Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

4-methylpentan-2-one; isobutyl methyl ketone

EC Number: 203-550-1
CAS Number: 108-10-1

CLH-O-0000001412-86-295/F

Adopted

20 September 2019

20 September 2019

CLH-O-0000001412-86-295/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **4-methylpentan-2-one; isobutyl methyl ketone**

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

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

The proposal was submitted by **Austria** and received by RAC on **25 September 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **12 November 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **25 January 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **Daniel Borg**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **20 September 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	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* STOT SE 3 Eye Irrit. 2	H225 H332 H335 H319	GHS02 GHS07 Dgr	H225 H332 H335 H319	EUH066		
Dossier submitters proposal	606-004-00-4	4-methylpentan-2-one; isobutyl methyl ketone	203-550-1	108-10-1	**Retain** Flam. Liq. 2 Eye Irrit. 2 STOT SE 3 **Modify** Acute Tox. 4 **Add** STOT SE 3 Carc. 2	**Retain** H225 H319 H335 **Modify** H332 **add** H336 H351	**Retain** GHS02 GHS07 Dgr **Add** GHS08	**Retain** H225 H319 H335 **Modify** H332 **add** H336 H351	EUH066	**Add** Inhalation: ATE = 11 mg/L (vapours)	
RAC opinion	606-004-00-4	4-methylpentan-2-one; isobutyl methyl ketone	203-550-1	108-10-1	**Retain** Flam. Liq. 2 Eye Irrit. 2 **Modify** Acute Tox. 4 **Add** STOT SE 3 Carc. 2 **Remove** STOT SE3	**Retain** H225 H319 **Modify** H332 **Add** H336 H351 **Remove** H335	**Retain** GHS02 GHS07 Dgr **Add** GHS08	**Retain** H225 H319 **Modify** H332 **Add** H336 H351 **Remove** H335	EUH066	**Add** Inhalation: ATE = 11 mg/L (vapours)	
Resulting Annex VI entry if agreed by 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 STOT SE 3 Eye Irrit. 2	H225 H351 H332 H336 H319	GHS02 GHS07 GHS08 Dgr	H225 H351 H332 H336 H319	EUH066	**Add** Inhalation: ATE = 11 mg/L (vapours)	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

In the CLH dossier, "4-methylpentan-2-one", "isobutyl methyl ketone" and "MIBK" were used synonymously.

4-methylpentan-2-one is an aliphatic ketone used as a solvent and denaturant. The substance has an existing Annex VI entry to CLP regulation. The proposal from the dossier submitter (DS) addressed all human health endpoints except respiratory sensitisation.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - Oral route

Two acute oral (gavage) toxicity studies in rats were available in the dossier. The studies were similar to OECD TG 401 (non-GLP). 4-methylpentan-2-one was tested as a 20 % emulsion (in Terginol 7 surfactant) in the first study and undiluted in the second study. The LD_{50} were 2 080 mg/kg (1 910-2 270 mg/kg confidence interval) in the first study and 2 980 mg/kg in the second study (Smyth *et al.* 1951; Anonymous, 1976). In both studies, the reporting was limited (e.g. unknown dose levels). Based on the study from Smyth *et al.* 1951 (which was scored as the most reliable study), supported by other reported LD_{50} values in rats and mice (Anonymous, 1976; ECETOC, 1987), the DS proposed no classification for 4-methylpentan-2-one.

Acute toxicity - Dermal route

For acute dermal toxicity, one study in rats was documented as reliable and similar to OECD TG 402 (Anonymous, 1996a). In this study, no deaths occurred at 2 000 mg/kg. On this basis, no classification was proposed by the DS.

Acute toxicity - Inhalation

The substance is currently classified as Acute Tox. 4; H332. Four acute inhalation toxicity studies were included in the dossier: two in rats, one in mice and one in guinea-pigs. In the rat study, similar to OECD TG 403, considered as key study by the DS (Smyth *et al*., 1951), no mortality was observed in six rats at 8.2 mg/L whereas all six rats died at 16.4 mg/L following 4-hour exposure to saturated vapour of 2-methylpentan-2-one. In the second rat study, an LC_{50} > 17.2 mg/L was reported following 6-hour exposure (Eastman Kodak, 1956). In mice, an LC_{50} of 20.5 mg/L was reported following 2-hour exposure (Batyrova, 1973). Due to missing study conditions, these two studies were only considered as supportive. In guinea-pigs, no LC_{50} was calculated due to limited available information on the test method and excessive dosing (Specht, 1938 and 1940). In human, no relevant information were available on potential lethal concentration. Overall, based on Smyth *et al.*, 1951, the DS proposed to classify 4-methylpentan-2-one as Acute Tox. 4; H332 with an ATE of 11 mg/L (conversion values from table 3.1.2. of CLP Regulation).

Comments received during public consultation

Two MS agreed with the DS's proposal.

For acute toxicity, one Member State (MS) commented that based on the LC_{50} range (between 8.2 and 16.4 mg/L), found in Smith *et al*. 1951, it cannot be excluded that the LC_{50} was below 10 mg/L, leading to a category 3 classification. The DS agreed with the MS but responded that based on LC_{50} reported in other studies (> 10 mg/L), a classification of 4-methylpentan-2-one as Acute Tox. 4 is more appropriate than Acute Tox. 3.

Assessment and comparison with the classification criteria

Acute toxicity - Oral route

The assessment of acute oral toxicity was based on two studies in rats. In the first study, the substance was tested as a 20 % emulsion in a non-ionic surfactant whereas in the second study, undiluted 4-methylpentan-2-one was used. In both studies, the LD_{50} was found to be above 2 000 mg/kg. The CLH dossier also quoted ECETOC, 1987, that reported LD_{50} between 1 900 and 2 850 mg/kg in mice (no further information). RAC noted that there were limited information on test designs and unknown impact of the use of a 20 % emulsion in Smyth *et al.* 1951. Nevertheless, based on a weight-of-evidence approach, RAC agrees with rapporteur's proposal for **no classification**.

Acute toxicity - Dermal route

In an OECD TG 402 study, the LD_{50} of 2-methylpentan-2-one in rats was greater than 2 000 mg/kg in both sexes. A rabbit study (Klimish score 3: unreliable) supported a LD_{50} above 2 000 mg/kg. Overall, RAC agrees with DS that **no classification** is warranted.

Acute toxicity - Inhalation

RAC agrees that the study from Smyth *et al.* 1951 is the key study for classification. Lack of reporting of study conditions in the three other studies did not allow an assessment of the quality of the studies. In Smyth *et al.* 1951, rats were exposed to saturated vapour of 2-methylpentane-2-one and the LC_{50} was found between 8.2 mg/L (no death) and 16.4 mg/L (6/6 killed animals). Although the lowest range value of LC_{50} was below the cut-off of 10 mg/L for classification in category 3 (LC_{50} between 2 and 10 mg/L for vapour), RAC considers category 4 more appropriate (LC_{50} between 10 and 20 mg/L) as no animals were found dead at 8.2 mg/L. Therefore, RAC agrees with DS's proposal to classify 4-methylpentan-2-one as **Acute Tox. 4; H332**.

For the converted acute toxicity point estimate, based on the conversion value obtained from table 3.1.2 of CLP regulation, **RAC agrees with the proposed ATE of 11 mg/L.**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

4-methylpentan-2-one is currently classified as STOT SE 3; H335 for respiratory irritation. Sensory irritation was reported in humans at 25 ppm and above (human volunteer studies, industry health records). The DS highlighted that interpretation of the results was difficult due to subjective indication of effects and low odour threshold (< 1 ppm) that influence the perceived irritative response. Transient respiratory tract irritation was also documented in animals (mice,

guinea-pigs). Overall, the DS considered that the classification STOT SE; H335 was appropriate for respiratory tract irritation.

Based on acute narcotic effects reported observed after acute and repeated exposure in animal studies (guinea-pigs, mice, rats) and industry health records, a classification STOT SE 3; H336 was proposed by the DS.

Comments received during public consultation

Two MS and one industry representative agreed to classify 4-methylpentan-2-one as STOT SE 3; H336. One MS noted that reduced immobility time in mice swimming test in De Ceaurriz *et al.* 1984 was not a good basis for classification of the substance as STOT SE 3; H336 for narcotic effects. The DS responded that the endpoint was only used to indicate potential behavioural changes.

Concerning STOT SE 3 for respiratory irritation (H335), no specific comments were received.

Assessment and comparison with the classification criteria

Central nervous system (CNS) effects

Animal data

No indication of narcotic effects were reported in the acute toxicity studies except in Specht, 1938 and 1940. Nevertheless, in most of these studies, only LD_{50} was reported. In Specht, 1938 and 1940, decreased respiratory rate was observed in guinea-pigs during the first 6-hour of exposure at 1 000 ppm. Excessive higher exposure concentration (> saturated concentration in air) produced ataxia, progressive narcosis and death.

In the study from De Ceaurriz *et al.* 1981, a decrease in respiratory rate was noted in mice following 5-minute exposure to 662, 757, 807 or 892 ppm of 4-methylpentan-2-one. This effect was considered indicative of respiratory tract irritation or narcosis. In De Ceaurriz *et al.* 1984, a behavioural effect was seen in mice (decreased immobility of mice swimming in a narrow cylinder from which they cannot escape). RAC noted that the reliability of such results are difficult to assess as low number of animals were used (6/group), short duration of exposure and lack of details in the study protocol.

In the study of Geller *et al.*1979, four male baboons, were maintained for 24-hour in an exposure chamber during 7-day. Increased response time was observed on every behavioural tests in baboons at 50 ppm. Minimal effects on accuracy of performance of tasks was noted (94 %, 100 %, 96.5 % and 94 % in four baboons). This was attributed to an acute effect.

In repeated-dose toxicity studies performed by inhalation, CNS symptoms were observed during exposure (but not after cessation of exposure) in several studies. Accustoming effects were noted in some repeated-dose toxicity studies.

- 2-generation study (Nemec, 2004): decreased response to a sound stimulus in F0 and F1 adults (≥ 1 000 ppm). Animals appeared normal 1-hour post-exposure.
- Prenatal developmental toxicity study in rats (Tyl *et al.* 1987): loss of coordination, paresis (partial hindlimb paralysis), negative tail and/or toe pinch, muscular weakness in hindlimbs (≥ 3 000 ppm) during exposure only.
- Prenatal developmental toxicity study in mice (Tyl *et al.* 1987): irregular gait, paresis, hypoactivity, ataxia during exposure only (≥ 3 000 ppm).

With regards to other routes of exposure, transient anaesthesia was observed at 200 mg/kg following intra-peritoneal administration (Krasavage *et al.*1982) during the first four-week of exposure. In Anonymous, 1986, reversible lethargy was seen for few hours following exposure to 4-methylpentan-2-one (1 000 mg/kg, 13-week gavage study).

Human data

In a human male volunteer study (Hjelm *et al.*1990), eight volunteers were exposed to 2.4, 24 and 49 ppm of 4-methylpentan-2-one, during 2-hour, under conditions of light exercise, on four occasions. Questionnaires and performance tests were used to investigate CNS symptoms and function. The degree of CNS symptoms increased with exposure levels and decreased rapidly after cessation of exposure (Headache, nausea, vertigo) as shown in table below. No other effects were seen (rating of mood, simple reaction time task and mental arithmetic). Biological sampling during the study may have influence some of the results (e.g. fatigue).

	2.4 ppm	24 ppm	49 ppm
Headache	0/8	2/8	2/8
Nausea	0/8	0/8	1/8
Vertigo	1/8	2/8	2/8

Iregren *et al.*1993 further investigated the increased time observed in human volunteers. Twelve volunteers (both sexes) were exposed during 2-hour to 2.4 or 49 ppm (2.4 ppm served as control) under the conditions of light exercises. Heart rate, performance tests, rating scale for local irritation, CNS symptoms and mood were investigated. Prevalence and intensity of neurological symptoms (ex: discomfort such as fatigue due to exposure) was significantly increased in the group exposed to 49 ppm compared to 2.4 ppm. The same pattern was observed for symptoms of irritation. No effects from exposure on performance of a reaction time task or an arithmetic test could be demonstrated.

In Dick *et al.* 1992, 13 adult male and 12 adult female volunteers were exposed to 200 ppm (410 mg/m^3) of 4-methylpentan-2-one for 4-hour. No effects were seen on any performance test used to measure neurobehavioral changes (choice reaction time, simple reaction time, visual vigilance, dual task, one sensorimotor test, and mood). In this study, the conditions did not include light exercises during exposure.

In industry health records (Armeli, 1968 and Linari, 1964), headache, nausea and vomiting were reported in 19 workers exposed to 500 ppm of 4-methylpentan-2-one, for 20-30 min. Insomnia, somnolence and eye burn were also noted in some workers. After reducing exposure to 100 ppm, some workers still complained about CNS symptoms.

Mechanism

In vitro effects of 4-methylpentan-2-one on isolated mouse synaptosomes (inhibition of receptor binding and enzyme activity) were documented by Huang, 1993.

Comparison with criteria

According to human volunteer studies and human health records, CNS depression were observed suggestive of a narcotic effect (vertigo, fatigue, headache, somnolence). A transient narcotic effect (lethargy, ataxia, paresis) were also observed in several studies in rats, mice and guinea-pigs.

In human volunteer studies, no impact on CNS function was demonstrated as no effects on performance tests were seen up to 100 ppm in the conditions of the studies. In rodents, CNS symptoms were transient and only weak evidence of behavioural changes (De Ceaurriz *et al.*

1984) or accuracy in performance test (Geller *et al.* 1979) were noted. When histopathology was performed on CNS (only in repeated-dose toxicity studies) no effects were revealed except in one study from Spencer *et al.* 1975. In this study, minimal axonal changes were noted. Nevertheless, contamination with methyl n-butyl ketone, also used in the study, may have explained the minimal observed effects. As CNS symptoms were transient and as no severe or significant changes in CNS were noted, RAC considers that classification of the substance as STOT SE 1 or 2 is not appropriate. STOT RE was also not considered to be appropriate as findings were considered acute even in repeated-dose toxicity studies.

Overall, RAC agrees with the DS's proposal to classify 4-methylpentan-2-one as **STOT SE 3; H336 for narcotic effects** based on narcotic effects seen in humans, supported by animal data. As narcotic effects are expected by any route of exposure, none needs to be specified.

Respiratory tract irritation

Animal data

In guinea-pigs (Specht, 1939 and 1940), little or no nasal irritation were observed in animals up to 1 000 ppm. Only higher dose levels produced obvious signs of nose irritation. Decreased respiratory rate in De Ceaurriz *et al.* 1981, considered by the authors as potential signs of nasal irritation or narcosis was also observed in mice (50 % decrease observed at 3 195 ppm). In other acute toxicity studies, clinical signs or histopathological examinations were not reported. Clinical signs (red perioral encrustation) suggestive of nasal irritation were also seen at 3 000 ppm in the prenatal developmental toxicity study in female rats (Tyl *et al.* 1987).

Human data

Several human volunteer studies and industry health records reported nose irritation complaints. Nose irritation was observed following various exposure times (15 minutes to 2 hours) and exposure concentrations starting from 24 ppm (Hjelm, 1990). Nevertheless, as investigated by Dalton *et al.* 2000, odour perception of 4-methylpentan-2-one (odour threshold at 10 ppm) significantly affected the perceived irritancy of the substance in human. The authors found that when affective responses to the odour or sensory properties of 4-methylpentan-2-one can be expressed in an "annoyance judgment", weak sensations of intranasal irritation was only observed at 8 874 ppm (mean value).

Comparison with criteria

The substance is currently classified as STOT SE 3; H335 for respiratory irritation. According to the CLP guidance, the evaluation of this classification should be mainly based on human data.

RAC agrees with the DS that, although the studies were poorly reported, nasal irritation was reported in most of the human studies (subjective assessment). Nasal irritation was also observed in some animal studies at high dose levels. Nevertheless, the ECHA guidance on the application of CLP criteria (V. 5.0) state that STOT SE 3; H335 for respiratory irritation is limited to local cytotoxic effects and not to sensory irritation. 4-methylpentan-2-one produced a strong odour sensation that may contribute to rating of perceived irritation. Moreover, there is no evidence of cytotoxic nasal irritation of the substance. Therefore, as there is only weak evidence nasal irritation, RAC is of the opinion that **no classification is warranted and that the present classification as STOT SE 3; H335 for respiratory irritation should be removed**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In a GLP study performed according to OECD TG 404, 3 rabbits were exposed for four hours to undiluted 4-methylpentan-2-one under semi-occlusive conditions (Anonymous, 1996b). No signs of toxicity and no erythema or oedema were observed up to 72-hour after treatment. Based on this study, no classification was proposed by the DS.

ECETOC, 1987, reported slight irritation in guinea-pigs and rabbits following 24-hour exposure under occlusive dressing (no further details). Moreover, daily application of 10 mL on 10 cm^2 of shaved skin for 7 days caused drying and flaking of the surface.

Overall, no classification was proposed by the DS. Nevertheless, the DS proposed to retain EUH066 currently applied as the substance has degreasing properties.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

According to the more recent study performed according to OECD TG and GLP, no classification is warranted. Other studies quoted in ECETOC, 1987 do not allow a direct comparison with CLP criteria due to study deviations but supported also a low irritating potential of the substance. Skin irritation was not reported in human health records. Therefore, RAC agrees with the DS that **no classification** is warranted for 4-methylpentan-2-one for skin corrosion/irritation.

Concerning EUH066, according to the DS, the statement was previously adopted based on a rabbit study where seven daily dermal applications (2 400 mg/kg) induced drying of the skin with some exfoliation (no further details). No practical observation of cracking or flaking were reported in human. Nevertheless, RAC agrees with DS that as a vapour degreasing solvent, skin irritation may be expected following frequent and prolonged exposure. Therefore, RAC agrees with DS' proposal to **retain EUH066.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The substance is currently classified as Eye Irrit. 2; H319. In an OECD TG 405 study performed (under GLP) in 3 rabbits, undiluted 4-methylpentan-2-one caused slight eye irritation in rabbits following instillation of the substance (Anonymous, 1996c). The mean scores for 24-72h in all rabbits were 0 (corneal opacity, iritis and conjunctivae Chemosis/erythema). In two other studies performed in rabbits (Bagley, 1992 and Anonymous, 1992) with a method similar to OECD TG 405, slight irritation was observed. Individual mean scores for 24-72h were between 0.3 and 1.2 and thus, did not meet the classification criteria. Eye irritation effects were reversible. Based on these *in vivo* animal studies, the substance did not meet the classification criteria for eye irritation.

The dossier also presented a bovine corneal opacity and permeability test (Gautheron, 1994). In this test, the mean *in vitro* irritancy score was determined to be 19.9. This score being between > 3 and ≤ 55, according to OECD TG 437 guideline, no prediction can be made.

In humans, several human volunteers studies were available. In particular, two studies (Silvermann, 1946 and Esso; 1965), gives a LOAEC for eye irritation of 340 ppm and 200 ppm. Based on human observations, a classification as Eye Irrit. 2 was confirmed by the DS.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

The substance is presently classified as Eye Irrit. 2; H319.

RAC agrees that based on *in vivo* animal data and the *in vitro* study, no classification is warranted.

In humans, volunteer studies and industry health records were available.

In Heljm *et al.* 1990, in human male volunteers exposed for two hours, eye irritation was not increased with exposure levels (1/8 at 2.5 ppm, 1/8 at 24 ppm and 0/8 at 49 ppm) based on a questionnaire. In the follow-up human volunteer study published by Iregren *et al.* 1993, local irritation symptoms (eyes and airways) were also evaluated using a questionnaire. Perceived irritation was not significantly different between the two exposure levels (2.5 and 49 ppm). Indeed, the irritation level at 2.5 ppm (used as control group in the study) was already high. The authors concluded that this observation may be interpreted as an indication of a high irritation potential of 4-methylpentan-2-one already at low concentration. In the study of Dick *et al.* 1992, following subjective assessment, no symptoms of irritation were attributable to 4-methylpentan-2-on up to 100 ppm (no further information).

Higher exposure levels were tested in older human volunteer studies. Silverman, 1946 reported irritation to the eyes at 200 ppm following 15 minutes exposure. No further information was available. In Esso Research and Engineering Company and Hazelton Laboratories Inc., 1965, eye irritation (subjective assessment) was reported to be generally increased with exposure levels starting from 340 ppm. The volunteers were exposed for 7 minutes via full face mask (two exposures at 2-weeks interval). Industry Health records also reported eye irritating effects (burning in the eyes) in some workers exposed to concentrations up to 500 ppm for 20-30 minutes. Such findings were not reported when the concentration was reduced to 100 ppm.

In summary, eye irritation was noted in several old human volunteer study studies and industry health records, already after 7 minutes, at high exposure levels (> 100 ppm). Although only few details were available in these studies, eye irritation at high exposure levels in human supports the existing classification of **Eye Irrit. 2; H319** for 4-methylpentan-2-one.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised in the CLH report a guinea Pig maximisation Test (Anonymous, 1989). The assay was conducted with a protocol similar to OECD TG 429 (GLP status unknown). In this study, 4-methylpentan-2-one was not found to be a skin sensitiser since no positive response was observed after challenge. Based on this study, the DS proposed not to classify 4-methylpentan-2-one as a skin sensitiser.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

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, 2×4 cm filter paper, 48h). The challenge exposure was conducted with 30 % 4 methylpentan-2-one (2×2 cm filter paper, 24h) in vehicle under occlusive conditions. RAC consider the Guinea-Pig Maximisation Test reliable with limitation, as only few details on the test method were available (e.g. no information on preliminary results for dose selection, no indication if a positive control was used). Nevertheless, as no positive reactions were observed in test animals and as no case reports were published, RAC agrees with the DS that **classification of 4-methylpentan-2-one for skin sensitisation is not warranted**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The kidney, the liver and the CNS were identified as target organs in repeated-dose toxicity studies.

Oral route

The DS based the evaluation of STOT RE on 2 studies in rats, one 90-day study by gavage (similar to OECD TG 408) and one 120-day study in drinking water (including a preliminary study). This second study did not follow OECD TG.

Liver effects (weight and clinical chemistry changes) were observed mainly at 1 000 mg/kg in both male and female in the gavage study. No histopathological changes were observed.

In the gavage study, kidney findings, such as weight changes in both males and females, were observed at ≥250 mg/kg bw, serum clinical chemistry changes were seen in both males and females at 1 000 mg/kg bw. Nephropathy in male rats were also increased compare to control. In the drinking water study, female only were used (n = 5) and were treated with estimated 1 041 mg/kg bw 4-methylpentan-2-one. An increase in kidney was observed. Additionally, renal tubular hyperplasia was seen in 1 out of 5 females.

Reversible lethargy was observed in both sexes at 1 000 mg/kg bw (gavage study). Incidence and severity decreased with study duration.

Inhalation route

The evaluation of the STOT RE hazard was based on six studies in rats, one study in mice, one study in dogs and two studies in monkeys (non-GLP, non-guideline studies). In some studies, a very low number of animals were used. The carcinogenicity studies performed in rats and mice, the 2-generation toxicity study conducted in rats (Nemec *et al.* 2004) and the prenatal developmental toxicity studies performed in rats and mice by inhalation (Tyl *et al.* 1987) were also considered relevant for evaluation of STOT RE.

Kidney effects, consisting of increased weight and hyaline droplet degeneration, were predominantly seen in male rats at doses ≥ 500 ppm. An αu-globulin-mediated mode of action (MoA) was assumed by the DS. Nevertheless, the DS considered that other MoA were plausible as nephropathy in female rats were also observed in the carcinogenicity study (This MoA is discussed in the carcinogenicity section).

Liver effects consisting of weight changes were considered as an adaptative physiological response by the DS.

CNS symptoms were also observed in repeated-dose toxicity studies but were considered transient, relative to narcosis and relevant for classification STOT SE 3; H336 but not for STOT RE (See discussion on STOT SE classification). In humans, five surveys or case studies reported neurotoxic effects. Nevertheless, it was not possible in these studies to exclude co-exposure and exposure concentration were unclear.

Besides, the DS also summarised enhancing effects observed in animals with 4-methylpentan-2-one. In animal studies, the substance potentiate cholestasis, nephrotoxicity or neurotoxicity induced by other substances. A possible MoA behind these effects was the induction of cytochrome P-450 enzyme species.

Other routes

No nervous system damage was observed in a cat study using subcutaneous injection (Spencer *et al.* 1976).

Overall, no classification as STOT RE was proposed by the DS.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

In the repeated-dose toxicity studies reported in the CLH dossier, 4-methylpentan-2-one caused effects in liver, kidney and central nervous system in rats and mice by oral or vapour inhalation route of exposure. In monkeys, effects in kidney and CNS symptoms were observed following inhalation.

Kidney effects

Kidney effects were observed in several repeated-dose toxicity studies (including carcinogenicity and reproductive toxicity studies) in rats, mice and monkeys.

By inhalation, in rats, the table below describes kidney effects observed at concentration relevant for classification as STOT RE 2 (0.2 mg/L < C ≤ 1 mg/L/6h for a 13-week study and 0.64-2 mg/L < C ≤ 3.2-10 mg/L/6h for 9 to 28-day studies, vapour inhalation in rats). These effects were found to be reversible (Mac Ewen *et al.* 1971).

Effects	Concentration (sex)	Study duration	Reference
↑ kidney relative weight	≥ 3.6 mg/L (m+f) ≥ 3.6 mg/L (m+f) ≥ 8.2 mg/L (m) ≥ 0.4 mg/L (m) ≥ 0.4 mg/L (m) ≥ 3.1 mg/L (m)	1-week 4-week 9-day 2-week 13-week 13-week	Borghoff, 2015 Borghoff, 2015 Phillips, 1987 Mac Ewen, 1971 Mac Ewen, 1971 David, 1999
↑ Hyaline droplets in proximal tubular cells	≥ 3.7 mg/L (m) ≥ 3.7 mg/L (m) ≥ 2 mg/L (m) ≥ 1 mg/L (m) ≥ 0.4 mg/L (m)	1-week 4-week 9-day 14-week 13-week	Borghoff, 2015 Borghoff, 2015 Phillips, 1987 Phillips, 1987 Mac Ewen, 1971
Epithelial regeneration of proximal convoluted tubules / Foci of tubular necrosis	≥ 8.2 mg/L (m) ≥ 0.4 mg/L (m)	9-day 13-week	Phillips, 1987 Mac Ewen, 1971
Tubules (representing precursor of granular cast)	≥ 7.4 mg/L (m)	4-week	Borghoff, 2015
Chronic progressive nephropathy (slight exacerbation)	≥ 3.7 mg/L (m)	4-week	Borghoff, 2015

Values in the table have been converted from ppm: 1 mg/m^3 = 0.244 ppm; m=males; f= females

In rats, following oral administration, kidney effects (weight and histopathological findings) were observed only at dose above guidance value criteria.

In mice, increase in kidney weight was observed only at dose above the guidance values for classification STOT RE.

In monkeys, Mac Ewen *et al.* 1971, observed that following 90-day continuous inhalation exposure, of 0.4 mg/L, one of the two male monkeys used in the study exhibited focal chronic inflammation of the kidney. Nevertheless, the result is difficult to interpret, as only 2 monkeys were used.

No kidney effects were seen in dogs at 0.4 mg/L (Mac Ewen *et al.* 1971).

Overall, kidney effects observed at concentration in excess of guidance values for classification included kidney weight change (in males and females) and increase in hyaline droplet proximal tubular cells in males only. The effects were reversible following cessation of exposure. As no significant effects were seen, RAC agrees with the DS that no classification as STOT RE is warranted for kidney.

Liver effects

Liver weight changes and some serum chemistry changes (e.g. serum cholesterol) have been observed in both mice and rats by oral or inhalation route of exposure. No histopathological findings were observed within the concentration range recommended in the guidance. Therefore, RAC agrees with the DS that no classification for liver findings are warranted.

Central nervous system effects

CNS symptoms were mainly related to narcosis and were discussed in the above section "STOT SE". Following repeated-dose, an adaptive effect was observed. Histopathological changes

performed did not revealed effects except in one study from Spencer *et al.* 1975. In this study, minimal axonal changes were observed but contamination with methyl n-butyl ketone also used in the study may have explained the minimal effects observed. Overall, RAC agrees with the DS that no classification CNS is warranted.

Overall, RAC agrees that **classification for STOT RE is not warranted.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

No classification for germ cell mutagenicity was proposed by the DS for 4-methylpenta-2-one. This was based on negative results in two Ames assays, a gene mutation assay in *saccharomyces cerevisiae*, an *in vitro* chromosome aberration study in rat liver cells, an *in vitro* unscheduled DNA synthesis and an *in vivo* micronucleus test following ip. administration (O'Donogue *et al.* 1988 or Brooks *et al.* 1988). The only exception was an equivocal result in a mammalian cell gene mutation test without metabolic activation (O'Donogue *et al.* 1988). Moreover, negative results were supported by lack of mutagenic activity of the two main metabolites of 4-methylpentan-2-one found in rats, mice and guinea-pigs: 4-hydroxy-4-methyl-2-pentanone and 4-methyl-2-pentanol (genotoxicity results as available in ECHA dissemination site).

Comments received during public consultation

One MS agreed that no classification is warranted for 4-methylpentan-2-one. Nevertheless, the MS pointed out that the classification is not warranted due to insufficient data. The following limitations were highlighted by the MS:

- Ames tests: no valid positive control (Brooks *et al.* 1988), missing cytotoxicity at the highest tested concentration (O'Donoghue *et al.* 1988);
- *In vitro* chromosome aberration test: no metabolic activation, missing cytotoxicity;
- *In vivo* micronucleus: no proof of exposure, ip. route not recommended in OECD TG 474.

Moreover, the MS considered that an indication of mutagenicity was provided in the *in vitro* gene mutation assay. The *in vivo* micronucleus test was not an appropriate follow up. Indeed, the study investigated cytogenic aberrations whereas an *in vivo* test of gene mutation would have been necessary to rule out a positive *in vitro* result (e.g. TGR or comet assays).

The DS agreed that the studies had limitations and were not "clear negative" as, in most of the studies, study designs were inappropriate. Nevertheless, the DS concluded that the overall picture showed a lack of mutagenicity. With regard to proof of exposure in the ip. study, the DS responded that based on a study by DiVincenzo *et al.* 1976, exposure to the two main metabolites of 4-methylpentan-2-one were demonstrated in guinea-pig serum.

Assessment and comparison with the classification criteria

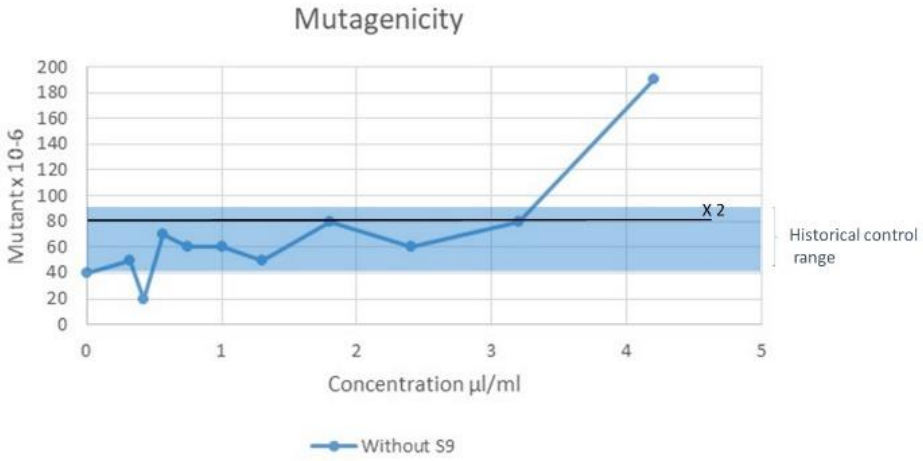
The outcome of two bacterial gene mutation assays were negative. The first test (O'Donoghue *et al.* 1988) was equivalent to OECD TG 471 and performed according to GLP. The test was performed in sealed glass containers to prevent escape of the test material up to 4 µL per plate. According to the published study, a preliminary cytotoxicity assay has been performed for dose selection. Although data of the preliminary study were not shown, at the highest dose level, bacterial lawn was slightly reduced in all tested strain with and without metabolic activation suggesting that cytotoxicity has been detected. The following two main limitations were noted by RAC:

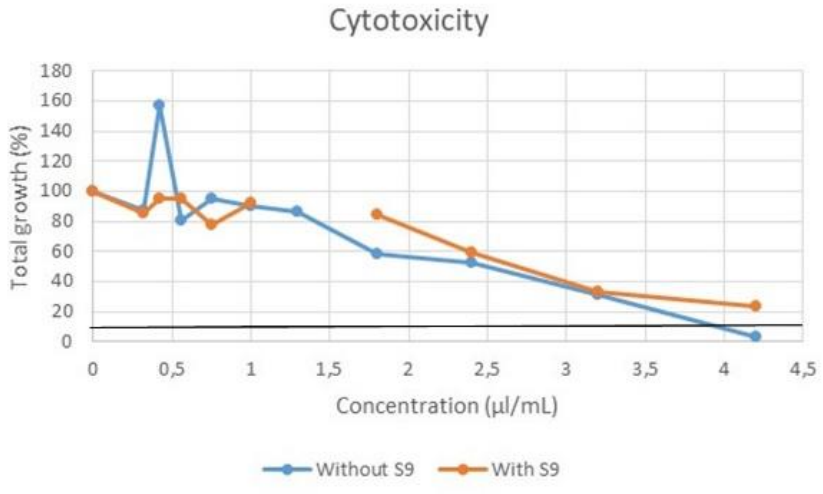
- The test did not include *S. typhimurium* TA102 or *E. coli* WP2;
- Only 2-aminoanthracene was used as positive control with metabolic activation whereas at least a second positive control is recommended in the test guideline.

Overall, RAC considered this negative study reliable with limitations.

In the second Ames assay from Brooks *et al.,* 1988 (non-GLP)*,* negative results were obtained in 5 strains including *S. typhimurium* TA100 and *E.coli* WP2. Nevertheless, it is stated in the dossier that positive controls did not result in increased reverse mutation rates. Therefore, RAC considered the study unreliable.

With regard to *in vitro* gene mutation assay in mammalian cells, a statistically significant increase in mutant frequency (MF) was observed at three dose levels: 1.8, 2.4 and 4.2 µg/L. Nevertheless, the increase was not dose-related and except at 4.2 µg/L, the MF was inside the range of value of negative historical controls of the laboratory (based on 8 assays). Moreover, at the highest tested concentration of 4.2 µg/L, excessive cytotoxicity was noted (3 % total growth compare to control). Mutagenicity and cytotoxicity are reported in the figures below. RAC considered the results of the study equivocal as the dose just behind the highest tested dose did not give enough cytotoxicity (no data between 10 and 30 % total growth) and as a second independent experiment was not performed.





With regard to the negative *in vitro* chromosomal aberration study in rat liver cells, the study was similar to OECD TG 473 but no metabolic activation was used and no cytotoxicity was reported. RAC considered this study insufficient to conclude on the cytogenicity of the substance.

Negative results were obtained in an *in vitro* gene mutation assay in *saccharomyces cerevisiae* and in an *in vitro* unscheduled DNA synthesis test. These studies have lower weight in the overall weight-of-evidence than other available *in vitro* studies.

In vivo, negative results were obtained in a micronucleus test performed in mice (ip. administration). The study was acceptable with limitations as a low number of polychromatic erythrocytes were scored (1 000 instead of 2 000 recommended). Moreover, only one dose (0.73 mL/kg bw) was tested and the dose is excessive as mortality was observed at this dose level. Proof of exposure was not observed in the study. Nevertheless, according to the available toxicokinetic data, the substance was identified in blood and brain after i.p. administration in mice (Granvil *et al.* 1994).

In vitro studies available on the two main metabolites identified in animals were negative (Ames, *in vitro* gene mutation studies in mammalian cells and in vitro chromosomal aberration studies).

With regard to structure-activity relationships, no alerts have been observed for DNA binding or genotoxicity endpoints (OECD QSAR toolbox, v.4.2).

Overall, RAC agrees with the DS that **no classification for 4-methylpentan-2-one is warranted** as negative results were mostly observed. Nevertheless, RAC noted that the overall database is limited and particularly on direct gene mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The assessment of carcinogenicity was based on a carcinogenicity study performed by NTP in 2007 in mice and rats. Moreover, human relevance was discussed in the dossier based on available *in vivo* and *in vitro* mechanistic data.

The DS proposed to classify 4-methylpentan-2-one as Carc. 2. This was based on a weight-of-evidence analysis which took into account the following:

- An increased incidence of hepatocellular tumours was seen in male and female mice. Mechanistic data were supportive of a CAR-mediated MoA, considered as not relevant to human. Nevertheless, uncertainties remained, as the MoA was not investigated in human cells.
- An increase in kidney tubular cell tumours was observed in male rats. The DS considered that a αu-globulin nephropathy MoA, specific to male rats, was plausible. Nevertheless, progressive chronic nephropathy seen in female rats leads to uncertainties on the proposed MoA.
- An increase in a rare malignant kidney mesenchymal tumour was observed in female rats. Nevertheless, the incidence was low (Two animals (2/50) bearing tumours).
- An increase in the incidence of pheochromocytoma in adrenal gland and mononuclear cell leukaemia in male rats were reported. Nevertheless, the incidences were at the upper limit or only slightly above historical control range of the testing laboratory.
- 4-methylpentan-2-one was not genotoxic;
- Negative results were obtained in an *in vitro* cell transformation assay;

Comments received during public consultation

One MS agreed with the DS's proposal.

One MS considered the case borderline with no classification but supported classification as Carc. 2. The increase in rare malignant mesenchymal tumours in female rats was considered by the MS as the decisive effect in determining the classification. Nevertheless, due to low incidence

and as the increase was only slightly above the historical control range, no classification was also considered as an option by the MS.

An individual disagreed with the DS's proposal. The individual was concerned about the potential presence of a relevant impurity, mesityl oxide, which is an intermediate in the manufacture of the substance. This impurity may have a carcinogenic potential although it has no such classification. The individual thus considered that the study was not reliable enough to classify the substance.

Three comments from industry representatives disagreed with the DS proposal and provided the following justification:

- Since the IARC assessment and conclusion (Category 2B "possibly carcinogenic to human"), new mechanistic studies (Borghoff *et al.* 2015, Hughes *et al.* 2016) have been provided to support CAR-mediated MoA of liver mice tumours and αu-globulin nephropathy MoA of kidney rat tumours. They also quoted published papers supporting the non-relevance of these tumours in human and referred to previous ECHA's opinion on human relevance of these MoA.
- Adrenal gland tumours were within the upper limit of historical control range;
- Mononuclear cell leukaemia is not a reliable endpoint for risk assessment due to excessive variability of this effect in F344 rats and as no human correlate exist.

Moreover, a copy of the letter sent by industry to IARC for reopening the discussion on 4-methylpentan-2-one, in light of the new mechanistic data, was provided in the public consultation.

Assessment and comparison with the classification criteria

Two carcinogenicity assays were included in the CLH report, one in B6C3F1 mice and one in F344 rats (NTP 2007). Additionally, mechanistic studies were available in the dossier.

IARC classification as "possibly carcinogenic to human" was mainly based on liver tumours in mice in both sexes and renal kidney tumours in rats in both sexes. These tumours were discussed by RAC in light of new mechanistic data. Moreover, the increase incidence of tumours at other sites in male rats (adrenal gland and hematopoietic system) were discussed.

Kidney tumours

In male rats, a statistically significant increase in renal tubule adenoma and combined adenoma or carcinoma was observed in the high dose group (at 1 800 ppm equivalent to 7.4 mg/L). The increases exceeded the historical control range of the laboratory (consisting of eight NTP studies performed by inhalation between 1995 and 2005).

In female rats, two females were found with a very rare malignant mesenchymal kidney tumour at termination of the study. This type of tumour was not found in NTP historical control.

In the carcinogenicity study, survival was affected in the high dose group in males but not in females. Body weight changes were not observed. Therefore, RAC noted that no excessive general toxicity was observed in the high dose groups. Kidney was the target organ in the study. A statistically significant dose-related increase in renal tubule hyperplasia and papilla mineralisation was observed in males. Additionally, a dose-related increase in chronic progressive nephropathy was observed in both males and females with increasing severity with dose levels. Kidney weight was not investigated in the NTP study. Nevertheless, increased kidney relative weight was observed in both males and females in repeated dose toxicity studies, in the 2-generation study or in the developmental toxicity studies.

The incidences of kidney tumours in male and female rats are shown in the table below:

	Kidney tumour incidence (%)				
Dose (ppm)	**0**	**450**	**900**	**1 800**	**HC**
Males: renal tubule[1]					
Adenoma	4	6	6	20**	0-2
Carcinoma	0	2	0	4	0-2
Carcinoma or adenoma	4	8	6	22**	0-2
Females mesenchymal tumours					
Malignant	0	0	0	2/50 4 %	0

HC: historical control; ** $p < 0.01$; [1]incidence of tumours of combined single section and step section.

In the table below, selected non-neoplastic kidney findings at termination are provided (combined single and step section):

	n = 50							
	Males				Females			
Dose (mg/kg bw/d)	**0**	**450**	**900**	**1 800**	**0**	**450**	**900**	**1 800**
Nephropathy (severity)	42 (2)	45 (2.6)	47 (2.4)	50* (3.1)	19 (1.4)	35** (1.5)	38** (1.5)	44** (1.9)
Renal tubule hyperplasia*	1	14*	7*	21**	1	1	-	1
Pelvis transitional epithelium hyperplasia	1	5	6*	19**	1	1	-	1
Papilla mineralization	1	6*	22**	29**	3	5	3	3

**p ≤ 0.01, *p ≤ 0.05 (poly-3 test)

To clarify a potential α2u-globulin nephropathy MoA of the observed renal tubule adenomas and carcinomas, specific to male rats, mechanistic studies have been provided.

The postulated MoA is that the accumulation of a chemical- α2u-globulin complex resistant to lysosomal degradation in male rats results in renal tubular cell death and compensatory cell proliferation and neoplasms. This MoA has been considered not relevant to humans, as this protein does not exist in humans (ECHA guidance on CLP criteria, 2017).

- <u>MoA in male rats</u>

The following key events were considered by the DS (taken from US EPA, 1991 and IARC, 1999):

- reversible binding or metabolite to α2u -globulin;
- increased number and size of hyaline droplets in renal proximal tubule cells;
- the hyaline droplets contained α2u -globulin;
- Histopathological changes in shorter-term studies, renal tubular cell proliferation and induction of tumours.

In Borghoff *et al.* 2015, 4-methylpentan-2-one was shown to have medium affinity to α2u-globulin in males as measured by partition coefficient of the substance in kidneys with and without addition of a known binder (D-limonene oxide) (*in vitro* model). This affinity was not observed in females. No information on potential binding activity of the metabolites of 4-methylpentan-2-one were available.

In Borghoff *et al.* 2015, male and female F344 rats were exposed to dose levels similar to those used in the carcinogenicity study by inhalation: 0, 450, 900 and 1 800 ppm for 1 week or 4 weeks. Protein droplets accumulation, with increasing concentration of α2u-globulin, was observed in the proximal tubules of all exposed males from 900 ppm after 1 week or 4 weeks. Tubules considered as precursors of granular casts were also observed in some males at 1 800 ppm. Chronic progressive nephropathy was slightly exacerbated at ≥ 900 ppm male rats compared to controls and to the positive control. Mitotic index in cortical tubule cells was increased in male following 1-week or 4-weeks at 1 800 ppm. These findings were not observed in females.

In a previous study, Borghoff *et al.* 2009, reported an increase in the severity of droplet accumulation in male rats following oral administration of 4-methylpentan-one during 10 days (1 000 mg/kg bw). In the 13-week toxicity study (at 100 ppm) from MacEwen *et al.* 1971, hyaline droplet degeneration with increasing size over time was reported. Foci of tubular necrosis was also noted in some males. In the 2-generation reproductive toxicity study, nephropathy were observed with droplets in renal cortical tubular epithelium in exposed F1 males (Nemec, 2004). Increased regenerative tubular epithelial was seen in male rats in Phillips *et al.* 1987 (at ≥ 500 ppm).

In the carcinogenicity study, minimal hyaline droplet accumulation was observed in male rats who died early (two at 900 ppm and two at 1 800 ppm).

Based on these data, RAC agrees with the DS that there is supporting evidence of an exposure-related correlation between kidney α2u-globulin concentration, protein droplet accumulation and renal cell proliferation in male rats and not in females.

- Exclusion of alternative MoA

Two other criteria have been established by IARC, 1999:

- Lack of genotoxicity: no evidence of genotoxicity was observed based on the available data;
- Specificity in male rat for nephropathy and renal tumorigenicity: tumours occurred in male rats but not in female rats. Nevertheless, in the carcinogenicity study, a statistically significant dose-related increase in chronic nephropathy was observed in females at ≥ 450 ppm with increased severity compare to control at the top dose. Moreover, in repeated dose toxicity studies, renal tubular cell hyperplasia was noted in one out of six females following 120-day exposure to 1 041 mg/kg bw of the substance in drinking water (Carnegie-Mellon Institute of Research, 1977a). Moreover, kidney was a target organ in monkeys as, following 90-day continuous exposure, one of the two male monkeys showed focal chronic inflammation of the kidney. Although these results are difficult to interpret due to the very low number of animals involved and the low dose used in the monkey study, these results support that nephropathy may not be specific to male rats. Therefore, the relation between chronic nephropathy MOA and tumour formation may involve other mechanisms.

According to the review of Doi *et al.* 2007, looking on NTP studies with 3 substances and their suspected α2μ-globulin MOA, the severity of chronic nephropathy correlated best with the pattern in tumour kidney response in male rats. As severity of nephropathy was also increased in female rats without correlated increase in tubular kidney tumours, these results support further that other MoA may be involved in kidney tumours.

Overall, RAC agreed with DS conclusion that α2u-globulin mediated MoA of male renal tubule tumours is plausible. Nevertheless, RAC noted that other MOA could not be excluded.

About renal mesenchymal tumours observed in female rats, these tumours are very rare and were not seen in concurrent NTP studies. A survey from Hard *et al.* 2016, on NTP studies, observed that this type of tumour was usually seen only as single tumour incidence in a single dose group even when more than 2 incidences occurred (tumour not restricted to the highest dose). This type of tumour when induced by genotoxic carcinogens was very invasive. Spontaneous tumours occurring with epigenetic carcinogens were also malignant. Overall, the occurrence of 2 cases of renal mesenchymal tumours could be considered of concern due to their malignancy and their very rare occurrence.

Adrenal gland tumours in rats

A dose-related increase in male pheochromocytomas (benign and malign) was observed in rats (8/50, 9/48, 11/50, 14/50 in 0, 450, 900 and 1 800 ppm, respectively). The incidences of tumours were not statistically significant and felt within the upper range of historical control range (10-28 %). Separate historical controls for malignant or benign pheochromocytomas in males were not reported. As the carcinogenicity study was performed in 2005, laboratory data from NTP (1995-2005) were slightly outside of the preferred 5-year period (reducing the relevance). A statistically significant increase in adrenal hyperplasia was also observed in male rats at 1 800ppm. An increase in adrenal weight was also seen in a sub-chronic gavage study in rats (Anonymous, 1986).

	Incidence in adrenal medulla (males) n=50			
Dose (mg/kg bw/d)	**0**	**450**	**900**	**1 800**
Hyperplasia	13	18	18	24*
Pheochromocytomas, malignant	0	2	1	2
Pheochromocytomas benign	8	7	10	12
Pheochromocytomas malign or benign	8	9	11	14

*statistically significant

Overall, in view of the low incidences of malignant tumours and as the tumours were in the range of historical controls, RAC considered that adrenal pheochromocytomas do not provide sufficient evidence of a carcinogenic effect of 4-methylpentan-2-one and are not considered further in this assessment.

Hematopoietic system tumours in rats

A positive trend in the incidence of mononuclear cell leukaemia was observed in male rats (50 %, 52 %, 64 % and 70 % at 0, 450, 900 and 1 800 ppm, respectively. The tumour incidence was statistically significant in the highest dose group and was marginally above the historical control range. Indeed, the HCD for this strain of rats indicate a rather wide range going up to 66 % (47 % mean, 32-66 % range) for mononuclear cell leukaemia. This type of tumour is thus commonly seen in F344 rats. The day of first incidence was similar among the control and in the high dose group. The NTP report concluded that the tumours may have been related to 4-methylpentan-2-one but pointed out that the strength of the response was insufficient to allow a definite conclusion.

	Mononuclear cell leukaemia in males				
Dose (mg/kg bw/d)	**0**	**450**	**900**	**1 800**	**HC**
Overall rate (incidence, n=50)	25/50 50 %	26/50 52 %	32/50 64 %	35/50 70 %	16/50 to 33/50
Estimated adjusted rate for intercurrent mortality (%)	52 %	59.1 %	67 %	72.6 %	
Terminal rate (incidence (%))	13/32 41 %	16/28 57 %	13/25 52 %	9/19 47 %	
First incidence (days)	468	595	205	544	

Overall, as the tumour is commonly seen in this strain of rat, was only observed in one sex and as the increase was only slightly above the HCD range, RAC considered that the increase of mononuclear cell leukaemia, as sole, does not provide sufficient evidence to justify classification.

Liver tumours in mice

An increase in hepatocellular adenoma and combined adenoma or carcinoma were observed in mice in both sexes. The increase was statistically significant at terminal sacrifice in the high dose group and a trend was identified in both males and females (poly-3 test). Although this is a common tumour in this strain of rat, the incidences in both male and females were increased

well above NTP concurrent historical control range. Moreover, although not statistically significant, an increase in hepatocellular carcinoma was observed above historical control range in female mice.

No effect on survival was observed in the study and only a slight decrease in body weight was seen in females at the top dose. No hepatocellular hypertrophy was noted in the livers of mice. No increase in liver cytotoxicity (e.g. necrosis) was either observed. Nevertheless, eosinophilic foci, suggestive of pre-neoplastic lesions were increased in both male and female mice.

Dose (mg/kg bw/d)	**Incidence (%)**									
	Males					**Females**				
	0	**450**	**900**	**1 800**	**HC**	**0**	**450**	**900**	**1 800**	**HC**
No. of animals	50	50	50	50		50	50	50	50	
Eosinophilic focus	6	8	10	16		8	22	20	28	
Hepatocellular adenoma	34	50	46	68**	30-46	26	30	40	46*	12-35
Hepatocellular carcinoma	24	24	20	18	18-32	12	10	12	22	8-12
Combined hepatocellular adenoma or carcinoma	54	68	56	74*	50-68	34	34	44	54*	22-39

** $p < 0.01$; *$p < 0.05$;

Potential human non-relevance of these tumours were assessed by the DS. Mechanistic studies were provided to investigate a potential CAR-mediated MoA in mice. The postulated MoA was that the activation of CAR and PXR nuclear receptors in rats results in hepatic cell proliferation leading to hepatocellular tumours.

Five key events have been considered by the DS:

- CAR activation;
- Altered gene expression;
- increased cell proliferation, inhibition of apoptosis;
- Clonal expansion leading to altered foci;
- Liver adenoma/carcinoma.

In high throughput assays (TOXCAST), 4-methylpentan-2-one and 4-hydroxy-4-methyl 2-pentanone were positive in 1 out of 4 assays on PXR and 1 out of 5 assays on PXR, respectively. No positive assay for CAR activity was found.

In vivo, CAR activation has been investigated by Hughes *et al.* 2016, in a 10-day toxicity study in male and female rats. A single dose by inhalation was tested (equivalent to the top dose used in the carcinogenicity study). Altered gene expression was noted as hepatic CYP 2B10 and CYP 3A11 mRNA levels were significantly increased (about 200-fold and 4-fold, respectively) in females and (980-fold and 235-fold) in males. Enzyme activity was not reported in this study. Associated events to CAR/PXR activation were noted. Indeed, increased liver weight and slight hypertrophy were noted in both males and females. A statistically significant increase in hepatocellular proliferation was found in B6C3F1 male and female mice (strain used in the carcinogenicity study). The increase was not statistically significant in female C57BL/6 mice also used in the study.

In a previous study (Anonymous, 2009), male mice were treated at 1 800 ppm, for 7 days. Increased CYP 2b10 transcript levels and enzyme activity was observed (4-fold) and slight hypertrophy and hepatocyte proliferation were noted.

CYP P450 enzymes were not evaluated in longer-term studies. Liver weight was not investigated in the carcinogenicity study from NTP study. Nevertheless, liver weight changes were seen in the prenatal-developmental toxicity study in mice at 3 000 ppm.

Although hepatocellular proliferation was not investigated in longer-term studies, an increase in a pre-neoplastic lesion (altered foci) was observed in both males and females at the top dose in the mice carcinogenicity study.

- Exclusion of alternative MOA

An *in vivo* mice CARKO/PXRKO double knockout study (Hughes *et al.* 2016) showed that the presence of functional CAR and/or PXR appeared essential for the initial hepatic proliferative response from 4-methylpentan-2-one in both sexes. Indeed, in contrast with the results observed in the *in vivo* study performed with wild-type rats, no cell proliferation was observed at 1 800 ppm in both males and females. Enzyme gene expression were in all treated groups comparable to control. Nevertheless, increase in liver weight and very slight hypertrophy were noted in CARKO/PXRKO knockout mice.

No evidence of genotoxicity is available for 4-methylpentan-1-one.

In vitro, an embryo cell transformation assay was available in mice (O'Donogue *et al.* 1988). The results of the assay was considered negative by the authors. Nevertheless, a positive result was observed in one experiment that was not confirmed in a second experiment.

No evidence of activation of PPARα or AhR activation was noted in the 10-day study (based on CYP 1A1 and CYP 4A10 gene expression profile.

There is no data in the dossier suggesting that other MoA such as Porphyria, statins/altered cholesterol synthesis, estrogenic activity and immunosuppression would be likely for 4-methylpentan-2-one.

No histopathological findings suggestive of cytotoxicity were observed in the carcinogenicity study in mice.

Overall, RAC agrees with the DS that the proposed MoA is plausible in male and female mice. Nevertheless, the MoA is not sufficiently investigated. Some limitations were noted in the studies and some uncertainties remain:

- Absence of dose-response data for CAR/PXR activation (single dose tested);
- No activation of CAR in high throughput assay data;
- no positive control in the *in vivo* Hughes *et al.* 2016 study;
- enzyme activity was not measured in the *in vivo* mice study (Hughes, 2016);
- No *in vivo* CAR/PXR knock out animals were used to confirm the *in vitro* results;
- Increase in liver weight and hypertrophy in CAR KO mice indicates uncertainties whether CAR activation is the exclusive MOA.
- Human relevance has not been investigated (e.g. *in vitro* studies using human hepatocytes, humanized mice). Potential quantitative differences in the activation of CAR has thus not been investigated.
- In rats, 4-methylpentan-2-one has been showed to increase the total amount of CYP liver and (CYP2E, CYP1A, CYP2B) kidney enzymes according to the summary report from IARC. This increase has been associated with a potentiating effect of the substance on hepatotoxicant, neurotoxicant and nephrotoxicant. Hepatocellular hypertrophy and liver weight changes were also observed in rats. No tumour induction were observed in rats. Potential rodent species differences has not been investigated.

Comparison with CLP criteria

As there is no evidence of carcinogenicity in human reported in the dossier, category 1A is not appropriate. There is evidence of tumour formation in rodent species (mice and rats). Thus, a classification may be appropriate. The following factors, including human relevance, can influence the outcome of the classification:

Factor	Evidence with 4-methylpentan-2-one	Conclusion
Tumour type Considering background incidence and HCD	Liver adenoma and carcinoma in B6C3F1 mice High spontaneous tumour Above historical control	Supportive of classification
	Mesenchymal renal tumours in F344 rats Small increase. Uncommon tumours. Not found in HC.	Supportive of classification
	Tubular kidney tumours Above HCD range	Supportive of classification
Multi-site responses	Systemic tumours were produced at the different sites in rats	Supportive of classification
Progression of lesions to malignancy	Malignant tumours (kidney mesenchymal tumours, hepatocellular carcinoma) were reported in rats and mice	Supportive of classification
Reduced tumour latency	Not investigated	
Whether responses are in single sex or both	Both sexes in rats and mice reported tumours	Cat. 1B
Whether responses are in a single species or several	Tumour formation occurred in rats and mice	Cat. 1B
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	Not investigated	-
Routes of exposure	Inhalation routes of exposure produced tumours.	Supportive of classification
Comparison of ADME between test animals and humans	Not species specific differences identified	Supportive of classification
The possibility of a confounding effect of excessive toxicity at test doses	No excessive toxicity was found in mice or rat	Supportive of classification
Mode of action and its relevance for humans	CAR-mediated MoA of liver tumours in mice Non relevance to human	MoA plausible but unresolved question Cat. 2
	α2μ-globulin nephropathy MoA of kidney tumours in male rats	MoA plausible but unresolved question Cat. 2
	Mesenchymal renal tumours in F344 rats	Relevant to human

4-methylpentan-2-one is not genotoxic.

CAR-mediated MoA of liver tumours in male and female mice is seen as plausible. Nevertheless, human relevance has not been investigated (in vitro test) leading to some uncertainties on the MoA.

α2u-globulin nephropathy MoA of kidney tumours in male rats is plausible but other MoA could have been involved in tumour formation.

Mesenchymal malignant kidney tumours in female rats were not statistically significant and only observed in 2/50 animals. Nevertheless, as the tumours were malignant and very rare, this type of tumour could be of concern.

Overall, RAC agrees with the DS's proposal to **classify 4-methylpenta-2-one as Carc. 2; H351.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

Based on a two-generation reproductive toxicity study (Nemec, 2004), no classification was proposed by the DS as no treatment-related effects were observed on sexual function or fertility.

Developmental toxicity

Two prenatal developmental toxicity studies, performed via inhalation, were available in the dossier, one in rats and one in mice (Tyl, 1987). In these studies, foetal body weight was decreased at the top dose (3 000 ppm equivalent to 12.3 mg/L) in both mice and rats. Statistically significant increase in delayed ossification was also observed in both species at the top dose. No malformations were observed. The developmental toxicity was seen in presence of maternal toxicity: reduced body weight and food consumption, mortality in mice. In the 2-generation reproductive toxicity study, no developmental toxicity was observed. Based on these studies, no classification was proposed by the DS.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

Fertility

In a 2-generation reproductive toxicity study, no effects of reproductive parameters were observed up to 2 000 ppm (whole body inhalation exposure of vapour). At this dose level, parental toxicity was observed (liver hepatocellular hypertrophy in males, nephropathy and CNS symptoms). In this study, absolute and relative seminal gland weight in F0 males and ovary weight in F0 females were increased at 2 000 ppm. These findings were not correlated with histopathological findings and were not seen in subsequent generations. Moreover, in repeated dose toxicity studies, no effects on reproductive organs were seen. Overall, RAC agrees with the DS that no classification is warranted for 4-methylpentan-2-one for effects on sexual function and fertility.

Lactation

No relevant effects were seen.

Developmental toxicity

The potential for 4-methylpentan-2-one to induce effects on development was investigated in two guideline developmental toxicity studies, one in rats and one in mice (Tyl *et al.* 1987) *via* vapour inhalation (whole body exposure).

<u>Rats</u>

In rats, there were no statistically significant increases in the incidence of external, visceral, skeletal, or total malformations in the foetuses. A significant decrease in foetal body weights and skeletal variations (delays in ossification) were seen at the high dose 3 000 ppm. Some statistically significant delay in ossification was also seen at 300 ppm but was not considered treatment related as no dose-response was observed. A notable finding was that the delayed

ossification were associated with a dose-related increase in foetal skeleton appearing fragile (0 in control and low dose, 5 in 1 litter at 1 000 ppm and 116 in all litters at 3 000 ppm). Although the exact significance of this finding is unclear, this is consistent with developmental delay observed in the high dose group. Maternal toxicity at 3 000 ppm consisted of reduced body weight during treatment and body weight loss (GD6-9), food consumption and clinical signs (including piloerection, loss of coordination, partial hindlimb paralysis, negative tail and/or toe pinch).

ppm	0	300	1 000	3 000
Foetal body weight per litter (g)	4.46	4.33*	4.39	4.18***
Cervical centrum 6, poorly ossified, % in foetuses (% in litters)	40 (79.2)	55 (100)	41 (92)	51 (87)
Anterior arch of atlas, unossified, % in foetuses (% in litters)	3.6 (16.7)	9 (34.6)	2.4 (12)	35 (73.9)*
Thoracic centrum 13, bilobeb	17 (63)	24 (69)	29 (80)	39 (91)*
Proximal phalanges, unossified	13 (46)	11 (46)	15 (44)	47 (87)*
Metatarsal of hindlimb, poorly ossified	2.7 (8.3)	5.2 (15)	8.7 (32)	25 (52)*
Sternebrae5, unossified	0.9 (4.2)	2.2 (12)	3.2 (16)	5.1 (26)*
Unilateral rudimentary ribs	2.7 (12.5)	1.5 (7.7)	3.2 (16)	13.6 (43.5)*

* $p > 0.05$

Mice

In mice, a statistically significant increase in the mean number of dead foetuses per litter at 3 000 ppm was observed (0.6 per litter compared to 0.1 in controls). Foetal body weight was significantly reduced at 3 000 ppm. An increase in the incidence of dilated lateral ventricles of the cerebrum and of dilated renal blood vessels were observed at 3 000 ppm. Moreover, an increased incidence of reduced ossification was observed at 3 000 ppm including vertebrae, sternebrae, limbs and skull plates. There was no statistically significant increase in the number of foetuses or of litters with one or more foetuses with individual malformations, pooled external, visceral, skeletal, or total malformations in any treatment group relative to controls.

Maternal toxicity at 3 000 ppm consisted of mortality (3 out of 25 mice died on GD 6 following first exposure), two dams at 300 ppm and 3 at 1 000 ppm delivered early (no further details). No maternal body weight differences were observed at any time point (absolute or corrected for gravid uterine weight). Increased body weight gain was observed at 3 000 ppm at the interval GD 6-9. Effects on food consumption was not reported. CNS symptoms were noted at 3 000 ppm only during exposure. Marked liver increase was noted in mice at this dose level.

ppm	0	300	1 000	3 000
Live foetuses per litter (means)	10	10.6	10.4	10.9
Early resorption per litter (means)	1.4	1	0.9	0.9
Late resorption per litter (means)	0.1	0	0.1	0.1
Dead foetuses per litters (means)	0.1	0.2	0	0.6*
Foetal body weight (means)	55.2	51.7	53	50

* $p > 0.05$ in comparison with control

Comparison with criteria

RAC supports the conclusion of the DS that no classification for developmental toxicity is warranted. The developmental effects observed in the top dose in rats represent variations and developmental delays rather than malformations, occurring together with maternal toxicity. In mice, the effects at the top dose (increased number of dead foetuses, skeletal variations) occurred together with severe toxicity (e.g. mortality).

Summary

Overall, RAC agrees with the DS that **no classification is warranted for reproductive** toxicity for 4-methylpentan-2-one.

RAC evaluation of Aspiration hazard

Summary of the Dossier Submitter's proposal

Aspiration hazard has been identified in two animal studies in rats. In the study of Panson *et al.* 1980, all animals (6/6) died following single intratracheal administration of the substance. In the study from Exxon chemical company, 1982, deaths (unknown number) were observed following placing the substance in the oral cavity of the animals.

In human, no evidence of aspiration hazard were available.

As the criteria for aspiration hazard are based on reliable human evidence or physico-chemical properties of the test substance, no classification was proposed by the DS.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC agrees that no relevant data in humans were available in the dossier and the substance is not a hydrocarbon. Therefore 4-methylpentan-2-one **does not fulfil the CLP criteria for aspiration hazard.**

Additional references

Hard, G *et al.* A survey of mesenchyme related tumours of the rat kidney in the national toxicology program Archives, with particular references to renal mesenchymal tumour. Toxicologic Pathology 2016, vol. 44(6) 2016.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).