Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde

EC Number: 203-600-2
CAS Number: 108-62-3

CLH-O-0000001412-86-171/F

Adopted

22 September 2017

22 September 2017

CLH-O-0000001412-86-171/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: **2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde**

EC Number: **203-600-2**

CAS Number: **108-62-3**

The proposal was submitted by **Austria** and received by RAC on **14 June 2016.**

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 **9 August 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 September 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

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 **22 September 2017** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	605-005-00-7	2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde	203-600-2	108-62-3	Flam. Sol. 2 Acute Tox. 4*	H228 H302	GHS07 GHS02 Wng	H228 H302			
Dossier submitters proposal	605-005-00-7	2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde	203-600-2	108-62-3	**Retain**: Flam. Sol. 2 **Modify**: Acute Tox. 3 **Add**: STOT RE 2	**Retain**: H228 **Modify**: H301 **Add**: H373 (Oral)	GHS02 GHS06 GHS08 Dgr	**Retain**: H228 **Modify**: H301 **Add**: H373 (Oral)			
RAC opinion	605-005-00-7	2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde	203-600-2	108-62-3	**Retain**: Flam. Sol. 2 **Modify**: Acute Tox. 3 **Add**: Repr. 2 Aquatic Chronic 3	**Retain**: H228 **Modify**: H301 **Add**: H361f H412	GHS02 GHS06 GHS08 Dgr	**Retain**: H228 **Modify**: H301 **Add**: H361f H412			
Resulting Annex VI entry if agreed by COM	605-005-00-7	2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; metaldehyde	203-600-2	108-62-3	Flam. Sol. 2 Repr. 2 Acute Tox. 3 Aquatic Chronic 3	H228 H361f H301 H412	GHS02 GHS06 GHS08 Dgr	H228 H361f H301 H412			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Metaldehyde is a molluscicide for the control of slugs and snails. It was approved in 2008 for Annex I listing as a 3A Review compound under Council Directive 91/414/EEC, with Austria as Rapporteur Member State.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Information from humans

Numerous case reports and notifications to Poison Centers on poisonings after accidental or suicidal intake of metaldehyde were documented by the Dossier Submitter (DS). The reported incidents involved 189 children and 24 adults. Of these, 122 intoxications were due to ingestion of metaldehyde tablets (pure metaldehyde), and 101 cases to snail pellets (containing 5 - 7% metaldehyde). The severity of the poisoning was known in 128 cases: 87 cases were mild, 25 moderately severe, 14 severe and 2 were fatal. From these cases, 22 case histories were presented in the CLH dossier. The course of the intoxication is characterised by a first phase involving gastrointestinal effects such as nausea, salivation, vomiting and later abdominal pain and diarrhoea. This phase may be followed by convulsions, somnolence, coma, apnoea, cyanosis, memory loss and decreased blood pressure.

Acute toxicity

Oral

The substance has an existing minimum classification for acute oral toxicity as Acute Tox. 4*.

The Dossier Submitter presented 3 acute oral toxicity studies. Two were OECD TG 401 compliant using rats and mice (Jones and Collier, 1987 and Coles, 1990, respectively) and the third was a TG 425 compliant study (Durando, 2009).

The two acute oral toxicity tests in rats showed that metaldehyde is of moderate acute oral toxicity. In mice, moderate acute oral toxicity (LD_{50} values of 411 mg/kg bw and 443 mg/kg bw in males and females, respectively) was demonstrated. The lowest LD_{50} value of 283 mg/kg bw for acute oral toxicity was found in rats (calculated for both sexes, Jones and Collier, 1987). At dose levels from 100 mg/kg bw and above, non-specific symptoms such as reduced activity and lethargy, hunched posture and decreased respiratory rate, but also convulsions and ataxia were observed in both species tested. Surviving rats and mice recovered by day 8 after treatment. Target organs in these studies were the lungs, liver and gastrointestinal tract.

Dermal

In a dermal acute toxicity study of limited validity (only partly compliant with OECD TG 402), groups of 5 rats/sex received a topical application of metaldehyde (without data on purity or batch no.) suspended in water at dose levels of 0 (vehicle control) and 5000 mg/kg bw (Davies and Collins, 1974). No mortality was observed during the 14-day observation period. The only signs of clinical toxicity were slight lethargy and piloerection on the day of treatment (the number

of animals affected was not given). There were no local reactions, such as erythema or oedema. Terminal autopsy revealed darkening of the liver and spleen together with pale or mottled kidneys (incidences were not reported)

Comments received during public consultation

Two MSCAs agreed with the proposed classification for acute oral toxicity.

Assessment and comparison with the classification criteria

Human information

Data was available on numerous cases of human intoxications after single accidental or suicidal oral intake. These case reports do not provide sufficient detail to enable the dose swallowed to be estimated, except in one case report stating that 29 g (appr. 330 mg/kg bw) was ingested (Longstreth and Pierson, 1982). Thus, it cannot be assessed whether human data would justify a lower category than category 3 resulting from the lowest LD_{50} estimated in animals (see below).

Oral

The lowest LD_{50} value of 283 mg/kg bw for acute oral toxicity was found in an OECD TG 401 compliant study in male/female rats dosed at 100-800 mg/kg bw in arachis oil (Jones and Collier, 1987). According to Regulation (EC) No. 1272/2008, metaldehyde therefore meets the criteria for classification in acute toxicity category 3 (50 < ATE ≤ 300 mg/kg bw);H301 "Toxic if swallowed".

The most recent, OECD TG 425 compliant study (Durando, 2009) was considered to provide supportive information. It was conducted on female rats only with the highest dose of 1110 mg/kg bw (in water) and with two test groups given 350 mg/kg bw using water and corn oil as vehicle. The LD_{50} was estimated to be 654 mg/kg bw.

The classification proposal is supported by observations from two acute neurotoxicity studies of mortalities in rats after single oral administration of 250 mg/kg bw (Haferkorn, 2009; Jones, Finn, Mullee, 2003).

Additionally, in a developmental toxicity study (Neeper-Bradley and Chun, 1990), mortality (6/25) was observed in pregnant dams in the initial 1-2 days of treatment (150 mg/kg bw by oral gavage).

Inhalation

The available acute inhalation study (Berczy *et al.*, 1973) was of limited quality and did not allow to estimate a LC_{50} value. Based on this information, no conclusion on the need for classification can be drawn for this endpoint.

Dermal

Taking the limitations of the acute dermal study in rats into account and the absence of mortalities during the 14 days observation period, it is concluded that these data do not support a need for classification for this endpoint.

Overall, RAC agrees with the proposal of the DS, and considers that **metaldehyde should be classified as Acute Tox. 3; H301**.

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

Summary of the Dossier Submitter's proposal

The Dossier Submitter concluded that there was no evidence of any specific, non-lethal target organ toxicity arising from a single exposure to metaldehyde. Reversible clinical signs of toxicity were observed after single exposures to metaldehyde, but these were considered to be non-specific signs related to general acute toxicity.

The CLH report evaluates neurotoxic effects from acute and repeated neurotoxicity studies in Chapter 4.12.1.1 (Other effects). The DS concluded that neurofunctional effects (e.g. tremor, convulsions, ataxia, paresis) following exposure to metaldehyde are considered to occur only at doses which are clearly acutely toxic. These effects did not persist (with the exception of hind limb paresis following spinal cord injury) and were not consistent with sustained dysfunctions normally induced by classical neurotoxins. Because of the distinct differences between metaldehyde and classical neurotoxins, the term "neurotoxic effect" was not considered adequate to characterize the toxicity profile of metaldehyde. Therefore in the EFSA peer review, it has been concluded that metaldehyde does not require classification as a neurotoxicant. The conclusion drawn by EFSA is as follows: "*Acute toxic effects following metaldehyde administration include partly pronounced neurological symptoms, without specific neurotoxic mechanism leading to degeneration or other toxic damage to the central or peripheral nerve tissue. Therefore, these reversible effects at high doses are not relevant for classification and labelling as neurotoxicant*."

One acute inhalation toxicity study of limited validity (Berczy, 1973) showed some effects suggesting irritation of the respiratory system: eye irritation, dyspnoea, sneezing, discomfort and increased nasal and oral secretion were seen in animals exposed to high dust concentrations. After exposure, these signs of irritation to the respiratory system disappeared within one hour.

No classification was proposed for STOT SE.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification for STOT SE.

Assessment and comparison with the classification criteria

No effects other than reversible non-specific clinical signs were reported in the acute toxicity studies at doses without mortalities.

Neurological abnormalities at the lethal dose of 250 mg/kg bw and at the non-lethal dose of 150 mg/kg bw from two acute neurotoxicity studies may be indicative of neurotoxicity. However, as 150 mg/kg bw is close to the lethal dose (factor < 2) and comparable abnormalities were seen at lethal/non-lethal doses, the neurotoxic effects are considered to be covered by the acute toxicity classification.

RAC considers that dyspnoea, sneezing, discomfort and increased nasal and oral secretion could be indicative of an transient irritative response of the respiratory tract. However, 15 mg/L was tested in the acute inhalation study and at this high dose it remains uncertain whether the high particle load caused the effects or whether it could be a substance-related (sensory) irritation. No information on lower test concentrations were available. RAC agrees with the Dossier Submitter that **no classification for STOT SE is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The Dossier Submitter summarised data from Lisi *et al.* (1987) indicating that 442 persons (a third of them were current or former agricultural workers) tested with 1% metaldehyde in patch tests showed neither irritant nor allergic reactions.

A study on skin irritation/corrosion in rabbits (similar to OECD TG 404) with occlusive administration of 0.5 g metaldehyde did not reveal any skin reactions after 60 minutes or 1, 2 and 3 days after treatment (Jones, 1983). The Dossier Submitter proposed no classification.

Comments received during public consultation

One MSCA agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agrees with the proposal of the Dossier Submitter that based on the available data **no classification as a skin irritant is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In an acute eye irritation test, which was compliant with OECD TG 405, slight irritation to the eyes of rabbits were observed. Iridial inflammation (grade 1) was noted in all treated animals 1 h after treatment but no longer at 24, 48 and 72 h. Minimal conjunctival irritation (grade 1) was noted in all treated eyes 1 and 24 h after treatment. Conjunctival chemosis and discharge were found only 1 h after treatment. All treated eyes appeared normal after 48 h. No corneal effects were noted during the study.

The Dossier Submitter proposed no classification as the estimated scores (24 – 72 h) were 0 (conjunctival chemosis), 0.33 (conjunctival redness) and 0 (iritis) and 0 (corneal opacity), and these scores did not meet the criteria for classification in the CLP Regulation.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agrees with the Dossier Submitter's proposal that based on the low severity grades of irritation and oedema of the conjunctiva and the reversibility of the mild effects after 1 h or 24 h, **no classification for eye irritation/eye damage is warranted**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The Dossier Submitter referred to the publication of Lisi *et al.* (1987), who reported that 442 persons (a third of them were current or former agricultural workers) tested with 1% metaldehyde in patch tests showed neither irritant nor allergic reactions.

The CLH report presented data from a Buehler test of limited validity conducted in guinea pigs (Nitka, 1984) and two Local Lymph Node Assays (LLNA) in mice (Bull, 2007; Dreher, 2008).

In the main study of Bull (2007), doses of 25 µL metaldehyde in 5%, 10% or 25% w/v preparations in acetone: olive oil (4:1 v/v), were administered onto the dorsal surface of the ear. This resulted in isotope incorporation of 3H-methylthymidine marker at ratios of 0.4, 0.9 and 1, while the positive control (10, 25, and 50% hexyl cinnamic aldehyde) revealed stimulation indices (SI) of 7.9 and higher.

SI of 0.6, 1.0 and 1.5 at concentrations of 1, 2.5 and 5% metaldehyde in DMSO were seen in the second LLNA (Dreher, 2008).

The Dossier Submitter concluded that metaldehyde was not sensitising in two LLNA and one Buehler test of limited validity and no classification was proposed.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agreed with the Dossier Submitter that the SI of ≤ 1.5 from the two LLNA are below the SI of 3 that may trigger classification.

The Buehler test cannot be considered for classification purposes as insufficient detail information was provided in the CLH report.

The negative Patch test findings of the Lisi study were consistent with the negative LLNA results.

No classification for skin sensitization is warranted.

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

Summary of the Dossier Submitter's proposal

The DS summarised the metaldehyde effects observed in repeated dose oral toxicity studies on rats, mice, dogs and rabbits in comparison with guidance values (see the Table below and Table 43, CLH report).

Mortalities and testicular toxicity seen in chronic studies in dogs were found by the DS to be adverse effects to justify classification in Category 2 for Specific target organ toxicity – repeated exposure, H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)".

The study results were summarised as follows:

In the histopathological examination in the 26-week dog toxicity study, diffuse atrophy of the testes was found in the mid (2/6 males) and high dose group (4/6 males). A histopathological re-examination was performed in 2009, when also severity scores were investigated. The re-examination confirmed the pattern of testicular findings: the severity score of focal findings was increased at 60 mg/kg bw/day while at the high dose of 90 mg/kg bw/day, a clear increase in diffuse atrophic changes was found.

In the 52-week study in Beagle dogs (10, 30 and 90 mg/kg bw/day), mortality occurred in the mid and high dose group which was considered treatment-related. All animals showed histological changes of testes at the highest dose (90 mg/kg bw/day), which consisted of mainly moderate to marked diffuse atrophy and/or degeneration of the germinative epithelium. At the two lower dose levels (10 and 30 mg/kg bw/day), findings were more focal and of minimal to mild severity and were thus considered to be spontaneous in nature and morphologically distinct from the treatment related findings seen at 90 mg/kg bw/day. Historical background data for atrophy and degeneration of the germinative epithelium demonstrated that incidences observed at the low and mid dose levels were comparable to historical control data.

No data on repeated inhalation toxicity was available.

No treatment-related effects were found at any dose in a 21-day dermal study in rabbits (Hermansky and Wagner, 1991). Slight local irritation was observed in control and treatment groups which was attributed to dosing and occlusion procedures.

Comments received during public consultation

Two comments from Industry expressed disagreement with the classification proposal, the interpretation of the effects in the dog studies and the adjustment of cut-off levels for the effects seen in dogs.

In one comment it was indicated that the pathology report of the 52-week dog study speculated that the cause of deaths were secondary to pulmonary exposure as a result of emesis. Effects on the testes were interpreted to be unrelated to treatment.

Two MSCAs agreed with the proposal to classify the substance as STOT RE 2; H373.

Assessment and comparison with the classification criteria

The available studies on repeated oral toxicity were presented in Table 43 of the CLH report and these data are presented in modified form in the Table below.

Table: Summary of effects observed in rats, mice, dogs and rabbits in comparison to guidance values (corresponding to Table 43 in the CLH Dossier, modified)

Species-Route (Reference)	Study duration	Guidance value Cat 1 STOT RE (1272/2008) [mg/kg bw/day]	Guidance value Cat 2 STOT RE (1272/2008) [mg/kg bw/day]	Effects at or below guidance value	Significance of toxicological effect (1272/2008) below guidance value
Rat- oral (van Miller, 1989) 0, 2500, 5000, 10000 and 20000 ppm/diet (≈ 0, 197, 382, 761 and 1547 mg/kg bw/day for	28 days	30	300	≥ 197 (M)- 233 (F) mg/kg bw/day: ↑ liver weights, hepatocellular	Changes in liver weight, no evidence of organ dysfunction (to the extent examined by clinical chemistry) Dose finding study, some deviations (limited

M; 0, 233, 454 and 875 mg/kg bw/day for F)					histopathology at ≥ 10000 ppm only), supplementary information only
Rat- oral (Thomas et al., 1998) 0, 250, 750 and 2500 ppm/diet (≈ 0, 21, 65 and 215 mg/kg bw/day for M and F)	90 days	10	100	≥ 65 mg/kg bw/day centrilobular hepatocyte enlargement (minimal at 65 mg/kg bw/day, slight at 215 mg/kd bw/d)	No evidence of organ dysfunction (from haematology, clinical chemistry and urinalysis)
Mouse- oral (Gill and Wagner, 1990) 0, 100, 300, 1000, 3000 and 10000 ppm/diet (≈ 0, 19, 54, 178, 560 and 1919 mg/kg bw/day for M; 0, 24, 70, 235, 743 and 2996 mg/kg bw/day for F)	90 days	10	100	≥ 19 (M)-24 (F) mg/kg bw/day: hepatocellular hypertrophy, necrosis, inflammation, anisokaryosis - ≥ 54 (M)-70 (F) mg/kg bw/day: ↑ liver weight, vacuolisation, bilary hyperplasia Liver lesions were minimal at 100 and 300 ppm	Liver lesions of minimal severity at 100 and 300 ppm - No conclusion on organ dysfunction can be drawn Dose range finding study for the oncogenicity study, limited investigations (no haematology, clinical chemistry or urinanalysis, histopathology on 10 animals of the control and 10000 ppm group), Supplementary information only
Dog- oral (diet) (Leuschner,2002) Escalating doses in 2 M and 2 F: 30, 60, 75 and 90 mg/kg bw/day, (each dose administered on 3 consecutive days followed by 2 wash-out days) Fixed doses (2 M and 2F/each dose): 75 and 90 mg/kg bw/day, 28 days Dose finding study, no histopathologic examination	28 days	30*	300*	- ≥ 60 mg/kg bw/day: reduced motility, clonic convulsions, increased respiratory rate, emesis - ≥ 75 mg/kg bw/day: tonoclonic convulsions, mydriasis, inflated stomach, slight tremor - 90 mg/kg bw/day ataxia, salivation, abdominal/lateral position, pale gingiva; moribund condition of 1 female (of the escalating dose group): 4 h post adm. shaking of the head, lateral position, difficulty in breathing - no pathological (macroscopic) findings after necropsy	The intensity/ severity of the symptoms of the fixed dose group declined with time and had almost disappeared towards the end of the 4-week treatment. All the effects were observed starting from 20-60 min after administration and lasted up to 6 hours. Moribund condition (dosing stopped after first administration) of 90 mg/kg of the escalating dose group which started with 90 mg/kg): already covered by acute toxicity classification
Dog- oral (diet) (Neumann, 1980, 1991, Leuschner, 2009) 0, 20, 60 and 90 mg/kg bw/day	26 weeks	5*	50*	- ≥ 60 mg/kg bw/day: **diffuse atrophy pf the prostate (4/6, 2/6)**, **moderate atrophy of the testes (1/6), mild to moderate focal**	**Severe organ damage (prostata and testis)**

Re-evaluation of histopathological findings in the testes Leuschner, 2009				**atrophy of the germinative epithelium (3/6),** parasitic granuloma in mesenteric lymph nodes - 90 mg/kg bw/day: mild to moderate diffuse atrophy of the germinative epithelium, follicular hyperplasia mesenteric Lymph nodes	
Dog- oral (diet) (Leuscher, 2003) 0, 10, 30 and 90 mg/kg bw/day Re-evaluation of Histopathological findings in the testes, (Leuschner and Drommer, 2009)	52 weeks	2.5*	25*	- ≥ 30 mg/kg bw/day: **mortality (1 M and 1F at 30 mg, 1 F at 90 mg/kg), days 260-322** ≥ 30 mg/kg bw/day **Prostate mild atrophy 1/4M, 3/4M** **Testes: minimal to moderate atrophy/ degeneration in 2/4M at 30 mg/kg** and mild to marked diffuse atrophy and/or degeneration of the germinative epithelium in 4/4 M at 90 mg/kg - 90 mg/kg bw: ataxia, reduced motility, emesis, tremor, twitching, salivation -, transient hearing loss, ↑AP	Incidence and severity of clinical signs at 90 mg/kg declined from study week 19 onwards. No other changes in histopathology. **Mortality** **Severe organ damage (testes/prostate)**
Dog oral (Gauvin, 2010) 0, 1, 3.5 and 15 mg/kg bw/day	52 weeks	2.5*	25*	≤ 15 mg/kg bw/day: no effects	No clinical macroscopic or microscopic signs (no information on the list of organs examined)
Rat oral (diet) (Jones, Finn, Mullee, 2003) 0,100, 500, 2500 ppm (0, 8, 39, 185 mg/kg/d)	90 days	10	100	2500 ppm: In 1 female, loss of hind limb function together with an increased respiratory rate	OECD TG 242 (Neurotoxicity) study
Rat** oral (diet) (Gauvin, 2010) 0, 250, 750 and 2500 ppm	90 days	10*	100*	2500 ppm: 1 death in 1/10 males Day 7 liver weight changes (M) and microscopic hepatocellular centrilobular hypertrophy (M+F)	Liver effects OECD TG 242 (Neurotoxicity) study
Rabbit- oral (gavage) (Neeper-Bradley, 1990a)	Develop mental (12 days of dosing)	30	300	Dams: 100 mg/kg bw: 1/5 tremor, 1/5 hypoactive and death	No gross lesions observed. No dose response relationship to mortality after administration of repeated doses

*values for experimental animals as described in 1272/2007. The DS noted in the CLH Dossier: for guidance values in dog studies, the only available document is ECBI/64/06 "Dose limits for classification with R48 based on dogs studies", 2006. In this document it was proposed that the cut off values for dog studies should be below the limit dose for the rat.
** This study was mentioned in Table 43 (CLH report) as a dog study, but no study report on dogs was found in the CLH report. It is assumed that the data are from a combined toxicity and neurotoxicity study in Sprague-Dawley rats of the author, Gauvin (2010).

As has been the practice for other substances, RAC proposes to use the guidance values of the rodent studies also for the dog studies (unless there is a rationale to deviate from this practice). For the oral route, the guidance values to assist in Category 2 classification are 10 < dose ≤ 100 mg/kg bw/day based on 90-day studies (values adjusted for study duration in the Table above).

- As mortalities and testes/prostate atrophy/degeneration were seen in the repeated dose studies at dose levels above the guidance values, classification as STOT RE is not warranted.

Mortalities in repeated dose studies should be considered only if related to repeated dosing within the range of guidance values:

- In the 28-day study, the moribund status seen in one female at 90 mg/kg bw/day (clinical symptoms appeared 4 h after administration, the animal was killed 6 h after administration) is considered as an acute toxic effect.
- The neurological signs in rats that received doses of 30 mg/kg bw/day for 3 days (after two days of wash-out the next highest dose was applied) were reduced motility/convulsions/ataxia. These symptoms are also interpreted as neurotoxic effects (at lethal and non-lethal doses) of an acute nature.
- The interpretation of its acute nature is supported by the observations from the second part of the study (with a fixed dose regime). Similar neurological signs were reported to occur starting from 20-60 min after administration and these lasted up to 6 h.

In the 52-week dog study, the mortalities that occurred (2 at 30 mg/kg bw/day, 1 at 90 mg/kg bw/day, on days 260-322) were clearly unrelated to acute toxicity, but doses were above the guidance value for classification for STOT-RE and no dose-response relationship was seen.
Pneumonia may have contributed to the deaths. The female dogs that died prematurely (1 at 30 mg/kg bw/day and 1 at 90 mg/kg bw/day) showed moderate interstitial pneumonia and bronchopneumonia which were considered in the monograph as contributing to the deaths. Although the study authors regarded the deaths as substance-related, no other obvious cause of death could be determined.

- Diffuse atrophy of the prostate and atrophy of the testes were considered as serious organ damage that could be considered for classification if occurring within the range of guidance values.

As these adverse effects occurred at doses above the guidance value of 50 mg/kg bw/day for a 26 week study (25 mg/kg bw/day for 52 week), at 60 mg/kg bw/day in the 26 week study and at ≥ 30 mg/kg bw/day in the 52 week study, classification as STOT RE is not warranted. In addition, severity grades were minimal to mild in dogs at 10 and 30 mg/kg bw/day (except the one that died earlier with moderate testis toxicity) in the 52-week study and minimal to mild at 60 mg/kg bw/day in the 26-week study.

- No clear dose-response was observed for the diffuse prostate atrophy (4/6 males at 60 mg/kg bw/day, 2/6 males at 90 mg/kg bw/day) in the 26 week-study (see Table 31, CLH Report).

However, it is to be noted that the leading effect is the testes atrophy (with prostate atrophy as a secondary effect that could be more sensitive). While small foci of focal atrophy may occasionally occur in control animals, no clear increase in the fraction of animals affected but an increased mean severity of focal atrophy was seen at 60 mg/kg bw/day (see re-evaluated findings in Table 31, CLH report).

Increased rates of bilateral diffuse atrophy of the testes are the strongest evidence of a treatment-relationship (compared to indirect effects on accessory glands and focal testis atrophy). If observed at higher incidences than in controls (rarely seen as a spontaneous lesion), this lesion can be assumed to be treatment related. Four out of 6 male dogs at 90 mg/kg bw/day (26 week study, Leuschner 2009) showed diffuse bilateral testes atrophy with at elevated severity scores. This dose was clearly above the guidance value.

The low severity of focal testes atrophy (0.17 in Table 31, CLH report) given for 90 mg/kg bw/day in the 26 week study may be misleading. If 4 animals show a diffuse atrophy, the incidence of 1 animal with focal atrophy cannot be divided by the total number of males in the 90 mg/kg bw/day group. Animals presenting diffuse atrophy cannot show focal atrophy at the same time.

In the 52 week study, mild atrophy of the prostate in 1/4 males and minimal to moderate atrophy/degeneration of the testes in 2/4 males were seen at 30 mg/kg (Table 37, CLH report). Although the incidences/severity increased at 90 mg/kg bw/day, this effect does not warrant classification for STOT RE as 30 mg/kg bw/day and higher doses are above the guidance values (see also section 'Additional key elements' on reproductive toxicity).

- Several repeated dose toxicity studies in rats showed spinal injuries such as haemorrhage and necrosis of the spinal cord and most of these animals were found in moribund state with hind limb paralysis/paresis. These were explained as secondary traumatic lesions to the clinical signs of tremor and convulsions and were not considered as relevant for STOT RE classification.

In conclusion, the combination of minimum to mild testis/prostate toxicity and some mortalities with unclear relationship to the treatment at doses above the guidance values **do not warrant classification for STOT RE**.

The effects on testis toxicity were taken into consideration for the endpoint reproductive toxicity.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS came to the conclusion that a proposal to classify metaldehyde for mutagenicity is not justified on the basis of the available genotoxicity data.

In vitro tests

Metaldehyde was tested in a battery of *in vitro* genotoxicity testings including bacterial gene mutation tests (Ames tests), testing on induction of DNA damage in *Escherichia coli*, a mouse lymphoma assay (L5178Y mouse lymphoma cells) and a chromosomal aberration test (CHO cells). None of these *in vitro* tests indicated genotoxicity of metaldehyde.

***In vivo* test**

In addition, an *in vivo* micronucleus assay in mice showed no genotoxic potential of metaldehyde.

In conclusion, there was no indication that metaldehyde was genotoxic *in vitro* or *in vivo*. Therefore, the DS proposed that no classification as germ cell mutagen is required.

Comments received during public consultation

One MSCA agreed with the proposal for no classification for metaldehyde.

Assessment and comparison with the classification criteria

RAC concludes in agreement with the proposal of the DS that no classification for germ cell mutagenicity is warranted.

The available *in vitro* genotoxicity tests are negative. Based on the negative *in vivo* study (micronucleus test) no mutagenicity was induced in somatic cells (criterion for classification as Category 2). Taking into account its systemic availability metaldehyde is considered to be non-mutagenic *in vivo*. Information on induction of germ cell mutagenicity (criterion for classification as Category 1B) is not available.

RAC considers that metaldehyde does not meet the criteria for classification for mutagenicity as defined in the Regulation (EC) No. 1272/2008. Accordingly, **no classification as germ cell mutagen is warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In a Chronic toxicity/oncogenicity study (Gill and Wagner; 1992) in Sprague Dawley CD rats, which was compliant with OECD TG 453, the increase in hepatocellular adenomas was within the HCR.

In another oncogenicity study in CD-1 mice (Chun and Wagner, 1993), compliant with OECD Guideline 451, no carcinogenic potential of metaldehyde was assumed based on the results. Also, in the follow-up GLP compliant study (Beyrouty, 1998), toxicity and an increase in benign hepatocellular adenoma was observed but again no carcinogenic potential of metaldehyde was assumed.

Based on the studies mentioned above, the Dossier Submitter concluded that metaldehyde demonstrated no carcinogenic potential.

NB. No effects on survival, clinical signs, body weights, food consumption, organ weights, gross and microscopic pathology were reported unless indicated here as being treatment-related.

In a 104-week OECD TG 453 (diet) study on Sprague-Dawley (SD) rats (Gill and Wagner, 1992), absolute and relative liver weights were increased in female rats at the high dose of 5000 ppm. Dose related increases in the incidence and severity of hepatocellular hypertrophy were observed for male and female rats in the 1000 and 5000 ppm treatment groups. Hepatocellular adenomas were observed in 6/60 females (*please note that the table below indicates 7 adenomas in the initial liver histopathology*) of the high dose group compared with incidences of 1/60 and 0/60 in the two control groups, being statistically significant when compared to the second control group. In males, no hepatocellular adenomas were observed in metaldehyde treated animals.

Hepatocellular carcinomas were observed for some males and females in treated and control groups. The incidences were not dose-related. In males of the mid dose group, incidences of carcinomas reached statistical significance in the animals sacrificed in study week 104. When the combined incidences of hepatocellular adenomas and carcinomas were analysed statistically, females of the high dose group had a significantly higher incidence of tumours when compared to the second control group. Historical control data were supplied from two studies conducted at the same laboratory, using the same study design and source of animals. In study no. 1 incidences of hepatocellular carcinomas and adenomas were in the same range as in the present study while in study no. 2 the incidences were somewhat lower.

The results from initial and peer reviewed liver histopathology were summarised as shown in the table below:

Table: Initial and peer reviewed liver histopathology findings in SD rats (corresponds to Table 54, CLH Report)

	Dose group level ppm (mg/kg bw/day)									
	Males					Females				
	01* (0)	02** (0)	50 (2)	1000 (44)	5000 (224)	01 (0)	02 (0)	50 (3)	1000 (60)	5000 (314)
Initial liver histopathology#										
Adenoma	3	1	4	3	5	1	-	1	2	7
Carcinoma	-	-	-	2	1	1	-	1	-	-
Adenoma + carcinoma	3	1	4	5	6	2	-	2	2	7
Peer review liver histopathology										
Adenoma	2	-	-	-	-	1	-	1	-	5
Carcinoma	1	-	4	4	3	1	-	1	-	3
Adenoma + carcinoma	3	-	4	4	3	2	-	2	-	8

* Control group 1, ** Control group 2
# The incidences differ from the incidences in Table 53 of the CLH Dossier.

Some data on chronic toxic effects (increased relative liver weights in high dose male rats, lordosis and traumatic lesions in the spinal cord secondary to posterior paralysis in 5/25 high dose female rats, 1/25 male and 1/25 female of the mid dose groups, 1/25 low dose male), but no information on tumour data were presented from the second chronic study in rats with test concentrations of 200, 1000 and 5000 ppm in the diet over 107 weeks which was judged as of limited validity (Verschuuren *et al.*, 1975). Increased relative liver weights were observed in males (10-16 %) and females (21-32 %) in the 5000 ppm treatment groups.

In the first mouse study (Chun and Wagner, 1993), there was a seemingly dose related increase incidence of hepatocellular adenomas in male CD-1 mice (receiving metaldehyde in diet at concentrations of 0, 25, 100 or 300 ppm), but without statistical significance. The incidence slightly exceeded the HCD at the high dose of 300 ppm. No carcinogenic potential of metaldehyde was assumed based on the results of this study.
In this study, liver weights (absolute and relative to body weight and brain weight) was slightly but not significantly increased in metaldehyde treated males. The only non-neoplastic change which was attributed to treatment was an increase in the incidence of hepatocellular hypertrophy in males and females of the highest dose group.

Table: Carcinogenicity study in CD-1 mice; Histopathology findings in the liver (see Table 58, CLH Report)

	Dose group level ppm (mg/kg bw/day)									
	Males					Females				
	01* (0)	02** (0)	25 (4)	100 (16)	300 (49)	01 (0)	02 (0)	25 5)	100 (20)	300 (60)
Liver histopathology										
Adenoma	8	8	4	9	15	0	1	1	0	0
Carcinoma	1	3	6	3	3	0	0	0	1	0
Adenoma + carcinoma	9	10#	10	12	17#	0	1	1	2	0

* Control group 1; ** Control group 2
#Including one mouse with adenoma and carcinoma

In the second (supplementary) 18-month mouse study (Beyrouty, 1998), in both male and female CD-1 mice (from the same supplier) there was a statistically significant increase in hepatocellular adenomas at 1000 ppm (single dose study) compared to the concurrent control groups. No HCD is available for the second mouse study.

In this study, an increased incidence of metaldehyde-treated male mice with liver masses (15/60) was observed, compared to the control groups (6/60 and 9/60, respectively). Similarly, an increase in the incidence of metaldehyde-treated male mice with enlarged liver (7/60) was seen compared to controls (0/60 and 1/60, respectively). Both metaldehyde-treated male and female mice showed significant increases in liver weight (absolute, relative to body weight and relative to brain weight). The average magnitude of the increase in absolute liver weight was approximately 46 % for males and 14 % for females compared to the control groups.

Treatment-related histopathological lesions in the liver were observed for male mice, and to a lesser extent for female mice. Histopathological lesions included hepatocellular hypertrophy in male and female mice and single cell necrosis, focal or multifocal necrosis, pigment accumulation, sinusoidal histiocytosis and benign hepatocellular adenoma in male mice. It should be noted that in 3 males, both hepatocellular adenoma and carcinoma were found concurrently. A small increase in the incidence of female mice in the metaldehyde treatment group with hepatocellular eosinophilic cell foci or hepatocellular adenoma was observed. Hepatocellular hypertrophy, eosinophilic cell foci and hepatocellular adenoma observed in this study often shared similar cellular morphology and were considered to represent a continuum of changes over time. These changes are consistent with an adaptive hypertrophic response of the liver to an increase in metabolic demand, with subsequent development of proliferative changes and hepatocellular toxicity. Historical control data from two other studies conducted at the same laboratory show that hepatocellular adenomas have been reported in control group mice of the same strain, sex and source (Charles River Laboratories, Portage, MI) in incidences ranging from 6/60 to 13/60.

Table: Supplementary Oncogenicity Study in CD-1 mice; Non-neoplastic and neoplastic lesions in the liver (see Table 62, CLH Report)

	Dose group level ppm (mg/kg bw/day)					
	Males			Females		
	01* (0)	02** (0)	1000 (135)	01 (0)	02 (0)	1000 (163)
Non-neoplastic lesions						
Necrosis, single cell	15	12	43a,b	4	9	8
Necrosis	7	7	17a,b	5	6	9
Pigment accumulation	10	7	26a,b	21	25	16

Hypertrophy, hepatocellular	9	15	55a,b	3	3	17a,b
Histiocytosis, sinusoidal	1	7	55a,b	3	4	6
Eosinophilic cell focus	1	3	3	0	0	5a,b
Neoplastic lesions						
Hepatocellular adenoma (benign)	4	5	14a,b	1	0	5a,b
Hepatocellular Carcinoma (malign)	2	3	4	0	0	0

* Control group 1; ** Control group 2
a statistically significant difference relative to control group 1 ($p<0.05$)
b statistically significant difference relative to control group 2 ($p<0.05$)

The CLH Report referred to the PRAPeR 79 (Pesticide Risk Assessment Peer Review) expert meeting (July, 2010), where the experts decided not to propose classification for metaldehyde with the risk phrase 'R40' (limited evidence of a carcinogenic effect) based on the hepatocellular adenomas found in CD-1 mice (majority after vote). A summary of a Position Paper from Herder *et al.* (2010) interpreted hepatocellular adenomas as a common finding in male mice of this strain, referred to the incidence range in the control mice, the lack of dose-dependence and questioned the biological relevance of this tumour type in mice.

Comments received during public consultation

One MSCA supported no classification and addressed the lack of mechanistic data.

Assessment and comparison with the classification criteria

Two chronic studies in CD-1 mice (from the same supplier) revealed increased incidences of hepatocellular adenomas in male mice. Statistical significance was not gained at 300 ppm (49 mg/kg bw/day) and was only seen in male mice at 1000 ppm (135 mg/kg bw/day) in a supplementary study of the same design. The study control groups showed lower incidences in this supplementary study (4/60 and 5/60) than in the first mouse study (8/60 in both control groups). The incidences in the study controls of the supplementary study were slightly below the given historical range (6/60 to 13/60) and thus the statistical significance may be explained by the rather low control incidences.

However, it was not documented whether the historical control ranges came from contemporary studies.

Based on the CLP Guidance, liver tumours in B6C3F1 mice were generally accepted as being an example of a mouse strain with high spontaneous tumour rates that limit the biological significance of substance-related increased incidences in carcinogenicity studies. The reported historical control range of 6/60 to 13/60 corresponds to findings in published data on CD-1 mice. From a total of 49 carcinogenicity studies from the 1990's to more recently conducted studies (until 2012) Charles River Laboratories reported spontaneous rates in CD-1 mice at 15% and about 3% in males females, respectively[1]. The same breeder reported in 1995 (close to the time of study on metaldehyde) ranges of 6-12% for males and 0-2% for females[2]. Chandra and Frith (1992) reported spontaneous incidences of 11% in 725 male mice and 1.8% in 725 female mice. These data show that at least a moderately high level of spontaneous liver adenomas are known for the CD-1 male mouse.

[1] http://www.criver.com/files/pdfs/rms/cd1/act_2012_cd1_mice_carcinogenicity_study_data.aspx

[2] http://www.criver.com/files/pdfs/rms/cd1/cd1-mouse-tox-data-1995.aspx

Increased incidences of hepatocellular adenomas in female CD-1 mice were statistically significantly increased at 1000 ppm only (5/60 in comparison to 0/60 and 1/60 in control groups), but no dose-related effect was seen at concentrations up to 300 ppm and the absolute incidence of adenoma at the high dose was low. Although information on the spontaneous incidences in female mice at the source is not given, it remains uncertain whether 5/60 adenomas in female CD-1 mice at 1000 ppm should be considered to be treatment-related.

In female rats (see Table "Initial and peer reviewed liver histopathology findings in SD rats", above), there was a slightly higher incidence of liver cell adenomas in the high dose group in comparison to the internal control groups (Gill and Wagner, 1992). However, the incidence was only significantly different from one of the two control groups and did not show dose-dependence. A non-significantly increased incidence of liver cell carcinomas was seen in male rats from 50 ppm onwards. Tumour data were not presented in the CLH report from the second rat study (Verschuuren *et al*., 1975), which was of limited validity (low number of animals/group, summarised information only from a publication). According to the original publication, no tumours were observed in the livers of control and treated rats in this study.

No metaldehyde-related increase of hepatocellular carcinomas or on other types of tumours in the liver or elsewhere were observed in studies on mice. The slight increase in liver carcinomas in all dose groups in male rats were not significantly different from controls .In addition, no dose-response relationship was obvious, nor a clear increase in incidence over a rather large dose-range (one hundred-fold difference between the lowest and the highest dose). Thus, it may be considered questionable whether this is a treatment-related effect.

Few mechanistic data on the tumourgenesis of hepatocellular adenomas in CD-mice are available. Based on this information, it is assumed that metaldehyde is not acting via a genotoxic mechanism. Increased incidences of necrosis and single cell necrosis, as observed in the second supplementary mouse study, may be indicative of a regenerative proliferative response (however no hepatocellular hyperplasia was reported). The pigment accumulation and histiocytosis could be interpreted as secondary to cytotoxic effects. Verschurren *et al.* (1975) found increased activities of liver enzymes aniline hydroxylase ((AH) and aminopyrine demethylase (APDM).

A statistically significant increase in liver adenomas in CD-1 mice was observed. However, uncertainties about the causality to metaldehyde treatment remain as it cannot be excluded that the increase in liver adenomas in male mice resulted from low incidences in the laboratory control groups and whether the marginal increase in female mice were treatment-related at all. Hepatocellular adenomas did not progress to increased rates of malignant liver tumours in the concentrations tested up to 1000 ppm in CD-1 mice. These considerations do not support a firm conclusion that metaldehyde is carcinogenic in this species. The higher incidence in male mice than the marginal increase in female mice corresponds to the sex differences seen in untreated mice from the same breeder.

As to the rat data, RAC takes note of the overall limited data and uncertainties from the available studies. The significant increase in liver cell adenomas as seen in female rats at 5000 ppm in comparison to one of the two control groups together with the increased incidences of liver cell carcinomas in male rats could potentially justify a classification as a carcinogen. RAC is in particular concerned about the increased numbers of liver cell carcinomas in the male rat without any mechanistic explanation. Although no clear dose response relationship was seen in these dose groups (despite a very large dose range), their incidences were clearly (but not statistically significantly) above the control incidence. The information on historical control data given in the CLH report was not documented in sufficient detail to decide whether the observed control incidences were within the expected ranges. The CLH report referred to control data from two studies (60 rats/sex/group) conducted at the laboratory using the same source of animals and concluded that the elevated incidences seen in female rats were within the historical background

range. The timely relationship to the Gill and Wagner study (1992) is unknown and the overall amount of historical control animals (2 studies only, documented in Table 53 of the CLH report) was too small as a basis for comparison.

Moreover, RAC observes that the marked differences between the initial liver histopathology findings and the findings after internal peer review (Table 54 in the CLH report) created notable uncertainties. In addition, the incidences of liver tumours in Tables 53 and 54 of the CLH report showed inconsistencies. Definitions on the diagnostic terms and justifications why the differences occurred were missing.

The increased incidences and severity of liver cell hypertrophy in the mid and high dose groups did not give any hint on the mode of action. RAC notes the insufficient considerations and the lack of additional investigations on the mode of action.

The overall impression was that the reliability of the rat study and its documentation is limited. Whereas this leaves some concern on the slight increase in liver tumours, RAC in the end considers that due to the identified uncertainties and inconsistencies, the low reliability of the rat data does not allow a firm conclusion that metaldehyde has a carcinogenic potential in rats. Combining this with the similar conclusion for mice, RAC concludes that **no classification for carcinogenicity** is appropriate.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on fertility

Two two-generation studies were submitted. The newer study (*Chun and Neeper-Bradley, 1993*) was conducted according to international test guidelines and GLP and is considered valid. The older study (Verschuuren *et al., 1975*) was not conducted according to recognised guidelines or GLP.

In the newer two generation study (OECD TG 416) conducted in CD rats, no effects on reproductive performance (see Table 66, CLH report) were noted throughout the study. The NOAEL for reproductive toxicity therefore is proposed to be higher than the highest dose tested: > 2000 ppm (equivalent to 134 mg/kg bw/day bw/day in males and 160 mg/kg bw/day in females). Systemic parental toxicity was evident at the high dose level (2000 ppm) when three females of the F0 generation developed paralysis (both hind legs) and were sacrificed moribund. Histopathology showed haemorrhage and necrosis of the spinal cord. Such findings were also observed in the older two generation study (Verschuuren *et al*., 1975), where 50-75% of the females of the 5000 ppm dose group and up to three females of the 1000 ppm dose group developed posterior paralysis with transverse lesions of the spinal cord. None of the males was affected in any generation in either study. The finding of hind limb paralysis was also observed in repeated dose toxicity studies at high dose levels, however, the increased body weight of the pregnant females may have contributed to this effect. Further signs of systemic toxicity were reduced body weight in F1 females receiving 1000 or 2000 ppm and increased liver weight in both sexes receiving 2000 ppm. Offspring toxicity was evident at 2000 ppm and resulted in decreased body weight (day 14 and day 21) and body weight gain (day 7-14). In conclusion, the NOAEL for parental systemic toxicity was 50 ppm (equivalent to 3.2 mg/kg bw/day in males and 4.0 mg/kg bw/day in females) based on reductions in body weight. For offspring toxicity, the NOAEL was 1000 ppm (equivalent to 65 mg/kg bw/day in males and 81 mg/kg bw/day in females), again based on the reductions in body weight and body weight gain.

In the 26 week (Neumann, 1980, 1991; Leuschner, 2009) and 52 week dog toxicity studies (Leuschner, 2002; Leuschner, Drommer, 2006), diffuse atrophy of the testes and/or degeneration of the germinative epithelium were observed from 60 mg/kg bw/day onwards. However as fertility was not affected in the two generation study in rats this effect in dogs was not considered relevant for reproductive toxicity, but should be covered by classification as STOT RE 2.

Developmental effects

In the rat developmental toxicity study (OECD TG 414; Neeper-Bradley, Chun, 1990) severe maternal toxicity was observed at the highest dose level of 150 mg/kg bw/day, including mortality, clinical signs and reduced body weight and body weight gain. No effects regarding maternal toxicity were observed at the low and mid dose. No effects on gestational parameters or on foetal toxicity were observed at any dose. In conclusion, the NOAEL for maternal toxicity in rats was 75 mg/kg bw/day and the NOAEL for foetal toxicity and teratogenicity was greater than 150 mg/kg bw/day.

In the main developmental toxicity study in rabbits (Neeper-Bradley, 1990b) no maternal toxicity or effects on gestational parameters and development were observed up to the highest dose tested of 80 mg/kg bw/day.

The DS concluded that no specific effects on fertility and no developmental toxicity was observed following metaldehyde administration to test animals. No classification was proposed.

Comments received during public consultation

One MSCA agreed with the proposal for no classification.

Assessment and comparison with the classification criteria

RAC agreed with the DS that no classification on developmental toxicity is warranted.

Regarding fertility, the concern from testicular toxicity in dogs has to be assessed together with the clinical signs of toxicity (ataxia, tremor) seen at 90 mg/kg and the late deaths at 30 and 90 mg/kg bw/day. While no mortality occurred in male dogs at 90 mg/kg during 52 weeks of exposure (a female dog at this dose died with pneumonia), one male dog at 30 mg/kg that died prematurely showed moderate testis toxicity. However, 2 other dogs with minimal to mild testes (prostate) toxicity did not show any other signs of toxicity. This indicates that testis toxicity was a direct effect of metaldehyde treatment.

The evidence for testis toxicity was supported by the findings of increased severity of focal testis atrophy at 60 mg/kg bw/day and increased severity and incidence of bilateral diffuse atrophy in 4 out of 6 male dogs at 90 mg/kg bw/day treated for 26 weeks. As no clinical symptoms or other signs of general toxicity were reported for doses up to 90 mg/kg bw/day, these effects were considered as direct effects of exposure to metaldehyde.

RAC discussed the data on fertility dysfunction and the overall data available that may warrant classification for reproductive toxicity, category 2, or no classification. Since there was no evidence that the testis toxicity seen in dogs is less relevant than the (negative) data from rats, that did not show testis toxicity in the repeated dose studies and where no impact on fertility parameters were seen in the two-generation study, RAC considers that the testis toxicity seen in dogs is relevant and may pose a hazard to humans. Toxicokinetic data that could be helpful to identify species differences and peculiarities were only available for the rat. Thus the testis

toxicity observed in dogs in the absence of general toxicity supports the need for classification as toxic for reproduction, category 2, for effects on fertility.

RAC therefore concluded that **classification as Repr. 2; H361f is warranted**.

A specific concentration limit (SCL) was not proposed by the DS, nor was it discussed by RAC. As the effective dose levels (and their ED_{10}) could be estimated to be above 4 mg/kg bw/day, there was no need to further discuss a SCL.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Metaldehyde is a molluscicide for the control of slugs and snails, it was approved under Council Directive 91/414/EEC in 2008. Metaldehyde is already listed in Annex VI of the CLP Regulation with harmonised classifications as Flam. Sol. 2 and Acute Tox. 4*.

Degradation

The DS proposed not to consider metaldehyde as rapidly degradable for classification purposes. The basis for this proposal was that metaldehyde is hydrolytically stable at a temperature of 25 °C and pH values of 5 – 9 (Carpenter, 1989a), photolytically stable at pH 7 and a temperature of 25 °C (Carpenter, 1989b), not readily biodegradable following OECD TG 301 E (Wüthrich, 1990a) and OECD TG 301 F (Lebertz, 2008), and not inherently biodegradable following OECD TG 302 B (Wüthrich, 1990b). In addition, metaldehyde was shown to have long residence times under OECD TG 308 (Kane, 2009) and dissipated from the water phase with a DT_{50} value >1000 days in silt loam system and 473 days in sand systems. DT_{50} values for the dissipation from the total system were >1000 days in silt loam system and 714 days in sand system (these values were incorrectly reported in the CLH dossier). In a BBA (German, Federal Biological Research Centre for Agriculture and Forestry) Guideline water/sediment-study (Möllerfeld *et al*., 1993), the DT_{50} values were calculated to be 30.98 and 19.01 days with sandy and loamy system, respectively. However, these were based on acetaldehyde, the major degradation product of metaldehyde.

Aquatic Bioaccumulation

The DS proposed to not consider metaldehyde as being bioaccumulative in the aquatic environment for classification purposes. The basis for this proposal was an OECD TG 107 (Shake flask method) test with a measured partition coefficient n-octanol/water log K_{ow} of 0.12 (19.9 - 20.1 °C, pH: 6.7) (Cardinaals, 1988b) and an OECD TG 305 E test with a measured BCF value of 11 (whole fish at steady state) (Unpublished, 1992).

Acute Toxicity

The DS proposed to not classify metaldehyde as acutely hazardous to the aquatic environment. The basis for this proposal was that the available short-term (acute) aquatic toxicity test results are >1 mg/L, showing toxicity of 75 mg/L for fish (96 h LC_{50}), 90 mg/L for crustacea (48 h EC_{50}), >200 mg/L for algae (72 h ErC_{50}) and > 200 mg/L for *Planorbarius corneus* (Great Ramshorn Snail) (48 h EC_{50}; immobility 24 h after termination of exposure and after recovery in clean media). The results indicated that fish were the most sensitive taxon and that the most sensitive acute endpoint is a 96 h LC_{50} = 75 mg/L (nominal concentration) for *Oncorhynchus mykiss* (Rainbow Trout).

Chronic Toxicity

The DS originally proposed to not classify metaldehyde as chronically hazardous to the aquatic environment. The basis for this original proposal was that the available long-term (chronic) aquatic toxicity test results are > 0.1 mg/L for a non-rapidly degradable substance, showing toxicity of 37.5 mg/L for fish (21 d NOEC), 90 mg/L for Crustacea (21 d NOEC) and 25 mg/L for algae (NOEC).

Comments received during public consultation

Four MSs commented on the proposal, with three agreeing with the proposal to not classify metaldehyde for aquatic environmental hazards. The fourth MS pointed out that the OECD TG 204 "Prolonged Toxicity Test: 14-Day Study" (Unpublished, 1990b) is not considered suitable for generating chronic (long-term) toxicity data (Information requirements Chapter R.7b: Endpoint specific guidance, Version 4.0, June 2017, page 30f) and that the test guideline has been withdrawn by the OECD. The MS argued that as such a data gap on chronic toxicity exists and acute fish data should be used in conjunction with degradation data under the surrogate approach to derive the potential chronic classification. In their response to this comment, the DS agreed to withdraw the NOEC value of the 21 d prolonged fish test and to base the chronic hazard assessment on the lowest LC_{50} for fish and environmental fate data via the surrogate approach, resulting in a classification as Aquatic Chronic 3; H412.

The same MS also commented on the acute toxicity study in snails (Egeler *et al*., 2007) and indicated that the EC_{50} should be based on immobility after 48 h (exposure termination) and not 24 h after exposure termination and recovery (when the snails had been transferred into vessels containing clean medium and a piece of cucumber as a food source). The DS agreed and estimated that, instead of the proposed endpoint of 48 h EC_{50} > 200 mg/L, the correct 48 h EC_{50} would be between 19 and 41 mg/L. However, the DS did not provide a new statistical evaluation of the study. Instead, the DS emphasised this would not change the proposal for acute aquatic classification of metaldehyde.

The same MS also pointed out that given the test species (*P. corneus*) is the only available ecotoxicity data for a mollusc species and metaldehyde is a molluscicide, this test result is important for classification. The MS proposed to include the EC_{50} value as a surrogate for chronic classification. Also, given ecotoxicity data is only available for one snail species and other gastropod molluscs may be more sensitive, the classification should be reconsidered in the future if further data becomes available. This might include data from efficacy studies if usable or snail studies following new OECD TGs 242 or 243.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal and argumentation of the DS to not consider metaldehyde as rapidly degradable for classification purposes.

Aquatic bioaccumulation

RAC agrees with the proposal and argumentation of the DS that metaldehyde is not bioaccumulative and therefore does not meet the criteria for bioaccumulation.

Acute Toxicity

RAC agrees with a commenting MS and with the response of the DS that the derived endpoint from Egeler *et al*. (2007) is not valid and instead an EC_{50} at the end of exposure at 48 h must be used. In general, RAC assesses this study as relevant since metaldehyde is a molluscicide. It is also important to note that the study was considered relevant by the RMS (Rapporteur Member State) under Reg. (EC) No 1107/2009 and by the DS when preparing the dossier. RAC assesses the test methodology as acceptable (according to Section 1 of Annex XI to the REACH Regulation (EC) No 1907/2006) and suitable for classification (CLP Regulation, Annex I, section 4.1.2.7.1). RAC considers the study to be reliable and concludes that immobility of *P. corneus* is an adverse effect which shall be taken into account for the purpose of classification. Consequently, this study is given the same weight as standard tests, meaning it is appropriate to consider it in an overall weight of evidence approach (CLP Regulation, Annex I, section 4.1.2.1). RAC has reassessed the study by Egeler *et al*. (2007) and concludes that the measured values show a clear concentration-response relationship at the end of the exposure period (48 h). As a result, RAC concludes that a 48 h EC_{50} of 78.2 mg/L (nominal), as re-calculated by RAC, should be used for classification.

In addition, RAC has reassessed the OECD TG 204 study (Unpublished, 1990b) as a prolonged acute study. No lethality was observed and only a 21 d EC_{50} (body weight) of 89.1 mg/L (nominal) for *O. mykiss* was derived from this study. However, as this endpoint is not equivalent to the available standard acute toxicity test results on fish, RAC concludes that the study is not relevant for the purpose of acute classification.

The following data for acute (short-term) aquatic toxicity are relevant for the purpose of acute classification:

	fish	**Crustacea**	**algae**	**gastropod molluscs**
included in dossier	75 mg/L (96 h LC_{50})	90 mg/L (48 h EC_{50})	>200 mg/L (72 h ErC_{50})	-
new after RAC evaluation	-	-	-	78.2 mg/L (48 h EC_{50} immobility)

RAC concludes that, besides fish, gastropod molluscs are the most sensitive group for acute (short-term) aquatic toxicity of metaldehyde, with Crustacea as the third tropic level with results in the same range. Overall - and including the reassessment of the study by Egeler *et al*. (2007) – all acute toxicity results are > 1 mg/L and RAC agrees with the proposal of the DS that metaldehyde does not meet the criteria for aquatic acute hazard classification.

Chronic Toxicity

RAC agrees with a commenting MS and with the DS's response that adequate long-term fish toxicity data cannot be derived from OECD TG 204 and as such the data from the study (Unpublished, 1990b) must not be considered for chronic classification. This is in line with ECHA guidance (Information requirements Chapter R.7b: Endpoint specific guidance Version 4.0 June 2017). RAC concludes that this study is not relevant for chronic classification.

RAC assessed the new chronic toxicity fish study OECD 210 (Unpublished, 2016) and accepts the results presented in the study report.

The following data for chronic (long-term) aquatic toxicity are relevant for the purpose of classification:

	fish	Crustacea	algae	gastropod molluscs
included in dossier	-	90 mg/L (21 d NOEC)	25 mg/L (NOEC)	-
newly available	> 25 mg/L (NOEC)	-	-	-

RAC notes that data are available for the three standard trophic levels from chronic aquatic toxicity studies with metaldehyde. These NOECs for fish, Crustacea and algae are all > 0.1 mg/L and do not meet the criteria for aquatic chronic classification of a non-rapidly degradable substance. However, fish (along with gastropods) were the most sensitive group under acute testing and the fish species used under chronic testing is not the same as that from the acute data set, so it is unclear if the chronic fish data truly represents the long-term hazard for this trophic level.

Furthermore, as metaldehyde is a molluscicidal substance and the Egeler *et al*. study (2007) is considered suitable for consideration under acute aquatic classification, RAC notes that no chronic toxicity data for molluscs are available.

RAC concludes that a weight of evidence approach is appropriate and that all information in addition to the standard dataset for the three trophic levels should be taken into account for a full description of the hazard of metaldehyde. As a consequence, RAC concludes it is appropriate to use a combination of environmental fate and acute toxicity data to derive aquatic chronic toxicity by applying the surrogate approach. In doing so, taking into account that the substance is not rapidly degradable and the aquatic snail 48 h EC_{50} (immobility) of 78.2 mg/L, a classification of metaldehyde as Aquatic Chronic 3; H412 is derived. The surrogate approach provides the worst case outcome and forms the basis for classification (the classification derived from the standard long-term toxicity data provided is 'no classification').

In conclusion, following a weight of evidence approach and using acute toxicity data from molluscs in combination with environmental fate data, metaldehyde meets the criteria for classification as **Aquatic Chronic 3; H412**.

RAC further notes that the classification may be reconsidered in the future if further data on chronic (long-term) aquatic toxicity for gastropod molluscs becomes available. This might include data from efficacy studies, if usable for classification purposes, or gastropod mollusc studies following the new OECD Test Guidelines 242 or 243.

Additional references

Unpublished (2016) Metaldehyde Technical: A study on the toxicity to Early-Life Stages of Zebrafish. According to Guideline No. 210 "Fish, Early-life Stage Toxicity Test"

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).