CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

4-methylpentan-2-one; isobutyl methyl ketone

EC Number: **203-550-1**

CAS Number: **108-10-1**

Index Number: **606-004-00-4**

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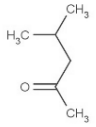
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4-methylpentan-2-one
Other names (usual name, trade name, abbreviation)	2-pentanone, 4-methyl- 4-methyl-2- pentanone Isobutyl methyl ketone Methyl isobutyl ketone / MiBK Isopropyl acetone
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	203-550-1
EC name (if available and appropriate)	4-methylpentan-2-one; isobutyl methyl ketone
CAS number (if available)	108-10-1
Other identity code (if available)	-
Molecular formula	$C_6H_{12}O$
Structural formula	
SMILES notation (if available)	CC(C)CC(=O)C
Molecular weight or molecular weight range	100.161 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	*Not relevant*

1.2 Composition of the substance

Not relevant for the classification of the substance.

Details on the test substance (if available) are given in the study summaries.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2: Proposed harmonised classification and labelling of isobutyl methyl ketone according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	606-004-00-4	4-methylpentan-2-one; isobutyl methyl ketone	203-550-1	108-10-1	Flam. Liq. 2 Acute Tox. 4 * Eye Irrit. 2 STOT SE 3	H225 H332 H319 H335	GHS02 GHS07 Dgr	H225 H332 H319 H335	EUH066		
Dossier submitters proposal	606-004-00-4	4-methylpentan-2-one; isobutyl methyl ketone	203-550-1	108-10-1	**Retain** STOT SE 3 **Modify** Acute Tox. 4 **Add** STOT SE 3 Carc 2	**Retain** H335 H332 **Add** H336 H351	**Retain** GHS02 GHS07 Dgr **Add** GHS08	**Retain** H335 H332 **Add** H336 H351	**Retain** EUH066	inhalation: ATE = 11mg/l[1]	
Resulting Annex VI entry if agreed by RAC and COM	606-004-00-4	4-methylpentan-2-one; isobutyl methyl ketone	203-550-1	108-10-1	Flam. Liq. 2 Carc 2 Acute Tox. 4 Eye Irrit. 2 STOT SE 3 STOT SE 3	H225 H351 H332 H319 H335 H336	GHS02 GHS07 GHS08 Dgr	H225 H351 H332 H319 H335 H336	EUH066	inhalation: ATE = 11mg/l[1]	

[1] Converted acute toxicity point estimate from Table 3.1.2 of CLP

Table 3: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via dermal route	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via inhalation route	*change in harmonised classification proposed*	Yes
Skin corrosion/irritation	*data conclusive but not sufficient for classification*	Yes
Serious eye damage/eye irritation	*harmonized classification*	Yes
Respiratory sensitisation	*Data lacking*	No
Skin sensitisation	*data conclusive but not sufficient for classification*	Yes
Germ cell mutagenicity	*data conclusive but not sufficient for classification*	Yes
Carcinogenicity	*harmonised classification proposed*	Yes
Reproductive toxicity	*data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-single exposure	*harmonised classification proposed*	Yes
Specific target organ toxicity-repeated exposure	*data conclusive but not sufficient for classification*	Yes
Aspiration hazard	*data conclusive but not sufficient for classification*	Yes
Hazardous to the aquatic environment	*hazard class not assessed in this dossier*	No

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

4-methylpentan-2-one (code Q028) has been discussed at the Commission Working Group on the Classification and Labelling of Dangerous Substances in 1997/1998. Available summary records document an agreed classification with F; R11: Xn; R20: Xi; R36/37: R66. Symbols F and Xn. R-phrases 11-20-36/37-66 (ECBI/27/98). It was also agreed that the substance should not be classified for dangers to the environment. The classification was introduced with Commission Directive 98/98/EC adapting to technical progress for the 25th time Council Directive 67/548/EEC.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

In addition:

There is a harmonised classification entry in Annex VI to CLP containing a minimum classification and it is concluded that a refinement of the classification based on available data is justified.

Further detail on need of action at Community level on other endpoints than CMR or respiratory Sensitisation:

4-methylpentan-2-one is an important chemical with wide dispersive use and exposure of professional workers and consumers. To ensure a high level of protection of human health all human health endpoints have been evaluated. Toxicological data provided by the registrants as well as open literature has been considered.

The current classification for 4-methylpentan-2-one has been introduced by Commission Directive 98/98/EC (25th ATP). This harmonized classification has been translated into harmonized CLP classification but the DSD criteria sometimes did not fully correspond to a classification according to the CLP criteria. A minimum classification for acute inhalation toxicity category 4 (Acute Tox 4*) was introduced. To minimize further uncertainty in classification of 4-methylpentan-2-one this endpoint has been evaluated as well and revised in this proposal.

5 IDENTIFIED USES

4-methylpentan-2-one has 27 active registrations under REACH, 1 Joint Submission. The substance has been registered in a tonnage band of 10,000-100,000 tpa (ECHA dissemination website, accessed September 2018).

4-methylpentan-2-one is registered for manufacture, formulation and use at industrial sites, by professionals and by consumers (Table 4). This substance is used in the following products: lubricants and greases, biocides (e.g. disinfectants, pest control products), coating products, anti-freeze products, fillers, putties, plasters, modelling clay and finger paints.

Table 4: Registered uses of 4-methylpentan-2-on (according ECHA dissemination site)

Uses at industrial sites	Use in rubber production and processing
	Use in polymer processing
	Use in cleaning
	Functional Fluids – Industrial
	Manufacture of substance, use as intermediate (not subject to strictly controlled conditions) and as processing aid
	Industrial Use in Adhesives/Sealants
	Water treatment chemicals
	Use in coatings/paints/primers
	Use in Oil and Gas field drilling and production operation – Industrial
	Lubricants
	Industrial use in inks/toners
Uses by professional workers	Metal working fluids / rolling oils
	Use in coatings
	Use in cleanings
	Lubricants
	Polymer processing
	Use in adhesives/sealants
	Functional fluids
	Use in agrochemicals
	Use in laboratories
Consumer uses	Use in coatings
	Use in cleanings
	Use in agrochemicals
	Use as ethanol denaturant

6 DATA SOURCES

Data sources and searches used to compile this CLH report:

ECHA-Dissemination site, 4-methylpentan-2-on: https://echa.europa.eu/registration-dossier/-/registered-dossier/14866/1

C&L inventory https://echa.europa.eu/information-on-chemicals/cl-inventory-database accessed November 2017

ToxPlanet https://toxplanet.com/

Pubmed https://www.ncbi.nlm.nih.gov/pubmed

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	liquid	Registration data	
Melting/freezing point	-84 °C	Registration data	review article or handbook
Boiling point	116 to 118°C	Registration data	review article or handbook
Relative density	0.801 at 20°C	Registration data	review article or handbook
Vapour pressure	2.64 kPa at 25°C	Registration data	review article or handbook
Surface tension	-	-	surface activity is neither expected or predicted from structure
Water solubility	14.1 g/L at 20°C and pH 5.4.	Registration data	measured
Partition coefficient n-octanol/water	1.9 at pH 6.7	Registration data	measured
Flash point	23°C	Registration data	review article or handbook
Flammability	-	-	-
Explosive properties	-	-	no chemical groups associated with explosive properties present in the molecule
Self-ignition temperature	448-460°C	Registration data	review article or handbook
Oxidising properties	-	-	no chemical groups associated with oxidising properties
Granulometry	-	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	-	-	no functional groups which are associated with dissociation behaviour
Viscosity	0.545 mPa s at 25ºC 0.406 mPa s at 50ºC	Registration data	review article or handbook

1mg/m^3 = 0.244 ppm

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 6: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Human data			
PBPK modelling	4-methylpentan-2-one was predicted to be rapidly eliminated from blood after terminating the exposure; not likely to accumulate in workers exposed to 50 ppm	Based on data from Hjelm (1990)	Saghir (2008)
Human volunteers (n=8) chamber exposure 2h, light physical exercise 2.4, 24.4, 48.8 ppm	- pulmonary uptake was about 60% - total uptake increased linearly with increasing exposure conc. - 0.04% of the total dose was eliminated unchanged via the kidneys, 3h post exposure - metabolites below detection limit in the urine	-	Hjelm (1990)
Human volunteers – chamber exposure 4h exposures, 100 ppm	Steady-state blood levels were attained after 2 hours of exposure		Dick (1990) [cited in IARC, 2003]
Humans GC, GC/MS	Detection of 4-methylpentan-2-one in brain, liver, lung, vitreous fluid, kidney and blood	exposure of two persons to methyl ethyl ketone, 4-methylpentan-2-one, toluene and the three isomeric xylenes	Bellanca (1982)
Humans GC/MS analysis of cord blood and maternal blood samples		-	Dowty (1976)
Animal data			
Sprague-Dawley rats Inhalation exposure: 4h/d, 3 days 200, 400, 600 ppm Oral: 2 days, 150, 300, 601 mg/kg /day	-plasma and tissue concentrations increased in a dose-related manner with the administered dose irrespective of the route of administration - inhal: parent compound and metabolites (4-hydroxy-4 methyl-2-pentanone and 4-methyl-2-pentanol) detected in plasma, liver, lung - oral: parent compound and metabolite 4-hydroxymethyl isobutyl ketone detected in plasma, liver, lung plasma 4-methylpentan-2-one conc (inhal): 5.0, 8.1, 14.3µg/ml plasma 4-methylpentan-2-one conc (oral): 5.3, 8.4, 16.1µg/ml	-	Duguay (1995)
Sprague-Dawley rats 5mmol/kg oral gavage solution in corn oil	- 4-methylpentan-2-one was readily absorbed after oral administration, the Cmax occurring at 0.25 h - major metabolite in the blood: 4-	-	Gingell (2003)

Method	Results	Remarks	Reference
	hydroxy-4-methyl-2-pentanone (Cmax reached at 9h) - 4-methyl-2-pentanol was a very minor component (<0.1% of the total AUC).		
Rat ip, single dose 100 mg/kg, 200 mg/kg, 300 mg/kg	- concentration of 4-methylpentan-2-one in the exhaled air attained its maximum within 0.5 hour - decreased with a half-life of 0.6 hour - total amount injected was exhaled within 24 hours - concentration of 4-methylpentan-2-one in the urine attained its maximum within 3 hours after injection - half-life of 1.8 hours -total amount administered was excreted in 18 hours. - 4-methyl-2-pentanol in the urine attained its maximum in 3-6 hour -total amount was excreted in 12 hours.	-	Hirota (1991)
CD-1 mice ip administration, 5mmol/kg	- Metabolites identified were 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone - Detection in blood and brain	-	Granvil (1994)
Guinea pig, male ip, single dose 450 mg/kg	Half-life in serum: 66min Clearance time 6h (4-methylpentan-2-one) 16h (4-hydroxy-4-methyl-2-pentanone)	-	DiVincenzo (1976)
Modelling			
dermal penetration rate (flux), predicted from physical properties	The penetration rate (predicted from the solubility and the octanol-water partition coefficient (log P = 1.38)) is 0.95 mg/cm^2/h	-	Fiserova-Bergerova (1990)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

A short overview on the available toxicokinetic information is given by IARC (2003):

Toxicokinetic data for 4-methylpentan-2-one indicate that pulmonary uptake and blood concentrations of this chemical increase linearly with the dose in human volunteers who were exposed via inhalation (Hjelm, 1990; Dick, 1990). Steady-state blood levels were attained after 2 hours of exposure. The major route of elimination was exhalation, and only a tiny fraction of 4-methylpentan-2-one (0.04%) was excreted in the urine. Metabolites in the urine were below the detection limit. Analysis of blood and breath samples collected after exposure indicated that most of the absorbed 4-methylpentan-2-one had been eliminated from the body within 2 hours. The compound was detected in the brain, liver, lung, vitreous fluid, kidney and blood in autopsy samples of two workers who had been exposed to organic solvents (Bellanca, 1982). There is evidence that 4-methylpentan-2-one may enter the umbilical cord and cross the placenta (Dowty, 1976).

Data from single-dose inhalation exposure studies were used to simulate the repeated-dose kinetics of 4-methylpentan-2-one in humans (Saghir, 2008). The two-compartment pharmacologically based pharmacokinetic model predicted the kinetics and accumulation for repeated exposures. It correctly

simulated the experimental data measured after single exposures and demonstrated a rapid rise in blood concentration within 1 hour and rapid elimination from the blood after cessation of exposure. On the basis of these results, 4-methylpentan-2-one is not likely to accumulate in workers exposed to 50 ppm.

4-methylpentan-2-one was rapidly absorbed after oral administration or inhalation exposure of male rats. It was detected in the lung, liver and plasma within 1 hour after an oral dose (Duguay, 1995). In mice, 4-methylpentan-2-one administered by intravenous injection was quickly distributed and eliminated (Granvil, 1994). A clearance time of 6 hours and a half-life in serum of about 1 hour were measured in guinea pigs after a single intraperitoneal dose of 4-methylpentan-2-one (DiVincenzo, 1976). No data are available on the metabolism of 4-methylpentan-2-one in humans. In rats, the parent compound and two metabolites — 4-hydroxy-4 methyl-2-**pentanone and 4-methyl-2-pentanol** — were identified in the plasma, liver and lung following inhalation. After an oral dose, the parent compound and the hydroxylated product were detected in these tissues, but not 4-methyl-2-pentanol (Duguay, 1995). These data are consistent with metabolism that involves alcohol dehydrogenase and cytochrome P450 mono-oxygenases (Vezina, 1990). Similar patterns of metabolism were seen in mice and guinea-pigs.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

Acute toxicity - oral route

4-methylpentan-2-one is not classified for this endpoint. Relevant available studies are presented in Table 7.

Table 7: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (Acute Oral Toxicity)	Rat, male n=6/group	4-methylpentan-2-one (20% emulsion in Terginol 7 surfactant)	not reported gavage	LC_{50} = 2080 mg/kg bw	Smyth (1951)
OECD 401 (Acute Oral Toxicity)	Rat, Harlan-Wistar (f) - (no info available)	4-methylpentan-2-one	not reported	LC_{50} = 2980 mg/kg bw	Anonymous (1976) [Echa dissemination site]

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In a study by Smyth (1951) six male rats per group were exposed to different concentrations of 4-methylpentan-2-one resulting in an acute oral LD_{50} of 2080 mg/kg. The study is reliable with restrictions as the reporting is limited. However, Smyth and his group at the Carnegie-Mellon Institute of Research published a series of papers (“Range finding toxicity data”) testing chemicals for acute toxicity under the same experimental conditions being the basis for current toxicity testing.

In another acute toxicity study (Anonymous, 1976) female rats were exposed via gavage to unknown concentrations (dosage levels differed by a factor of 2 in a geometric series) of 4-methylpentan-2-one. The number of animals was not reported. The LD_{50}, calculated by the moving average method based on a 14-day observation, was 3.73 mL/kg bw (equal to 2980 mg/kg bw based on a density of 797.8 g/L).
ECETOC, 1987 further lists LD_{50} values for rats = 4600 mg/kg bw and mice =2850 mg/kg bw and 1900 mg/kg bw (Batyrova, 1973 and Zakhari, 1977; original literature not available).

10.1.2 Comparison with the CLP criteria

According to the CLP criteria, classification for Acute Toxicity 4 (oral) needs to be assigned if the acute toxicity value expressed as LD_{50}/ATE value is between 300 and 2000 mg/kg bw.

All available studies are non GLP studies but documented as equal or similar to OECD 401, however, study conditions are not well documented. Smyth (1951) is scored as the most reliable study with an LD_{50}/ATE of 2080 mg/kg bw. In addition other available values are in the same order of magnitude or above supporting this value.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the criteria 4-methylpentan-2-one has not to be classified for acute oral toxicity.

10.2 Acute toxicity - dermal route

4-methylpentan-2-one is not classified for this endpoint so far. The relevant available studies are presented in Table 8.

Table 8: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402	Rat /Crl:CD.BR 5 animals per sex 24h	4-methylpentan-2-one	2000 mg/kg bw (2.5ml/kg bw) semiocclusive	LC_{50} > 2000 mg/kg bw	Anonymous (1996a) [ECHA dissemination site]
OECD 402	Rabbit 4h	4-methylpentan-2-one	20ml/kg bw	LC_{50} > 20ml/kg bw	Anonymous, 1976 [ECHA dissemination site]

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

The acute dermal toxicity of 4-methylpentan-2-one was assessed by Anonymous (1996a) in Crl:CD. BR rats. In a limit test, 5 male and 5 female rats were treated with 2000 mg/kg bw of undiluted 4-methylpentan-2-one with a semiocclusive covering for 24 hours. After removal dermal reactions were recorded from day 2 to day 14. Organ weights and histopathology were documented. No

animals died during the test or the observation period and no clinical signs of toxicity were noted. No irritation or other dermal changes at the sites of application. The acute dermal LD_{50} in rats was determined to be > 2000 mg/kg bw.

Based on a study from Anonymous (1976) in rabbits dermal LD_{50} was greater than 20 mL/kg (equal to 15950 mg/kg bw based on a density of 797.8 g/L). However this study was assigned not to be reliable in the registration data. No further details available.

10.2.2 Comparison with the CLP criteria

According to the CLP criteria, classification for Acute Toxicity 4 (dermal) needs to be assigned if the acute toxicity value expressed as LD_{50}/ATE value is between 1000 and 2000 mg/kg bw.

The LD_{50}/ATE value was determined to be > 2000 mg/kg bw.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the criteria 4-methylpentan-2-one has not to be classified for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

4-methylpentan-2-one is currently classified for this endpoint as Acute Tox. 4 *, H332 (Harmful if inhaled) (minimum CLP classification). The relevant available studies are presented in Table 9.

4-methylpentan-2-one is a liquid at room temperature (20°C). In the ambient atmosphere 4-methylpentan-2-one is expected to exist solely in the vapour phase (Bidleman, 1988 cited in US-EPA, 2003). The vapour pressure at 20°C is 15.3mm Hg and the critical temperature is about 300°C (NIST Chemistry WebBook).

Table 9: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 (acute inhalation toxicity)	Rat 6 animals per dose	4-methylpentan-2-one (98.5%) impurities: Mesityl Oxide; Methyl Amyl Alcohol; Methyl Butyl Ketone; Acetone; 3-Methyl-2-Butanone	2000, 4000 ppm 4h	LC_{50}> 2000 ppm and < 4000 ppm	Smyth (1951)
-	Guinea pig 10f/group	4-methylpentan-2-one (99%)	1000, 3000, 10000, 16800, 28000 ppm chamber exposure 24h	28000 ppm: animals died within 45 min 10000 ppm: animals died within 4 h	Specht (1938) and Specht (1940) [ECHA dissemination site and EHC, 1990]

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
-	mice no further info available	4-methylpentan-2-one	- (no info available) 2h	LC_{50} = 5000 ppm	Batyrova (1973) [cited in ECETOC, 1987]
-	rats no further info available	4-methylpentan-2-one (98.5%, impurities: Mesityl Oxide; Methyl Amyl Alcohol; Methyl Butyl Ketone; Acetone; 3-Methyl-2-Butanone)	- (no info available) 6h	LC_{50}>4277ppm 21,662 ppm (calculated) killed 3/3 4,227 ppm (calculated) killed 0/3.	Eastman Kodak, 1956 [cited in OECD, 2009]

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Smyth (1951) investigated the acute inhalation toxicity of 4-methylpentan-2-one in rats. Six rats per dose level were exposed to 2000 (8.2 mg/l) or 4000 ppm (16.4 mg/l, saturated vapour) for 4 hours. No mortality was observed at 2000 ppm, but all animals died (6/6) at 4000 ppm within 14 days. Smyth (1951) also reported that the maximum time for which rats could be exposed to a saturated atmosphere of 4-methylpentan-2-one without dying was 15 min.

In studies by Specht (1938) and Specht (1940), female guinea-pigs were exposed to 4-methylpentan-2-one concentrations of 1000, 3000, 10000, 16800 and 28000 ppm for up to 24 h. In view of the method used for generating the atmosphere (allowing measured amounts of 4-methylpentan-2-on to evaporate freely to one cubic meter volume of air at 25-26 °C), the two higher levels must be greatly exaggerated because the saturation concentration in air for 4-methylpentan-2-on at 25 °C is 40,000 mg/m3. The 1000 ppm level caused little or no ocular or nasal irritation in the animals. There was a decreased respiratory rate during the first 6 h of exposure, which was attributed to a narcotic effect. The higher levels produced obvious signs of eye and nose irritation, followed by salivation, lacrimation, ataxia, progressive narcosis, and death. At 16800 ppm the respiratory rate fell of very abruptly from about 117 breaths per minute to 35 and less. At the highest concentration (28000 ppm) animals died within 45 min. At 10000 ppm animals died within 4h. Autopsy and histopathological investigations in some animals showed fatty livers and congestion of the brain, lungs, and spleen, but no damage to the heart and kidneys was observed (EHC 117, 1990). Survivors of the exposure have not indicated any gross pathology. Based on the limited available information no LC50 can be derived.

ECETOC, 1987 cites a study with 2h exposure of mice and a resulting LC50 of 5000 ppm (Batyrova, 1973 – study not available).

In a study cited by OECD, 2009 (Eastman Kodak, 1956 – study not available) rats were exposed for 6h. 21,662 ppm (calculated) killed 3/3 rats within in 53 minutes and 4,227 ppm (calculated) killed 0/3 rats. The LC50 value can be estimated to be >4200ppm.

Human exposed to 100 ppm 4-methylpentan-2-one for 4h showed significant odour sensation and irritant effects (Dick, 1992). A 2h exposure of humans (n=12) to 2.5 and 50 ppm showed no effects

on heart rate or reaction time tasks but irritation to the airways and CNS-symptoms like fatigue (Iregren, 1993). Hjelm (1990) exposed human volunteers 2h to 2.4, 24.5 and 48.4 ppm. Irritative and CNS symptoms (headache and/or vertigo and/or nausea) occurred during exposure, which increased during exposure to 24.5 and 48.4 ppm compared with 2.4 ppm. There were no significant effects on the performance of a simple reaction time task or a test of mental arithmetic. These irritant and CNS effects are further discussed in Chapter 10.11 (specific target organ toxicity).

10.3.2 Comparison with the CLP criteria

According to Table 3.1.1 of Regulation (EC) No. 1272/2008 a substance shall be classified as

- **Acute Tox 4 (inhal) if the LC_{50} values are > 2.0mg/l and ≤ 10mg/l (4h exposure)**
- **Acute Tox 3 (inhal) if the LC_{50} values are > 10.0mg/l and ≤ 20.0 mg/l (4h exposure)**

The LC_{50} [mg/l] derived from the available studies are:

species	duration	LC_{50} value	reference
rat	4h	8.2 mg/l < LC_{50} > 16.4 mg/l	Smyth (1951)
rat	6h	>17.2 mg/l	Eastman Kodak (1956)
guinea pig	24h	[derivation not possible]	Specht (1938) and Specht (1940)
mice	2h	20.5mg/l	Batyrova, 1973

Smyth (1951) is used as key study for the evaluation of this endpoint as this group of scientists at the Carnegie-Mellon Institute of Research published a series of papers (“Range finding toxicity data”) testing chemicals for acute toxicity under the same experimental conditions being the basis for current toxicity testing. In addition for other studies information on study conditions is not available or the derivation of an LC_{50} was not possible.

The LC_{50} in rats was determined to be > 8.2 mg/l and < 16.4 mg/l (Smyth, 1951). Based on this toxicity range a classification as Acute Tox 4, H332 is indicated. As there is no exact experimentally-derived LD_{50} value the appropriate conversion from CLP, Table 3.1.2 to a converted acute toxicity point estimate that relates to a classification category is used. Therefore the ATE was determined to be 11 mg/l.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

According to the criteria 4-methylpentan-2-one has to be classified as Acute Tox 4, H332. Currently 4-methylpentan-2-one is harmonized classified as Acute Tox 4* (H332) for the inhalatory route of exposure. A removal of the asterisk (*) is proposed. The asterisk indicates a minimum CLP classification which is no longer necessary since the data confirm the classification. An ATE of 11mg/l has to be indicated.

10.4 Skin corrosion/irritation

The substance has no harmonized classification for this endpoint but a suppl. hazard statement code EUH066 (Repeated exposure may cause skin dryness or cracking).

A labelling with EUH066 has been agreed by the Commission Working Group on the Classification and Labelling of Dangerous Substances in 1997/98 based on information in handbooks and safety data sheets where it is stated that “prolonged or repeated exposure may cause drying and flaking of the skin and/or dermatitis”. One rabbit study was cited where seven daily dermal applications (2400mg/kg) induced drying of the skin with some exfoliation (McOmie, 1949 – study not available). In the former CLH proposal (ECBI/43/95 –Add.37) classification as R38 (including EUH066) was proposed, however, the group decided not to classify for skin irritation (not supported by available toxicological information) but to label with EUH066.

Table 10: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
OECD 404 GLP	Rabbit, New Zealand White n=3	0.5ml undiluted 4-methylpentan-2-one	semi-occlusive 4h exposure	No erythema or oedema at 1h, 24h, 48h and 72h after treatment No signs of toxicity	Anonymous (1996b)
OECD 404 -	rabbit		Open 24h	not reported	Anonymous (1976)
-	Rabbit	undiluted 4-methylpentan-2-one	occlusive 10h exposure	erythema which was evident immediately after the exposure and persisted for 24 hours	ECETOC (1987) [unpublished study]
	Rabbit		occlusive 24h exposure	Slight irritation	
	Guinea pig		10ml/day for 7days of exposure	Drying and flaking of the surface	

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Anonymous (1996b) conducted a skin irritation study (according OECD 404, GLP) with 4-methylpentan-2-one. 3 New Zealand White rabbits were exposed to 0.5 ml of undiluted 4-methylpentan-2-one on the shaved dorsal region. Animals were exposed for 4 hours under semi-occlusive conditions and observations were recorded at 1, 24, 48 and 72 hours after removal of the patch and residual test substance. No dermal response to treatment was observed in any animal throughout the observation period. No other signs of toxicity were observed.

In the registration data another study is mentioned (Anonymous, 1976) but aside from the species and the kind of application not further information and results are given.

ECETOC (1987) presented an unpublished study were a single application of 4-methylpentan-2-one to the shaved skin of rabbits under occluded conditions for a period of 10h produced erythema which was evident immediately after the exposure and persisted for 24 hours. Daily applications of

10 ml on 10 cm^2 skin for 7 days caused drying and flaking of the surface. Undiluted 4-methylpentan-2-one (5 and 10 ml, occlusive) held in contact with the depilated skin of guinea pigs for 24h produced slight irritation.

10.4.2 Comparison with the CLP criteria

A substance has to be classified as Skin Irrit Cat. 2 if the mean scoring values are ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions.

For substances and mixtures which may cause concern as a result of skin dryness, flaking or cracking but which do not meet the criteria for skin irritancy (based on either practical observations or relevant evidence concerning their predicted effects on the skin) additional labelling as EUH066 “Repeated exposure may cause skin dryness or cracking” is foreseen.

In the available GLP study (Anonymous, 1996b) no erythema or oedema were reported at 1h, 24h, 48h and 72h after treatment.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

According to the CLP criteria no classification for skin corrosion/irritation for the substance 4-methylpentan-2-one is proposed.
4-methylpentan-2-one has been labelled with EUH066; this shall further apply based on the degreasing property of 4-methylpentan-2-one.

10.5 Serious eye damage/eye irritation

4-methylpentan-2-one is harmonized classified as Eye Irrit. 2, H319.

Table 11: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 GLP	Rabbit New Zealand White n=3	4-methylpentan-2-one	0.1ml undiluted 4-methylpentan-2-one	Slightly irritating Ocular changes were assessed immediately, 1/2h, 1 and 4 hours after treatment and 24, 48, and 72 hours after instillation Scores: 0 at 24, 48 and 72h for all endpoints Changes seen directly after treatment (conjunctival irritation, chemosis, ocular discharge) were reversible within 24h.	Anonymous (1996c)
OECD 405 GLP	Rabbit n=4	4-methylpentan-2-one	0.1ml undiluted 4-methylpentan-2-one	Slightly irritating Observation at 24, 48 and 72h	Bagley (1992) [ECETOC

		(purity 98%)		Mean scores (n=4; 24, 48,72h) Cornea 0.08 (of max.4) Iris 0 (of max. 2) Conjunctiva redness 0.8 (of max. 3) Chemosis 0.17 (of max. 4) Discharge 0 (of max. 3)	(1998)]
OECD 405 GLP compliance not specified	Rabbit n=3	4-methylpentan-2-one	0.1ml undiluted 4-methylpentan-2-one	Mean scores (n=3; 24, 48,72h) Cornea opacity 0.1 (of max.4) Iris 0 (of max. 2) Conjunctiva redness 0.87 (of max. 3) Chemosis 0.2 (of max. 4)	Anonymous (1992) [ECHA dissemination site]
OECD 405	Rabbit n=5	4-methylpentan-2-one	24h	No results reported	Anonymous (1976)
Bovine corneal opacity and permeability test (BCOP) *in vitro*	6 eyes per substance 3 eyes for control	4-methylpentan-2-one	0.75ml undiluted 4-methylpentan-2-one for 10min	Mild irritating Mean in vitro irritancy score (IVIS) = 19.9	Gautheron (1994)

Table 12: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Human volunteer study	4-methylpentan-2-one	n=6 per group 1st exposure: Full face mask exposure to 402, 915, 1393, 1680, 2301, or 2827 mg/m^3 7min exposure duration 2nd exposure (two weeks later): 845, 1493, or 2066 mg/m^3 7min exposure duration	nose, eye, and throat irritation generally increased with exposure level estimated thresholds: odour 402 mg/m^3 irritation 1393 mg/m^3 (LOAEL)	Esso Research and Engineering Company (1965); Hazleton Laboratories, Inc. (1965) [cited in US EPA, 2003]
Human volunteer study	4-methylpentan-2-one	n=12 (m, f) 15min exposure	100 ppm was found to be the sensory response limit Eye irritation at 200 ppm (for nose and throat >200 ppm)	Silverman (1946)

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In an eye irritation study (Anonymous, 1996c) with 4-methylpentan-2-one according to OECD No. 405 and in compliance with GLP 0.1 ml of undiluted substance was instilled into one conjunctival sac of each of 3 New Zealand White rabbits. Ocular changes were assessed 0.5, 1, 4, 24, 48, 72 hours after treatment. No systemic toxicity was observed in any rabbit during the course of the study. Instillation of 4-methylpentan-2-one caused slight or practically no initial sting response. All rabbits developed conjunctival irritation (not exceeding a crimson appearance), slight chemosis, and an occular discharge during the 4-hour period following instillation of the test substance. All conjunctival reactions were reversible within 24 hours after treatment. The iris remained unaffected. No corneal opacities developed in any rabbit. A small part of the cornea of one rabbit was permeable to applied fluorescein 24 hours after instillation of the test article, indicative of a minor disruption of the corneal epithelium; however, the cornea was proved to be impermeable to applied fluorescein on the following day. All individual mean scores (corneal opacity, iris, conjunctive, chemosis) over 24, 48 and 72 h were 0.

Bagley (1992) presents results from an OECD 405 test where 4 rabbits were treated with 0.1ml of undiluted 4-methylpentan-2-one in the conjuctival sac. Observations were made at 24, 48 and 72h. The mean scores are presented in Table 11. The substance was only slightly irritating.

In another OECD 405 test 0.1ml of undiluted 4-methylpentan-2-one was applied into the conjunctival sac of rabbits and removed after 1h (Anonymous, 1992). Obervations were made at 1 hour, 24h, 48h, 72h and 4, 7, 14 days. Scoring was done according to Draize scale. A slight conjunctival irritation was observed, which cleared in 4 days, as well as slight corneal opacity which cleared within 2 days. The mean individual scores over 24, 48 and 72 hours are presented in Table 13. The maximal average score (MAS) (Kay and Calandra, 1962) was determined to be 20.3**.**

Table 13: Individual mean Draize scores over 24/48/72h (Anonymous, 1992).

Mean scores over 24/48/72h	Animal #1	Animal #2	Animal #3
Chemosis (2)	0.3	0.3	0
Conjunctivae score (1)	0.7	0.7	1.2
Iris score	0	0	0
Cornea opacity (2)	0.3	0	0

(1) fully reversible within 74 h, (2) fully reversible within 2 days

The study by Anonymous, 1976 is documented insufficiently and therefore cannot be applied.

In an inter-laboratory study the bovine corneal opacity and permeability assay (BCOP) was validated against results from in vivo studies (Gautheron, 1994). The correlation between the BCOP scores and the Draize MAS values was 0.73. In the meantime, the test method is defined in OECD Test No. 437 (OECD, 2013). The test method is recommended as initial step within a testing strategy to identify chemicals inducing serious eye damage or for chemicals that do not require classification for eye irritation or serious eye damage. A potential shortcoming of the BCOP test method is high false positive rates for alcohols and ketones. However, since not all alcohols and ketones are over-predicted by the BCOP test method and some are correctly predicted as UN GHS Category 1, these two organic functional groups are not considered to be out of the applicability domain of the test method (OECD TG 437). Undiluted 4-methylpentan-2-one was applied for

10min at 32°C to 6 bovine eyes, control group included 3 eyes. Measurement of opacity was done after removal of the test substance and 2h afterwards. Permeability values (OD_{490}) were determined 2h post exposure. Mean in vitro irritancy score (IVIS) was determined to be 19.9 (mean score over 12 laboratories). According to Gautheron (1994) this score indicates mild irritation. According to the OECD TG 437 (2017) for scores between >3 and ≤55 no prediction can be made and subsequent testing with other validated test is necessary. An IVIS > 55 should be accepted as indicative of a response inducing serious eye damage that should be classified. A negative result (IVIS ≤ 3) should be accepted as indicative that no classification is required. Therefore, based on this *in vitro* assay no conclusion can be drawn.

Sensory irritation has been seen in several human volunteers after inhalatory exposure to 4-methylpentan-2-one. Eye irritation in particular is reported by Silverman (1946) and Esso (1965).
Groups of six adult volunteers were exposed for 7 minutes via full face mask to 402, 915, 1393, 1680, 2301 or 2827 mg/m^3 of 4-methylpentan-2-one, followed 2 weeks later by a second 7-minute exposure to 845, 1493, or 2066 mg/m^3 (Esso Research and Engineering Company, 1965; Hazleton Laboratories Inc., 1965 cited in US EPA, 2003). Volunteers indicated the presence and disappearance of eye, nose and throat irritation throughout the exposures, which provided a continuous subjective assessment of irritation relative to known exposure levels. The incidence of volunteers reporting nose, eye, and throat irritation generally increased with exposure level; the thresholds for odour and irritation were reported to be 402 and 1393 mg/m^3 (98 and 340 ppm), respectively, estimated from graphs of the number of individual reports of irritation at various exposure levels.
Silverman (1946) investigated the sensory response to industrial solvent vapours. 12 persons were exposed for 15 minutes to various vapour air concentrations. 4-methylpentan-2-one was found to have a sensory response limit of 100 ppm. A majority of subjects found the odour objectionable at 200 ppm and the vapour was irritating to the eyes. Concentration relevant for eye and throat irritation was determined to be >200 ppm.
Industry health records (Armeli, 1968 and Linari, 1964) also report irritating effects. Workers (n=19) exposed to concentrations up to 500 ppm near a centrifuge for a duration of 20-30 min complained of weakness, loss of appetite, headache, burning in the eyes, stomach ache, nausea, vomiting, sore throat.

10.5.2 Comparison with the CLP criteria

A substance has to be classified as irritating to eyes (Category 2) if, when applied to the eye of an animal, a substance produces at least in 2 of 3 tested animals, a positive response (mean scores: corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2). The effects have to be fully reversible within an observation period of 21 days.

Although in a guideline study all rabbits developed conjunctival irritation, slight chemosis, and an ocular discharge during the 4-hour period following instillation of the test substance the resulting mean scores after 24, 48 and 72h were 0 for all endpoints (Anonymous, 1996c). Two other studies (Anonymous, 1992; Bagley, 1992) resulted in scores below 1.
A study with human volunteers gives an estimated LOAEL (irritation) of 340 ppm (Esso, 1965). Another study indicates 200 ppm as irritant to eyes (Silverman, 1946). Anonymous (1965) and Armeli (1986) determined a sensory irritation threshold in humans at 340 ppm and 8874 ppm respectively (see Chapter 10.11). Industry health records document eye irritation.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the results of 3 animal studies according OECD TG 405 no classification is indicated. However human data indicate sensory irritation at 200 ppm and above. The current harmonized classification for eye irritation is based on experience from human exposure. Based on available human data a classification as Eye Irrit 2 is confirmed.

10.6 Respiratory sensitisation

Data lacking – no relevant information available

10.7 Skin sensitisation

Table 14: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 406 (GPMT)	Guinea pig albino Bor: DHPW female n=20 (control n=10)	4 methylpentan-2-one vehicle: corn oil	1st induction: 5 % test substance (TS) in corn oil, intradermal 2nd induction: 100% TS, 48h, epicutaneous Challenge: 30% TS in corn oil, 24h, epicutaneous	Not sensitizing (0/20 positive after 24h and 48h) Local reactions (redding, swelling) at site of induction	Anonymous (1989)

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

In a Guinea Pig Maximisation Test (Anonymous, 1989) 20 female albino Bor: DHPW guinea pigs were exposed to 4-methylpentan-2-one. The vehicle (corn oil) control group consisted of 10 animals. The intradermal induction was performed with 0.1 ml of 5% 4 methylpentan-2-one in corn oil followed by epicutaneous induction with undiluted 4 methylpentan-2-one in the shoulder area (occlusive, 2x4 cm filter paper, 48h). The challenge exposure was conducted with 30% 4 methylpentan-2-one (2x2cm filter paper, 24h) in vehicle under occlusive conditions. Skin reactions were observed and recorded 24 and 48 hours after the challenge exposure. No reactions (0/20 positive) have been documented in the study protocol 24h and 48h after exposure (score 0). Test and control animals displayed normal body weight gain throughout the investigation. Local skin reactions at site of induction (swelling or reddening) were observed in all treated animals. Some irritation reactions were also observed in control animals.

10.7.2 Comparison with the CLP criteria

Substances shall be classified as skin sensitisers (category 1) I (1) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons or (2) if there are positive results from an appropriate animal test.

A GPMT with 4-methylpentan-2-one was negative.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the available GPMT no classification for skin sensitisation is warranted.

10.8 Germ cell mutagenicity

4-methylpentan-2-one has no harmonized classification for this endpoint.

Table 15: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
bacterial reverse mutation assay (AMES test) equivalent to OECD 471 GLP	4-methylpentan-2-one (99.6% pure) Vehicle: DMSO preliminary study: 0.015, 0.05, 0.15, 0.5, 1.7, 5.2, 17, 50, 100, or 150 µl/plate main study: with 0.04, 0.1, 0.4, 1, or 4 µl/plate pos control: 2-Aminoanthracene, 4-Nitro-O-phenylenediamine, sodium azide, 9-Aminoacridine	TA 1538, TA 1535, TA 1537, TA 98 and TA 100 **± S9** preliminary study for dose selection (cytotoxicity at 5.2 µL/plate and above) 1 (reliable without restriction)	negative No cytotoxicity No increase in the reverse mutation rate	O´Donoghue (1988)
bacterial reverse mutation assay (AMES test) equivalent to OECD 471	4-methylpentan-2-one (98.5%) Vehicle: DMSO 0, 31.25, 62.5, 125, 250, 500, 1,000, 2,000, or 4,000 µg/plate	TA 1538, TA 1535, TA 1537, TA 98, TA 100, WP2 and WP2 uvrA 2 (reliable with restrictions)	Negative No valid positive control for TA 98, TA 100, TA 1537, TA 1538 – S9 and WP2 + S9	Brooks (1988) [ECHA dissemination site]
In Vitro Mammalian Cell Gene Mutation Test in L5178Y mouse lymphoma	4-methylpentan-2-one (99.6% pure) Vehicle: DMSO 1st test: 0.32, 0.42, 0.56, 0.75,	Preliminary toxicity test	1st test: +S9: negative, no cytotoxicity -S9:pos results and cytotoxicity (97%) at 4.2 µl/ml; at 3.2 and 1.8µg/ml mutant frequency 2-fold of control level with cytotoxicity	O´Donoghue (1988)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
cells similar to OECD Guideline 476	1.0, 1.3, 1.8, 2.4, 3.2 and 4.2µl/ml (+ and – S9) 2nd test: 0.6, 1.4, 2.1, 2.9, or 3.7 µl/ml (-S9) 1.4, 1.9, 2.5, 3.0, or 3.4 µl/ml (+S9). Pos control: ethylmethanesulfonate (-S9), 7,12-dimethylbenz [a]anthracene (+S9)	1 (reliable without restriction)	of 69 and 42%, respectively) 2nd test (done in duplicate): +S9: negative, no cytotoxicity -S9: pos results and cytotoxicity (96-99%)) at 3.7 µl/ml without dose-response relationship Pos result (2-fold of control level) in one experiment at 2.9µg/ml (68% cytotoxicity) and in one at 2.1µg/ml (69% cytotoxicity)	
Unscheduled DNA Synthesis in Mammalian Cells *in vitro* Similar to OECD 482	4-methylpentan-2-one (99.6% pure) Vehicle: DMSO Preliminary cytotoxicity test: 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 50, 100 µl/ml Main test: 0.010, 0.10, 1.0, 10, 100 µl/ml Pos control: 2-acetylaminofluorene	Preliminary test: relative toxicity of 66.18% at the highest concentration of 100 µl/ml. 1 (reliable without restriction)	Negative No cytotoxicity No increases in the average nuclear grain count	O´Donoghue (1988)
Gene Mutation Assay Saccharomyces cerevisiae Similar to OECD 480	4-methylpentan-2-one (98.5%) 0, 10, 100, 500, 1000, or 5000 µg/ml Pos control: 4-Nitroquinoline-N-oxide (- S9), cyclophosphamide (+ S9)	2 (reliable with restrictions)	negative	Brooks (1988) [ECHA dissemination site]
chromosome aberration study in mammalian cells rat liver cells	4-methylpentan-2-one (98.5%) 0, 250, 500, or 1000 µg/ml	2 (reliable with restrictions)	negative	Brooks (1988) [and ECHA dissemination site]

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
similar to OECD 473	Pos control: 7,12-dimethylbenzanthracene			

Table 16: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus assay Equivalent to OECD 474 CD1 mice 5m+5f per group and timepoint	4-methylpentan-2-one (99.6% pure) 0.7ml/kg bw, i.p. Vehicle: corn oil Pos control: triethylene melamine	Preliminary toxicity study Only one dose tested 1 (reliable without restriction)	negative	**O'Donoghue (1988)** [ECHA dissemination site]

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

For an Ames assay O´Donoghue (1988) used Salmonella strains TA 1538, TA 1535, TA 1537, TA 98 and TA 100. TA102 or E. coli strain WP2 uvrA were not included. Based on a preliminary toxicity assay (0.015, 0.05, 0.15, 0.5, 1.7, 5.2, 17, 50, 100, or 150 µl/plate (± S9)) and a resulting cytotoxicity at 5.4 µl/plate the Ames assay was done with 0.04, 0.1, 0.4, 1, or 4 µl/plate (± S9) (vehicle DMSO). The assay was conducted in triplicate. No increase in the reverse mutation rate and no cytotoxicity were observed at any concentration ± S9. Positive control substances were included.

Another bacterial reverse mutation assay (non GLP) (Brooks, 1988) also gave negative results with tested doses of 4-methylpentan-2-one in DMSO (0, 31.25, 62.5, 125, 250, 500, 1,000, 2,000, or 4,000 µg/plate) in S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 and in E. coli strains WP2 andWP2 uvr A ± S9. The assay was conducted in triplicate and in an independent repeat experiment. No increase in the reverse mutation rate and no cytotoxicity were observed at any concentration ± S9. As positive control substances did not always result in increased reverse mutation rates (TA 98, TA 100, TA 1537, TA 1538 –S9 and WP2+S9) the study is reliable with restrictions.

In an *in vitro* Mammalian Cell Gene Mutation Test in L5178Y mouse lymphoma cells 4-methylpentan-2-one was tested in concentrations of 0, 0.32, 0.42, 0.56, 0.75, 1.0, 1.3, 1.8, 2.4, 3.2

and 4.2µl/ml with and without metabolic activation (O'Donoghue, 1988). 4-methylpentan-2-one was negative when tested with metabolic activation but resulted in equivocal results when tested at high concentrations without activation (significant increase at 4.2µg/ml and lethality of 97%). Therefore, a second study was conducted at doses of 0, 0.6, 1.4, 2.1, 2.9 and 3.7 µl/ml (-S9) and at doses of 0, 1.4, 1.9, 2.5, 3.0, or 3.4 µl/ml (+S9). This experiment was conducted in duplicate. No cytotoxicity and no increase in the mutant frequency were observed at any concentration in the presence of metabolic activation. However, cytotoxicity and equivocal genotoxicity (defined as a two-fold increase in the mutation frequency over solvent control levels at one or more dose levels but the absence of a dose-response) were noted at 3.7 µl/ml (the highest concentration tested) in the absence of metabolic activation. Response was observed at doses which resulted in 96-99% lethality and DMSO control cultures were within the historical control ranges but showed also a high variation. Sporadic positive results were seen at 2.1 and 2.9µg/ml. Results of tests without metabolic activation are presented in Table 17. Study authors concluded negative for the mutagenic potential of 4-methylpentan-2-one as according to Clive (1979) doses which result in 90-100% lethality are not relevant. Positive control substances were included resulting in expected increase in mutation frequencies.

Table 17: Mouse lymphoma assay with 4-methylpentan-2-one, results without metabolic activation (O´Donoghue, 1988)

1st test (-S9)				2nd test (done in duplicates) (-S9)			
µg/ml	Mean mutant colonies/plate	Mutant freq (/10^4 cells)	Total growth (%)	µg/ml	Mean mutant colonies/plate	Mutant freq (/10^4 cells)	Total growth (%)
4.2	136	1.9*	3	3.7	70	28.0*	1
3.2	82	0.8*	31	3.7	43	3.4*	4
2.4	62	0.6	52	2.9	56	1.3	42
1.8	82	0.8*	58	2.9	52	1.6*	32
1.3	61	0.5	86	2.1	46	1.9*	31
1.0	76	0.6	90	2.1	47	1.2	51
0.75	74	0.6	95	1.4	43	1.1	60
0.56	70	0.7	81	1.4	42	1.2	46
0.42	45	0.2	157	0.6	41	1.0	68
0.32	49	0.5	87	0.6	43	0.8	80
DMSO	42	0.4	100	DMSO	43	0.8	100
Pos. control 1µg/ml	184	15.2	6	Pos. control 1µg/ml	40	4.4	4
Pos. control 0.5µg/ml	-	Culture lost	-	Pos. control 0.5µg/ml	174	8.3	24

* Significant increase in mutant frequency – 2-fold control level

For the Unscheduled DNA Synthesis in Mammalian Cells *in vitro* primary rat hepatocytes were used (O´Donoghue, 1988). In a preliminary test cytotoxicity was tested at concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 50, 100 µl/ml. For the DNA repair test 0.010, 0.10, 1.0, 10, 100 µl/ml were used. Positive control was valid. No cytotoxicity and no significant increased nuclear grain count was observed.

In a gene mutation assay in *Saccharomyces cerevisiae* 4-methylpentan-2-one was tested at concentrations of 0, 10, 100, 500, 1,000, 5,000 µg/ml (+ and -S9). Incubations at each concentration were done in quadruplicate and in addition an independent repeat experiment was performed. Deviating from the standard protocol the post-treatment incubation period was only 3 days. Positive controls were valid but a vehicle control experiment was not reported. No cytotoxicity and no increase in the rate of mitotic gene conversion at any concentration + or –S9 was reported (Brooks, 1988).

4-methylpentan-2-one was tested in a mammalian chromosome aberration test at doses of 0, 250, 500, 1000 µg/mL in rat liver cells. No exogenous metabolic activation was used. Incubations were done in triplicate but independent repeat experiment was not performed. The positive control was valid, however, the use of a vehicle control was not reported. No cytotoxicity and no chromosome damage was seen at any concentration tested (Brooks, 1988).

In an *in vivo* genotoxicity study (O´Donoghue, 1988) male and female CD1 mice were exposed intraperitoneally to 4-methylpentan-2-one at a dose of 0.73ml/kg bw. Mice were sacrified 12, 24, 48h after exposure (5 mice/sex/time). Animals appeared heavily sedated and two male mice and 4 female mice died following administration. In other animals no signs of toxicity were observed. No significant increases in the number of micronucleated polychromatic erythrocytes at any time point were reported; positive control was valid.

In general metabolism of 4-methylpentan-2-one via alcohol dehydrogenase and cytochrome P450 (CYP) mono-oxygenases resulted in the two metabolites 4-hydroxy-4 methyl-2-pentanone and 4-methyl-2-pentanol (Vezina, 1990). Metabolic activation via S9 used for mutagenicity testing therefore also reflects possible effects of metabolites. Study details submitted for the registration of 4-hydroxy-4 methyl-2-pentanone (CAS 123-42-2) and 4-methyl-2-pentanol (CAS 108-11-2) also show a lack of mutagenic activity[1].

10.8.2 Comparison with the CLP criteria

Classification for germ cell mutagenicity in Category 2 is based on positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

– Somatic cell mutagenicity tests in vivo, in mammals; or
– Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

4-methylpentan-2-one was negative in two bacterial reverse mutation assay (O`Donoghue, 1988; Brooks, 1988) and it did not induce unscheduled DNA syntheses in mammalian cells *in vitro* (O`Donoghue, 1988). In addition, a Gene Mutation Assay in Saccharomyces cerevisiae was negative as well as a chromosome aberration study in rat liver cells (Brooks, 1988). An *in vitro* Mammalian Cell Gene Mutation Test in mouse lymphoma cells gave equivocal results (positive response without dose-response relationship) without metabolic activation (O`Donoghue, 1988). In the micronucleus cytogenetic assay in mice i.p. administration of 4-methylpentan-2-one was negative.

[1] Source: ECHA dissemination site (accessed September, 2018)

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification for germ cell mutagenicity is proposed due to clear negative results in the Ames tests, the UDS test and the micronucleus test in combination with equivocal results in the mouse lymphoma assay.

10.9 Carcinogenicity

For the evaluation of a possible carcinogenic potential of 4-methylpentan-2-one a carcinogenicity study with two species (inhalation) as well as studies investigating possible α2u-globulin mode of action and CAR-mediated mode of action are available. No oral or dermal carcinogenicity studies are available.

Table 18: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD Guideline 451 OECD Guideline 451 Carcinogenicity Study rat (F344/N) male/female no vehicle n=50m+50f/group	Test material : 4-methylpentan-2-one inhalation: vapour (whole body) 450, 900, or 1800 ppm (nominal conc.) 1843, 3686 and 7373 mg/m^3 (analytical conc.) Exposure: 2 years (6h/day, 5 days per week)	NOAEC (carcinogenicity) = 450 ppm (analytical) (male/female) (= 1840 mg/m^3) neoplastic and non-neoplastic lesions at higher doses (hyperplasia of the kidney and adrenal gland, renal adenoma/carcinoma, mononuclear cell leukemia, renal mesenchymal tumors) Survival rates [0, 450, 900, 1800 ppm]: Males: 32/50, 28/50, 25/50, 19/50 Females: 35/50, 34/50, 26/50, 32/50 2 (reliable with restrictions)	NTP (2007) Stout (2008b)
OECD Guideline 451 Carcinogenicity Study mouse (B6C3F1) male/female no vehicle n=50m+50f/group	Test material: 4-methylpentan-2-one inhalation: vapour (whole body) 450, 900, or 1800 ppm (nominal conc.) 1843, 3686 and 7373 mg/m^3 (analytical conc.) Exposure: 2 years (6h/day, 5 days per week)	NOAEC (carcinogenicity): 450 ppm (male/female) neoplastic effects in the liver of mice – CAR mediated mechanism Survival rates [0, 450, 900, 1800 ppm]: Males: 40/50, 42/50, 35/50, 37/50 Females: 35/50, 37/50, 39/50, 38/50 2 (reliable with restrictions)	NTP (2007) Stout (2008b)

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Groups of 50 male and 50 female rats (F344/N) were exposed to 4-methylpentan-2-one at concentrations of 0, 450, 900, or 1,800 ppm for 6h/day, 5 days per week for 104 weeks (NTP, 2007).
Body weight and clinical findings were recorded during the whole study period. Complete necropsies and microscopic examinations were performed on all rats. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved, processed and trimmed for microscopic examination2. Extended evaluation was done for investigation of renal proliferation. Haematology, clinical chemistry and urinalysis were not done.

Survival was decreased in male rats at 1800 ppm (see Table 18). Body weight gains were decreased in male rats at 900 and 1800 ppm after weeks 97 and 89, respectively. Mean body weights of exposed female rats were generally similar to chamber controls (see Table 20). No information on organ weights is available. During the second year of exposure seizures were observed sporadically (males: 2/50 at 0 ppm, 3/50 at 450 ppm, 4/50 at 900 ppm, 5/50 at 1800 ppm; females: 12/50 at 0 ppm, 4/50 at 450 ppm, 6/50 at 900 ppm, 14/50 at 1800 ppm). Most seizures were mild characterized by an abnormal hunched posture and chewing movements. They lasted approx. 30 sec. with a rapid recovery. Uncommon seizures of greater severity lasted up to 60 sec with recovery of 2min. Neither incidence nor number of episodes per rat was dose related.
The primary target site of 4-methylpentan-2-one in rats was the kidney. Nephropathy of the kidney (similar to that which occurs in aged rats) was observed in most male rats including chamber controls. Incidences and severities increased with increasing exposure concentration. Also in female rats, the incidences of chronic nephropathy were significantly increased in all exposed groups. Incidences of papillary mineralization (of minimal to mild severity) were significant in all exposed groups of males (see Table 19). Such papillary mineralization of the renal papilla oriented in a linear fashion is characteristic of α2u-globulin inducers in 2-years studies. The incidences of transitional epithelial hyperplasia (minimal to mild severity) in the renal pelvis of male rats were increased in all exposed groups of male rats (significant at 900 and 1800 ppm). The increased incidences of epithelial hyperplasia in the current study may reflect the enhanced nephropathy. The incidences of renal tubule hyperplasia were significantly increased in male rats exposed to 450 or 1,800 ppm, and the severities in these groups were greater than that of the chamber controls. Additionally there were slightly increased incidences of renal tubule adenoma, carcinoma, and adenoma or carcinoma (combined) in male rats. The incidence was not statistically significant but exceeded the historical control data (see Table 19). Because the increased incidences of benign and malignant renal tubule neoplasms and hyperplasia indicated the possibility of a treatment-related carcinogenic effect in male rats, an extended evaluation of the kidney was performed in male rats. Results are presented in Table 19.
Minimal hyaline droplet accumulation was observed in two 900 ppm and two 1800 ppm male rats that died relatively early in the study. However, the increase in hyaline droplets diminishes with age and is not expected to be detectable in aged rats in a 2-year study (US EPA, 1991).
Two female rats in the 1,800 ppm group had renal mesenchymal tumors.
In addition, a significantly increased incidence of mononuclear cell leukemia in male rats at 1,800 ppm was seen.

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

There also was a significant increased incidence of adrenal medulla hyperplasia in male rats of the 1,800 ppm group.
Exposure-related increased incidences of benign or malignant pheochromocytoma (combined) occurred in male rats with the high dose lying at the upper range of the historical control data (28% vs 16% in current control and 17% in historical control data).
The incidence of alveolar/bronchiolar carcinoma was slightly increased in male rats exposed to 1,800 ppm 4-methylpentan-2-one (0/50, 0/49, 0/50, 2/50). Although the two carcinomas seen in the 1,800 ppm group in this study exceeded the historical control rate [5/399 (1% ± 1%), range, 0%-2%], they were not statistically significant compared to the concurrent control group and were considered not related to 4-methylpentan-2-one.
A compilation of incidences of adverse effects as well as information on available historical control incidences is given in Table 19. The NTP historical database contains all studies that use the NTP-2000 diet (equal to this study) with histopathology findings completed up to date. For the present study the historical database contained the control groups of 8 studies[3] (F344/N rats, inhalation, 50 animals/control).

Groups of 50 male and 50 female B6C3F1 mice were exposed to 4-methylpentan-2-one at concentrations of 0, 450, 900, or 1800 ppm for 6h/day, 5 days per week for 105 weeks (NTP, 2007). Complete necropsies and microscopic examinations were performed on all mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues[2] were fixed and preserved, processed and trimmed for microscopic examination. Haematology, clinical chemistry and urinalysis were not done.
Survival of male and female mice was similar to that of the chamber controls. After week 17, body weights of 1800 ppm females were less than those of the chamber controls (see Table 20). No clinical findings were observed. No information on organ weights is available.
The liver was the primary site of toxicity. The incidences of eosinophilic foci were increased in all exposed groups of female mice, and the differences from the chamber controls were significant in the 450 and 1800 ppm groups (Table 19). According to the study authors the exact role of these foci in hepatocarcinogenesis is uncertain. Gad (2016) state that foci of cellular alteration are occasionally seen in control mice but also have been seen in mice exposed to carcinogens progressing to adenomas and carcinomas. Foci of cellular alteration are common in rodent studies greater than duration of twelve months and they represent a localized proliferation of hepatocytes that are phenotypically different from surrounding hepatocyte parenchyma. Foci of cellular alteration are not necessarily preneoplastic (non-neoplastic end stage lesions). However, a number of models have linked specific types of foci of cellular alteration with carcinogenesis (e.g. the nitrosomorpholine model is linked with eosinophilic and clear cell foci as precursors). Most importantly, types of foci in controls should be compared to those found in treated animals (Thoolen, 2010).

The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were increased in all exposed groups of males and in 900 and 1800 ppm females, and the incidences

[3] NTP Toxicology and carcinogenesis studies of Decalin, Divinylbenzene, Indium phosphide, Methyl isobutyl ketone , Naphthalene, Propylene glycol mono-t-butyl ether, Stoddard solvent (Type IIC) , Vanadium pentoxide (inhalation studies)

in the 1800 ppm groups were significantly greater than those in the chamber controls (exceeding the historical control range) (see Table 19). The incidence of hepatocellular carcinoma in females was increased at 1800 ppm; the result was not statistically significant but the incidence exceeded the historical control range.
Although hepatocellular adenoma is the most frequent spontaneous liver neoplasm in B6C3F1 mice, the number of neoplasms detected in mice exposed to 1800 ppm and the positive trends in the multiplicity observed in exposed males and females provide some evidence of a carcinogenic effect of the test substance in mice.

In addition exposure to 4-methylpentan-2-one resulted in significantly decreased incidences alveolar/bronchiolar adenoma in 900 ppm males (9/50, 5/50, 1/50, 5/50) and of alveolar/bronchiolar adenoma or carcinoma (combined) in 450 and 900 ppm males (14/50, 5/50, 3/50, 10/50). These decreased incidences occurred only in male mice and were considered to be spurious and not related to exposure to 4-methylpentan-2-one [Historic control data: adenoma: 74/349 (21% ± 6%), range 12%-26%; adenoma or carcinoma (combined): 115/349 (33% ± 6%), range 26%-44%].

The NTP historical database contains all studies that use the NTP-2000 diet (equal to this study) with histopathology findings completed up to date. For the present study the historical database contained the control groups of 7 studies[4] (B6C3F1 mice, inhalation, 50 animals/control).

Table 19: Summary of effects seen in the 2-year carcinogenicity study in rats and mice (chamber control, 450, 900 and 1800 ppm) [NTP historical incidences of control animals] (NTP, 2007).

	male F344/N rats	**female F344/N rats**	**male B6C3F1 mice**	**female B6C3F1 mice**
Nonneoplastic effects	Kidney: renal tubule hyperplasia (standard evaluation) (1/50, 11/50**, 3/50, 18/50**); standard and extended evaluation# combined (1/50, 14/50*, 7/50*, 21/50**) nephropathy (42/50, 45/50, 47/50, 50/50*); severity (2.0, 2.6, 2.4, 3.1*) pelvis transitional epithelium hyperplasia (1/50, 5/50, 6/50*, 19/50**) papilla mineralization (1/50,	Kidney: nephropathy (19/50 (38%), 35/50** (70%), 38/50** (76%), 44/50** (88%) (no historical control data given in the study report; based on the studies used for the compilation of the historic control data (see footnote 3) a historic incidence of 275/396 (69.4% ± 23.8% SD) was deviated by the dossier submitter)	Liver: eosinophilic foci (3/50 (6%), 4/50 (8%), 5/50 (10%), 8/50 (16%))	Liver: eosinophilic foci (4/50 (8), 11/50* (22%), 10/50 (20%), 14/50**(28%))

[4] NTP Toxicology and carcinogenesis studies of Decalin, Divinylbenzene, Indium phosphide, Methyl isobutyl ketone , Propylene glycol mono-t-butyl ether, Stoddard solvent (Type IIC) , Vanadium pentoxide (inhalation studies)

	6/50*, 22/50**, 29/50**) Adrenal Gland: adrenal medulla hyperplasia (13/50, 18/48, 18/50, 24/50*)			
Neoplastic effects	Kidney: renal tubule adenoma (standard evaluation) (0/50, 0/50, 2/50, 3/50) standard and extended evaluation# combined (2/50 (4%), 3/50 (6%), 3/50 (6%), 10/50 (20%)) [→historical incidence: 3/399 (0.8%; 0.8%±1.0% SD, range 0-2%] renal tubule carcinoma (standard evaluation (0/50 (0%), 1/50 (2%), 0/50 (0%), 2/50 (4%)); [→historic incidence: 1/399 (0.3%; 0.3%±0.7% SD, range 0-2%] renal tubule adenoma or carcinoma (combined) (standard evaluation) (0/50, 1/50, 2/50, 4/50) standard and extended evaluation# (2/50 (4%), 4/50 (8%), 3/50 (6%), 11/50 (22%)) [→ historical incidence 4/399 (1.0%; 1.0%±1.1% SD, range 0-2%]] **→possible α2u-globulin nephropathy MOA** Adrenal Gland: Pheochromocytoma (benign+malign) (8/50 (16%), 9/48	- Kidney: mesenchymal tumor malignant (0/50 (0%), 0/50 (0%), 0/50 (0%), 2/50 (4%)) [→historical incidence 0/396]	Liver: hepatocellular adenoma (17/50 (34%), 25/50 (50%), 23/50 (46%), 34/50** (68%)); [→historic incidence: 134/350 (38.3% ± 6.3%); range, 30%-46%] hepatocellular carcinoma (12/50, 12/50, 10/50, 9/50) [→historical incidence: 85/350 (24.3% ± 4.8%); range, 18%-32%] hepatocellular adenoma or carcinoma (27/50 (54%), 34/50 (68%), 28/50 (56%), 37/50*(74%) [→historical incidence: 196/350 (56.0% ± 6.2%); range, 50%-68%] **→possible CAR-mediated MOA**	Liver: hepatocellular adenoma (13/50 (26%), 15/50 (30%), 20/50 (40%), 23/50*(46%)); [→historical incidence: 78/347 (22.5% ± 8.1%); range, 12%-35%] hepatocellular carcinoma (6/50, 5/50, 6/50, 11/50) [→historical incidence: 37/347 (10.7% ± 1.8%); range, 8%-12%] hepatocellular adenoma or carcinoma (17/50 (34%), 17/50 (34%), 22/50 40%), 27/50*(54%)) [→historical incidence: 108/347 (31.1% ± 6.8%); range, 22%-39%0 **→possible CAR-mediated MOA**

	(19%), 11/50 (22%), 14/50 (28%)) [→historical incidence. 69/398 (17% ± 7%), range 10-28%] Mononuclear cell leukemia: (25/50 (50%), 26/50 (52%), 32/50 (64%), 35/50*(70%)) [→historical incidence 188/399 (47.1%; 47,1%±10.3% SD, range 32-66%]]	Mononuclear cell leukemia: (14/50 (28%), 21/50 (42%), 12/50 (24%), 16/50 (32%))		

#For extended evaluation of renal proliferative lesions in kidneys of male rats 3 to 4 additional sections were obtained from each kidney. * Significantly different from control ($p \leq 0.05$) ** $p \leq 0.01$

Table 20: Mean body weights of rats and mice (m, f) (NTP, 2007)

weeks	control	450 ppm	% from control	900 ppm	% from control	1800 ppm	% from control
Male rats							
1-13	**250**	**251**	**101**	**254**	**102**	**257**	**103**
14-52	**432**	**433**	**100**	**431**	**100**	**438**	**101**
53-103	**515**	**516**	**100**	**496**	**97**	**497**	**97**
Female rats							
1-13	**159**	**160**	**101**	**161**	**101**	**163**	**103**
14-52	**242**	**241**	**100**	**244**	**101**	**242**	**100**
53-103	**344**	**338**	**98**	**347**	**101**	**335**	**98**
Male mice							
1-13	**30.8**	**29.6**	**96**	**29.5**	**96**	**29.8**	**97**
14-52	**46.6**	**44.6**	**96**	**44.1**	**95**	**42.9**	**92**
53-103	**52.0**	**52.5**	**101**	**51.3**	**99**	**50.5**	**97**
Female mice							
1-13	**25.1**	**25.5**	**101**	**25.6**	**102**	**25.6**	**102**
14-52	**42.1**	**42.4**	**101**	**41.3**	**98**	**37.5**	**90**
53-103	**56.9**	**58.9**	**104**	**55.4**	**97**	**50**	**88**

Investigation of a possible α2u-globulin nephropathy MoA:

To clarify a possible α2u-globulin nephropathy mode of action of the observed renal tubule adenomas and carcinomas in rats Borghoff (2015) exposed F344 rats (m+f) for 1 week (4 days/week, 6h/day) or 4 weeks (5days/week, 6h/day) to concentrations of 0, 450, 900 and 1800 ppm (n=10 animals/group). 18h after the last exposure rats were euthanized. 3.5 days prior to euthanasia rats were subcutaneously implanted with an osmotic pump containing BrdU to measure cell proliferation. D-limonene (300mg/kg/day, oral for 4 consecutive days) was used as positive control showing prominent hyaline droplet accumulation (grad 12) which was positive for α2u.

Inhalative exposure to 4-methylpentan-2-one resulted in significant increase in absolute kidney weights in male and female rats exposed to 900 and 1800 ppm for 1 and 4 weeks. Protein droplet accumulation in the proximal convoluted tubules of all exposed males was seen after 1- and 4-weeks of exposure. With increasing exposure there was increasing prominence of hyaline droplets in terms of size and autofluorescence (grade 1 in control to grade 10.75 in high exposure males). Due to the short study duration the formation of granular casts were not observed. However tubules representing precursors of granular casts were observed in some male rat kidneys (6/8) at 1800 ppm and 4 weeks of exposure. Chronic progressive nephropathy was slightly exacerbated at 900 and 1800 ppm in males only. Protein droplets accumulating in male rats were positive for α2u (immunhistochemical staining). Concentration of α2u in the kidney homogenate showed an exposure related increase in male rats at 1 and 4 weeks of exposure. The concentration of α2u in females was 250-fold lower compared to males.
In a separate in vitro study a two compartment model (method according to Poet, 1997) was used to assess the interaction between 4-methylpentan-2-one and α2u. The binding affinity[5] for 4-methylpentan-2-one to α2u was estimated to be 1.27×10^{-5}M (medium affinity). The apparent binding affinities for a number of chemicals known to cause α2u-mediated nephropathy vary over three orders of magnitude. Compounds with high binding affinity include 2,4,4-trimethyl-2-pentanol and d-limonene oxide (7.6×10^{-7}M and 5.1×10^{-7}M respectively). The binding affinity of 1,4-dichlorobenzene (shown to cause α2u mediated nephropathy), however, is in the order of 5.2×10^{-4} M similar to 2.2×10^{-4}M reported for methyl tert-butyl ether (Poet, 1997). According to the authors this investigation provides indirect evidence that 4-methylpentan-2-one interacts reversibly with α2u.
Renal cell proliferation, measured via BrdU labelling, was statistically significantly increased in male rats exposed for 1 week to 450 ppm and male rats exposed for 4 weeks to 450, 900 and 1800 ppm, but not in female rats. Mitotic figures were counted in kidney sections showing approximately a 10-fold increase in males but not in females exposed to 1800 ppm for 1 or 4 weeks. The latter two results demonstrate the ability of 4-methylpentan-2-one to cause a compensatory increase in renal cell proliferation in males (Borghoff, 2015).

Borghoff (2009) exposed male and female F-344 rats for 10 consecutive days (oral) to 4-methylpentan-2-one (1000 mg/kg bw) or corn oil and male rats to d-limonene (300 mg/kg bw). 4 male and 4 female animals were used per group. Approximately 24h after the final dose the kidneys were excised and the left one was evaluated for histological changes including protein (hyaline) droplet accumulation, immunohistochemical staining for α2u-globulin, and proliferating cell nuclear antigen (PCNA) to quantitate renal cell proliferation. The right kidney was prepared for quantitation of total protein and α2u using an ELISA.
4-methylpentan-2-one elicited an increase in protein droplets in male rats. The severity of droplet accumulation was graded as mild in 3/4, and moderate in 1/4 male rats. In males there was also a minimal increase in mitotic activity in the cortex and a minimal number of cell debris-containing pars recta tubules at the OSOM/ISOM junction – precursors of granular casts according to Hard (2008). Female rat kidneys were judged to be within normal limits. Immunohistochemical staining of kidney sections for α2u from both 4-methylpentan-2-one and d-limonene treated male rats were more intense and occupied a greater area of the renal cortex compared to control rats. No positive staining for α2u was observed in the kidneys from control rats or 4-methylpentan-2-one administered female rats. PCNA immunohistochemistry in the renal cortex showed a statistically significant threefold increase in male rats administered 4-methylpentan-2-one, but not in female rats. 4-methylpentan-2-one produced identical histopathological changes in the male rat kidney

[5] dissociation constant, K_d, for 4-methylpentan-2-one to α2u.

when compared to the positive control d-limonene, however, the grade of severity tended to be slightly lower with 4-methylpentan-2-one.

To decide whether the effects observed in the kidney may be related to α2u-globulin nephropathy, a syndrome commonly seen in male rats, a comparison with available criteria (cited from Doi, 2007) is done:

Criteria	Study results
US EPA (1991)	
Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats	- Minimal hyaline droplet accumulation in male rats who died early in the study (two at 900 ppm, two at 1,800 ppm) [hyaline droplets are not expected to be detectable in aged rats] (NTP, 2007) - Hyaline droplet degeneration of the proximal tubules with occasional foci of tubular necrosis [100 ppm]; trend towards a linear progression of hyaline droplet degeneration over time; hyaline droplets tended to be larger with time; recovery: gradual reversion of kidney tubular damage with time, completely reversed 60 days postexposure. (MacEwen, 1971, 90-day study) Short term exposure: - Droplet accumulation in all exposed males (Borghoff, 2015) - increase in protein droplets, accumulation of α2u at 1000 mg/kg bw (Borghoff, 2009) - Nephropathy and homogeneous, developed acidophilic and spherical inclusions/droplets in the renal cortical tubular epithelium were observed in the exposed males (F1) (Nemec, 2004, Two-generation study) - increases in regenerative tubular epithelia and hyaline droplets in the kidneys of male but not female rats exposed to 500 or 2000 ppm (Phillips, 1987)
Protein in the hyaline droplets is α2u-globulin	Positive staining of droplets for α2u-globulin (Borghoff, 2015)
Additional pathological sequence of renal tubule lesions	Hyaline droplets accumulation in males, male specificity of mineralization, sustained increases in cell proliferation in the renal cortex and the induction of combined adenomas and carcinomas in the kidney (NTP, 2007; Borghoff, 2015)
IARC (1999)	
Tumors occur only in male rats.	Renal tubule adenoma and carcinoma in male rats only (NTP, 2007) But chronic progressive nephropathy was also seen in female rats (NTP, 2007)
Acute exposure exacerbates hyaline droplet formation	Exposure related increase in hyaline droplet accumulation in male rats after 1 and 4 weeks of exposure (Borghoff, 2015)
α2u-globulin accumulates in hyaline droplets	- Minimal hyaline droplet accumulation in male rats which died early in the study (two at 900 ppm, two at 1,800 ppm) [hyaline droplets are not expected to be detectable in aged rats], α2u-globulin not measured (NTP, 2007)

	- Positive staining of droplets for α2u-globulin (Borghoff, 2015) - Exposure related increase of α2u concentration in kidneys of male rats (homogenate) (Borghoff, 2015)
Subchronic lesions include granular casts and linear papillary mineralization	Exposure-related and statistically significant increase in incidences of minimal to mild linear mineralization of the renal papilla tubule epithelium were seen in all groups of exposed male rats (NTP, 2007).
Absence of hyaline droplets and other histopathological changes in female rats and mice	- Absence of droplets in female rats (NTP, 2007; Borghoff, 2009; Borghoff, 2015). - But: Incidences of chronic nephropathy were significantly increased in all exposed females (NTP, 2007).
Negative for genotoxicity	No evidence for genotoxicity
Reversible binding of chemical to α2u-globulin *	in vitro binding affinity for 4-methylpentan-2-one: $1.27x10^{-5}$M (medium affinity); not clear if any of the metabolites also bind to α2u (Borghoff, 2015)
Increased sustained cell proliferation in proximal tubule (P2 segment) *	- Significant increase of renal cell proliferation (BrdU, renal cortex) in male rats (Borghoff, 2015) - Mitotic figures showed a 10-fold increase in males **but not in females exposed to 1800 ppm for 1 or 4 weeks** (mainly proximal tubule cells where droplets accumulate) (Borghoff, 2015)
Similarities in dose-response relationship of the tumour outcome with the histopathological end-points (protein droplets, α2μ-globulin accumulation, cell proliferation) *	Dose–response consistency and male specificity of mineralization, sustained increases in cell proliferation in the renal cortex and the induction of combined adenomas and carcinomas in the kidney. However, the study found that chronic progressive nephropathy was not male-specific. α2u-Globulin levels were not evaluated in this study (NTP, 2007)

* additional supporting evidence

Like the authors of the NTP-study also Phillips (1987) and Nemec (2004) suggested an association between the α2u-globulin syndrome and nephrotoxicity in male rats.
However exposure-related increased incidences of chronic nephropathy in female rats indicate that exposure-related nephropathy also may occur independent of the α2u-globulin mechanism. But no tumours were seen in females.
IARC (2012) concluded that the relevance of the tumour response to humans cannot be excluded as the strength of the evidence that male rat kidney tumours arose through a α2u-globulin nephropathy mechanism is weak. This is based on a publication by Doi (2007) reviewing the linkage between endpoints that are typically considered to support an α2u-globulin mode of action, where recent NTP studies demonstrated inconsistencies with this proposed mechanism, including, in some cases, kidney tumour responses that were far weaker than expected based on the extent of α2u -globulin nephropathy. Doi (2007), looking on NTP study results with 3 substances and their suspected α2μ-globulin MOA, revealed no or at best weak associations of tumour responses with renal α2u-globulin concentrations, indices of cell turnover, or microscopic evidence of α2u-associated nephropathy in prechronic studies. However, tumour responses corresponded somewhat with a measure of cumulative α2u-associated nephropathy (linear mineralization of the papilla) at the end of the 2-year studies. They concluded, while α2u-globulin nephropathy may contribute to the renal

tumour response, the critical component(s) of the nephropathy most closely associated with the development of tumours could not be clearly identified in this review.

Investigation of a possible CAR/PXR-mediated MOA:

Due to the lack of a mutagenic potential of the substance the possibility of a CAR/PXR[6] nuclear receptor activation mode of action (MoA) in the murine liver, a common mechanism in rodents, was investigated by Hughes (2016) based on five key events for CAR activators published by Elcombe (2014):

#1 CAR activation
#2 Altered gene expression specific to CAR activation
#3 Increased cell proliferation, inhibition of apoptosis
#4 Clonal expansion leading to altered foci
#5 Liver adenomas/carcinomas

Associated events are hypertrophy, CYP2b induction, decreased apoptosis and altered epigenetic changes.

Hughes (2016) exposed $B6C3F_1$ (8m+8f/group), C57BL/6 (8m+8f/group) and CAR/PXR Knockout (8m+5f/group) mice in a chamber to 0 or 1800 ppm for 6h/day, 5d/week for a total of 10 days. An osmotic pump containing BrdU was implanted on day 1 for histopathological determination of DNA-synthesis (proliferation) in the liver. Mean relative liver weights in exposed mice were statistically significantly higher in male $B6C3F_1$ and CAR/PXR knockout mice as well as in female $B6C3F_1$, C57BL/6 and CAR/PXR knockout mice. In male C57BL/6 mice mean relative liver weights were also increased but statistical significance was not reached. Hepatocytes in exposed $B6C3F_1$ and C57BL/6 male and female mice showed slight hypertrophy of centrilobular/midzonal regions. In knock-out mice a very slight hypertrophy in combination with increased vacuolization was described.
Clinical chemistry parameters at day of termination showed no clear trend. While one the one hand there was no statistical difference for AST or GGT serum levels between exposed and control groups in any strain on the other hand ALT was increased in exposed female $B6C3F_1$ and C57BL/6 mice and males of $B6C3F_1$ and CAR/PXR knockout mice (see Table 21). The results indicate that exposure to 4-methylpentan-2-one has some effect on the liver but no exposure related difference between wild type and knock-out mice was seen. CAR receptor activation has been shown to play a role in regulation of lipogenesis, β-oxidation of fatty acids, gluconeogenesis and cholesterol/bile acid metabolism (Yang, 2014). Therefore it is likely that alterations in triglycerides and cholesterol are also a consequence of CAR activation.

Table 21: Clinical chemistry parameters after 10d exposure of mice to 4-methylpentan-2-one (Hughes, 2016).

Parameter	Dose [ppm]	$B6C3F_1$		C57BL/6		CAR/PXR KO	
		male	female	male	female	male	female
AST [U/L]	0	**61**±**13**	**104**±26	**125**±105	**132**±71	**90**±10	**145**±54
	1800	**80**±31	**81**±35	**102**±55	**124**±46	**116**±36	**180**±59
ALT [U/L]	0	**46**±13	**52**±18	**5**±19	**53**±12	**47**±11	**65**±12
	1800	**79**±22*	**80**±30*	**67**±14	**89**±29*	**62**±17*	**76**±16
AP	0	**122**±7	**130**±17	**101**±10	**150**±9	**83**±11	**79**±34

[6] CAR…constitutive androstane receptor, PXR…pregnane X receptor

[U/L]	**1800**	**106**±12*	**122**±11	**107**±25	**143**±10	**96**±14*	**133**±39*
GGT	**0**	**3**±2	**8**±3	**6**±4	**6**±3	**3**±3	**5**±2
[U/L]	**1800**	**6**±4	**5**±4	**4**±4	**4**±3	**4**±3	**4**±3
Total bilirubin	**0**	**0.1**±0.1	**0.2**±0.1	**0.1**±0.1	**0.2**±0.1	**0.1**±0.0	**0.2**±0.2
[mg/dL]	**1800**	**0.1**±0.0	**0.1**±0.0	**0.2**±0.2	**0.1**±0.1*	**0.2**±0.1	**0.3**±0.2
Total protein[a]	**0**	**5.2**±0.1	**5.4**±0.2	**5.1**±0.1	**5.0**±0.2	**5.2**±0.2	**5.0**±0.3
[g/dL]	**1800**	**5.3**±0.2	**5.3**±0.2	**5.3**±0.2*	**5.3**±0.2*	**5.4**±0.3	**5.4**±0.2*
Albumin (A)	**0**	**4.1**±0.1	**4.7**±0.2	**4.0**±0.1	**4.5**±0.2	**4.2**±0.3	**3.9**±0.6
[g(gL]	**1800**	**4.2**±0.1	**4.5**±0.2	**4.3**±0.2*	**4.5**±0.3	**4.5**±0.2	**4.9**±0.4*
Globulin (G)	**0**	**1.1**±0.1	**0.7**±0.1	**1.1**±0.2	**0.5**±0.1	**1.0**±0.2	**1.0**±0.5
[g/dL]	**1800**	**1.2**±0.1	**0.9**±0.2*	**1.0**±0.1	**0.7**±0.2*	**1.0**±0.4	**0.6**±0.3
A/G ratio	**0**	**3.6**±0.4	**6.9**±0.9	**3.8**±0.6	**9.3**±2.1	**4.3**±1.4	**4.8**±2.9
	1800	**3.5**±0.3	**5.5**±1.3*	**4.3**±0.7	**6.4**±1.4*	**5.2**±1.8	**11.8**±7.9
Triglycerides[b]	**0**	**66**±9	**48**±3	**58**±13	**51**±8	**62**±14	**57**±19
[mg/dL]	**1800**	**74**±17	**62**±10*	**71**±15	**62**±12*	**51**±13	**43**±8
Cholesterol	**0**	**118**±13	**96**±8	**97**±6	**83**±10	**88**±5	**65**±6
[mg/dL]	**1800**	**128**±7	**123**±6*	**116**±9*	**99**±7*	**85**±5	**70**±6

***Significant at p<0.05; [a]log transformed; [b]inverse transformed; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; AP=Alkaline phosphatase; GGT=γ-glutamyl-transpeptitase**

At 1800 ppm $B6C3F_1$ (m+f) and C57BL/6 (m) mice showed a statistically significant increase in BrdU labelled cells in the centrolobular, midzonal and periportal regions. For female C57BL/6 the results were not statistically significant. In WT mice an increase in mitotic figures was seen in H&E stained liver sections. 4-methylpentan-2-one did not induce hepatocellular proliferation in CAR/PXR KO mice as there were no statistically significant increases in BrdU labelling (Table 22).
To investigate nuclear receptor-mediated pathways in the hepatic tissue gene expression responses for CYP1a1 (AhR-responsive transcript), CYP2b10 (specific for CAR activation), CYP3a11 (PXR inducible transcript) and CYP4a10 (PPAR-α responsive transcript) were examined as biomarkers. Results are presented in Table 22. Significant elevation of gene expression (CYP2b10 and CYP 3a11) indicative for the CAR/PXR pathway was seen and no evidence for induction of AhR and PPAR-α pathways can be derived (Table 22).
According to the authors gene expression data, histopathology and hepatocellular proliferation provide evidence for a CAR/PXR nuclear receptor MoA for 4-methylpentan-2-one induced liver tumours in mice. Initiating events seems to be activation of the CAR and PXR nuclear transcriptors resulting in hepatocellular proliferation and leading to rodent liver tumours.

Table 22: Responses in the liver of 4-methylpentan-2-one treated mice (Hughes, 2016).

	Ratio of gene expression responses in the liver				Mean relative liver weights	Histopathological changes	Hepatocellular proliferation (BrdU labelling)
	CYP1a1	CYP2b10	CYP3a11	CYP4a10			
$B6C3F_1$ mice							
0 ppm	1	1	1	1		-	-
1800 ppm **male**	1.3	981.9*	2.3*	1.2	↑*	slight hypertrophy	↑*
1800 ppm **female**	1.3	234.8*	4.3*	0.6	↑*	slight hypertrophy	↑*
C57BL/6 mice							
0 ppm	1	1	1	1		-	-
1800 ppm	1.8	599.2*	2.2*	0.8	↑	slight	↑*

male						hypertrophy	
1800 ppm **female**	2.7	269.8*	4.8*	0.6	↑*	slight hypertrophy	↑
CAR/PXR KO mice							
0 ppm	1	1	1	1		-	-
1800 ppm **male**	0.9	0.9	1.3	2.2	↑*	very slight hypertrophy, increased vacuolization	no
1800 ppm **female**	0.8	2.2	1.3	3.6	↑*	very slight hypertrophy, increased vacuolization	no

The effects of 4-methylpentan-2-one on the mouse liver were also evaluated by Anonymous (2009; cited from ECHA dissemination site). Male B6C3F1 mice were implanted with 5-bromo-2'deoxyuridine (BrdU) pumps and then exposed to 0 or 1800 ppm (n=6/group) for 6 hours/day for 7 days (whole body). Clinical signs and body weights were recorded. Mice were euthanized and assessed for clinical chemistry, gene expression analysis of the upper third of the left liver lobe, liver histopathological examination and BrdU proliferation analysis, and liver enzyme activity. There were no treatment-related effects noted for clinical signs, body weights, liver weights, or clinical chemistry assessments. Treatment-related findings included very slight hepatocytes hypertrophy with increased cytoplasmic eosinophilia in the centrilobular/midzonal regions of the hepatic lobule which were consistent with increased smooth endoplasmic reticulum and induction of cytochrome P450 enzymes. CYP2b10 transcript levels increased 4-fold and CYP4a10 decreased 5.56-fold. This was verified by increased CYP2b10 enzyme activity (PROD) and hepatocyte proliferation.
According to Ueda (2002) CAR may be a transcription blocker that prevents CYP4a10 and CYP4a14 genes from being induced.

Evidence for a CAR-mediated MoA can be summarized as following (according Elcombe, 2014):

Key event	**Study results**
#1: the activation of the CAR nuclear receptor	**Studies in CAR knock -out mice:** **In CAR/PXR KO mice no induction of CYP2b10 and CYP3a11 expression, only very slight hypertrophy; no hepatocellular proliferation in CAR/PXR KO mice (BrdU labelled nuclei) (Hughes, 2016).**
#2: Altered gene expression specific to CAR activation	**Induction of CYP2b10, CYP3a11 in m and f WT mice (Hughes, 2016; Anonymous, 2009)** **Block of induction of CYP4a10 (Anonymous, 2009), slight induction in CAR/PXR KO mice (Hughes, 2016)**
#3: increased cell proliferation	**demonstrated via BrdU proliferation analysis and increase in mitotic figures in H&E staining (Hughes, 2016)**
#4: clonal expansion, altered foci	**eosinophilic foci seen in mice; increase with dose (NTP, 2007)**
#5: liver adenomas/carcinomas	**Seen in mice (NTP, 2007) (Table 19)**
Associated events:	

Hypertrophy	**$B6C3F_1$ and C57BL/6 male and female mice showed slight hypertrophy of centrilobular/midzonal regions (Anonymous, 2009; Hughes, 2016). Hepatocytes in male and female CAR/PXR KO mice showed a decreased response i.e. very slight hypertrophy in the centrilobular/midzonal regions of the liver (Hughes, 2016).**
CYP2b induction	**Observed increase in CYP2b10 enzyme expression after treatment with 4-methylpentan-2-one (Hughes, 2016; Anonymous, 2009). No induction in CAR/PXR KO mice**
decreased apoptosis and altered epigenetic changes	**-**
Altered epigenetic changes specific to CAR activation	**-**

Liver effects of 4-methylpentan-2-one were seen in mice but not in rats in the NTP-study. For Phenobarbital, the model substance for this CAR-mediated-MoA, the rat appears to be more resistant than the mouse regarding tumour formation (Elcombe, 2014).

Several substances (including phenobarbital) are known to co-activate the CAR and PXR pathways as they have overlapping genes and overlapping functions (Elcombe, 2014). Phenobarbital, the model substance for CAR/PXR nuclear receptor activation induced a 474.4-fold increase of CYP2b10 and a 2.3-fold increase of CYP3a11 at a dose of 150mg/kg/day (Geter, 2014). Results for 4-methylpentan-2-one are in the same order of magnitude.
According to Elcombe (2014) CAR in particular can be activated by ligand binding or without direct ligand binding by an indirect/ligand-independent mechanism. Phenobarbital activates CAR by a dephosphorylation reaction. For 4-methylpentan-2-one no investigation on this initial step is known.

There are clear species differences in CAR activation which severely hamper the extrapolation of animal data to humans. The expression level of CAR, as response to xenobiotics and endogenous chemicals, differs between humans and rodents as well as males and females. Eg. TCBOPOP is a potent ligand of murine CAR, but cannot activate or bind to human or rat CAR. Phenobarbital, a non-ligand activator, activates both human and mouse CAR. CITCO is an effective agonist of human CAR but not mouse CAR (Nassar, 2009).
Species differences in ligand selectivity also exist between human and rodent PXR (Bunce, 2010).
CAR activation can also occur in humans but there is no evidence that subsequent effects on other key events (induction of DNA-syntheses, proliferative response) would occur in humans. Elcombe (2014) therefore concluded that this MoA would be qualitatively not plausible for humans.

***In vitro* studies**

Table 23: in vitro tests on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
In vitro Mouse embryo cell transformation assay BALB/3T3	4-methylpentan-2-one (99.6% pure) 1st experiment: Dose (–S9): 2.4, 3.6, 4.8 µl/ml Dose (+S9): 1, 2, 4 µl/ml 2nd experiment (repeat assay due to equivocal results in 1st experiment): Dose (–S9): 4, 5, 6, 7 µl/ml Dose (+S9): 2, 3, 4, 5 µl/ml Pos. control: (-S9): N-Methyl-N'-nitro-N-nitrosoguanidine 0.5µg/ml (+S9): benzo[a]pyrene 12.5µg/ml	1st experiment: (-S9): 4 .8 µl /ml: type III foci in 3/15 dishes, reduced cloning efficiency (+S9): no transforming activity. 2nd experiment: (-S9): 5 µl/ml resulted in 2 type III foci for 15 plates with 100% cell survival, transformation frequency not statistically increased negative in both the presence and absence of exogenous metabolic activation as the results of the first assay (-S9) could not be confirmed	O´Donoghue (1988)

In vitro cell transformation assays have been proposed for predicting the carcinogenic potential of chemicals by measuring the phenotypic conversion from normal to malignant characteristics in mammalian cells. BALB/c-3T3 mouse embryo cells form normally a monolayer culture and get contact-inhibited after reaching confluence. Upon treatment with chemical agents, some cells do not stop proliferation and grow as morphologically aberrant foci over the monolayer of normal cells. Such assays are capable of detecting non-genotoxic as well as genotoxic carcinogens (Sakai, 2007).
In an *in vitro* cell transformation assay BALB/3T3 mouse embryo cells ($2x10^6$) were incubated for 2h with the test substance 4-methylpentan-2-one (see Table 23) in phosphate-buffered saline with or without Aroclor 1254-induced rat liver S9 fraction (O´Donoghue, 1988). Doses for the study were chosen based on a preliminary cytotoxicity. Doses of 4-methylpentan-2-one were 2.4, 3.6 and 4.8µl/ml without activation and 1, 2 and 4 µl/ml with activation. In a repeat assay doses were 4, 5, 6 and 7 µl/ml without activation and 2, 3, 4 and 5µl/ml with activation. After removal of the test substance the cells were incubated for 4-6 weeks, then the transformation plates were fixed, stained and scored for type II and type III loci.
The transformation frequency for each treatment condition was expressed as the number of transformed loci per surviving cell. The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control and called “relative cloning efficiency”.
In a first assay at the highest concentration tested a positive response was seen in the non-activated system (3 type III foci in 15 dishes, reduced cloning efficiency of 51%) and is presented in detail in Table 24). The number of type III foci, coupled with a reduced cloning efficiency, resulted in a

positive statistical analysis in the non-activated system. With metabolic activation, there was no transforming activity. In a repeat assay a concentration of 5 µl/ml resulted in 2 type III foci for 15 plates with 100% cell survival. The resulting transformation frequency was not statistically increased over the negative control. Higher test concentrations also gave negative results. Thus the results of the first BALB/3T3 assay of 4-methylpentan-2-one, in the absence of metabolic activity, could not be confirmed in a repeat assay (O´Donoghue, 1988).

Table 24: Cell transformation assay results (O´Donoghue, 1988).

	4-methylpentan-2-one (µl/ml)	Total foci/dishes		Transformation frequency ($x10^{-4}$)	Relative cloning efficiency (%)
		Type II	Type III		
Without metabolic activation	4.8	0/15	3/15	0.87*	51
	3.6	0/15	3/15	0.56	80
	2.4	1/15	2/15	0.34	87
	0 (PBS control)	1/15	0/15	<0.15	100
	Pos. control (0.5µg/ml MNNG)	7/15	19/15	12.67*	22
	7	1/15	0/15	toxic	<1
	6	2/15	0/15	<0.95	17
	5	0/15	2/15	0.33	100
	4	0/14	0/14	<0.19	93
	0 (PBS control)	1/14	0/14	<0.17	100
	Pos. control (0.5µg/ml MNNG)	5/15	11/15	5.64*	32
With metabolic activation	4	1/14	0/14	<0.22	66
	2	0/13	1/13	0.21	76
	1	1/14	1/14	0.17	84
	0 (PBS control)	2/15	0/15	<0.14	100
	Pos. control (12µg/ml BaP)	8/15	12/15	3.08*	53
	5	0/15	1/15	0.18	75
	4	1/15	1/15	0.16	82
	3	1/15	4/15	0.59	88
	2	0/15	1/15	0.14	96
	0 (PBS control)	0/14	0/14	<0.14	100
	Pos. control (12µg/ml BaP)	0/15	5/15	1.23*	53

High-throughput assay data for 4-methylpentan-2-one and its metabolites have been screened via TOXCAST7 (US EPA, 2018) with special focus on CAR and PXR. 4-methylpentan-2-one was positive for 1/4 assays on PXR and 4-hydroxy-4-methyl-2-pentanone for 1/5 assays on PXR. An

[7] *https://www.epa.gov/chemical-research/toxcast-dashboard retrieved 27th August, 2018.*

overview on available and positive assays is given in the table below. In general substances did not show non-specific cytotoxicity.

Table 25: high-throughput assays for CAR/PXR in TOXCAST

substance	Available qHTS assays for CAR and PXR	Positive tests	Total number of assays/positive assays
4-Methylpentan-2-one CA 108-10-1	ATG_CAR_TRANS_up ATG_CAR_TRANS_dn ATG_PXRE_CIS_up ATG_PXRE_CIS_dn ATG_PXR_TRANS_up ATG_PXR_TRANS_dn	ATG_PXRE_CIS_up	339/2
4-hydroxy-4 methyl-2-pentanone [metabolite] CAS 123-42-2	ATG_CAR_TRANS_up ATG_CAR_TRANS_dn NVS_NR_hCAR_Agonist NVS_NR_hCAR_Antagonist ATG_PXRE_CIS_up ATG_PXRE_CIS_dn ATG_PXR_TRANS_up ATG_PXR_TRANS_dn NVS_NR_hPXR	ATG_PXRE_CIS_up	874/17
4-methyl-2-pentanol [metabolite] CAS 108-11-2	ATG_CAR_TRANS_up ATG_CAR_TRANS_dn NVS_NR_hCAR_Agonist NVS_NR_hCAR_Antagonist ATG_PXRE_CIS_up ATG_PXRE_CIS_dn ATG_PXR_TRANS_up ATG_PXR_TRANS_dn NVS_NR_hPXR	-	881/6

A compilation on the available carcinogenicity data and identified MoAs relevant for the evaluation of the carcinogenic potential of 4-methylpentan-2-one is given in the Table 26 below.

Table 26: Compilation of factors to be taken into consideration in the hazard assessment (according "Guidance on the application of the CLP criteria", Chapter 3.6.2.3.2).

Species and strain	Tumour type [8]	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat F344/N	Males: renal tubule adenoma or carcinoma Pheochromocytoma (incidence in the 1,800 ppm group was at the upper limit of the historical range)	yes	yes	-	both sexes but with different tumours	No excessive toxicity (mean body weights of the 900 and 1,800 ppm males were less than those of the chamber	inhalation	Kidney: α2u-mediated nephropathy in male rats

[8] Background incidences are presented in Table 19

Species and strain	Tumour type [8]	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	Mononuclear cell leukemia (high background incidence but dose dependent increase and statistical significance in the high dose) <u>Females</u>: Mesenchymal tumour, malignant (low incidence at high dose but rate tumour)					controls after weeks 97 and 89, respectively; bw reduced up to a values of 92% of control)		
Mice B6C3F1	hepatocellular adenoma or carcinoma	no	yes	-	both sexes	No excessive toxicity (females: reduced bw in 1,800 ppm group after week 17, bw reduced up to a value of 84% of control)	inhalation	CAR/PXR mediated MoA

10.9.2 Comparison with the CLP criteria

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A (known to have carcinogenic potential for humans, classification is largely based on human evidence)

Category 1B (presumed to have carcinogenic potential for humans, classification is largely based on animal evidence)

The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from: (1) human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or (2) animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

The mutagenicity studies for 4-methylpentan-2-one gave negative results.
The primary toxicity targets of 4-methylpentan-2-one in the available carcinogenicity study were the kidney in rats and the liver in mice.
Carcinogenic effects in the kidney seen in male rats may be the result of a α2µ-globulin related mechanism - a mechanism that is not considered to be a predictor of carcinogenic risk to humans as an analogous protein in humans is missing. Mechanistic studies are available and criteria defined by IARC, 1999 are met. However, uncertainties concerning this MoA were identified in a review by Doi (2007). In addition the finding of chronic nephropathy in exposed female rats shows that an additional mechanism inducing renal toxicity is present and could also be involved in the formation of tumours seen in males.
Hepatocellular adenomas and carcinomas were seen in mice but not in rats (under the same testing conditions). The possibility of a CAR/PXR -mediated mechanism was investigates in detail. Based on available data a CAR-mediated MOA has to be assumed. The hepatocellular adenomas/carcinomas are therefore not considered relevant for humans. Investigating high-throughput assay (TOXCAST9; US EPA, 2018) 4-methylpentan-2-one was positive for 1/4 assays on PXR and 4-hydroxy-4 methyl-2-pentanone for 1/5 assays on PXR.

Two cases of renal mesenchymal tumours were seen in female rats (2/50) of the high dose (1800ppm) only (4% vs 0.0% in current control and 0.0% in historical control data).
There was high background incidence of mononuclear cell leukemia, however, in male rats a dose dependent increase of mononuclear cell leukemia (above historical control) was observed reaching statistical significance in the high dose (70% vs 50% in current control and 47.1% in historical control data).
In male rats a dose-dependent increase of pheochromocytoma was observed with the high dose lying at the upper range of the historical control data (28% vs 16% in current control and 17% in historical control data).

An *in vitro* cell transformation assays was negative.
No human data are available.

10.9.3 Conclusion on classification and labelling for carcinogenicity

In a weight of evidence approach it cannot be excluded that 4-methylpentan-2-one has a carcinogenic potential relevant for humans. Liver tumours seen in mice appear to be not relevant for humans as they are CAR-mediated, however, mechanistic tests in human cells are missing.
There are some indications that kidney tumours seen in male rats are caused by a α2µ-mediated MoA, however, as there is also some kidney toxicity seen in female mice another mechanism may also be involved in the tumour formation. A recent review identified some uncertainties regarding the link between α2µ and kidney tumour formation.

Tumours were also seen at other sites (renal mesenchymal tumours, adrenal gland, leukemia), but were restricted to one sex and one species and were only slightly above or in the upper range of historical control data.

Based on a limited evidence of carcinogenicity in animal studies classification as Carc. Cat. 2 is proposed. Due to a non-genotoxic mechanism a threshold can be presumed.

9 *https://www.epa.gov/chemical-research/toxcast-dashboard retrieved 27th August, 2018.*

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 27: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two-Generation Reproduction Toxicity Study (OECD 416) GLP Rat, Sprague Dawley Crl:CD, m+f 30 animals/sex/group	4-methylpentan-2-one (99.93%) inhalation, whole body 0, 500, 1000, 2000 ppm 6 hours/day 7 days/week	500 ppm: - **centrilobular hepatocellular hypertrophy 3/30 (m)** 1000 ppm: - **absent or decreased response to a sound stimulus (F0,– m,f; F1-m), reversible** - **centrilobular hepatocellular hypertrophy 15/30 (F0 - m), 18/30 (F1 – m)** - **absolute and relative kidney weights ↑ (F0 - m) [absolute weight +12%]** - **nephropathy (basophilic tubules with variable inflammation and thickening of the tubular basement membrane) (F0 – m)** 2000 ppm: - **absent or decreased response to a sound stimulus (F0, F1 – m,f), reversible** - **body weight gains ↓ in week 1, 2↓ (F0 - f)** - **food consumption ↓ in week 1 (F0, F1 – m,f)** - **absolute liver weight ↑ (F0 – m +20%, f +10%; F1)** - **centrilobular hepatocellular hypertrophy 26/30 (F0- m) , 20/30 (F1 – m)** - **absolute and relative kidney weights** ↑ (F0 - m) [**absolute weight +28%]** - **relative kidney weight ↑ (F1-f)** - **nephropathy (basophilic tubules with variable inflammation and thickening of the tubular basement membrane) (F0 – m)** - acidophilic inclusions/droplets in kidney (F1 – m) - **absolute and relative seminal vesicle/coagulating gland weight** ↑ (F0 – m) - **increased absolute and relative ovary weight** ↑ F0 – f) - adrenal gland weight ↑ (F0 – f) - clinical signs of neuro- or neuromuscular toxicity (F1 - m,f, PND 22) NOAEL (parental systemic toxicity) = 500 ppm NOAEL (reproductive toxicity) = 2000 ppm	Nemec (2004)

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In the Two-Generation Reproduction Toxicity study reported by Nemec (2004) Sprague Dawley rats were exposed to 4-methylpentan-2-one concentrations of 0, 500, 1000 and 2000 ppm for 6h/day and 7days/week. F_0 and F_1 males were exposed for 10 weeks prior to mating and throughout mating until one day prior to euthanasia. F_0 and F_1 females were exposed for 10 weeks prior to mating and throughout mating, gestation and lactation until one day prior to euthanasia. Exposure of the F_0 and F_1 dams was suspended for 5 days following parturition. Exposure resumed on postnatal day (PND) 5 and dams were removed from litters for the daily 6h exposure during lactation. The F1 and F2 pubs were potentially exposed to 4-methylpentan-2-one in utero, via milk through nursing during PNDs 0 to 21 and for F1 pups, via direct exposure following weaning. These F1 weanlings were first directly exposed to 4-methylpentan-2-one for 6 h beginning on PND 22. In the 2000 ppm groups exposures were suspended on PND 22 due to the death of one male pup and clinical signs of CNS depression indicative of a sedative effect. Exposures were reinitiated on PND 28.
All animals were observed twice daily. On each exposure day (at the midpoint of exposure) the response to a loud noise/novel stimuli on the front glass of the exposure chamber was classified. To assess estrous cyclicity, vaginal smears from each F0 and F1 female was evaluated. On the day of parturition pups were examined for external malformations, and the numbers of stillborn and live pups were recorded. Samples of sperm from the right epididymis were collected from each adult F0 and F1 male and evaluated for the percentage of progressively motile sperm. Sperm morphology was evaluated by light microscopy. Microscopic evaluations were performed on the following tissues for F0 and F1parental animals (10/sex/group) from the control and high-dose groups and for all adult animals found dead or euthanized in extremis: adrenal glands, prostate, brain, spleen, thymus, liver, kidneys, lung, pituitary, seminal vesicles, the right epididymis (caput, corpus and cauda), the right testis, vas deferens, vagina, cervix, coagulating gland, uterus, oviducts, ovaries.

The following observations have been made for the F_0-generation:

- no exposure-related mortalities or clinical signs of toxicity noted during the study
- absent or decreased response to a sound stimulus in the 1000 and 2000 ppm groups suggesting a sedative effect during exposure. Animals appeared normal at 1 h postexposure observation
- Regularity and duration of estrus were not affected by exposure. The mean lengths of estrous cycles were 4.2, 4.1, 5.0, and 4.2 days in the 0, 500, 1000, and 2000 ppm groups, respectively.
- no effect on F_0 spermatogenic endpoints (mean testicular and epididymal sperm numbers, sperm production rate, and sperm motility and morphology)
- Statistically significant reductions in body weight gains in the 2000 ppm F_0 females during weeks 0 to 1 and 1 to 2 (no details on % reduction)
- Statistically significant reductions in food consumption for both sexes during weeks 0 to 1 at 2000 ppm (no further details)
- Exposure-related increases in liver weights (absolute and relative to final body weights) in the 2000 ppm group (m+f) (males: absolute weight +20%; females: absolute weight +10%)
- centrilobular hepatocellular hypertrophy was noted in 0, 3, 15, and 26 F_0 males in the 0, 500, 1000, and 2000 ppm groups, respectively
- Increased absolute and relative kidney weights in all exposed male groups in correlation with an increased occurrence of nephropathy characterized by basophilic tubules with variable inflammation and thickening of the tubular basement membrane in the 1000 and 2000 ppm groups (absolute weight +12% at 1000ppm and +28% at 2000ppm). Kidney weights in females were unaffected.

- Increased absolute and relative seminal vesicle/coagulating gland weight in males at 2000 ppm. No correlating histopathologic findings observed.
- Increased absolute and relative ovary and adrenal gland weights in females at 2000 ppm (absolute weight +10%). No correlating histopathologic findings observed.

Observations in the F_1-generation:

- The number of pups born, live litter size, sex ratio at birth, pup survival at various intervals and pup body weights were unaffected by parental exposure
- Sexual maturation (balanopreputial separation, vaginal patency) was not affected
- No internal findings in pubs found dead or euthanized in extremis
- absent or diminished response to a sound stimulus in the 1000 ppm males and the 2000 ppm males and females
- 1 h post exposure on PND 22: 7 males and 11 females in the 2000 ppm group exhibited clinical signs of neuro- or neuromuscular toxicity (rocking, lurching, swaying, prostrate, halfclosed eyelids, lacrimation); death of one male pub at 2000 ppm.
- The regularity and duration of estrus were not affected by exposure. The mean lengths of estrous cycles were 5.1, 4.5, 4.7, and 4.3 days in the 0, 500, 1000, and 2000 ppm groups, respectively.
- no effects on gestation lengths or reproductive performance
- no effects on F1 spermatogenic endpoints
- Weekly body weights were slightly reduced throughout the study in the 2000 ppm group males and throughout the prebreeding and postlactational phases in the 2000 ppm group females
- Statistically significant reductions in food consumption were observed for both sexes in the 2000 ppm group during the first week of measurement
- absolute and relative liver weights were significantly increased for males and females in the 2000 ppm group; centrilobular hepatocellular hypertrophy was noted in 18/30 F_1 males in the 1000 ppm group and in 20/30 F1 males in the 2000 ppm group
- Nephropathy and droplets (up to 4-5 μm diameter) in the renal cortical tubular epithelium were observed in exposed males; correlating increases in absolute and relative kidney weights
- Relative kidney weights were statistically increased in 2000 ppm females; no correlating histopathologic findings

Observations in the F2-generation:

- number of pups born, live litter size, sex ratio at birth, pup survival at various intervals, and pup body weights were unaffected
- transient reduction in pup body weights on PND 14 in the 2000 ppm group
- No exposure related internal findings were observed at necropsy
- No effects on absolute or relative (to final body weight) brain, spleen, or thymus weights

α2μ-globulin–mediated nephropathy seen in male rats was not considered relevant for human hazard identification. Exposure–related centrilobular hepatocellular hypertrophy was considered as an adaptive physiological response to an intensified metabolic liver burden. 4-methylpentan-2-one-exposure did not affect any reproductive parameters nor offspring growth or development. The NOAEL for parental systemic toxicity was considered to be 500 ppm (acute sedative effect). The NOAEL for neonatal toxicity (based on clinical signs of neuro- or neuromuscular toxicity) was also considered to be 1000 ppm. The NOAEL for reproductive toxicity was considered to be 2000 ppm, the highest concentration tested (Nemec, 2004).

10.10.3 Comparison with the CLP criteria

Known or presumed human reproductive toxicant substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Suspected human reproductive toxicant substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

4-methylpentan-2-one was studied in a Two-Generation Reproduction Toxicity Study (GLP). Rats exposed to 0, 500, 1000 or 2000 ppm via vapour inhalation (6h/d, 7d/w) showed effects on liver (increased liver weight at 2000 ppm, hepatocellular hypertrophy in males), kidney (increased kidney weight in males of all exposure groups, alpha2µ-mediated mechanism) and CNS (depressive effect in F1 pubs at 2000 ppm, reduced reaction to a noise stimulus in F0/F1 at 1000 and 2000 ppm) but no effects on the reproductive parameters or the development of offsprings. Based on these findings the NOAEL for parental systemic toxicity and neonatal toxicity was considered to be 1000 ppm. The NOAEL for reproductive toxicity was considered to be 2000 ppm, the highest dose tested.

10.10.4 Conclusion on classification and labelling for adverse effects on sexual function and fertility

No effects on reproductive parameters were seen in a two-generation-GLP-study. The NOAEL for reproductive toxicity was considered to be 2000 ppm, the highest concentration tested. No classification is warranted.

10.10.5 Adverse effects on development

Table 28: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Developmental Toxicity study (OECD 414) Rat, Fischer F344 35 females/group	4-methylpentan-2-one inhalation, whole body 0, 300, 1000, 3000 ppm 6h/days, GD 6-15	NOAEL (maternal toxicity) = 1000 ppm NOAEL (fetal toxicity) = 1000 ppm NOAEL (teratogenicity) = 1000ppm (ossification) /3000 ppm	Tyl (1987)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two-Generation Reproduction Toxicity Study (OECD 416) GLP Rat, Sprague Dawley Crl:CD, m+f 30 animals/sex/group	4-methylpentan-2-one (99.93%) inhalation, whole body 0, 500, 1000, 2000 ppm 6 hours/day 7 days/week	NOAEL (parental systemic toxicity) = 500 ppm NOAEL (neonatal toxicity - CNS effects) = 1000 ppm NOAEL (teratogenicity) > 2000 ppm (ossification not examined)	Nemec (2004)
Prenatal Developmental Toxicity study (OECD 414) Mouse, CD-1 30 females/group	4-methylpentan-2-one inhalation, whole body 0, 300, 1000, 3000 ppm 6h/days, GD 6-15	NOAEL (maternal toxicity) = 1000 ppm NOAEL (fetal toxicity) = 1000 ppm NOAEL (teratogenicity) = 1000(ossification) /3000 ppm	Tyl (1987)

10.10.6 Short summary and overall relevance of the provided information on adverse effects on development

In a Prenatal Developmental Toxicity Study (Tyl, 1987) pregnant F344 rats and CD-1 mice were exposed to 0, 300, 1000 or 3000 ppm 4-methylpentan-2-one from GD 6-15 in exposure chambers. Vapour concentration was monitored via GC and all concentrations were within ±10% of the target concentration. Animals were observed daily for clinical signs. Maternal body weights were taken on GD 0, 6, 9, 12, 15 and 18; for rats also on GD 21. Mice were sacrificed on GD 18 and rats on GD 21. Ovarian corpora lutea of pregnancy were counted. Maternal liver, kidney, and gravid uterine weights were determined. Statuses of all implantation sites and resorption sites were recorded. Nongravid uteri were especially examined for early resorptions. Live fetuses were weighted, sexed and examined for external malformations. One-half of the fetuses in each litter were examined for thoracic and abdominal visceral abnormalities and the other half was examined for skeletal alterations.
In rats maternal toxicity at 3000 ppm was evident by significant reductions in body weight on GD 9 ($p<0.01$), 12 and 15 ($p<0.001$) and 18 ($p<0.05$) accompanied by significantly reduced food consumption in these periods. As no effect on body weight was seen on GD 21 this effect was classified as transient. At 3000 ppm clinical signs like loss of coordination, negative tail and/or toe pinch, paresis (partial hindlimb paralysis), muscular weakness in hindlimbs, piloerection, lacrimation, and red perioral encrustation were observed. At sacrifice on GD 21 maternal relative kidney weight was slightly (104% of controls) but significantly elevated at 3000 ppm. Absolute kidney weight, relative and absolute liver weight, gravid uterine weights were unaffected. Gestation parameters were not affected. Fetal body weight per litter was significantly reduced at 3000 ppm ($p<0.001$) and slightly reduced at 300 ppm ($p<0.05$). There were no statistically significant increases in the incidence of external, visceral, skeletal or total malformations in rat foetuses.

Increased incidence of skeletal variations, indicative of toxicity, was observed at 3000 ppm, involving the vertebrae, sternebrae, and distal limbs. Skeletal preparations of almost all of the fetuses (116/118) at 3000 ppm exhibited a fragility (less cleared tissue surrounding the skeleton). Details are presented in Table 29.

Table 29: Skeletal variations observed in Fischer 344 rat fetuses after exposure to 4-methylpentan-2-one on GD 6-15 (Tyl, 1987).

	Fetuses[a]				Litters			
Skeletal variations	0 ppm	300 ppm	1000 ppm	3000 ppm	0 ppm	300 ppm	1000 ppm	3000 ppm
Number of foetuses/litters examined	112	134	126	118	24	26	25	23
Cervicel centrum 6, poorly ossified	45	73	52	60	19	26*	23	20
Anterior arch of atlas unossified	4	12	3	41	4	9	3	17*
Cervical centra 1-3 and/or 4, split	6	1	2	0	5	1	2	0*
Thoracic centrum 2, bilobed	8	5	2	4	7	4	1*	4
Thoracic centrum 13 bilobed	19	32	36	46	15	18	20	21*
Rudimentary rib, lumbar arch 1, unilateral	3	2	4	16	2	3	4	10*
Bone island associated with lumbar arch 1, unilateral	5	1	0	3	5	1	0*	2
Sternebra 1, poorly ossified	3	9	6	5	2	9*	4	5
Sternebra 5, unossified	1	3	4	6	1	3	4	6*
Proximal phalanges, poorly ossified	75	93	85	96	20	26*	22	23
Proximal phalanges, poorly ossified	14	15	19	55	11	12	11	20*
Metatarsals of hindlimb, poorly ossified	3	7	77	29	2	4	8	12*
Fetal skeleton appears fragil (less cleared tissue)	0	0	5	116	0	0	1	23*

*$p<0.05$, [a] only live foetuses were examined for defects

Three pregnant females mice (out of 25, 12%) died at 3000 ppm on GD 6 after the first exposure. Two dams at 300 ppm and three at 1000 ppm delivered early. There were no treatment-related changes in maternal body weight at any time point evaluated. Clinical observations were only noted in dams at 3000 ppm, and only during the exposure period irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, lacrimation). A treatment-related significant increase in absolute (117.8% of controls) and relative (104.5% of controls) maternal liver weight was seen at 3000 ppm. No treatment related effects on gestational parameters are documented with the exception of a significant increase in the number of dead fetuses (but not early or late resorptions) per litter at 3000 ppm ($p<0.05$). Total male and female fetal body weight per litter was significantly reduced at 3000 ppm relative to controls ($p<0.001$).

There was no statistically significant increase in the number of foetuses/litters with individual malformations. An increased incidence of reduced ossification in a number of skeletal districts indicative of toxicity was observed at 3000 ppm. These variations are presented in Table 30.

Retardation of skeletal ossification can be an indication of a delayed development. However, the study authors also considered that reduced skeletal ossification and fetal body weight, reliable indicators of toxicity, may be affected by litter size. Therefore further statistical analysis were done indicating that fetal body weights for both large and small litters at 3000 ppm differed significantly from control values.

Table 30: Skeletal variations observed in CD-1 mice fetuses after exposure to 4-methylpentan-2-one on GD 6-15 (Tyl, 1987).

	Fetuses[a]				Litters			
Skeletal variations	0 ppm	300 ppm	1000 ppm	3000 ppm	0 ppm	300 ppm	1000 ppm	3000 ppm
Number of foetuses/litters examined	111	111	117	117	21	21	22	22
Cervical centra 1-3 and/or 4, unossified	6	3	7	24	5	2	7	13*
Cervical centrum 5, unossified	2	3	4	23	2	2	4	12*
Cervical centrum 6, unossified	2	3	3	22	2	2	3	12*
Cervical centrum 7, poorly ossified	7	8	19	31	5	7	11	13*
Cervical centrum 7, unossified	1	0	2	10	1	0	2	8*
All cervical centra unossified	0	1	1	14	0	1	1	6*
Thoracic centrum 1, poorly ossified	1	2	2	20	1	2	2	8*
Sternebra 6, bilobed	1	3	5	23	1	3	4	15*
Sternebra 6, split	0	0	0	7	0	0	0	5*
Proximal phalanges (hindlimb), unossified	6	13	8	54	5	7	4	18*
Proximal phalanges (forelimb), poorly ossified	9	7	8	45	5	4	4	16*
Proximal phalanges (forelimb), unossified	0	3	3	12	0	2	2	10*
Intermediate phalanges (hindlimb), poorly ossified	58	54	62	17	20	19	17	11*
Some ossification in the tarsal region	87	92	98	53	20	21	21	15*
Metatarsals, poorly ossified	0	4	1	8	0	3	1	5*
Distal phalanges (forelimb) poorly ossified	4	9	7	21	3	6	4	10*
Distal phalanges (hindlimb) unossified	0	4	2	6	0	4	2	5*
Supraoccipital, split	0	2	0	14	0	2	0	8*

Supraoccipital, bilobed	2	4	2	41	2	3	2	15*

* $p<0.05$, [a] only live foetuses were examined for defects

The Two-Generation Reproduction Toxicity study (Nemec, 2004) is presented in detail in Chapter 10.10.1. Pregnant female rats were exposed during whole gestation to **0, 500, 1000, 2000 ppm 4-methylpentan-2-one.** No internal findings and malformation have been documented for offsprings of F_0 and F_1. Ossification was not examined in this study.

10.10.7 Comparison with the CLP criteria

Known or presumed human reproductive toxicant substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Suspected human reproductive toxicant substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

For the evaluation of a possible developmental toxic effect of 4-methylpentan-2-one two studies are available. In a Two-Generations study (Nemec, 2004) no malformations in offsprings of F_0 and F_1 were seen (NOAEL=2000 ppm). The NOAEL for parental toxicity was 500 ppm.
In a Prenatal Developmental Toxicity Study (Tyl, 1987) 4-methylpentan-2-one showed maternal toxicity in rats (CNS symptoms, reduced bw, kidney weight) and mice (death, CNS symptoms, liver weight) at 3000 ppm. Fetal toxicity was manifested by reduced fetal body weights at 3000 ppm in mice and rats and by poorly ossified or unossified skeletal elements in both species (NOAEL=1000 ppm). These effects were considered secondary to maternal toxicity. No effects on gestational parameters and no malformations were documented.

No classification is warranted**.**

10.10.8 Adverse effects on or via lactation

Not relevant

10.11 Specific target organ toxicity - single exposure

4-methylpentan-2-one is harmonized classified as STOT SE 3, H335. For the assessment of specific target organ toxicity, respiratory irritation and acute neurotoxic effects after single exposure relevant animal and human studies are presented in Table 31 and Table 32.

For acute toxicity tests, presented in Chapters 10.1, 10.2. and 10.3, no effects on specific organs are reported or documented. Therefore these tests are not presented again. One exception is Specht (1938), which shows irritation after inhalation and is documented below.

Table 31: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
- Guinea pig	4-methylpentan-2-one (99%) Inhalation (chamber exposure) 1000, 3000, 10000, 16800, 28000 ppm up to 24h	1000 ppm: Irritation of conjunctival and nasal mucosa in operator (human), little inconvenience in guinea pigs, slight effects on reflexes and temperature, respiratory rate fell in the first 6h to a level suggesting a low grade narcosis Higher concentrations: marked irritation, narcosis occurred resulting in reduced respiratory rate, death Complete recovery of irritant effects when removed 10000 ppm: animals died within 4 h 28000 ppm: animals died within 45 min Bias: exposure concentrations - not reliable	Specht (1938), Specht (1940)
- Swiss OF1 mice	4-methylpentan-2-one Inhalation (chamber exposure) 4 different exposure concentrations n=6/dose 5min exposure	Concentration associated with a 50% decrease in the respiratory rate (RD50) RD50=3195 ppm	de Ceaurriz (1981) [Registration data, ECHA dissemination site]
- Swiss OF1 mice	4-methylpentan-2-one Inhalation (chamber exposure) 0, 662, 757, 807, 892 ppm 4h exposure	Concentration associated with a 50% decrease in immobility (ID_{50}) ID_{50}=803 ppm	de Ceaurriz (1984) [Registration data, ECHA dissemination site]
OECD 408 Sprague-Dawley rats (30m/30f per dose)	4-menthylpentan-2-one in corn oil Gavage 0, 50, 250, 1000 mg/kg bw/day 7 days/week, 13 weeks	1000 mg/kg bw: reversible lethargy for a few hours following exposure; decreased in incidence and severity during the study.	US EPA (2003) (citing Anonymous, 1986) [REACH registration data]

Two-Generation Reproduction Toxicity Study (OECD 416) GLP Rat, Sprague Dawley Crl:CD, m+f 30 animals/sex/group	4-methylpentan-2-one (99.93%) inhalation, whole body 0, 500, 1000, 2000 ppm 6 hours/day 7 days/week	increase in the number of observations of F_0 rats having an absent or decreased response to a novel sound stimulus (a single loud noise at the midpoint of exposure) was observed in the 1000- and 2000 ppm groups. However, the animals appeared normal at the 1 h postexposure observation	Nemec, 2004

Table 32: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Human volunteer study	4-methylpentan-2-one	Inhalation, n=12 (m+f) 15min exposure	4-methylpentan-2-one was found to have a sensory response limit of 100 ppm irritation to eyes at 200 ppm; irritation of nose or throat at >200 ppm.	Silverman (1946)
Human volunteer study	4-methylpentan-2-one	n=6 per group 1st exposure: Full face mask exposure to 402, 915, 1393, 1680, 2301, or 2827 mg/m³ (98, 220, 340, 410, 560, 690 ppm) 7min exposure duration 2nd exposure (two weeks later): 845, 1493, or 2066 mg/m³ (206, 360, 505 ppm) 7min exposure duration	nose, eye, and throat irritation generally increased with exposure level estimated thresholds: odour 402 mg/m³ irritation 1393 mg/m³ (LOAEL)	US EPA, 2003 (citing Esso Research and Engineering Company, 1965; Hazleton Laboratories, Inc., 1965)
Human volunteer study	4-methylpentan-2-one	n=6 7min exposure duration Full face mask	sensory irritation threshold: 340 ppm odour threshold: 98 ppm	Anonymous (1965)

Human volunteer study	4-methylpentan-2-one	n=8 2h on four different occasions 10, 100 and 200 mg/m^3 (2.4, 24, 49 ppm) chamber exposure exposure under conditions of light exercise	degree of irritative and CNS symptoms increased during exposure (questionnaire)	Hjelm (1990)
Human volunteer study	4-methylpentan-2-one	n=25 (13m, 12f) 410 mg/m^3 (100 ppm), chamber exposure 4h	Psychomotor test, sensorimotor test and test of mood inconspicuous → no significant neurobehavioral effect strong odour sensation (significant) irritant effects	Dick (1992)
Human volunteer study	4-methylpentan-2-one	n=12 10 and 200 mg/m^3 (2.5 and 50 ppm) 2h, chamber exposure (1-week intervals for an unspecified total number of exposures) exposure under conditions of light exercise for the first 90min Performance tests and questionnaire	no significant elevated sensory irritation at 50 ppm as irritation at 2.5 ppm already was rather high. prevalence and intensity of neurol. symptoms was signif. increased at 200 mg/m^3 according to questionnaire	Iregren (1993)
Human volunteer study	4-methylpentan-2-one	n=25 evaluation of brief sniffs (1-2sec) of different concentrations	Odour detection threshold: 10 ppm Irritation threshold: 8874 ppm	Dalton (2000)
Health records industry	4-methylpentan-2-one	n=19 80ppm room level, up to 500 ppm near a centrifuge, 20-30 min	Symptoms in 16/19 workers: weakness, loss of appetite, headache, burning in the eyes, stomach ache, nausea, vomiting, sore throat, Some workers: insomnia, somnolence, heartburn, intestinal pain and unsteadiness Slightly enlarged livers (n=4) and colitis (n=6)	Armeli (1968) Linari (1964) [Registration data, ECHA dissemination site]

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Studies on acute toxicity of 4-methylpentan-2-one after oral, dermal and inhalation exposure (**Chapters 10.1, 10.2. and 10.3)** do not document or showed organ toxicity.

However, 4-methylpentan-2-one has neurotoxic and irritating effects to the respiratory tract after inhalation. As an index of sensory irritation De Ceaurriz (1981) measured the reflex decrease in respiratory rate in male Swiss OF1 mice. This model is based on data showing that sensory irritation of the upper respiratory tract is accompanied by a reflex pause in the expiratory phase of

the respiration resulting in a decrease in the respiratory rate. During a five minute exposure 4-methylpentan-2-one caused a concentration-dependent decrease in respiratory rate. A 50% decrease in respiratory rate (RD_{50}) was seen at 13,100 mg/m^3 (3195 ppm). However, it has to be considered that a decrease of the respiratory rate may also be the result of a narcotic effect which also has been seen with this substance (e.g. Spencer, 1975). In another publication by De Ceaurriz (1984) a neurobehavioral effect was determined by measuring the duration of immobility in mice swimming in a narrow cylinder from which they cannot escape. According to the method by Porsolt (1977) the unexposed mouse adopts a characteristic immobile posture after a brief period of vigorous activity. The decrease in immobility time (ID_{50}) was used as an indicator of the behavioural toxicity. After 4h of exposure (662, 757, 807, 892 ppm) each mouse was placed in a vertical glass cylinder containing water. Transient periods of immobility were accompanied by periods of intensive swimming activity and the total duration of immobility during the first 3 min was measured. Results are presented in Table 33. The ID50 was calculated to be 803 ppm.

Table 33: Duration of immobility after treatment with 4-methylpentan-2-one (De Ceaurriz, 1984).

Concentration [ppm]	Duration of immobility [sec]		Decrease of immobility [%]
	Dosed animals	Control animals	
662	57.3±7.7	76.1±6.4	25
757	49.3±7.7*	79.6±6.1	38
807	42.6±8.2*	79.6±6.7	46
892	26.3±2.5*	87.7±7.7	70

*significantly different from control value ($p<0.05$)

In studies by Specht (1938) and Specht (1940), female guinea-pigs were exposed to 4-methylpentan-2-one concentrations of 1000, 3000, 10000, 16800 and 28000 ppm for up to 24 h. In view of the method used for generating the atmosphere (allowing measured amounts of 4-methylpentan-2-on to evaporate freely to one cubic meter volume of air at 25-26 °C), the two higher levels must be greatly exaggerated because the saturation concentration in air for 4-methylpentan-2-on at 25 °C is 40 000 mg/m^3. The 1000 ppm level caused little or no ocular or nasal irritation in the animals but irritation of nasal and conjunctival mucosa of the operator. There was a decreased respiratory rate during the first 6 h of exposure, which was attributed to a narcotic effect. The higher levels produced obvious signs of eye and nose irritation, followed by salivation, lacrimation, ataxia, progressive narcosis, and death. At 16800 ppm the respiratory rate fell of very abruptly from about 117 breaths per minute to 35 and less. At the highest concentration (28000 ppm) animals died within 45 min. At 10000 ppm animals died within 4h. Autopsy and histopathological investigations of animals died during exposure showed fatty livers and congestion of the brain, lungs, and spleen, but no damage to the heart and kidneys was observed (EHC 117, 1990). Survivors of the exposure have not indicated any gross pathology.

US EPA (2003) cites a study (Anonymous, 1986), where exposure of rats to 1000mg/kg bw 4-methylpentan-2-one resulted in reversible lethargy for a few hours following exposure. Incidence and severity decreased during the study (less effective over time, accustoming effect).

In a two-Generation study (Nemec, 2004) an acute CNS-effect was seen during the exposure period. An increased number of F_0 and F_1 adult rats having an absent or decreased response to a sound stimulus was observed in the 1000 and 2000 ppm groups. However, the animals appeared normal at

the 1 h postexposure observation. As the response rate was unaffected at 500ppm the authors suggested a sedative effect during exposure at higher concentrations.
Anonymous (1986) showed reversible lethargy of rats for a few hours following exposure to 1000ppm and Tyl (1987) reported loss of coordination, irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, etc. in mice and rats during exposure periode only and Kasavage (1982) documented transient anaesthesia (see also Table 38).

Sensory irritation as well as neurological effects also have been seen in humans after inhalatory exposure to 4-methylpentan-2-one. The odour threshold is reported to be 1.64 mg/m^3 (0.4 ppm) (Ruth, 1986).
Groups of six adult volunteers were exposed for 7 minutes via full face mask to 402, 915, 1393, 1680, 2301 or 2827 mg/m^3 of 4-methylpentan-2-one, followed 2 weeks later by a second 7-minute exposure to 845, 1493, or 2066 mg/m^3 (Esso Research and Engineering Company, 1965; Hazleton Laboratories Inc., 1965 cited in US EPA, 2003). Volunteers indicated the presence and disappearance of eye, nose and throat irritation throughout the exposures, which provided a continuous subjective assessment of irritation relative to known exposure levels. The incidence of volunteers reporting nose, eye, and throat irritation generally increased with exposure level; the thresholds for odour and irritation were reported to be 402 and 1393 mg/m^3 (98 and 340 ppm), respectively, estimated from graphs of the number of individual reports of irritation at various exposure levels. No control group without chemical exposure was done.
Silverman (1946) investigated the sensory response to industrial solvent vapours. 12 persons were exposed for 15 minutes to various vapour air concentrations. During exposure motion pictures were shown for diversion. 4-methylpentan-2-one was found to have a sensory limit of 100 ppm. A majority of subjects found the odour objectionable at 200 ppm and the vapour was irritating to the eyes. Exposure at concentrations higher than 200 ppm resulted in throat/nose irritation. No further information available.
Irritation as well as CNS effects were studied by Hjelm (1990) in human male volunteers (n=8). They were exposed under conditions of light exercise in an exposure chamber for 2h on four different occasions to about 2.4, 24 and 49 ppm 4-methylpentan-2-one. No control exposure was done. The relative pulmonary uptake was 60%. Questionnaires (before and several times during exposure) and performance tests (before and after exposure) were used for the determination of symptoms. The degree of irritative and CNS symptoms increased with exposure level and decreased rapidly after cessation of exposure. None of the symptoms were experienced by more than 3 subjects at any exposure level. Details are presented in the following table. There was an increase of reaction time over test time. There were no significant effects on the performance of a simple reaction time task or a test of mental arithmetic. Additionally there were no effects in the rating of mood.

Table 34: Effects of human volunteer exposure (questionnaire) (Hjelm, 1990)

Symptoms	10mg/m³	100mg/m³	200mg/m³
Eye irritation	1/8	1/8	0/8
Nose irritation	1/8	3/8	3/8
Throat irritation	1/8	3/8	3/8
Headache	0/8	2/8	2/8
Nausea	0/8	0/8	1/8
Vertigo	1/8	2/8	2/8

Effects from acute exposure to 4-methylpentan-2-one were studied by Dick (1992) with special attention to neurobehavioral performance. A group of 13 adult male and 12 adult female volunteers was exposed in an environmental chamber to 100 ppm (410 mg/m^3) 4-methylpentan-2-one for two consecutive 2-hour exposure periods. Another group received placebo treatment during the exposure periods. Subjects underwent double-blind evaluations of performance on five psychomotor tests, one sensorimotor test, and a test of mood on the day before exposure, immediately prior to exposure, during each of the two consecutive 2-hour exposure sessions, immediately after exposure, and on the day following exposure. Irritation and other symptoms were surveyed by subjective assessment. No effects attributable to 4-methylpentan-2-one were seen in any of the performance tests or with respect to the percentage of subjects experiencing various neurological or irritation symptoms. A significant increase in percentage of subjects detecting a strong odour sensation was reported in the treated group.

Iregren (1993) studied a possible narcotic impact on CNS function during a 2h-chamber exposure to 10 and 200 mg/m^3 (2.5 and 50 ppm) 4-methylpentan-2-one in groups of six male and six female volunteers (19-47 years old). 10 mg/m³ was used as control exposure. Volunteers performed light exercise during the first 90 minutes and rested during the final 30 minutes of each exposure. Performance tests were conducted immediately prior to and following exposure, heart rate was monitored throughout exposure, and central nervous system (CNS) and irritation symptoms were assessed using a questionnaire. Sensory irritation ratings were not significantly different between the two exposure levels as irritation ratio at 10 mg/m^3 already was elevated, however there was a clear trend towards higher irritation at higher concentration. No consistent effect on heart rate was found. Index of prevalence and intensity of neurological symptoms (determined by questionnaire) was significantly increased in the group exposed to 200 mg/m^3 as compared to the 10 mg/m^3 group.

Odour and sensory irritation threshold for 4-methylpentan-2-one was investigated by Dalton (2000). The mean odour detection threshold for 4-methylpentan-2-one was 10 ppm, and mean irritation threshold was 8874 ppm. Calculating the fifth percentile for lateralization thresholds revealed that 95% of the sample population did not experience sensory irritation at or below 1802 ppm. Odour and irritation intensity ranking increased with increasing concentration. The authors concluded that the best predictors of perceived irritation to high concentrations of 4-methylpentan-2-one were those measures related to its odour, not to the threshold for sensory irritation, suggesting that negative responses to 4-methylpentan-2-one involve reactions to olfactory properties.

Sensory irritation threshold was also investigated by Anonymous (1965). Six human volunteers were exposed via full face mask to different concentrations of 4-methyl-pentan-2-one or negative control for 7 minutes. Volunteers had to indicate the presence of odour and eye, nose, throat irritation without information on current exposure concentration. The sensory irritation threshold was determined to be 340 ppm and the odour threshold was 98 ppm (cited from ECHA dissemination site).

Industry health records (Armeli, 1968 and Linari, 1964) also show irritating effects and neurotoxicity. Workers (n=19) exposed to concentrations up to 500 ppm near a centrifuge for a duration of 20-30 min complained of weakness, loss of appetite, headache, burning in the eyes, stomach ache, nausea, vomiting, sore throat. Also insomnia, somnolence, heartburn, intestinal pain and unsteadiness were reported by some workers. Slightly enlarged livers (n=4) and colitis (n=6) were seen in some workers. When the exposure concentration near the centrifuge was reduced to about 100 ppm and respiratory protection was worn by workers still some (4/14) complained of gastrointestinal and central nervous system effects.

In vitro:
To investigate the toxicological mechanism behind the seen transient neurotoxic effects Huang (1993) documented the effects of monoketones on isolated mouse synaptosomes. As a function of lipophilicity monoketones, including 4-methylpentan-2-one, penetrate synaptic membrane preparations, leading to conformational changes in membrane structure and increased ability to inhibit both neuroreceptor binding (β-adrenergic receptor binding) and enzyme activity (Na+-K+-ATPase activity). The authors concluded that monoketones increase the lipid fluidity in the synaptic membrane, thereby disrupting the function of receptor proteins and membrane enzymes. The IC_{50} (conc for 50% inh) values for 4-methylpentan-2-one inhibition of receptor binding and enzyme activity in the described in vitro system were 46 and 43 μM respectively.

10.11.2 Comparison with the CLP criteria

STOT SE – Category 1: Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure. Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: (a) reliable and good quality evidence from human cases or epidemiological studies; or (b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values used as part of weight-of-evidence evaluation are: oral C ≤ 300mg/kg bw, dermal C ≤ 1000mg/kg bw, inhal C ≤ 10 mg/l/4h.

STOT SE – Category 2: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure. Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are: oral 2000 ≥ C > 300 mg/kg bw, dermal 2000 ≥ C > 1000 mg/kg bw, inhal . 20 ≥ C > 10 mg/l/4h. In exceptional cases, human evidence can also be used to place a substance in Category 2.

STOT SE – Category 3 (Transient target organ effects): This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

- The criteria for classifying substances as Category 3 for respiratory tract irritation (H335) are (1) respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain), (2) subjective human observations supported by objective measurements of clear respiratory tract irritation (RTI), (3) useful information obtained from the single and repeated inhalation toxicity tests.
- The criteria for classifying substances as Category 3 for narcotic effects (H336) are central nervous system depression including narcotic effects in humans (drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included) and/or narcotic effects observed in animal studies (transient lethargy, lack of coordination, loss of righting reflex, and ataxia; transient nature).

No target organ toxicity after single exposure is documented in acute toxicity animal studies. Industry health records (Armeli, 1968 and Linari, 1964) document slightly enlarged livers (4/19) and colitis (6/19).

Sensory irritant in humans was seen at 25 ppm (Hjelm, 1990) and above (Dick, 1992; Silverman, 1946, US EPA, 2003; Armeli, 1968, Anonymous, 1965). Anonymous (1965) and Armeli (1986) determined a sensory irritation threshold in humans at 340 ppm and 8874 ppm respectively. However the interpretation of results is difficult due to subjective indication of effects in human studies and the fact that 4-methylpentan-2-one has a low odour threshold.
A possible narcotic impact of 4-methylpentan-2-one was investigated in human studies. No effects on CNS were seen by Dick (1992) at 100 ppm in performance tests and by Iregren (1993) at 50 ppm by questionnaire. Hjelm (1990) reported vertigo at 25 and 50 ppm. Industry health records showed weakness, loss of appetite, headache, and somnolence at an exposure concentration of 500 ppm. In guinea pigs a low grade of narcosis was detected at 1000 ppm (Specht, 1938). De Ceaurriz (1981) reported a 50% decrease in respiratory rate in mice at 3195 ppm which may be due to a narcotic effect or irritation of the respiratory tract.
Acute neurotoxic effects (narcosis, lethargy, reduced activity level, etc.) also have been seen in repeated dose studies. Effects occurred during exposure only and were transient. Anonymous (1986) showed reversible lethargy of rats for a few hours following exposure to 1000ppm and Nemec (2004) reported a sedative effect expressed as absent or diminished response to a sound stimulus. Loss of coordination, irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, etc. in mice and rats are reported by Tyl (1987) during exposure periode only and Kasavage (1982) documented transient anaesthesia (see also Table 38).
Effects of 4-methylpentan-2-one on isolated mouse synaptosomes (inhibition of receptor binding and enzyme activity) were documented by Huang (1993) *in vitro*.

10.11.3 Conclusion on classification and labelling for STOT SE

Based on the available information on acute toxicity testing in animals and the lack of effects on organs documented for human studies no classification for STOT SE 1 or 2 is warranted.

The substance is already classified for respiratory tract irritation (STOT SE 3, H335). Sensory irritation has been documented in animal and human studies.

Acute narcotic effects after acute and repeated exposure have been reported in animal studies (guinea pigs, mice, rats) and in industry health records. A classification as STOT SE 3, H336 is indicated.

10.12 Specific target organ toxicity-repeated exposure

Repeated dose studies via oral, inhalation and subcutane exposure are available. Duration of exposure varies from 7days up to 2 years. Relevant studies are presented in Table 35.

Table 35: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Oral exposure			
OECD 408 Gavage Sprague-Dawley rats (30m/30f per dose)	4-menthylpentan-2-one in corn oil 0, 50, 250, 1000 mg/kg bw/day 7 days/week, 13 weeks	NOEL=50 mg/kg/day 250 mg/kg bw:serum glutamic-pyruvic transaminase (SGPT)↑ (f), terminal absolute or relative kidney weights ↑ (m, f) 1000 mg/kg bw: reversible lethargy for a few hours following exposure; at terminal sacrifice: haemoglobin ↑ (f), haematocrit ↑ (f), lymphocyte count ↓(m), adverse liver effects, absolute and relative kidney weights ↑ (m, f), blood-urea-nitrogen↑ (m), serum potassium ↑ (m), decreased serum glucose ↓ (m), urinary protein and ketones ↑ (m, f), mild nephropathy (m), relative adrenal weights ↑ (m, f), relative testis weights ↑ (m)	US EPA (2003) (citing Anonymous, 1986) [REACH registration data]
- Oral, drinking water HLA Wistar rats (f) 5 animals per group	4-menthylpentan-2-one at saturation concentration (1.3% aqueous concentration) [1040 mg/kg bw estimated dose] control 120days	1040 mg/kg: mean absolute and relative kidney weights ↑, renal tubular cell hyperplasia (1/5)	US EPA (2003) (citing Carnegie-Mellon Institute of Research, 1977a, b)

Preliminary study HLA Wistar rats (f)	4-menthylpentan-2-on (0.5 and 1% aqueous concentration) [300 and 900 mg/kg bw/day estimated dose] No control 7days	300 mg/kg: Pale or mottled kidneys (3/3) 900 mg/kg: body weight gain ↓, Pale or mottled kidneys (2/3)	US EPA (2003) (citing Carnegie-Mellon Institute of Research, 1977a, b)
Inhalatory exposure			
Baboon (n=4) Inhalation Match-to-sample task	4-methylpentan-2-one [50 ppm] methyl ethyl-ketone [100 ppm] 4-methylpentan-2-one + Methyl ethyl-ketone [100 ppm+50 ppm] Acetone [500 ppm] 7day, 24h/day	<u>4-methylpentan-2-one :</u> - Accuracy of discrimination affected (100% correct responses in control period vs. 94.0, 96.5%, 98.5% and 100% in four exposed baboons) - Increased response time for all animals <u>methyl ethyl-ketone</u> - Accuracy (100% control vs, 96.0, 99.0, 99.0, 98.5) - Increased response time (with exceptions)) <u>4-methylpentan-2-one + methyl ethyl-ketone</u> - Accuracy (100% control vs, 99.5, 99.5, 97.5, 100) - minimal effects on response time (much less than additive) <u>Acetone</u> - Accuracy (100% control vs, 98.4, 96.0, 97.5, 98.0) - Increased response time (with exceptions))	Geller (1979)
subacute Inhalation study Rats, F344 (6m+f per group) Mice, B6C3F1 (6m+f per group)	6 hrs/day, 5 days/week for 9 days (5days, 2 days off, 4 days) 0, 101, 500, 2000 ppm	101 ppm: no adverse effects 500 ppm: liver weights ↑ in rats (m), hyaline droplet degeneration in rats (m) 2000 ppm: scattered lethargy, periocular wetness in rats, liver weights ↑ in rats (m), liver weights ↑ in rats (f) and mice (f), kidney weights ↑ in rats (m) and mice (f), kidney weight ↓ in mice (m), hyaline droplet degeneration in rats (m)	Phillips, 1987 (Bushy Run Research Center, 1982)
Charles River albino rats 10 m + 10 f per group 4 weeks (1100 ppm) 2 weeks (183ppm)	0, 1100 ppm 0, 183 ppm	1100 ppm (4 weeks): cytoplasmic eosinophilic droplets in proximal convoluted tubule epithelium of males (2/3) 183 ppm (2 weeks) relative liver weights ↑ (m), no renal histopathological lesions	US EPA, 2003 (citing Hazleton Laboratories, Inc., 1966, 1968)

TSCA/FIFRA guidelines for neurotoxicity Sprague-Dawley rat, m, n=20 subchronic inhalation	0, 250, 750, 1500 ppm 6h/day, 5d/w, 13 weeks. 2 groups: - food-restricted - ad-libidum fed	No effect on operative behaviour (no significant differences in SCOB testing) 750 ppm: transient reduced activity levels (reversible) rel/abs. liver weight↑, rel kidney weights↑ 1500 ppm: transient reduced activity levels (reversible), rel/abs. liver weight↑, rel kidney weights↑ no gross pathologies in various nervous system tissues	David (1999)
Rat 6animals/group inhalation	4-methylpentan-2-one (contaminated with 3% methyl n -butyl ketone): 1500 ppm 5 months [methyl n-butyl ketone:1300 ppm 4 months]	4-methylpentan-2-one (contaminated): minimal distal axonal changes, slight signs of narcosis [methyl n-butyl ketone: distal axonopathy]	Spencer (1975)
Preliminary study 14d Inhalation, chamber exposure Wistar Rats (n=50), Mice (n=40) Beagle dogs (n=8), Macaca mulatta (n=4)	4-methylpentan-2-one 0, 100 ppm, 200 ppm 14d continuous exposure	Rats: 100 ppm: kidney weights ↑ 200 ppm: kidney weights ↑, liver weights ↑ Dogs, monkeys: no adverse effects Mice: not reported	Mac Ewen, 1971

90 day study Inhalation, chamber exposure Wistar Rats (n=100) , Beagle dogs (n=8), Macaca mulatta (n=2)	4-methylpentan-2-one 0, 100 ppm 90d continuous exposure	Rat: liver and kidney weights ↑, Hyaline droplet degeneration of the proximal tubules – reversible over time Monkey: focal chronic inflammation of the kidney (1/2) Dogs: no adverse effects	Mac Ewen, 1971
Fischer 344 rats B6C3F1 mice inhalation 14f+14m per group	4-methylpentan-2-one 0, 50, 252, 1002 ppm 6 h/day, 5 days/week, for 14 weeks	Rat: 50 ppm: hyaline droplet formation (m) 252 ppm: Terminal body weights ↑ (f), Serum cholesterol ↑ (m), Urine glucose ↑ (m), hyaline droplet formation (m) 1002 ppm: platelet numbers↑ (m), eosinophil number↓ (f), Serum cholesterol ↑ (m), absolute and relative liver weight ↑ rats (m) (m), Urine glucose ↑ (m+f), urine protein ↑ (m), hyaline droplet formation (m) Mouse: 252 ppm: absolute liver weight ↑ (m) 1002 ppm: absolute and relative liver weight ↑ (m)	Phillips, 1987

Two-Generation Reproduction Toxicity Study (OECD 416) GLP Rat, Sprague Dawley Crl:CD, m+f 30 animals/sex/group	4-methylpentan-2-one (99.93%) inhalation, whole body 0, 500, 1000, 2000 ppm 6 hours/day 7 days/week	NOAEL (parental systemic toxicity) = 1000 ppm NOAEL (neonatal toxicity - CNS effects) = 1000 ppm ➢ Absent or diminished response to a novel sound stimulus was noted during exposure at the 1000 and 2000 ppm concentrations (acute effect) ➢ CNS depression on PND22 in weanlings (2000 ppm) ➢ Elevated kidney weights (F0: 1000 and 2000 ppm males), F1: 2000 ppm females) ➢ Nephropathy and droplets (F1) (1000 and 2000 ppm) ➢ Elevated liver weights (2000 ppm, m+f) (F0, F1) ➢ Centrolobular hepatocellular hypertrophy (F0 males: **500, 1000, and 2000 ;** F1 males:1000 and 2000 ppm)	Nemec (2004)
Prenatal Developmental Toxicity study (OECD 414) (1) Rat, Fischer F33 35females/group (2) Mice, CD-1 30 females/group	4-methylpentan-2-one inhalation, whole body 0, 300, 1000, 3000 ppm 6h/days, GD 6-15	NOAEL (maternal toxicity) = 1000 ppm NOAEL (fetal toxicity) = 1000 ppm Rat 3000 ppm (dams) ➢ **loss of coordination, negative tail and/or toe pinch, paresis (partial hindlimb paralysis), muscular weakness in hindlimbs, piloerection, lacrimation, and red perioral encrustation** ➢ **relative kidney weight was slightly (104% of controls) but significantly elevated** Mouse **3000 ppm (dams):** ➢ **irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, lacrimation.** ➢ **significant increase in absolute (117.8% of controls) and relative (104.5% of controls) maternal liver weight**	Tyl (1987)
Subcutane exposure			

Cat sc injection, twice daily (150mg/kg)	(1) methyl *n*-butyl ketone (MBK, 99.66% purity), n=8 (2) methyl ethyl ketone (MEK, 99.98% purity), n=6 (3) 4-methylpentan-2-one (MIBK, 98.79% purity); n=4 (4) 9:1 mixtures of MEK/MBK, n=4 (5) 9:1 mixture of MEK/4-methylpentan-2-one, n=6 (6) control: saline, n=4 5d/week, 8.5 months	MBK: central-peripheral distal axonopathy: Nerve fiber pathological changes (multifocally swollen axons filled with neurofilaments, changes of myelin sheaths and overt fiber breakdown), degeneration first affected the distal parts of nerve tracts MEK: no detectable nervous system damage 4-methylpentan-2-one (+impurity): no detectable nervous system damage (distal portions of tibial and ulnar nerve showed evidence of increased dilated mitochondrial remnants) MEK/4-methylpentan-2-one: no detectable nervous system damage	Spencer (1976)

Table 36: Summary table of human data on STOT RE

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**
Case study	4-methyl-pentan-2one	6 years of repeated exposure to high levels inhalation	Cognitive impairment Persistent CNS dysfunction	Grober (2000)
survey - shipyard painters	Solvent mixture (including 10 ppm 4-methylpentan-2-one)	n=180 inhalation questionnaire + psychometric tests	neurobehavioral performance impaired	Lee (2005)
survey - shipyard painters	solvent vapour mixture (4-methylpentan-2-one, xylene, perchlorethylene, ethylene glycol, white spirit)	n=74 Inhalation	acute neurological symptoms decrements in neurobehavioral performance tests	Valciukas (1985)

survey – paint factory	low-level organic solvents (toluene, xylene, n-hexane, n-butyl acetate, 4-methylpenta n-2-one)	n=325 inhalation	prolonged response latencies impairment in continuous performance tests	Tsai (1997)
Case study	mixture exposure (acetone, dichloromet hane, methyl-ethyl keton, 4-methylpenta n-2-one, toluene, butanol)	16 year old male exposure: spraying several times	One week after exposure: burning paraesthesia acute decreased muscle strength segmental demyelination	AuBuchon (1979)

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Oral exposure:

Two studies with oral exposure are presented by US EPA (2003). Anonymous (1986) exposed groups of 30 female and 30 male Sprague-Dawley rats to 4-methylpentan-2-one by gavage in corn oil for 7days/week for 13 consecutive weeks. Animals exposed to 0, 50, 250 and 1000 mg/kg bw/day were evaluated for changes in body weight, food consumption, mortality, clinical signs, ophthalmological parameters, and terminal organ weights. Haematology, clinical chemistry, urinalysis and comprehensive gross pathology were done in week 7 and 13. Histopathology was done for high-dose and control rats. Kidney samples were also evaluated in mid-dose rats.
At 1000 mg/kg bw reversible lethargy was observed in both sexes following exposure. Incidence and severity decreased during the study - accustoming effect can be assumed. Decreased mean body weight gain (9%) was seen in males in the high-dose group in week 12 and 13. Female body weight gain was significantly increased during 5 of the last 6 weeks. Food consumption in females and males was significantly increased during the second half of exposure. In high-dosed females some significant haematological effects (haemoglobin +6%, haematocrit +8%) were observed. For high-dosed males a 15% decrease in lymphocyte count is documented. Adverse liver effects are summarised in Table 37.

Table 37: Adverse liver effects after subchronic oral exposure to 4-methylpentane-2-one (Anonymous, 1986).

Liver effects	male		female	
	after 7 weeks	after 13 weeks	after 7 weeks	after 13 weeks
250mg/kg bw/day				
serum glutamic-pyruvic transaminase (SGPT)				+39%

1000mg/kg bw/day				
serum glutamic-pyruvic transaminase (SGPT)			+73%	+34%,
serum alkaline phosphatase			+84%	
serum cholesterol	+30%		+59%	+65%
terminal absolute liver weights		+34%		+39%
terminal relative liver weights		+42%		+38%
albumin/globulin ratio	-16%			
serum total protein			+9%	+10%

Increased terminal absolute or relative kidney weights in males and females, ranging from 6 to 12% over controls, were seen at 250 mg/kg bw. At 1000 mg/kg bw the following adverse kidney effects were observed: increased terminal absolute and relative kidney weights (from 25 to 34% in males and from 20 to 22% in females) as compared to controls, increased blood-urea-nitrogen (BUN) in males (+37%, interim), increased serum potassium in males (+34%, terminal), decreased serum glucose in males (-27%, terminal), and a reported increase in urinary protein and ketones in males and females at terminal sacrifice (summary data were not provided).
At 1000 mg/kg bw histological examination of kidney tissues revealed an increased incidence of mild nephropathy (multifocally distributed swollen or hyperchromatic and flattened renal cortical tubular epithelial cells) in male rats (16/20) as compared to controls (4/20). No effect was seen in female rats. Significantly increased relative adrenal weights in male (+29%) and female (+11%) rats and slightly increased relative testis weights (+9%) in males were also observed at 1000 mg/kg day. No exposure-related histopathologic lesions were evident in the liver or adrenal glands nor in any other tissue examined. No treatment-related effects of any kind were observed at 50 mg/kg bw/day (NOEL=50 mg/kg/day) (US EPA, 2003).

In a second oral study female HLA wistar rats (n=5 per group) were provided drinking water ad libitum containing either no 4-methylpentan-2-one or 4-methylpentan-2-one at saturation concentration (1.3% aqueous concentration) for 120 days. The resulting dose was estimated to be 1041 mg/kg bw/day. Rats were evaluated for changes in food and water consumption, body weight, general appearance and behaviour, gross pathological examination, liver and kidney weights, histopathology (sciatic nerve, brachial plexi, lumbo-sacral spinal ganglia, anterior and posterior thigh muscles, larynx, nasal cavity, brain, spinal cord, heart, lymph nodes, lungs, spleen, liver, kidney), and performance in neurologic and neuromuscular function tests (balance, coordination, strength, behaviour). The only statistically significant finding was increased mean absolute and relative kidney weights in treated rats as compared to controls. No gross pathologies were observed in the kidneys. Histopathological examination revealed renal tubular cell hyperplasia in only one of five of the treated rats. No other histological lesions of the kidney were reported. No exposure related histological changes were found in other organs (Carnegie-Mellon Institute of Research, 1977a, b cited in US EPA, 2003).
A preliminary range-finding study female HLA Wistar rats exposure to 4-methylpentan-2-one at 0.5 and 1% in drinking water (without control) for 7 days were evaluated for changes in food and water consumption, general appearance and behaviour, body weight, and gross pathology of unspecified extent. The daily dose was estimated to be 300 and 900 mg/kg bw. Significantly reduced body weight gain was observed in females exposed to 1% 4-methylpentan-2-one. Pale or mottled kidneys were seen in 3/3 rats at 0.5% and 2/3 rats at 1% 4 methylpentan-2-one (Carnegie-Mellon Institute of Research, 1977a, b cited in US EPA, 2003).

Inhalatory exposure:

Geller (1979) investigated the effect of subacute inhalatory exposure to 4-methylpentan-2-one (50 ppm) on match-to-sample discrimination tasks in juvenile baboons. Four baboons were exposed for 24h/day over 7 days with experimental sessions of 2h duration. Percentages of correct responses obtained prior to exposure were compared to the correct responses during exposure. Minimal effects on the accuracy of the discrimation were observed (100% correct responses in control period vs. 94.0, 96.5%, 98.5% and 100% in four exposed baboons). In addition 4-methylpentan-2-one increased the response time on every behavioural test day for all animals. Authors concluded that this could be an early manifestation of incoordination and narcosis seen at higher concentrations. However it has to be considered that he same test animals had been exposed to 100 ppm methyl ethyl ketone (MEK) 1 month previously, which may bias the results.

In a previous study (Geller, 1978; cited from EPA, 2003) whole-body exposure of juvenile baboons (reportedly two per group) to 0, 25, 35, 50, or 75 ppm 4-methylpentan-2-one over 7 days was examined. No clear exposure related effects were observed in task performance, although one baboon exposed to 50 ppm 4-methylpentan-2-one consistently showed an increase in extra responses as compared to controls in five separate behavioural testing occasions during the 7-day exposure period.

Subacute exposure has been investigated by Phillips (1987). In a nine-day vapour inhalation study groups of six male and six female rats and mice were exposed for 6 h/day, 5 days/week to measured concentrations of 0, 101, 500, or 2000 ppm. Groups were evaluated for changes in clinical signs, body weight, organ weights (liver, lungs, kidneys, and testes), ophthalmology, gross pathology, and histopathology. Scattered incidence of lethargy and lacrimation was observed in rats exposed to 2000 ppm, increased relative liver weights in male rats at 500ppm (9%) and 2000 ppm (36%) and in female rats (8%) and female mice at 2000 ppm (13%), increased kidney weights in male rats (11%) and female mice at 2000 ppm (8%), and hyaline droplet degeneration in kidneys of male rats exposed to 500 and 2000 ppm, with epithelial regeneration of proximal convoluted tubules in the high-exposure group. No effects seen at 100 ppm.
Another study on subacute/subchronic exposure is cited by US EPA (2003). Hazelton Laboratories (1966/1968) investigated rats exposed to 0 or 1100 ppm for 6h/week, 5 days/week for a period of 4 weeks or exposed to 0 and 183ppm for a period of 2 weeks. Body weight, organ weights (lungs, liver, kidneys, adrenals, and spleen), haematological parameters, and gross appearance of organs were examined. Histopathology of lungs, liver, kidneys, adrenals, and spleen has been made. Increased relative liver weights in males were seen after exposure to 183 ppm after 2 weeks of exposure. Exposure to 1100 ppm for 4 weeks resulted in cytoplasmic eosinophilic droplets in the proximal convoluted tubule epithelium in 2 of 3 exposed males.

The effects of subchronic inhalatory exposure (6h/day, 5d/w, 13 weeks) to 4-methylpentane-2-one (0, 250, 750, 1500 ppm) on the behaviour of male rats were determined by David (1999). Exposure was done in two groups - food restricted or ad libitum-fed. The latter was included to assess systemic effects using indicators like body weight, food consumption or organ weight. Exposed animals on restricted diet were subject to a daily schedule-controlled operant behavioural testing (SCOB) from 4 days prior exposure till two weeks after cessation of exposure. No significant differences in SCOB between control group and treated groups were observed. Relative and absolute liver weights and relative kidney weights were significantly higher for 750 and 1500 ppm exposed ad libitum-fed rats. In the food-restricted group only absolute and/or relative liver weights were significantly higher for all doses. Reduced activity levels were observed during first 8-10

weeks of exposure to 750 and 1500 ppm but not after cessation of exposure. No exposure related gross pathology observed.
Spencer (1975) exposed whole-body six rats for four months (intermittent exposure) to 1300 ppm methyl n-butyl ketone and six rats for five months to 1500 ppm 4-methylpentan-2-one (contaminated with 3% methyl n -butyl ketone). A group of three rats served as controls. Histological evaluations were performed in the following CNS and peripheral nervous system tissues: tibial nerve of the hindlimb, ulnar nerve of the forelimb, peroneal and sural nerves of the lower thigh, lumbosacral dorsal root ganglion with dorsal and ventral roots, lumbar and cervical spinal cord, medulla, and cerebellum. Rats exposed to methyl n-butyl ketone developed severe symmetric weakness in the hindlimbs with massive focal axonal enlargements containing abnormally large numbers of neurofilaments and dying-back axonal degeneration in peripheral and central nerve fibers (distal axonopathy). Exposure to 4-methylpentan-2-one showed slight signs of narcosis and minimal distal axonal changes. Based on the knowledge of enhancing effects of 4-methylpentan-2-one (see below) minimal effects seen may be due to contamination with methyl n -butyl ketone.
Mac Ewen (1971) designed an inhalation toxicity study to evaluate the continuous inhalation toxicity of 4-methylpentan-2-one under space cabin conditions as the substance is/was a known spacecraft contaminant. First in a 2-week range-finding experiment rats (n=50), mice (n=40), dogs (n=8) and monkeys (n=4) were continuously exposed to mean concentrations of 100 and 200 ppm 4-methylpentan-2-one. 3 monkeys, 4 dogs, 20 mice, and 25 rats were used for the control group. For evaluation of central nervous system effects one monkey in each group had cortical electrodes implanted. Spontaneous activity measurement, symptomatology, mortality response were examined during exposure; body weight, organ to body weight ratios, EEG, clinical chemistry, hematology, pathology, blood pH and gases were examined at the end of the study. No signs of toxicity were detected during exposure. Only significantly increased kidney weights were seen in rats exposed to 100ppm and rats exposed to 200 ppm showed elevated liver and kidney weights.
Based on this range-finding study a 90-day-study male was conducted. Wistar rats (n=100), male beagle dogs (n=8) and male monkeys (n=2) were exposed continuously to 100 ppm (410 mg/m3). During exposure dogs were examined biweekly (body weight, blood samples). Liver function tests were performed preexposure and immediately postexposure. Serum acid phosphatase and serum glucuronide determinations were done preexposure and at 30 and 60 days. At termination a gross examination was done and samples of liver, brain, kidney, heart, lung, spleen, and endocrine glands were taken for histological evaluation. Rats were weighted biweekly and two rats/group were necropsied weekly for the first 3 weeks and the biweekly. To determine reversibility of possible adverse effects 10 rats were removed after 2 weeks of exposure and necropsied in groups of two biweekly. Also at the termination of the experiment 10 rats were removed for reversibility studies. Body weights, Organ weights and histopathology were determined at the end of the study using the remaining rats. Clinical chemistry and hematology tests on dogs and monkeys did not reveal any biologically significant differences between exposed and control animals. Liver function tests in dogs showed no significant differences. Histopathology of heart, lung, brain, liver, spleen, kidney, adrenals and pituitary glands showed no differences. One of the two exposed monkeys exhibited focal chronic inflammation of the kidney. Rats showed a statistically significant difference in the liver and kidney weights of the exposed animals with a corresponding increase in organ to body weight ratios for these tissues. Hyaline droplet degeneration of the proximal tubules with occasional foci of tubular necrosis was seen in all exposed rats exposed for 90-days. Rats removed from exposure after 15, 22, 28, 71 and 85 days also showed the same changes in kidney tubules. No pathological changes in the liver. Rats removed for reversibility studies after 15-days of exposure revealed a gradual reversion of kidney tubular damage with time. Toxic effects appeared to be completely reversed in rats held for 60 days postexposure. The rats retained and serially killed for

reversibility studies after 90-day exposure also exhibited recovery from the induced lesion but not as rapidly as those exposed for a shorter period.

Exposure of 14 male and 14 female Fischer 344 rats and B6C3F1 mice was investigated by Phillips (1987). After 14 weeks of exposure (6h/day, 5days/week) to 0, 50, 250, 1000 ppm 4-methylpentan-2-one clinical signs, body weights, organ weights (kidneys, heart, liver, lungs, and testes), urinalysis, haematology, serum chemistry (including glucose and hepatic enzyme levels), complete gross pathology, targeted histopathology (nasal cavity, trachea, liver, kidneys, lungs) and histopathology were evaluated. No effects were seen at 50 ppm in mice and rats. At 250ppm terminal body weights were significantly increased in female rats. At 1000 ppm platelet numbers in male rats were significantly (13%) increased. In female rats eosinophil numbers were significantly (57%) decreased. Serum cholesterol was significantly increased in male rats at the 250ppm (23%) and 1000 ppm (35%). Mouse haematology was unaffected. At 1000 ppm a significant increase in absolute and relative liver weight in male rats (13% and 9%) and mice (7% and 11%) was seen without histological lesions or changes in serum liver enzymes and bilirubin. Hyaline droplet formation in proximal tubular cells was seen in all exposed male rats increasing with exposure level.

The Two-Generation-Reproduction Toxicity Study by Nemec (2004) has been presented in detail in Chapter 10.10. Concerning CNS effects a dose-related increase in the number of F_0 and F_1 parental animals with absent or diminished response to a sound stimulus was noted during exposure to 1000 and 2000 ppm. The response rate was unaffected at 500 ppm, suggesting a sedative effect during exposure at higher concentrations. Higher susceptibility of weanling animals was seen i**n the 2000 ppm groups. Exposure had to be suspended on PND 22 due to the death of one male pup and clinical signs of CNS depression (rocking, lurching, swaying, prostrate, half-closed eyelids, lacrimation) indicative of a sedative effect. Furthermore increased liver weights were seen in the F0 and F1 2000 ppm group. Centrilobular hepatocellular hypertrophy was noted in F0 and F1 males in a dose-dependent manner. Kidney weights were increased in F0 and F1 males in combination with nephropathy and droplets in the renal cortical tubular epithelium.**

In a Prenatal Developmental Toxicity Study (Tyl, 1987) pregnant rats and mice were exposed to 0, 300, 1000 or 3000 ppm 4-methylpentan-2-one from GD 6-15 in exposure chambers. Study details are presented in Chapter 10.10. Clinical observations were noted in dams at 3000 ppm (only during exposure): irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, lacrimation). Also a treatment-related significant increase in absolute (117.8% of controls) and relative (104.5% of controls) maternal liver weight was seen at 3000 ppm.

Other routes of exposure:
Chronic exposure of cats (5d/week, 8 months) via subcutaneous injection to commercial solvents, amongst others 4-methylpentan-2-one (98.79% purity), was examined by light and electron microscopy of nerves, muscles and pacinian corpuscles (Spencer, 1976). In general 4-methylpentan-2-one was tolerated well. Animals were biopsied (hindfeet) after 45 and 135 days of exposure. The results indicate that the solvent methyl n-butyl ketone is neurotoxic to cats (multifocally swollen axons filled with neurofilaments, secondary changes of myelin sheaths and overt fiber breakdown). Cats treated with 4-methylpentan-2-one alone failed to develop detectable nervous system damage.

In a study by Krasavage (1982 - cited in WHO 1990) rats were given ip injections of 4-methylpentan-2-one, or a mixture of methyl ethyl ketone and 4-methylpentan-2-one (9:1 by volume), 5 times/week, for 35 weeks. The dose levels of 10, 30, and 100 mg/kg body weight were

doubled after 2 weeks of treatment. Transient anaesthesia was noted during the first 4 weeks in the highest dose group, but there was no evidence of peripheral neuropathy.
In dogs administered 300 mg 4-methylpentan-2-one /kg body weight per day subcutaneously (sc) for 11 months, electromyographic examination showed no evidence of neurotoxicity. In beagle dogs receiving similar treatment, there were no neurotoxic changes.

Enhancing effects of 4-methylpentan-2-one:

Beside direct toxic effects 4-methylpentan-2-one has some enhancing effects. In animal studies the substance potentiates cholestasis, hepatotoxicity, nephrotoxicity or neurotoxicity induced by other substances. A possible mode of action behind these effects may be the induction of cytochrome P-450 enzyme species.

The maximum motor-fibre conduction velocity in the tail nerve decreased markedly when male rats were treated with methyl *n* -butyl ketone (401 mg/kg, 5 times/week for 55 weeks) but not when they were treated with 4-methylpentan-2-one (601 mg/kg, 5 times/week for 55 weeks). However, treatment with 4-methylpentan-2-one (201 mg/kg) facilitated the neurotoxic effect of methyl *n* -butyl ketone (401 mg/kg) possibly due to the demonstrated ability of 4-methylpentan-2-one to increase the metabolic activity of 10 000 g liver supernatants towards both 4-methylpentan-2-one and methyl *n* –butyl ketone (Nagano, 1988 - cited in WHO, 1990).

Abou-Donia (1985) investigated the joint neurotoxicity of 4-methylpentan-2-one and n-hexane. Five hens per group were continuously exposed for 90 days to 4-methylpentan-2-one and/or n-hexane followed by a 30-day observation period. Hens continuously exposed to 1000 ppm 4-methylpentan-2-one developed leg weakness with subsequent recovery, while inhalation of the same concentration of n-hexane produced mild ataxia. Hens exposed to mixtures of n-hexane and 4-methylpentan-2-one developed clinical signs of neurotoxicity, the severity of which depended on the 4-methylpentan-2-one concentration. Thus, all hens exposed to 1000 ppm n-hexane in combination with 250, 500, or 1000 ppm 4-methylpentan-2-one progressed to paralysis. Hens continuously exposed to 1000/100 n-hexane/4-methylpentan-2-one showed severe ataxia which did not change during the observation period. The neurologic dysfunction in hens exposed simultaneously to n-hexane and 4-methylpentan-2-one was accompanied by large swollen axons and degeneration of the axon and myelin of the spinal cord and peripheral nerves. The results indicate that the 4-methylpentan-2-one synergized the neurotoxic action of the weak neurotoxicant n-hexane. In another experiment to further investigate the mechanism of this synergism hens were again continuously exposed to 4-methylpentan-2-one and/or n-hexane for 50 days. Exposure to 1000 ppm n-hexane had no effect on hen hepatic microsomal enzymes, whereas inhalation of 1000 ppm 4-methylpentan-2-one or a mixture of 1000 ppm of each of n-hexane and 4-methylpentan-2-one for 30 days significantly induced aniline hydroxylase activity and cytochrome P-450 contents in hen liver microsomes. The authors therefore concluded that the synergistic action of 4-methylpentan-2-one on n-hexane neurotoxicity may be related to its ability to induce liver microsomal cytochrome P-450, resulting in increased metabolic activation of n-hexane to more potent neurotoxic metabolites (2,5-hexanedione). This has been confirmed by Lapadula (1991) who exposed hens to n-hexane and 4-methylpentan-2-one showing that there was a dose-dependent increase in three different isoenzymes of cytochrome P450.

The effects of 4-methylpentan-2-one on the duration of ethanol-induced loss of righting reflex and on ethanol elimination in mice were studied by Cunningham (1989). 4-methylpentan-2-one was dissolved in corn oil and injected intraperitoneally 30 min before ethanol (4g/kg) was injected (ip). The tested concentration of 5mmol/kg prolonged significantly the duration of ethanol-induced loss of righting reflex. The concentrations of ethanol in blood or brain on return of the righting reflex

were similar in solvent-treated and control animals. The solvent reduced the activity of mouse liver alcohol dehydrogenase in vitro.

The substance also potentiates cholestasis induced by various chemicals. Studies by Duguay (1993, 1997) showed that 4-methylpentan-2-one potentiates both taurolithocholic acid and manganese/bilirubin cholestasis in a dose related fashion. The severity of the hepatotoxic response was dependent on the plasma concentration irrespective of the route of administration. This effect was not only seen with the substance itself but also with its metabolites 4-methyl-2-pentanol and 4-hydroxymethyl isobutyl ketone (Vezina, 1988). 4-methylpentan-2-one and both metabolites also significantly increased the liver damage induced by chloroform. Cytochrome P-450 content and the oxidation of aniline and 7-ethoxycoumarin were significantly increased with either a single or a multiple administration of 4-methylpentan-2-one. (Vezina, 1990).
4-methylpentan-2-one also potentiates carbon tetrachloride induced hepatotoxicity and chloroform induced nephrotoxicity in male Sprague-Dawley rats. 4-methylpentan-2-one significantly increased cytochrome P-450 content of liver and renal cortical microsomes (Raymond 1995 a, b).

Human data:

A case report (Grober, 2000) describes a 44-year-old man who became cognitively impaired during a 6-year period of repeated exposure to high levels of 4-methylpentane-2-one. Neuropsychological tests administered six times over 10 years demonstrated a stable pattern of cognitive impairment. Dynamic imaging studies suggested persistent CNS dysfunction. The authors conclude that chronic, high-level, occupational 4-methylpentan-2-one exposure can cause a persistent cognitive syndrome best explained by impaired working memory.
Shipyard painters exposed to solvent vapour mixtures (including 4-methylpentan-2-one) showed increased prevalence of acute neurological symptoms or decrements in neurobehavioral performance tests (Valciukas, 1985; Lee, 2005). However attribution to one chemical is not possible. Workers in a paint factory exposed to low-level organic solvents were examined by Tsai (1997). Neurobehavioral tests showed significant effects like prolonged response latencies, impairment of pattern comparison or pattern memory. A 16-year old man complained of burning paraesthesia in the extremities and decreased muscle strength in hands and feet one week after the use of a spray painting (AuBuchon, 1979). Nerve biopsy revealed acute segmental demyelination.

A clear attribution of the neurotoxic effects to the solvent 4-methylpentane-2-one in all of these studies is not possible due to mixture exposure and unknown exposure concentrations. The degree to which the chemical contributed to the observed effects is uncertain.

10.12.2 Comparison with the CLP criteria

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure are classified in Category 1 for target organ toxicity (repeat exposure). Classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur at or below the guidance values (C) (oral: 10mg/kg bw/day; dermal: 20mg/kg bw/day; inhal: gas 50 ppmv/6h/day, vapour 0.2mg/l/6h/day)

Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure are classified in Category 2 for target organ toxicity (repeat exposure). Classification in Category 2 is applicable,

when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the given guidance value ranges (oral: 10 < C ≤ 100 mg/kg bw/day; dermal: 20 < C ≤ 200 mg/kg bw/day; inhal: gas 50 < C ≤ 250 ppm V/6h/day , vapour: 0,2 < C ≤ 1,0 mg/litre/6h/day)

Studies with repeated exposure to 4-methylpentan-2-one (oral or inhalative) show effects on liver, kidney and the nervous system of tested animals. A rough compilation is given in Table 38. The effects were seen at rather high concentrations.

Table 38: Compilation of effects seen in testing animals after repeated exposure to 4-methylpentan-2-one.

Adverse effects [effects seen in rats if not specified otherwise]	**Citation** **[study length]**
Kidney effects	
1000 mg/kg: Absolute and relative kidney weights (m/f) ↑ (from 25 to 34% in males and from 20 to 22% in females) BUN (m) ↑ Serum potassium (m) ↑ Serum glucose (m) ↓ Urinary protein (m,f) ↑ Mild nephropathy (m)	Anonymous, 1986 [13 weeks]
2000 ppm: kidney weight ↑ (m – rat (11%), f – mice (5%)), hyaline droplet degeneration (m)	Phillips, 1987 [9days]
1041 mg/kg: kidney weights (f) ↑	Carnegie-Mellon Inst; 1977 - cited by US EPA, 2003 [120days]
750/1500 ppm: kidney weights (m) ↑	David, 1999 [13 weeks]
100 ppm: kidney weights (m) ↑ (+19%), hyaline droplet degeneration of the proximal tubules, gradual revision of damage with time	MacEwen, 1971 [90d]
250/1000 ppm: Hyaline droplet formation (m)	Phillips, 1987 [14weeks]
500/1000/2000 ppm: F0: kidney weights ↑ in males at 500 (+8%), 1000 (+12%) and 2000ppm (+28%) F1: kidney weights ↑ in males at 500 (+7%), 1000 (+12%) and 2000ppm (+22%) nephropathy and droplets in the renal cortical tubular epithelium (m)	Nemec, 2004 [Two generation study]
450/900/1800 ppm: nephropathy (f) dose dependant increase	NTP, 2007

	[2 years]
Liver effects (% indicated if available)	
250mg/kg bw: Liver weight ↑ (absolute (+34%, males; +39%, females) and relative (+42%, males; +38%, females)) 1000 mg/kg bw/d: Liver weight ↑(terminal absolute (+34%, males; +39%, females) and relative (+42%, males; +38%, females)) Several enzymes ↑	Anonymous, 1986 [13 weeks]
500ppm: relative liver weights ↑ (+9%) in male rats 2000 ppm: relative liver weight ↑ in male rats (+36%) and in female rats (+8%) and female mice at 2000 ppm (+13%) Increased number of mitotic figures (qualitative assessment) in 2/6 male rand 1/6 female rats [rat, mice]	Phillips, 1987 [9days]
750ppm: Liver weight (m) ↑	David, 1999 [13 weeks]
1002 ppm: absolute and relative liver weight in male rats ↑ (+13% and +9%) and mice ↑ (+7% and +11%) [rats+mice]	Phillips, 1987 [14 days]
183 ppm: Liver weight (m) ↑ 1100 ppm: eosinophilic droplets	Hazleton Laboratories, 1966/68 –– cited in US EPA, 2003 [2-4 weeks]
2000 ppm: F0: Liver weight ↑ in males (+20%) and females (+10%) F1: Liver weight ↑ in males (+14%) and females (10%) F0/F1: centrilobular hepatocellular hypertrophy (m)	Nemec, 2004 [Two generation study]
3000 ppm: liver weight (f) ↑ (absolute +18% and relative + 4.5%) [mice]	Tyl, 1987 [GD 6-15]
CNS effects	
200 mg/kg: Transient anaesthesia was noted during the first 4 weeks in the highest dose group (acute effect, accustoming effect?)	Krasavage, 1982 [35 weeks]
1000 mg/kg: reversible lethargy for a few hours following exposure; decreased in incidence and severity during the study (acute effect, accustoming effect?)	Anonymous, 1986 [13 weeks]
50 ppm: minimal effect on accuracy of performance	Geller, 1979

of tasks, increased response time on every behavioural test day [baboon] (acute effect)	[7 days]
750/1500 ppm: Reduced activity levels during first 8-10 weeks of exposure but not after cessation of exposure; no cross pathologies	David, 1999 [13 weeks]
1500 ppm: Signs of narcosis during the exposures, minimal axonal changes (contamination?) (acute effect)	Spencer, 1975 [5 months]
1000/2000 ppm: dose-related increase in the number of F0 and F1 parental animals with absent or diminished response to a sound stimulus (acute sedative effect) clinical signs of CNS depression in weanlings	Nemec, 2004 [Two generation study]
3000 ppm: loss of coordination, irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, lacrimation – only during exposure periode (acute effect) [rats, mice]	Tyl, 1987 [GD 6-15]
1000 ppm: leg weakness [hens]	Abou-Donia, 1985 [90 days]

Elevated kidney weights as well as hyaline droplet degeneration were seen predominantly in male rats. Though sometimes below the relevant guidance value a α2µ-mediated MOA can be assumed, which is not considered relevant for humans (see Chapter 10.9). **However exposure-related increased incidences of chronic nephropathy in the female rats indicate that exposure-related** nephropathy also may occur independent of the α2u-globulin mechanism.

Elevated liver weights were seen in male and female mice at doses not relevant for classification as STOT RE and are considered to be an adaptive physiological response to an intensified metabolic liver burden.

Most of the neurotoxic effects (narcosis, lethargy, reduced activity level, etc.) seen in repeated dose studies occurred during exposure only and were transient; therefore they are attributed to be acute effects of 4-methylpentan-2-one. This acute effect already has been addresses in Chapter 10.11 with a proposal for STOT SE, H336. Also an accustoming effect has been observed in some rat studies. Histological changes in the CNS have been investigated several times but no effects (David, 1999; Carnegie-Mellon Inst; 1977) or only minimal equivocal effects (Spencer, 1975) could be detected. Human case studies or surveys, most of them with mixture exposure and unknown exposure concentrations, show neurotoxic effects like cognitive impairment, prolonged response latencies or decreased muscle strength with acute segmental demyelination in one case. However, the degree to which the chemical contributed to the observed effects is uncertain.

10.12.3 Conclusion on classification and labelling for STOT RE

Effect on liver weights is considered to be an adaptive physiological response. Elevated liver weights in males are explained by a α2µ-mediated MOA, which has been investigated in detail in Chapter 10.9. Neurotoxic effects in repeated dose studies are acute reactions to 4-methylpentan-2-one. No classification for STOT RE effects is proposed.

10.13 Aspiration hazard

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
- Sprague-Dawley albino rats (3m, 3f)	Intratracheal single exposure 4-methylpentan-2-one (1ml/kg) Neg. control: water (n=36) pos control: Kerosene (n=10)	6/6 died within the 24h hemorrhagic areas in the lung (25%)	Panson (1980) [cited from ECHA dissemination site]
- albino rats (m, n=5)	4-methylpentan-2-one (0.2ml) Neg. control: water	deaths due to respiratory arrest and cardiac failure	Exxon Chemical Company (1982) [cited from ECHA dissemination site]

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Panson (1980) evaluated the aspiration hazard of 4-methylpentan-2-one using six Sprague-Dawley albino rats (three males, three females). Each anesthesized animal was given a single intratracheal dose (delivered into the rear portion of the mouth near the tracheal orifice) of 1ml/kg, the nostrils were closed with the fingers to force the animal to breathe through its mouth, and then rats were observed over a 24-h period. Necropsy was then performed. All of the rats died instantly. Lung weights ranged from 1.42 to 2.51 g (mean = 1.84) and the lung weight/body weight ratio ranged from 0.70 to 1.23 (mean = 0.88). In most of the animals, 25% of the lung tissue (all right lobes and caudal lobe included) was hemorrhagic. In one animal 50% of the lung tissue was hemorrhagic. A blood clot at the base of the heart was also noted in one animal. The author concluded that 4-methylpentan-2-one may be aspirated into the lungs when swallowed.

The Exxon Chemical Company (1982) evaluated the aspiration hazard and toxicity of 4-methylpentan-2-one using five male albino rats. The animals were anesthetized with diethyl ether vapour to the point of apnoea and 0.2 ml of the test substance was placed in the oral cavity of each. Next, the animals were held in a vertical position with mouths held open and nostrils closed at end of expiration phase of breathing cycle. The nostrils were closed to promote entry of the test material into the trachea. At 24h post-dosing the lungs were removed from animals that died and surviving animals that were killed under ether anesthesia by exsanguination from the abdominal aorta. Some of the animals (number not stated) died; all deaths were due to respiratory arrest, cardiac failure, or

both, rather than pulmonary edema. None of the negative-control animals died. It was concluded that 4-methylpentan-2-one presents a potential aspiration hazard. No further information available.

10.13.2 Comparison with the CLP criteria

A substance is classified for aspiration hazard if it is known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard. A substance is classified in Category 1: (a) based on reliable and good quality human evidence or (b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C.

The substance shows some evidence of aspiration hazard in two available animal studies, no evidence from human exposure is available.

10.13.3 Conclusion on classification and labelling aspiration hazard

The substance is a ketone and there is no human evidence for aspiration hazard. According to classification criteria no classification is indicated.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not relevant

12 EVALUATION OF ADDITIONAL HAZARDS

Not relevant

13 ADDITIONAL LABELLING

The already harmonized labelling with EUH066 “Repeated exposure may cause skin dryness or cracking” shall further apply based on the degreasing property of 4-methylpentan-2-one (see Chapter 10.4).

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