of the highest concentration group had a significantly increased incidence of interstitial cell adenoma (together with bilateral interstitial cell adenoma) (36/50, 38/50, 40/50, 46/50). A positive trend in incidences was seen in all exposed groups.

Table 3-5: Incidences of observed neoplastic and non-neoplastic lesions of male rats after exposure to different cumene concentrations (overall rates according to NTP). Results taken from NTP (2009).

Organs affected	0 ppm	250 ppm	500 ppm	1000 ppm
Nose				
Olfactory epithelium, hyperplasia, basal cell[a]	0/50 (0%)	19/50 (38%)**	27/49 (54%)**	26/50 (52%)**
Respiratory epithelium, hyperplasia	0/50 (0%)	15/50 (30%)**	16/49 (32%)**	23/50 (46%)**
Goblet cell, hyperplasia	3/50 (6%)	11/50 (22%)*	7/49 (14%)	5/50 (10%)
Glands, respiratory epithelium, adenoma	0/50 (0%)	0/50 (0%)	1/49 (2%)	0/50 (0%)

Respiratory epithelium, adenoma, multiple	0/50 (0%)	1/50 (2%)	2/49 (4%)	6/50 (12%)*
Respiratory epithelium, adenoma (includes multiple and all sites)[b,c]	0/50 (0%) P=0.004[d]	7/50 (14%)**	18/49 (37%)***	10/50 (20%)***
Kidney				
Renal tubule, hyperplasia[a]	0/50 (0%)	3/50 (6%)	8/50 (16%)**	6/50 (12%)*
Papilla, mineralisation	5/50 (10%)	35/50 (70%)**	44/50 (88%)**	41/50 (82%)**
Pelvis, transitional epithelium, hyperplasia	3/50 (6%)	5/50 (10%)	14/50 (28%)**	15/50 (30%)**
Nephropathy	47/50 (94%)	47/50 (94%)	47/50 (94%)	50/50 (100%)
Renal tubule, adenoma[b,e]	1/50 (2%) P=0.219[d]	4/50 (8%)	5/50 (10%)	4/50 (8%)
Renal tubule, carcinoma, bilateral	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Renal tubule, carcinoma (includes bilateral) [b,f]	1/50 (2%) P=0.180[d]	1/50 (2%)	3/50 (6%)	3/50 (6%)
Renal tubule, adenoma or carcinoma[b,g]	2/50 (4%) P=0.087[d]	5/50 (10%)	8/50 (16%)*	7/50 (14%)
Renal tubule, lipoma[h]	1/50 (2%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Testis				
Interstitial cell, hyperplasia[a]	12/50 (24%)	18/50 (36%)	19/50 (38%)	9/50 (18%)
Bilateral interstitial cell, hyperplasia	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Interstitial cell, adenoma	18/50 (36%)	14/50 (28%)	13/50 (26%)	9/50 (18%)
Bilateral interstitial cell, adenoma	18/50 (36%)	24/50 (48%)	27/49 (54%)	37/50 (74%)
Interstitial cell, adenoma (includes bilateral)[b,i]:	36/50 (72%) P=0.006[d]	38/50 (76%)	40/50 (80%)	46/50 (92%)**
Level of evidence of carcinogenic activity (according to NTP)	Clear evidence			

Significant difference from chamber control group determined by Poly-3 test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] Overall rate, number of animals with lesion per number of animals examined microscopically

[b] Overall rate, number of animals with neoplasm per number of animals with nose/kidney/testis examined microscopically (only with regard to the organ under investigation)

[c] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 1/447 (0.2% ± 0.7%), range 0%-2%

[d] For chamber control incidence, P value is given that is associated with the trend test determined by Poly-3 test (accounts for differential mortality in animals, which do not reach terminal sacrifice).

[e] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 4/449 (0.9% ± 1.0%), range 0%-2%

[f] Historical incidence for inhalation studies: 2/449 (0.4% ± 0.9%), range 0%-2%

[g] Historical incidence for inhalation studies: 6/449 (1.3% ± 1.4%), range 0%-4%

[h] Historical incidence for inhalation studies: 1/449 (0.2% ± 0.7%), range 0%-2%

[i] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 345/449 (76.8% ± 5.9%), range 66%-84%

Table 3-6: Incidences of observed neoplastic and non-neoplastic lesions of female rats after exposure to different cumene concentrations (overall rates according to NTP). Results taken from NTP (2009).

Organs affected	0 ppm	250 ppm	500 ppm	1000 ppm
Nose				
Olfactory epithelium, hyperplasia, basal cell[a]	0/50 (0%)	14/48 (29%)**	25/50 (50%)**	31/50 (62%)**

Respiratory epithelium, hyperplasia	0/50 (0%)	0/48 (0%)	4/50 (8%)	6/50 (12%)*
Respiratory epithelium, adenoma[b,c]:	0/50 (0%) P=0.320[d]	5/48 (10%)*	4/50 (8%)	3/50 (6%)
Kidney				
Nephropathy	38/50 (76%)	37/50 (74%)	41/50 (82%)	44/50 (88%)
Level of evidence of carcinogenic activity (according to NTP)	Some evidence			

Significant difference from chamber control group determined by Poly-3 test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

[a] Overall rate, number of animals with lesion per number of animals examined microscopically

[b] Overall rate, number of animals with neoplasm per number of animals with nose examined microscopically

[c] Historical incidence for inhalation studies: 0/496

[d] For chamber control incidence, P value is given that is associated with the trend test determined by Poly-3 test (accounts for differential mortality in animals, which do not reach terminal sacrifice).

3.9.1.2 [Study 2]

Study reference:

NTP, Toxicology and Carcinogenesis Studies of Cumene in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 542, 2009 (NTP, 2009).

Detailed study summary and results:

Test type

2-year carcinogenicity study in mice, similar to OECD TG 451, GLP compliance according to FDA Good Laboratory Practice Regulations (21 CFR, Part 58) is given.

Male and female mice (n=50/sex/concentration) were exposed to cumene concentrations at 0, 125 (females only), 250, 500, and 1000 (males only) ppm in air for 6 hours plus T_{90} (12 minutes) per day, 5 d/w, for a period of 105 weeks. Survival, clinical signs, and body weights were observed during the study. Surviving animals were sacrificed at study end and necropsy performed. Appearances of neoplasms and non-neoplastic lesions in exposed animals compared to controls was analysed.

A reliability of 2 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- Degree of purity: 99.9%
- Impurities: no impurities with an area percent greater than 0.1% determined by GC-chromatography
- Batch number: 200556852
- Stability under test conditions: stable over the test period, no degradation of test substance was detected

Test animals

- Mice/B6C3F1/ male and female

- 50 male and female animals /concentration group
- Age at study initiation: 5 to 6 weeks and weight at first day of exposure: 23.6-23.8 g (males) and 19.6-19.8 g (females)

Administration/exposure

- Route of administration: inhalation (vapour)
- Duration of test/exposure period: 6 hours plus T90 (12 minutes) per day
- Nominal concentration levels: 0, 125 (females only), 250, 500, and 1000 (males only) ppm, analytical concentration levels: 0, 125 ± 2, 250 ± 6, 501 ± 13, and 1007 ± 24 ppm, dose selection rationale was based on results from a 90-d study in which exposure to cumene (0, 62.5, 125, 250, 500, and 1000 ppm) led in males and females to: increase in absolute liver weight at 500 and 1000 ppm; in males to: slight decreases in body weights (500 ppm and greater) and minimal effects on organ weights (cauda epididymis decreased at 1000 ppm) and incidences of lesions (liver necrosis at 1000 ppm); in females: lower survival rate for 1000 ppm females, incidences of thymic necrosis at 1000 ppm, and incidences of liver (focal chronic inflammation increased at 62.5 ppm and greater) and forestomach lesions (inflammation and hyperplasia at 500 ppm and greater), thus lower concentrations were used for females in the carcinogenicity study)
- Frequency of treatment: 5d/w, 105 weeks
- Control group animals were chamber exposed
- Historical control data is available for the study
- No post exposure time
- Vehicle: unchanged (no vehicle)
- Type of inhalation exposure and test conditions: an inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) was used. Vapour was generated by evaporating the substance in glass column with glass beads. Conditions in chamber were: Temperature (°C): 24 ± 2 (75 ± 2°F), Humidity (%): 55 ± 15, Air changes (per h): 15 ± 2, Photoperiod (hrs dark / hrs light): 12/12. Each chamber had a total active mixing volume of 1.7 m^3.
- Method of exposure: whole body, animals were hold individually in exposure chambers
- Analytical verification of test atmosphere concentrations: Every 20 minutes, the concentrations in exposure chambers were monitored by on-line gas chromatography (GC-FID).
- Particle size: A condensation particle counter (Model 3022A, TSI, Inc., St. Paul, MN) was used. Particle counts greater than 200 particles/cm^3 were not detected.

Examinations:

- Cage side observations: yes, twice daily

- Detailed clinical observations: yes, weekly trough week 13, afterwards every 4 weeks through week 93, every 2 weeks thereafter and at the end of the study
- Body weight: yes, initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter and at the end of the study
- Food efficiency: No data
- Water consumption: No data
- Ophthalmoscopic examination: No data
- Clinical pathology: No
- Haematology: No
- Clinical chemistry: No
- Gross pathology: yes, all animals were examined for grossly visible lesions on all organs and tissues
- Histopathology: Yes, adrenal gland, bone with marrow, brain, clitoral gland, oesophagus, eyes, gallbladder, 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

Results and discussion

Incidences of concentrations are given in the following order: controls, 125 (females only), 250, 500, and 1000 (males only) ppm.

- Mortality and time to death: A significant decrease in survival rates in male mice (38/50, 34/50, 30/50, 23/50) was observed at 1000 ppm. Survival rates female (37/50, 36/50, 39/50, 35/50) mice were similar in comparison to controls.
- Clinical signs: Despite more frequently observed thinness and abnormal breathing in 1000 ppm males and 500 ppm females late in the study, no treatment-related clinical findings were noticed.
- Body weight gain: Body weights of exposed rats were similar to controls except mean body weights of males at 1000 ppm were decreased compared to controls after 8 weeks and females exposed to 500 ppm had decreased body weights from week 28 until week 76 of the study.
- Organ weights: no effects
- Necropsy findings: no effects
- Statistical methods were used for estimating the probability of survival, identifying treatment-related effects on survival, calculating incidences, determining incidences of neoplasm and non-neoplastic lesions, determining the significance of pairwise comparisons between exposed and control groups and are described in details in the study on pages 31 to 32.

HISTOPATHOLOGY: NON-NEOPLASTIC

Histopathological findings in form of incidences of neoplastic and non-neoplastic lesions are given in Table 3-7Table 3-7 for male and in Table 3-8 for female mice.

LUNG:

In male and female mice of all exposed groups, a significant increase in incidences of alveolar epithelial bronchiole metaplasia (males: 5/50, 43/50, 42/50, 39/50, females: 0/50, 42/50, 49/50, 47/50) and bronchiole hyperplasia were observed (males: 0/50, 11/50, 17/50, 18/50, females: 0/50, 17/50, 10/50, 14/50).

NOSE:

Effects in males: olfactory epithelium, atrophy (4/50, 13/50, 11/49, 38/48); olfactory epithelium, hyperplasia, basal cell (0/50, 0/50, 15/49, 33/48); olfactory epithelium, hyperplasia, atypical (0/50, 0/50, 5/49, 11/48); olfactory epithelium, glands, hyperplasia (3/50, 11/50, 9/49, 23/48); inflammation, suppurative (2/50, 2/50, 9/49, 6/48). Incidences were significantly increased in 500 and 1000 ppm males.

Effects in females: olfactory epithelium, atrophy (4/50, 11/50, 9/50, 18/50); olfactory epithelium, hyperplasia, basal cell (0/50, 1/50, 11/50, 25/50); olfactory epithelium, hyperplasia, atypical (0/50, 0/50, 2/50, 10/50); olfactory epithelium, glands, hyperplasia (1/50, 4/50, 4/50, 11/50); respiratory epithelium, metaplasia, squamous (0/50, 0/50, 1/50, 6/50); inflammation, suppurative (0/50, 1/50, 3/50, 7/50).

Incidences were significantly increased in 500 ppm females, except for incidences of basal cell hyperplasia, which were also significantly increased in 250 ppm females.

LIVER:

Incidences of eosinophilic foci were significantly increased in males mice (6/50, 5/50, 16/50, 14/50).

FORESTOMACH:

Higher incidences of ulceration (1/50, 4/50, 6/50, 6/49) and inflammation (0/50, 2/50, 1/50, 5/49) were significantly increased in 1000 ppm males and of epithelial hyperplasia (2/50, 7/50, 8/50, 13/49) in 500 and 1000 ppm males.

HISTOPATHOLOGY: NEOPLASTIC

LUNG:

Effects in males: alveolar/bronchiolar adenoma (13/50, 31/50, 31/50, 29/50); alveolar/bronchiolar carcinoma (9/50, 19/50, 32/50, 33/50); alveolar/bronchiolar adenoma or carcinoma (19/50, 38/50, 42/50, 43/50).

Effects in females: alveolar/ bronchiolar adenoma (1/50, 26/50, 36/50, 38/50); alveolar/bronchiolar carcinoma (3/50, 16/50, 20/50, 34/50); alveolar/bronchiolar adenoma or carcinoma (4/50, 31/50, 42/50, 46/50).

Incidences were seen in all exposed groups (males and females), which were significantly increased compared to controls.

LIVER:

In 500 ppm females, a significant increase in incidences of hepatocellular adenoma (18/50, 23/50, 27/50, 29/50) and hepatocellular adenoma or carcinoma (combined) (25/50, 26/50, 29/50, 36/50) were observed.

SPLEEN:

Neoplastic effects observed in spleen were equivocal. Significantly, increased incidences of hemangiosarcoma (0/50, 0/50, 0/49, 4/50) were seen in 1000 ppm male mice.

THYROID GLAND:

Neoplastic effects observed in thyroid gland were equivocal. Significantly, increased incidences of follicular cell adenoma (0/50, 0/50, 0/49, 3/50) were seen in 1000 ppm male mice.

Table 3-7: Incidences of neoplastic and non-neoplastic lesions of male mice after exposure to cumene concentrations (overall rates according to NTP). Results taken from NTP (2009).

Organs affected	0 ppm	250 ppm	500 ppm	1000 ppm
Lung				
Alveolar epithelium, bronchiole, metaplasia[a]	5/50 (10%)	43/50 (86%)**	42/50 (84%)**	39/50 (78%)**
Bronchiole, hyperplasia	0/50 (0%)	11/50 (22%)**	17/50 (34%)**	18/50 (36%)**
Alveolar/bronchiolar adenoma, multiple	1/50 (2%)	11/50 (24%)**	15/50 (30%)**	20/50 (40%)**
Alveolar/bronchiolar adenoma (includes multiple)[b,c]	13/50 (26%) P<0.001[d]	31/50 (62%)***	31/50 (62%)***	29/50 (58%)***
Alveolar/bronchiolar carcinoma, multiple	0/50 (0%)	8/50 (16%)**	20/50(40%)**	17/50 (34%)**
Alveolar/bronchiolar carcinoma (includes multiple)[b,e]	9/50 (18%) P<0.001[d]	19/50 (38%)*	32/50 (64%)***	33/50 (66%)***
Alveolar/bronchiolar adenoma or carcinoma[b, f]	19/50 (38%) P<0.001[d]	38/50 (76%)***	42/50 (84%)***	43/50 (86%)***
Nose				
Olfactory epithelium, atrophy[a]	4/50 (8%)	13/50 (26%)*	11/49 (22%)*	38/48 (79%)**
Olfactory epithelium, hyperplasia, basal cell	0/50 (0%)	0/50 (0%)	15/49 (31%)**	33/48 (69%)**
Olfactory epithelium, hyperplasia, atypical	0/50 (0%)	0/50 (0%)	5/49 (10%)*	11/48 (23%)**
Olfactory epithelium, glands, hyperplasia	3/50 (6%)	11/50 (22%)*	9/49 (18%)*	23/48 (48%)**
Inflammation, suppurative	2/50 (4%)	2/50 (4%)	9/49 (18%)*	6/48 (12%)
Liver				
Eosinophilic focus[a]	6/50 (12%)	5/50 (10%)	16/50 (32%)**	14/50 (28%)*
Hepatocellular adenoma, multiple	17/50 (34%)	20/50 (40%)	22/50 (44%)	26/50 (52%)

Hepatocellular adenoma (includes multiple)[g]	34/50 (68%)	33/50 (66%)	37/50 (74%)	35/50 (70%)
Hepatocellular carcinoma, multiple	3/50 (6%)	1/50 (2%)	4/50 (8%)	7/50 (14%)
Hepatocellular carcinoma, (includes multiple)[h]	13/50 (26%)	18/50 (36%)	21/50 (42%)	17/50 (34%)
Hepatocellular adenoma or carcinoma[b,i]	40/50 (80%) P=0.250[d]	42/50 (84%)	43/50 (86%)	41/50 (82%)
Forestomach				
Epithelium, hyperplasia[a]	2/50 (4%)	7/50 (14%)	8/50 (16%)*	13/49 (27%)**
Ulcer	1/50 (2%)	4/50 (8%)	6/50 (12%)	6/49 (12%)*
Inflammation	0/50 (0%)	2/50 (4%)	1/50 (2%)	5/49 (10%)*
Hemangiosarcoma				
Hemangiosarcoma, Spleen[b,j]	0/50 (0%) P=0.002[d]	0/50 (0%)	0/49 (0%)	4/50 (8%)*
Hemangiosarcoma, all organs[k,l]	0/50 (0%) P=0.015[d]	1/50 (2%)	2/50 (4%)	4/50 (8%)*
Thyroid gland				
Follicular cell, hyperplasia[a]	7/50 (14%)	7/50 (14%)	7/49 (14%)	11/50 (22%)
Follicular cell, adenoma[b,m]	0/50 (0%) P=0.010[d]	0/50	0/49 (0%)	3/50 (6%)[n]
Level of evidence of carcinogenic activity (according to NTP)	Clear evidence			

Significant difference from chamber control group determined by Poly-3 test: * $p \leq 0.05$, ** $p \leq 0.01$,*** $p \leq 0.001$

[a] Overall rate, number of animals with lesion per number of animals examined microscopically

[b] Overall rate, number of animals with neoplasm per number of animals with lung/liver/tissue/thyroid gland examined microscopically (only with regard to the organ under investigation)

[c] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 92/449 (20.5% ± 5.3%), range 12%-26%

[d] For chamber control incidence, P value is given that is associated with the trend test determined by Poly-3 test (accounts for differential mortality in animals, which do not reach terminal sacrifice).

[e] Historical incidence for inhalation studies: 64/449 (14.2% ± 4.6%), range 10%-24%

[f] Historical incidence for inhalation studies: 146/449 (32.5% ± 5.9%), range 26%-44%

[g] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 196/449 (43.7% ± 10.9%), range 30%-68%

[h] Historical incidence for inhalation studies: 107/449 (23.8% ± 4.6%), range 18%-32%

[i] Historical incidence for inhalation studies: 264/449 (58.8% ± 9.6%), range 50%-80%

[j] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 6/444 (1.4% ± 1.5%), range 0%-4%

[k] Overall rate, number of animals with neoplasm per number of animals necropsied

[l] Historical incidence for inhalation studies: 21/450 (4.7% ± 3.7%), range 0%-12%

[m] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 5/441 (1.1% ± 2.0%), range 0%-6%

[n] P = 0.095 (NTP, 2009), but according to NTP (2013) the significance is ** ($P \leq 0.01$) compared with chamber controls

Table 3-8: Incidences of neoplastic and non-neoplastic lesions of female mice after exposure to cumene concentrations (overall rates according to NTP). Results taken from NTP (2009).

Organs affected	0 ppm	125 ppm	250 ppm	500 ppm
Lung				
Alveolar epithelium, bronchiole, metaplasia[a]	0/50 (0%)	42/50 (84%)**	49/50 (98%)**	47/50 (94%)**

Bronchiole, hyperplasia	0/50 (0%)	17/50 (34%)**	10/50 (20%)**	14/50 (28%)**
Alveolar/bronchiolar adenoma, multiple	0/50 (0%)	13/50 (26%)**	20/50 (40%)**	30/50 (60%)**
Alveolar/bronchiolar adenoma (includes multiple)[b,c]	1/50 (2%) P<0.001[d]	26/50 (62%)***	36/50 (72%)***	38/50 (76%)***
Alveolar/bronchiolar carcinoma, multiple	0/50 (0%)	6/50 (12%)*	7/50(14%)**	19/50 (38%)**
Alveolar/bronchiolar carcinoma (includes multiple)[b,e]	3/50 (6%) P<0.001[d]	16/50 (32%)***	20/50 (40%)***	34/50 (68%)***
Alveolar/bronchiolar adenoma or carcinoma [b,f]	4/50 (8%) P<0.001[d]	31/50 (62%)***	42/50 (84%)***	46/50 (92%)***
Nose				
Olfactory epithelium, atrophy[a]	4/50 (8%)	11/50 (22%)*	9/50 (18%)	18/50 (36%)**
Olfactory epithelium, hyperplasia, basal cell	0/50 (0%)	1/50 (2%)	11/50 (22%)**	25/50 (50%)**
Olfactory epithelium, hyperplasia, atypical	0/50 (0%)	0/50 (0%)	2/50 (4%)	10/50 (20%)**
Olfactory epithelium, glands, hyperplasia	1/50 (2%)	4/50 (8%)	4/50 (8%)	11/50 (22%)**
Respiratory epithelium, metaplasia, squamous	0/50 (0%)	0/50 (0%)	1/50 (2%)	6/50 (12%)*
Inflammation, suppurative	0/50 (0%)	1/50 (2%)	3/50 (6%)	7/50 (14%)*
Liver				
Eosinophilic focus[a]	8/50 (16%)	11/50 (22%)	7/50 (14%)	14/50 (28%)
Hepatocellular adenoma, multiple	9/50 (18%)	13/50 (26%)	9/50 (18%)	10/50 (20%)
Hepatocellular adenoma (includes multiple)[b,g]	18/50 (36%) P=0.040[d]	23/50 (46%)	27/50 (54%)[h]	29/50 (58%)*
Hepatocellular carcinoma, multiple	2/50 (4%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Hepatocellular carcinoma, (includes multiple)[i]	10/50 (20%)	7/50 (14%)	6/50 (12%)	12/50 (24%)
Hepatocellular adenoma or carcinoma[b,j]	25/50 (50%) P=0.024[d]	26/50 (52%)	29/50 (58%)[h]	36/50 (72%)*
Hemangiosarcoma				
Hemangiosarcoma, Spleen[a,k]	0/49 (0%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Hemangiosarcoma, all organs[l,m]	1/50 (2%) P=0.518N[d]	3/50 (6%)	6/50 (12%)	1/50 (2%) P= 0.746N[d]
Level of evidence of carcinogenic activity (according to NTP)	Clear evidence			

Significant difference from chamber control group determined by Poly-3 test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

[a] Overall rate, number of animals with lesion per number of animals examined microscopically

[b] Overall rate, number of animals with neoplasm per number of animals with lung/liver/tissue examined microscopically (only with regard to the organ under investigation)

[c] Historical incidence for inhalation studies: 19/449 (4.2% ± 2.5%), range 2%-8%

[d] For chamber control incidence, P values is given that is associated with the trend test determined by Poly-3 test (accounts for differential mortality in animals, which do not reach terminal sacrifice). A negative trend or lower incidence in an exposed group is indicated by N.

[e] Historical incidence for inhalation studies: 15/449 (3.4% ± 3.9%), range 0%-12%

[f] Historical incidence for inhalation studies: 34/449 (7.6% ± 4.0%), range 2%-14%

[g] Historical incidence for inhalation studies: 109/447 (24.4% ± 8.7%), range 12%-36%

[h] One animal with adenoma also had hepatoblastoma

[i] Historical incidence for inhalation studies: 48/447 (10.7% ± 4.1%), range 6%-20%

[j] Historical incidence for inhalation studies: 145/447 (32.4% ± 8.8%), range 22%-50%

[k] Historical incidence for inhalation studies: 6/445 (1.3% ± 1.4), range 0%-4%
[l] Overall rate, number of animals with neoplasm per number of animals necropsied
[m] Historical incidence for inhalation studies: 16/449 (3.6 ± 2.2%), range 2%-8%

3.9.2 Human data

No data presented here.

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No data presented here.

3.9.4 Other data (e.g. studies on mechanism of action)

No data presented here.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 [Study 1]

Study reference:

Darmer Jr et al., Developmental Toxicity of Cumene Vapor in CD Rats and New Zealand White Rabbits, International Journal of Toxicology, 16:119- 139, 1997 (Darmer et al., 1997).

Detailed study summary and results:

Test type

Developmental toxicity study in rats, according to OECD 414, GLP compliance is given

Pregnant CD rats (n=25/sex) were exposed to cumene vapours (0, 100, 500, and 1200 ppm) by inhalation on gestation days 6 to 15 for 6 hours a day. Effects on fertility and sexual function of female rats and malformations (external, visceral, or skeletal) and variations of foetuses were evaluated.

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- Degree of purity (analytical): >99.9%
- Impurities: not given

Test animals

- Rats/Sprague-Dawley/female
- 25 female animals /concentration group
- Age at study initiation: 74 d (14 d quarantine included)
- Weight at study initiation: 213 - 251 g

Administration/exposure

- Route of administration –inhalation (vapour)
- Duration and frequency of test/exposure period: only females, 6 h/d from GD 6 to 15
- Nominal concentration levels: 0, 100, 500, and 1200 ppm, analytical concentration levels: 0, 99 - 2.4 ppm (99 % of target), 488 - 8.6 ppm (98 % of target), and 1,211 - 23.8 ppm (101 % of target) (data presented as indicated by the authors, presumably this means: 0, 99 ± 2.4, 488 ± 8.6, and 1,211 ± 23.8 ppm). In control chambers, no cumene was detected.
- Dose selection rationale was based on preliminary range-finding studies conducted in the same laboratory.
- Control group animals were chamber exposed
- Historical control data: not given
- Post exposure time: until GD 21
- Vehicle: unchanged (no vehicle)
- Type of inhalation exposure and test conditions: Exposure apparatus: 4320 L rectangular glass and stainless steel chambers (Wahmann Manufacturing, Timonium, MD, USA). A piston pump metered cumene in liquid form in one or two heated glass evaporators and by passing conditioned air through the evaporators the cumene vapour was carried into the test chambers. Test conditions in chamber were temperature (°C): 25.3 – 25.7, humidity (%): 43.2 – 52.6, air flow rate (mL/min): 900, air changes (per h): 14, and photoperiod (hrs dark / hrs light): 12/12.
- Method of exposure: whole body, animals were hold individually in exposure chambers
- Analytical verification of test atmosphere concentrations: Once every 30 minutes, the concentrations in exposure chambers were monitored by GC-FID for a 6-hour exposure period. At 5 different locations the cumene distribution in each chamber was tested. Due to a variation coefficient of 1.4 % or less, a uniform vapour distribution of cumene in test chambers was presumed.

Description of test design:

- Details on mating procedure: M/F ratios per cage: 1:1, length of cohabitation: not given, proof of pregnancy: vaginal plug referred to as day 0 of pregnancy
- Premating exposure period for males and females: none

- Groups of 25 plug-positive females were randomly assigned to 4 exposure groups and treated with cumene vapours 6 h/d from GD 6 to 15
- Standardization of litters: no
- Parameters assessed for P:
    - cage side observations: yes, daily
    - detailed clinical observations: yes, daily
    - body weight: yes, GD 0, 6, 9, 12, 15, 18, and 21
    - food consumption and compound intake: yes, daily
    - post-mortem examinations: yes
    - sacrifice on gestation day 21
    - organs examined: gravid uterus, ovaries (incl. corpora lutea), cervix, vagina, abdominal and thoracic cavities, upper and lower respiratory tracts incl. nasal turbinates, and liver weights were recorded
    - ovaries and uterine examinations: number of corpora lutea, number of implantations, number of early resorptions, number of late resorptions
- Parameters assessed for F1:
    - body weight: yes
    - sex: yes
    - external examinations: all per litter
    - soft tissue and skeletal examinations: half per litter
    - head examinations: half per litter
- Post exposure observation period: until GD 21

Results and discussion

- Statistical treatment of results: The data of treated female groups or litters were compared to the control group. For assessment of quantitative continuous variables, such as body weight, liver weight, etc., ANOVA and t test were used and for data obtained from laparohysterectomy Kruskal-Wallis test followed by Mann-Whitney U test were performed. Fisher's exact test was used for comparing incidences. The differences in the data were considered statistically significant at probability of $p<0.05$ (two-tailed).

For P:

- Number of animals at the start of the test and matings: 25 pregnant females
- Time of death during the study and whether animals survived to termination: all animals survived until termination

- Pregnancy: 3 dams were not pregnant in the 500 ppm group and 2 dams in the 100 ppm and 0 ppm group, respectively.
- Body weight data: reduced body weight gain at 1200 ppm during exposure period, food consumption intake was significantly decreased in 500 and 1200 ppm exposure groups (see Table 3-9).
- Body weight at sacrifice and absolute and relative organ weight data for the parental animals: The body weights at sacrifice were not significantly different to controls. In the highest concentration group (1200 ppm) an increase in relative liver weights was observed (see Table 3-9). Absolute liver weights were not affected.
- Body weight change and gravid uterine weight: The maternal corrected gestational weight change or gravid uterine weight were not significantly different compared to controls.
- Clinical observations: Perioral wetness and encrustation in the 1200 ppm group was observed.
- None of the investigated gestational parameters were affected (number of corpora lutea, number of total nonviable (early or late resorptions or dead foetuses) or viable implantations, percent pre- or post-implantation loss).
- Of the pregnant dams all had live litters and for each examined group the size of litters was between 22 and 25.
- Necropsy findings: none

For F1:

- Sex ratio: not affected
- incidences of malformations and variations are presented in Table 3-10

Table 3-9: Effects of cumene exposure on mortality, body weight gain, food consumption and relative organ weight of female rats.

Parameters	**0 ppm**	**100 ppm**	**500 ppm**	**1200 ppm**
Mortality/pregnant/ total number of females	0/23/25	0/23/25	0/22/25	0/25/25
Body weight gain (g)				
Days 6-9 (mean ± SD)	12.7 ± 5.4	9.3 ± 5.4	8.7 ± 6.8	2.3 ± 5.7**
Days 6-15 (mean ± SD)	49.2 ± 7.0	48.3 ± 9.9	48.4 ± 7.2	38.6 ± 12.2**
Food consumption (g/animal day^{-1})				
Days 0-6 (pre-treatment) (mean ± SD)	26.1 ± 2.1	25.6 ± 2.6	25.8 ± 2.0	26.3 ± 2.8
Days 6-15 (treatment) (mean ± SD)	27.2 ± 2.1	26.9 ± 2.1	25.3 ± 2.4*	23.1 ± 2.5**
Days 15-21 (post-treatment) (mean ± SD)	31.1 ± 2.9	32.4 ± 3.0	31.6 ± 2.5	31.1 ± 2.9
Mean relative liver weight[a] (%, mean ± SD)	5.22 ± 0.39	5.25 ± 0.36	5.50 ± 0.34	5.62 ± 0.49**

* Significantly different from control group (p≤0.05). **Significantly different from control group (p≤0.01). [a]Mean relative liver weight calculated as percentage of body weight at sacrifice minus gravid uterine weight. Results taken from Darmer et al. (1997).

Table 3-10: Incidences of malformations and variations in foetuses of rats exposed to cumene vapours. Data presented as affected foetuses (litters involved).

Parameters	0 ppm	100 ppm	500 ppm	1200 ppm
Number of foetuses (litter) examined				
External	363 (23)	328 (23)	338 (22)	378 (25)
Visceral	186 (23)	170 (23)	174 (22)	197 (25)
Skeletal	177 (23)	158 (23)	164 (22)	181 (25)
Total Malformations				
External Number/Percent	0 (0) /0.0 (0.0)	3 (1) /0.9 (4.3)	0 (0) /0.0 (0.0)	1 (1) /0.3 (4.0)
Soft Tissue Number/Percent	17 (12) /9.1 (52.2)	22 (12) /12.9 (52.2)	19 (10) /10.9 (45.5)	14 (9) /7.1 (36.0)
Skeletal Number/Percent	0 (0) /0.0 (0.0)	1 (1) /0.6 (4.3)	0 (0) /0.0 (0.0)	0 (0) /0.0 (0.0)
Total Malformations Number/Percent	17 (12) /4.7 (52.2)	23 (12) /7.0 (52.2)	19 (10) /5.6 (45.5)	15 (10) /4.0 (40.0)
Specific variations				
Visceral				
- dilated ureter, bilateral	22 (10)	6 (5)	9 (6)	3 (1)**
-urinary bladder distended	21 (10)	13 (6)	13 (9)	6 (3)*
Skeletal				
- thoracic centrum #11 bilobed	27 (17)	15 (9)*	15 (10)	52 (21)
- thoracic centrum #12 bilobed	35 (15)	9 (7)*	30 (15)	62 (20)
- parietal, poorly ossified	7 (5)	0 (0)*	7 (4)	0 (0)*
- sternebra #5 bilobed	14 (11)	10 (6)	4 (3)*	6 (5)
Total Variations				
External Number/Percent	18 (12)/5.0 (52.2)	14 (9)/4.3 (39.1)	21 (13) /6.2 (59.1)	18 (15) /4.8 (60.0)
Soft tissue Number/Percent	71 (21) /38.2 (91.3)	61 (20) /35.9 (87.0)	59 (20) /33.9 (90.9)	66 (24) /33.5 (96.0)
Skeletal Number/Percent	177 (23)/100.0 (100.0)	158 (23) /100.0 (100.0)	164 (22) /100.0 (100.0)	181 (25) /100.0 (100.0)
Total Variations Number/Percent	254 (23) /70.0 (100.0)	224 (23) / 68.3 (100.0)	229 (22) / 67.8 (100.0)	254 (25) / 67.2 (100.0)

* Significantly different from control group at 0.05 level (two-tailed Fisher's exact test). **Significantly different from control group at 0.01 level (two-tailed Fisher's exact test). Results taken from Darmer et al. (1997).

3.10.1.2 [Study 2]

Study reference:

Darmer Jr et al., Developmental Toxicity of Cumene Vapor in CD Rats and New Zealand White Rabbits, International Journal of Toxicology, 16:119- 139, 1997 (Darmer et al., 1997).

Detailed study summary and results:

Test type

Developmental toxicity study in rabbits, according to OECD 414, GLP compliance is given

Pregnant CD rabbits (n=15/sex) were exposed to cumene vapours 0, 500, 1200, and 2300 ppm by inhalation on gestation days 6 to 18 for 6 hours a day. Effects on fertility and sexual function of female rabbits and malformations (external, visceral, or skeletal) and variations of foetuses were evaluated.

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Cumene
- Degree of purity (analytical): >99.9%
- Impurities: not given

Test animals

- Rabbits/New Zealand White/female
- 15 female animals /concentration group
- Age at study initiation: 5.5 months + 14 d quarantine
- Weight at study initiation: at least 2.5 kg

Administration/exposure

- Route of administration –inhalation (vapour)
- Duration and frequency of test/exposure period: only females, 6 h/d from GD 6 to 18
- Nominal concentration levels: 0, 500, 1200, and 2300 ppm, analytical concentration levels: 0, 492 - 11.5 ppm (98 % of target), 1,206 - 23.4 ppm (101 % of target), and 2,297 - 65.2 ppm (100 % of target) (data presented as indicated by the authors, presumably this means: 492 ± 11.5, 1,206 ± 23.4, and 2,297 ± 65.2 ppm). In control chambers was no cumene detected, dose selection rationale was based on preliminary range-finding studies conducted in the same laboratory
- Control group animals were chamber exposed
- Historical control data: yes, for ecchymosis (haemorrhage) on the head
- Post exposure time: until GD 29
- Vehicle: unchanged (no vehicle)
- Type of inhalation exposure and test conditions: Exposure apparatus: 4320 L rectangular glass and stainless steel chambers (Wahmann Manufacturing, Timonium, MD, USA). A piston pump metered cumene in liquid form in one or two heated glass evaporators and by passing conditioned air through the evaporators the cumene vapour was carried into the test chambers. Test conditions in chamber

were temperature (°C): 20 – 22.2, humidity (%): 44 – 70, air flow rate (mL/min): 900, air changes (per h): 14, and photoperiod (hrs dark / hrs light): 12/12.

- Method of exposure: whole body, animals were hold individually in exposure chambers
- Analytical verification of test atmosphere concentrations: Once every 30 minutes, the concentrations in exposure chambers were monitored by GC-FID for a 6-hour exposure period. At five different locations, the cumene distribution in each chamber was tested. Due to a variation coefficient of 1.4 % or less, a uniform vapour distribution of cumene in test chambers was presumed.

Description of test design:

- Details on mating procedure: M/F ratios per cage: 2:1, length of cohabitation: not given, proof of pregnancy: date of copulation is referred to as day 0 of pregnancy
- Premating exposure period for males and females: none
- Groups of 15 mated females were randomly assigned to 4 exposure groups and treated with cumene vapours 6 h/d from GD 6 to 18
- Standardization of litters: no
- Parameters assessed for P:
    - cage side observations: Yes, daily
    - detailed clinical observations: Yes, daily
    - body weight: Yes, GD 0, 6, 12, 18, 24 and 29
    - food consumption and compound intake: Yes, daily
    - post-mortem examinations: Yes
    - sacrifice on gestation day 29
    - Organs examined: gravid uterus, ovaries (incl. corpora lutea), cervix, vagina, abdominal and thoracic cavities, upper and lower respiratory tracts incl. nasal turbinates, and liver weights were recorded
    - ovaries and uterine examinations: number of corpora lutea, number of implantations, number of early resorptions, number of late resorptions
- Parameters assessed for F1
    - body weight: yes
    - sex: yes
    - external and skeletal examinations: all per litter
    - visceral examinations: half per litter
    - head examinations: half per litter
- Post exposure observation period: until GD 29

Results and discussion

- Statistical treatment of results: The data of treated female groups or litters were compared to the control group. For assessment of quantitative continuous variables, such as body weight, liver weight, etc., ANOVA and t test were used and for data obtained from laparohysterectomy Kruskal-Wallis test followed by Mann-Whitney U test were performed. Fisher's exact test was used for comparing incidences. The differences in the data were considered statistically significant at probability of $p<0.05$ (two-tailed).

For P (see Table 3-11):

- Number of animals at the start of the test and matings: 15 mated females
- Time of death during the study and whether animals survived to termination: Two does died (one killed due to moribund, one found dead) and one aborted in the highest concentration group (2300 ppm) on GD 18.
- Pregnancy: all females were pregnant.
- Body weight data: In the 2300 ppm exposure group, body weight gain was significantly reduced during exposure from GD 6 to 18 and a subsequent increase in the post treatment period (GD 18-29) was observed. However, in periodic body weights a significantly difference between groups was not seen. Food consumption was significantly decreased in all exposed groups during exposure.
- Body weight at sacrifice and absolute and relative organ weight data for the parental animals: The body weights at sacrifice were not significantly different to controls. In the highest concentration group (2300 ppm) an increase in relative liver weights was observed. Absolute liver weights were not affected.
- Body weight change and gravid uterine weight: The maternal corrected gestational weight change or gravid uterine weight were not significantly different compared to controls.
- Clinical observations: Perioral wetness in the 2300 ppm group was observed.
- Nonviable implants were found in one doe of 500 and 1200 ppm group, respectively.
- None of the investigated gestational parameters were affected (number of corpora lutea, number of total nonviable (early or late resorptions or dead foetuses) or viable implantations, percent pre- or post-implantation loss).
- Necropsy findings: In 4 of 12 does at 2300 ppm, colour changes of lungs were seen.

For F1:

- Sex ratio: not affected
- Significant differences in the incidences of malformations were not observed. The observed, significantly higher incidences in skeletal and visceral variations were not treatment-related (see Table 3-12). A significant increase in the external variation of ecchymosis (small haemorrhage) on the head was seen at 500 ppm compared to controls. The number decreased with higher cumene

concentrations (1200 ppm: 28.6 %; 2300 ppm: 25.0 %). For this type of variation, historical control data from 24 developmental toxicity studies in rabbits performed in the same laboratory is present and reported a variation from 0 to 66.7 % of litters in controls. A consistency between the incidence of this variation in historical control data and the cumene-exposed groups was considered and therefore it is not treatment-related effect.

Table 3-11: Effects of cumene exposure on mortality, body weight gain, food consumption and relative organ weight of female rabbits.

Parameters	0 ppm	500 ppm	1000 ppm	2300 ppm
Mortality[a]/total number of females	0/15	0/15	0/15	2/15
Females pregnant/aborted	15/0	15/0	15/0	15/2
Body weight gain (g)				
Days 6-18 (mean ± SD)	81.6 ± 121.5	9.8 ± 201.7	6.7 ± 151.8	-178.1 ± 292.0**
Days 18-29 (mean ± SD)	162.9 ± 97.4	145 ± 93	164 ± 118	259 ± 73*
Food consumption (g/animal day^{-1})				
Days 0-6 (pre-treatment) (mean ± SD)	179.3 ± 43.8	165.5 ± 17.7	162.6 ± 53.2	159.9 ± 34.4
Days 6-18 (treatment) (mean ± SD)	139.5 ± 39.3	102.4 ± 39.6*	104.6 ± 35.9*	83.2 ± 27.2**
Days 18-29 (post-treatment) (mean ± SD)	130.4 ± 36.8	129.6 ± 36.2	130.1 ± 35.9	151.8 ± 24.1
Mean relative liver weight[b] (%, mean ± SD)	2.56 ± 0.33	2.61 ± 0.35	2.68 ± 0.43	2.99 ± 0.30**

* Significantly different from control group ($p \leq 0.05$). **Significantly different from control group ($p \leq 0.01$). [a]Animals found dead or sacrificed due to moribund signs. [b]Mean relative liver weight calculated as percentage of body weight at sacrifice minus gravid uterine weight. Results taken from Darmer et al. (1997).

Table 3-12: Incidences of malformations and variations in foetuses of rabbits exposed to cumene vapours. Data presented as affected foetuses (litters involved).

Parameters	0 ppm	100 ppm	500 ppm	1200 ppm
Number of foetuses (litter) examined				
External	129 (15)	130 (14)	107 (14)	82 (12)
Visceral	129 (15)	130 (14)	107 (14)	82 (12)
Skeletal	129 (15)	130 (14)	107 (14)	82 (12)
Total Malformations				
External Number/Percent	0 (0) /0.0 (0.0)	1 (1) /0.8 (7.1)	1 (1) /0.9 (7.1)	1 (1) /1.2 (8.3)
Soft Tissue Number/Percent	10 (7) /7.8 46.7)	12 (5) /9.2 (35.7)	9 (4) /8.4 (28.6)	4 (2) /4.9 (16.7)
Skeletal Number/Percent	4 (3) /3.1 (20.0)	2 (2) /1.5 (14.3)	2 82) /1.9 (14.3)	4 (3) /4.9 (25.0)
Total Malformations Number/Percent	14 (10) /10.9 (66.7)	13 (5) /10.0 (66.7)	11 (4) /10.3 (28.6)	8 (5) /9.8 (41.7)
Specific variations				
External				

- Ecchymosis, head	0 (0)	7 (5)*	4 (4)	4 (3)
Skeletal				
- rudimentary rib #13, first lumber arch, unilateral	23 (12)	26 (10)	12 (7)	7 (4)*
Total Variations				
External Number/Percent	0 (0) /0.0 (0.0)	7 (5)*/5.4 (35.7)	4 (4) /3.7 (28.6)	4 (3) /4.9 (25.0)
Soft tissue Number/Percent	41 (13) /31.8 (86.7)	38 (10) /29.2 (71.4)	25 (11)/23.4 (78.6)	20 (9) / 24.4 (75.0)
Skeletal Number/Percent	119 (15) /92.2 (100.0)	122 (14) /93.8 (100.0)	103 (14) /96.3 (100.0)	77 (12) /93.9 (100.0)
Total Variations Number/Percent	124 (15) / 96.1 (100.0)	124 (14) /95.4 (100.0)	103 (14) /96.3 (100.0)	78 (12) /95.1 (100.0)

* Significantly different from control group at 0.05 level (two-tailed Fisher's exact test). **Significantly different from control group at 0.01 level (two-tailed Fisher's exact test). Results taken from Darmer et al. (1997).

3.10.2 Human data

No data presented here.

3.10.3 Other data (e.g. studies on mechanism of action)

No data presented here.

3.11 Specific target organ toxicity – single exposure

Evaluation not performed for this substance

3.12 Specific target organ toxicity – repeated exposure

Evaluation not performed for this substance

3.13 Aspiration hazard

Evaluation not performed for this substance

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

Evaluation not performed for this substance

4.2 Bioaccumulation

Evaluation not performed for this substance

4.3 Acute toxicity

Evaluation not performed for this substance

4.4 Chronic toxicity

Evaluation not performed for this substance

4.5 Acute and/or chronic toxicity to other aquatic organisms

Evaluation not performed for this substance

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